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PLAGUE
PLAGUE

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CORRIGENDA

Chapter 2, page 101, table XI

Line 24 (Manchuria . . .), columns 3 and 4

delete   83   18
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Line 36 (Total . . .), columns 3 and 4

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Preface

In reviewing the history of plague research from the discovery of Pasteurella pestis, in 1894, to the present day, one cannot fail to perceive that, although work in this field of applied science was carried on assiduously the whole time, the greatest progress was made at the beginning and at the end of this sixty-year period. During the twenty years immediately following the isolation of the causative organism, the work of such pioneers as Yersin, Simond, Albrecht, and Ghon, together with the comprehensive studies of the Plague Research Commission in India, laid a secure foundation for future investigations; and it was also in this period that plague vaccination was introduced by Haffkine and, thus, the first milestone on the road to effective plague-control was reached. But it is only within the last decade that treatment with sulfonamides and antibiotics, on the one hand, and the application of potent insecticides—particularly DDT—on the other, have rendered plague both a normally curable and a thoroughly controllable disease.

The last publication in the English language to deal comprehensively with the plague problem was a manual compiled in 1936 by Wu Lien-teh, J. W. H. Chun, R. Pollitzer, and C. Y. Wu. Appearing as it did before the spectacular advances in the treatment and control of the disease had begun, this work, while still appreciated as a reference-book, has become rather outmoded. For this reason, and because he was the only one of the four authors who had remained active in plague research, the present writer was repeatedly urged to bring out a second edition. However, quite apart from the fact that the writer's work on plague and cholera control occupied the major part of his time both during and immediately after the second World War, several considerations militated against a re-edition of the 1936 manual. In the first place, the book was written primarily for workers in China, and contains some sections of little interest for the general reader. Secondly, as already indicated, the parts concerned with treatment and with the control of rats and fleas have become so obsolete as to be of historical rather than practical importance.
Hence, it became clear that it would be far better to publish an entirely new monograph, dealing with the plague problem as it exists today, than to attempt a revision of the 1936 manual.

It was therefore decided that, initially, a number of studies, dealing successively with all aspects of plague, should be published in the Bulletin of the World Health Organization, and that, eventually, these individual studies should be grouped and reprinted in book form. The first study was published in November 1951, the last in September 1953. Before publication in the present monograph, the studies were carefully revised and brought up to date. It is hoped that the final result will be of real assistance to workers engaged in plague research.
ACKNOWLEDGEMENTS

The writer's responsible task of compiling this monograph has been greatly alleviated through the advice and help generously given by many colleagues and friends. Invidious as it is to single out some of those who have thus earned his gratitude, the writer wishes to mention specifically: Mr. D. H. S. Davis of the Plague Research Laboratory, Union Department of Health, Johannesburg, Union of South Africa, who provided much useful information on plague-infected rodents and fleas in Africa; Mr. G. H. E. Hopkins of the British Museum (Natural History), Zoological Museum, Tring, England, whose assistance was of great value in connexion with the chapters on rodents and fleas; Dr. V. B. Link and Dr. C. O. Mohr of the Communicable Disease Center, Atlanta, Ga., USA, who supplied additional data on plague in the United States of America; Dr. A. Macchiavello, formerly Expert Consultant on Plague at the Pan American Sanitary Bureau, Washington, D.C., USA, who kindly made available unpublished lists of reservoirs and vectors of plague and furnished much valuable information on plague in South America; Dr. K. F. Meyer, Director of the George Williams Hooper Foundation, University of California Medical Center, San Francisco, USA, who was indefatigable in giving his generous assistance in many ways; Mr. F. G. A. M. Smit, Custodian of the Rothschild Collection of Siphonaptera at the Zoological Museum, Tring, who contributed an important annex—enriched by excellent drawings he had prepared himself—on the identification of fleas; and Dr. S. S. Sokhey, formerly Director of the Haffkine Institute, Bombay, and Dr. P. M. Wagle, the present Director of the Institute, who supplied most useful information on plague and its control in India.

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The George Williams Hooper Foundation, University of California Medical Center, San Francisco, Calif., USA (fig. 5-9, 15-23, and 32);

The Institut Pasteur, Paris, France (fig. 11 and 12) and the Institut Pasteur, Madagascar (fig. 33 and 34);

The Wellcome Museum for Medical Science, London, England, Dr. G. Robertson, Capetown, Union of South Africa, and Colonel P. V. Karanchandani, Madras, India (fig. 28-31);

The Haffkine Institute, Bombay, India (fig. 24-26); and

Ciba Aktiengesellschaft, Basle, Switzerland (fig. 1, 2 and 10).

Others have been reproduced, by kind permission of the editors, from the following publications:

Indian Journal of Medical Research, 1939/40, 27, 325, 326—fig. 13 and 14 (by permission of the Indian Council of Medical Research);

Rat-borne disease: prevention and control, 1949, pp. 55, 60, 61, 266 (United States Public Health Service, Communicable Disease Center, Atlanta, Ga.)—fig. 27, 36, 37, and 39;

Control of rats and mice, 1948, pp. 20, 25 (University of California, College of Agriculture, Agricultural Extension Service Circular 142)—fig. 35 and 38.

Finally, the author wishes to express his gratitude to Dr. G. Girard, Chef du Service de la Peste, Institut Pasteur, Paris, who, besides rendering valuable help in other ways, has undertaken the onerous task of translating the studies into French. Thanks to his work, the readership of the monograph will be greatly increased.
Chapter 1

HISTORY AND PRESENT DISTRIBUTION OF PLAGUE

HISTORICAL SUMMARY

Dealing with the historical aspects of the plague problem in 1936, Wu Lien-teh maintained that the disease had been present since time immemorial in the areas within or near the Central Asiatic plateau which he considered as the original home of the infection. He noted that some authors were inclined to place this in Central Africa but, though the focus existing there was undoubtedly of very old standing, Wu Lien-teh was in agreement with the statement of Payne that "possibly, if we could follow the history far enough back, we might find that the African was a colony of the Asiatic plague."

Basing his statement upon the authority of Sticker, Wu Lien-teh maintained that the first plague epidemic on actual record was the outbreak among the Philistines in 1320 B.C. which, as described in the Bible (1 Samuel, v and vi), was characterized by the appearance of "emerods in their secret parts."

As claimed by some recent writers, this interpretation of the biblical text has become untenable. Dealing with this subject in 1942, Neustätter pointed out the interesting fact that the identity of the emerods with plague boils had been mentioned already in a marginal note to the revised version of the Bible appearing in 1885, that is, at a time when people in Europe paid little, if any, attention to the subject of plague. It seemed, however, that the 1885 compilers had followed the lead of the Bible commentator Thenius who had asserted the plague nature of the Philistine outbreak about 50 years earlier. Thenius in his turn had probably been influenced by the writings of J. J. Scheuchzer (1672-1733), doctor of medicine and
professor of mathematics and physics at Zürich, Switzerland, referring to the 1720 epidemic in Marseilles, France.

While not definitely committing himself as to the real nature of the biblical outbreak, Neustätter concluded "that the version hemorrhoids, untenable as it is, comes nearer to . . . the truth than plague-boils". Shrewsbury and Girard felt certain that the emerods were really haemorrhoids because they considered that the disease decimating the Philistines was bacillary dysentery. However, this assumption was vigorously opposed by MacArthur, who asserted the plague nature of the Philistine outbreak on historio-epidemiological and philological as well as on clinical grounds and, in a further note, also adduced evidence putting "the great antiquity of the rat [Rattus rattus] in Palestine beyond question".

A further, generally accepted, record testifying to the existence of plague in the West during the pre-Christian era is contained in a fragment from the writings of Rufus, physician at Ephesus about A.D. 100, who noted the occurrence of fatal bubonic plague in Libya, Egypt, and Syria during and before his time, apparently as far back as about the end of the third century B.C. (Wu Lien-teh).

Whether this scanty information refers to occasional manifestations of the disease which remained localized, or whether some of these outbreaks were episodes of an early pandemic, it is impossible to decide. It is certain that the first really satisfactory evidence regarding the prevalence of plague concerns a pandemic commencing in the fifteenth year of the Emperor Justinian's reign (A.D. 542), which was voluminously dealt with by contemporaneous writers. In the opinion of most of these chroniclers, the pandemic had started at Pelusium in Lower Egypt, but probably this port served merely as the distributing centre of an infection derived from an endemic focus. The contention of Evagrius that the plague had come from Ethiopia might suggest a Central African origin of the pandemic during Justinian's reign (Wu Lien-teh).

Lasting for a period of fifty to sixty years, the pandemic gradually spread, as one of the chroniclers put it, "to the ends of the habitable world". Usually seaports were invaded first, the infection then progressing inland and eventually involving even the most sequestered localities (Procopius, quoted by Gibbon). It was estimated at the time that the number of victims might have reached a total of one hundred million and Gibbon, when scrutinizing the records of the contemporaneous writers, considered this figure as "not wholly inadmissible". Certainly the outbreak of plague in Justinian's time which, as deplored by the chronicler Warnefried, "depopulated towns, turned the country into a desert, and made the habitations of men to become the haunts of wild beasts" was one of the worst calamities that ever befell mankind. As Hirsch points out, it is possible that the simultaneous presence of other epidemic diseases partly accounted for this death-toll, but the contemporaneous records leave no
doubt that bubonic plague took the foremost part. The question of whether or not commensal rodents played an important role in the causation of these bubonic epidemics is much debated. In the opinion of many writers, because rats (R. rattus) were not imported into Europe before the 12th century through the ships of the returning crusaders, they were still absent at the time of the pandemic during Justinian's reign. It is generally admitted that commensal mice were already abundant in the West at that period, but, as stressed by Shrewsbury and Girard, usually these rodents, though apt to become secondarily involved in the course of rat epizootics, do not play an independent role in the causation of human outbreaks. The same view was strenuously advocated by Tricot-Royer who came to the conclusion that

"interhuman transmission formerly played a considerable if not predominant role in the spread of epidemics, and was often even the only factor concerned".

However, it deserves attention that this opinion was not shared by MacArthur, who adduced evidence pointing to the early presence of rats in Europe.

Though it is likely that plague outbreaks, due to fresh importation of the infection if not to its local persistence, continued to occur in the West as well as in the East, the information actually available is usually unsatisfactory until once more the evil culminated in a pandemic which, as Hirsch put it,

"arrested the attention of the chroniclers, poets, and physicians of these days; and that interest was awakened by the enormous diffusion that it reached over the whole of the then known world, by its victims reckoned in millions, and by the shock to the framework of society which it brought with it and left behind it".

While it is uncertain whether the pneumonic form of the disease played an important part in the plague outbreaks in Justinian's reign, this type figured prominently in the pandemic of the 14th century which, for not fully elucidated reasons (see Wu Lien-teh), became known under the name of the "Black Death". However, rats being without doubt implicated in the perpetuation of the infection, the bubonic form was also frequent, or even preponderant, as for instance in rural England (Greenwood).

As mentioned above, it is possible that in the case of the pandemic in Justinian's time the infection had been derived from the Central African plague focus. No doubt can exist that the Black Death originated in Central Asia. Circumstantial evidence for this assumption was brought forward by Wu Lien-teh who showed that this pandemic was not restricted to Europe and the Near East but was rampant in India and China as well: a spread of the infection from its original home in inner Asia southwards and eastwards as well as towards the west was therefore likely.

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\[a \text{ "dans l'épidémisation ancienne, la convection interhumaine joua un rôle considérable, sinon prédominant, et souvent même exclusif."} \]
Through a fortunate accident the present writer was able to obtain confirmation for this contention. In a book entitled *The Nestorian Missionary Enterprise* by Stewart a reference was found to the work of the Russian archeologist Chwolson near Issyk Kul Lake in the Semirechinsk district, an area now known to lie within the Central Asiatic plague focus. Chwolson found in old Nestorian graveyards three memorial stones dating back to the years 1338-9, the inscriptions on which showed that the persons referred to had succumbed to plague. It was, moreover, evident that during the period in question an exceptionally large number of burials had been made. It is certain, therefore, that plague was conspicuous in Central Asia a few years before the Crimean ports became infected (1346) and the disease was carried from there by ship to Europe.

In Great Britain, from half to two-thirds of the people are believed to have been killed by the Black Death. Hecker's estimate that 25 millions or one-fourth of the population fell victim to the pandemic in Europe was therefore perhaps conservative. Millions succumbed in Asia as well where, according to Gabriel de Mussis, an eyewitness of the outbreak in the Crimea,

"so great was the mortality that Arabs, Saracens and Greeks throughout the whole of the East gave themselves up to clamour ".

It was inevitable that in many places the infection introduced during the pandemic became firmly entrenched among the rats. As a result, many countries in Europe continued to have frequent or even perennial epidemics during the centuries following the Black Death. However, as described by Wu Lien-teh, in the course of the 17th century a decline set in, leading to the gradual disappearance of the disease first from western and then from eastern Europe until in 1841 Turkey, the last stronghold of the pest, became free.

The reasons for the cessation of plague in Europe have been the subject of much debate. Great stress was laid by some authors upon the change in the rat population by which *R. rattus* became largely superseded by the sewer-rat (*R. norvegicus*). However, as shown in a classical study by Jorge, the invasion of the latter rodent, taking place during the first half of the 18th century, occurred long after the retrogression of plague from the western part of the continent. Some writers considered the progress of civilization which led to higher standards of cleanliness, housing, and sanitation a possible cause for the disappearance of the infection. However, as pointed out by Wu Lien-teh, a lull during the period under consideration was conspicuous not only in Europe but in the Near East, India, and China as well. It seemed likely, therefore, that a natural decline of plague was responsible for the cessation of the outbreaks, rather than extrinsic factors which at best could have been of auxiliary importance only and became fully operative well after the disappearance of the evil from Europe.
Though, as discussed above, autochthonous plague ceased to exist in Europe and many parts of the East, occasional outbreaks due to an importation of the infection from still active foci continued to occur. Thus, while France had become generally free by 1668, an epidemic, believed to have been due to importation from Syria, and sweeping away 50,000 people (Gibbon 39), took place at Marseilles, France, in 1720; the infection even spread over a great part of Provence but disappeared in 1722. Likewise, while most parts of India seem to have become plague-free at the end of the 17th century, outbreaks, supposed by some to be due to importation from Persia, occurred during the period 1812-21 at Cutch, Gujarat, and Kattyawar, and in 1836-8 at Pali in Rajputana.

Far more important than these outbursts was that, with its deep-rooted tendency to become latent rather than to disappear altogether, plague continued to linger in quite a considerable number of endemic foci. As stated by Wu Lien-teh, 158 the most important of these were situated in and around the Central Asiatic plateau in Russian Turkistan, Semirechinsk, Chinese Turkistan, Inner Mongolia, Outer Mongolia, and Transbaikalia; in the foothills of the Himalayas in northern India; in Kurdistan as well as in Central Africa and possibly also in parts of North Africa.

It would seem that by the end of the 18th century plague outbreaks had become frequent in the north-east of Burma and that inroads of the infection into the adjacent part of Yun-nan Province in China took place. As discussed by Wu Lien-teh, 160 during the first half of the 19th century the disease appears to have become established in the extreme west of Yun-nan without, however, prevailing epidemically.

Quite possibly under normal conditions the infection would have continued to smoulder in west Yun-nan without spreading farther afield. Most unfortunately, however, the equilibrium was upset by a rebellion of the Mohammedans commencing in 1855, for the suppression of which troops had to be sent in. Their operations and possibly also movements of refugees provided suitable means for a propagation of the disease which was to prove disastrous not only for China but for many other parts of the world.

Progressing in general by slow stages, plague reached the provincial capital Yun-nan-fu (now Kunming) in 1866 and it took 28 years more before Canton and Hong Kong were reached in 1894. Rather surprisingly, however, the disease appeared at Pak-hoi in Southern Kwang-tung in 1867 already. Serious doubts were entertained as to whether the invasion of this port could have been due to a long-distance sprint of the infection from Yun-nan. However, since it is difficult to see by what other route Pak-hoi could have been reached, one has to accept the explanation of Simpson: 138

"An epidemic of plague occurs in Yunnanfu in 1866, which decimates the population while they are in the midst of war, and in 1867 Pakhoi, one of the homes of returning troops from Yunnan, is attacked."

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It is depressing to reflect that the situation confronting the world when Hong Kong was invaded was in some respects even worse than that at the onset of the pandemic of Justinian's time, in Pelusium. For while in A.D. 542 the means for transporting the infection were slow and comparatively inadequate, and the orbit within which it could spread was limited, in 1894 steamships and railways had replaced the small sailing-craft and caravans of Justinian's day and the new as well as the old world were open to the inroads of plague. It is true that progress in civilization and public health had made it impossible for the infection to gain a firm foothold in modern Europe, but in many other parts of the world ample fuel was available for its perpetuation and spread.

To draw even in broad lines an adequate picture of the progress of plague since 1894 would be a task far beyond the scope of the present survey. All that can be done, and at the same time all that is really needed for the purposes of this monograph, is to come to a general appreciation of the situation in those areas where plague continues to exist or was recently present.

In compiling the present survey, advantage has been taken of the reports on the prevalence of plague in recent years published by Stowman in 1945 and by Kaul in 1949, and for most African plague foci also of the results of an inquiry instituted by the WHO Expert Committee on Plague at its first session (1950). Valuable information on some of the plague foci in East Asia was found in a series of reports by Wilcocks, while in the case of the Americas the studies of Moll & O'Leary served as a useful guide.

**PRESENT DISTRIBUTION OF PLAGUE**

**Asia**

(1) **China**

When trying to assess the present plague situation in China it is necessary to consider:

(a) The incidence of the disease at Pak-hoi since 1867, and at Canton and Hong Kong since 1894 and the spread of infection into Kwang-tung Province of which Canton is the capital and to which Hong Kong belongs geographically;

(b) The invasion of other provinces from Canton and Hong Kong by the sea-route;

(c) Outbreaks due to recent entry of the infection by inland routes;

(d) The reappearance of plague in Yun-nan Province.

(a) **Kwang-tung Province.** Epidemics at Pak-hoi, the first port which had been reached by plague, continued to be frequent up to the year 1902
and again from 1910 to 1915, the apparent lull during the period 1903-9 being presumably due to mere lack of information.

Perennial outbreaks of varying intensity continued to occur at Hong Kong until 1923; after that year sporadic cases were seen in 1928 and 1929. As maintained by Uttley in a retrospective study on plague in Hong Kong, the decline of the infection could not be ascribed to the sanitary improvements made, because even before 1923 the severity of the outbreaks had diminished elsewhere in south China. In fact, as far as one is permitted to judge from incomplete figures, epidemics had ceased to be perennial in Canton as early as 1916 with subsequent outbreaks in 1923 and 1925 (Wu Lien-teh 160).

The invasion of Canton in 1894 led almost immediately to a contiguous spread of the infection into Kwang-tung Province, most parts of which became successively affected. This period of expansion was followed by a gradual decline becoming manifest about the end of the first World War. At present only some districts situated at or near the Lui-chow Peninsula in the south of the province and east of Pak-hoi continue to be involved, but it would seem that the situation there has become worse recently, 627 cases having been reported during the period January-September 1950 as against 186 cases in 1949. Presumably, plague also continues on the island of Hainan which was originally invaded in 1900 (Landauer 72).

(b) Spread of the infection from Kwang-tung to other coastal provinces. Amoy, the principal port of south Fu-kien, became affected in 1894 soon after the appearance of the disease at Canton and Hong Kong. In 1899 the infection made a long-distance sprint to the port of Newchwang in south Manchuria whereas Foochow, the capital of Fu-kien, was invaded in 1901.

The disease continued to reappear at Newchwang and in its immediate hinterland for some years only. The invasion of Amoy and Foochow on the contrary had serious and lasting consequences. Plague not only continued to appear perennially in these two cities for a number of years but, owing principally to the dense water-borne traffic to smaller seaports as well as up the rivers, most of the counties of Fu-kien Province became successively affected. Though the frequency and extent of the infection seemed to be subject to variation, the recorded incidence of plague being low in certain years, the situation during the decade from 1937 to 1946 was, in general, rather serious. 4,764 cases in 35 counties were recorded in 1943, 7,157 cases in 42 counties in 1946; in both years serious epidemics occurred in Foochow city. From 1947 to 1949 there was a gradual decrease in the case incidence, but the situation seems to have become worse again in 1950 when up to the end of September 988 cases were reported as against a total of 368 cases during 1949.

Amoy is now quite free from infection. Rat epizootics continue to occur at Foochow, but it has become possible to prevent the appearance
of human plague through the systematic use of DDT and measures aiming at a reduction of the rodent population.

Plague in Fu-kien Province has become increasingly rural in character. Though pneumonic manifestations are occasionally met with, the bubonic form of the disease is preponderant. Commensal rats alone appear to be responsible for the perpetuation of the infection with *Xenopsylla cheopis* as the sole practically important vector.

It has to be added that owing to intensified traffic over hitherto little-used routes during the second World War the two provinces of Che-kiang and Kiang-si, formerly quite plague-free, became affected in 1940 and 1941 respectively. While the latter province seems to have been free from the end of 1949, a slight incidence of the disease continued during 1950 in Che-kiang where Wenchow remains the only major port on the China coast still suffering from human plague.

(c) Outbreaks due to recent entry of the infection by inland routes. Since China may be said to have some of the regions composing the Central Asiatic focus at her back door, it is not surprising to find that the country remained open to inroads of plague from these endemic areas.

Mention must first be made in this connexion of north Manchuria which, becoming infected by human agency from the wild rodent foci in Outer Mongolia and Transbaikalia, suffered from pneumonic plague epidemics in 1910-1 and again in 1920-1. Though exerting a grievous toll in deaths, these outbreaks, because they did not involve the local rat population, disappeared as rapidly as they had come once the climatic conditions ceased to favour spread of the infection from man to man.

In 1917-8 pneumonic plague, apparently derived from a wild-rodent focus in the Ordos country of Inner Mongolia (now Suiyuan), invaded Chahar and Shan-si and even spread to a slight extent into Chihli, Shan-tung, An-whei, and Kiang-su Provinces, the number of victims amounting to about 16,000. As described by Wu Lien-teh, further outbreaks, some of them mainly or solely bubonic in character, continued to occur in the north-west, infection being derived either from the Ordos Country or from endemic foci which had become established in Shen-si. In 1931 there was an ominous spread of the infection in Shan-si and Shen-si where, it was claimed, at least 20,000 people succumbed to mainly bubonic affections. To judge from incomplete information, a decline seems to have set in since. A mainly pneumonic outbreak with a case incidence of 485 started at the end of 1941 in Suiyuan and spread in 1942 also to Ningsia, Shen-si, and Shan-si (Fan 28). The presence of an epidemic which, originally bubonic in character, later assumed pneumonic features was reported in 1949 from Chahar where 69 cases with 66 deaths occurred from July to November in 10 villages. However, since the northern part of the province was stated to have been involved, it is possible that the infection was derived from the foci in south Manchuria or Jehol dealt with below.
To trace the origin of the plague outbreaks proved to exist since 1927 in south-west Manchuria is difficult. Weighing the scanty available evidence, Wu Lien-teh assumed that the infection, the history of which could be traced back to about 1917, originated from a wild-rodent focus in Inner Mongolia, possibly in Chahar which, as noted above, had been involved in the 1917-8 outbreak. However, no doubt can exist that about the time when the presence of plague was confirmed in south-west Manchuria epizootics existed among the commensal rats of the affected localities, especially among *R. norvegicus* (Hsiao), and *X. cheopis* served as vector.

The affected area, at first restricted to the Tungliao region in the west, gradually extended. Even north Manchuria, which had been free from plague since 1921, became involved in the 1946 outbreak, while in 1947, the last year for which information is available, 200 fatal cases were reported from Kirin Province in the east of south Manchuria. Jehol, where an endemic focus existed at the close of the 19th century, again reported an outbreak in 1933 which was probably due to a fresh importation from south Manchuria. To judge from the scanty information recently available, the plague situation in Jehol became once more serious in 1946, when 15,000 cases seem to have been recorded. The case incidence in 1947, when an energetic anti-plague campaign was conducted, seems to have amounted to about 2,000. Only a few dozen cases were recorded in 1948.

While the epidemics in south Manchuria were preponderantly bubonic in character, occasional pneumonic manifestations were met with. An outbreak of this type (39 cases with 3 recoveries) in 1946 was described by Tieh et al. (d) Reappearance of plague in Yun-nan Province. As in the case of the historic plague invasion of Yun-nan dealt with above, it is impossible to state exactly when the recent re-entry of the infection into the province commenced. Presumably now as then the manifest appearance of the disease had been preceded by unnoticed invasions of villages just across the Burma border. It is certain that as the result of a serious outbreak at Nam Kham, Burma, in 1939 the infection not only reappeared early in 1940 in that area, but also spread across the Shweli River into Chinese territory (UNRRA Report), where, however, the Mungmao district alone became involved.

While no information is available for 1941 and 1942, plague was again observed in the autumn of 1943 in a locality about two days' walk from the Burma border. In 1944 the disease not only assumed epidemic proportions in that area, but also appeared in three other districts of Yun-nan, a total case incidence of 542 with 247 deaths being recorded.

A further extension of the affected area took place in 1946 when the infection, having crossed the Salween River, reached Paoshan, the principal city of west Yun-nan. The situation was not much changed in 1947 and
there seems to have been less plague in 1948. However, according to information received in 1949, the infection, having crossed the Me-kong River, appeared in Ta-li. At the same time plague was reported from three hitherto-unaffected counties and a traveller from Paoshan was found to be suffering from the disease upon arrival at Kunming. The total plague incidence in 1949 was 283 cases with 92 deaths as against 168 cases with 36 deaths in 1948.

As will be gathered from the account given above, rat-caused plague is entrenched in parts of south China as well as in the north-west of the country and recently perennial outbreaks of the same type occurred in Yun-nan as well. On the other hand, plague has been either altogether absent from the central provinces or the infection, if imported upon rare occasions, has failed to establish itself.

It should be noted in the latter connexion that plague was introduced into Shanghai in 1908 but, though infected rats continued to be found every year until 1916, no widespread epizootics resulted and human cases never became numerous. From 1917 onwards rat falls were few and far between or altogether absent and Shanghai has been quite free from plague since 1927.

Likewise, when plague, believed to have been introduced by bacterial warfare, appeared at Chang-teh, Hu-nan Province, in 1941 (King, 70 Fan 28), no permanent harm resulted even though in the following year a rat epizootic was rampant in that town and almost 100 human cases occurred. The infection disappeared early in 1943 and since then Hu-nan has been as free from plague as it was before the end of 1941.

The absence of plague from central China or its failure to establish itself cannot be ascribed to a paucity of commensal rats or of $X. \text{cheopis}$, both of which abound everywhere. Likewise, as confirmed by the observations in Hu-nan, there is no reason to assume that plague-resistant rat strains prevail in central China. Probably, however, peculiarities of the seasonal incidence of $X. \text{cheopis}$ go a long way to explain the freedom of central China. As pointed out by Wu, 157 the incidence of that flea in Shanghai was low during the months of the plague season in south China, which commences in early spring. He admitted that the autumnal incidence of the disease in the north exhibited a dangerous coincidence with the $cheopis$ season in Shanghai, but pointed out that under the existing conditions the southern plague foci alone were potentially dangerous for an importation of the infection into Shanghai.

(2) Burma

Though, as discussed above, the historic as well as the recent invasion of Yun-nan could be traced back to Burma, it would be rash to assert
that permanent endemic foci, comparable in standing to those in Central Asia, exist in the latter country. On the contrary, it seems more likely that there, as well as in west Yun-nan, prolonged periods during which rat epizootics continued, and epidemics were consequently frequent, alternated with quiescent periods. In conformity with this concept, Kaul considered an importation of plague into Rangoon in 1905 as responsible for the present wave of infection which led to the establishment of endemic foci in Meiktila (especially the town of Mahlaing), Pyawbwe town, and the northern Shan States near the Yun-nan border (Wilcocks). Though country districts as well as towns were involved, according to Wilcocks 70% of the plague deaths were recorded in Rangoon and in the towns on the main lines of communication by river or rail.
The incidence of the disease, which had increased to 3,517 cases with 2,743 deaths in 1946, decreased until 1950 but then rose again as shown by the following figures:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1947</td>
<td>1,518</td>
<td>1,192</td>
</tr>
<tr>
<td>1948</td>
<td>1,479</td>
<td>1,099</td>
</tr>
<tr>
<td>1949</td>
<td>799</td>
<td>641</td>
</tr>
<tr>
<td>1950</td>
<td>637</td>
<td>442</td>
</tr>
<tr>
<td>1951</td>
<td>900</td>
<td>583</td>
</tr>
<tr>
<td>1952</td>
<td>1,029</td>
<td>646</td>
</tr>
</tbody>
</table>

The usual plague season in Burma falls into the period November to April, but in Lower Burma there may be a secondary rise in July (Wilcocks 156). The bubonic type is preponderant but occasional pneumonic epidemics have been recorded, recently by Wynne-Griffith 161 at Rangoon.

Wilcocks 156 considered *R. concolor* and *R. rattus* of main importance in the perpetuation of plague in Burma; the former was more common. *R. norvegicus*, dwelling in fields and sewers, seemed less dangerous. Harrison & Woodville 47 found during a recent survey in Rangoon *R. exulans concolor* most frequent (45%), followed by *Bandicota bengalensis* (31%); *R. rattus* formed only 8% of the total, *R. norvegicus* 6%. However, plague infection was found only in two *R. rattus* and one bandicoot.

In some parts of Burma *Xenopsylla astia* was more commonly found than *X. cheopis*, but the latter was responsible for most instances of human infection (Wilcocks 156).

(3) Indochina

Dr. Robert, in a valuable report on the plague situation in Indochina presented to the second session of the Joint OIHP/WHO Study-Group on Plague, stated that plague was endemic in the following localities:

(a) Cochin-China (South Viet-nam), principally in Saigon/Cho-lon, the hotbed of the infection being located in the precincts round the central market of Saigon which had been invaded from Canton and Hong Kong in 1906;

(b) Cambodia, especially Pnom-Penh, where plague, probably imported from Saigon, had become established in 1907;

(c) Phan-thiet/Phan Rang region on the south Annam Coast, infected by ship from Saigon in 1908;

(d) Lang Bian plateau of south Indochina where the disease, imported with rice cargoes from Cho-lon, possibly also from Phan Rang, became established in 1943 or 1944.
The recent incidence of plague in the country was:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1946</td>
<td>52</td>
<td>24</td>
</tr>
<tr>
<td>1947</td>
<td>90</td>
<td>40</td>
</tr>
<tr>
<td>1948</td>
<td>355</td>
<td>105</td>
</tr>
<tr>
<td>1949</td>
<td>113</td>
<td>55</td>
</tr>
<tr>
<td>1950*</td>
<td>142</td>
<td>33</td>
</tr>
<tr>
<td>1951*</td>
<td>119</td>
<td>39</td>
</tr>
<tr>
<td>1952*</td>
<td>54</td>
<td>14</td>
</tr>
</tbody>
</table>

* Cambodia and Viet-nam.

While the bubonic type was most frequently met with, occasional epidemics with pneumonic features were observed (Wilcocks,156 Robert, communication to WHO).

*Rattus* played in general the most dangerous role with *X. cheopis* as the usual vector. Most interestingly, however, Herivaux & Toumanoff,53 investigating a limited outbreak at Saigon in 1943 (42 cases), proved the existence of an epizootic among domestic mice. These animals, while not harbouring any *Leptopsylla segnis*, were more heavily *cheopis*-infested than the rats. Only one specimen of the latter (*R. norvegicus*) was found infected.

(4) *Thailand*

Plague, probably imported by the sea-route from China (Bangxang 3), appeared at Bangkok in 1904 and it would seem that the inland town of Korat became infected in the same year.

Though persisting in both places and eventually also involving other localities, the infection seems to have caused comparatively little havoc in Thailand. According to Wilcocks156 a total of 1,722 cases occurred at Bangkok from 1905 to 1922. At Korat a severe outbreak (586 cases with 580 deaths) took place in 1917. Preventive measures, including house improvement, were then instituted and only 248 cases with 199 deaths were recorded from 1918 to 1934. Plague began to decline in the country in general from 1929 onwards and the disease seems to have been absent during the period from 1935 to 1937 (Bangxang 8). However, the infection reappeared towards the close of 1938 in the north-western province of Tak and, as described by Park,108 spread first eastwards and then to the south. Bangkok became re-infected early in 1940, but only a few cases were reported there and the port seems to have remained free from plague since that year.

Korat became once more infected in 1942, since when cases or small groups of cases have been noted every year. The recent incidence of
plague in Thailand may be gathered from the following:

<table>
<thead>
<tr>
<th>Year</th>
<th>Thailand Cases</th>
<th>Thailand Deaths</th>
<th>Korat Cases</th>
<th>Korat Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1944</td>
<td>57</td>
<td>29</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>1945</td>
<td>107</td>
<td>46</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>1946</td>
<td>72</td>
<td>33</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1947</td>
<td>71</td>
<td>23</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1948</td>
<td>122</td>
<td>36</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>1949</td>
<td>176</td>
<td>64</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1950</td>
<td>57</td>
<td>10</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>1951</td>
<td>5</td>
<td>1</td>
<td>(January-November)</td>
<td></td>
</tr>
<tr>
<td>1952</td>
<td>9</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dealing with the seasonal occurrence of the disease, Bangxang stated that the case incidence began to increase in September and reached its highest point in February and March. The disease was at a low level from May to September, particularly during the wet months of June and July. Human plague was almost invariably of the bubonic type, but Bangxang referred to one outbreak with pneumonic features described by Braddock in 1912.

As maintained by Park, R. rattus alexandrinus was principally involved; it formed over 60% of the Bangkok rats as against 14% R. norvegicus. Infected shrews were found and might, as Bangxang believed, play a role in the propagation of the infection. As everywhere in the Far East, X. cheopis was the principal vector.

(5) Java

Plague appeared in Java in 1911 but it is likely that the introduction of the infection, ascribed to the importation of rice cargoes into the port of Surabaya, went back to the previous year (November 1910). The interior of the eastern part of the island soon became invaded. While it became possible to terminate the outbreaks there through wholesale rat-proofing of the houses, the infection began to move westwards, successively affecting middle and western Java. During the period 1920-7 from 8,000 to 10,000 deaths occurred annually. A decrease of the infection-rate was noted from 1928 to 1931 but then the incidence rose again, reaching a maximum of 23,267 cases with 23,239 deaths in 1934. Then, as shown below, a gradual decline set in, which was ascribed to large-scale inoculation campaigns with Otten's live vaccine:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1935</td>
<td>13,022</td>
<td>1939</td>
<td>1,558</td>
</tr>
<tr>
<td>1936</td>
<td>6,227</td>
<td>1940</td>
<td>312</td>
</tr>
<tr>
<td>1937</td>
<td>3,834</td>
<td>1941</td>
<td>550</td>
</tr>
<tr>
<td>1938</td>
<td>2,107</td>
<td>1942</td>
<td>339</td>
</tr>
</tbody>
</table>

Little information is available for the period during the second World War; van Loghem maintained that generally speaking the situation...
was not alarming. However, though the case incidence reported for the period 1945-7 was low, incomplete figures for 1948 and 1949 indicated a disquieting increase of the infection-rate (3,422 cases with 3,365 deaths and 874 cases with 844 deaths respectively), the residency of Jogjakarta in central Java being most heavily affected.

The recent incidence of plague in Java is shown below:

<table>
<thead>
<tr>
<th>Year</th>
<th>Eastern Java</th>
<th>Middle Java</th>
<th>Western Java</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>8</td>
<td>3,450</td>
<td>129</td>
</tr>
<tr>
<td>Deaths</td>
<td>310</td>
<td>4,292</td>
<td>549</td>
</tr>
</tbody>
</table>

*Incomplete

It will be noted that the plague situation in eastern Java was not serious. In fact, this part of the island seemed to be free from the infection during the second half of 1952. On the contrary, middle Java, particularly the residencies of Jogjakarta and Surakarta were seriously involved. Plague caused less havoc in western Java, but there can be no doubt that there as well as in middle Java endemic foci exist.

As pointed out by Park, in Java, where the temperature remains fairly uniform throughout the year, no pronounced seasonal incidence of the disease was to be expected. Still, the plague mortality showed a tendency to increase during the third quarter of each year, reaching its maximum in December and January; then a decrease set in which lasted until July.

Human plague in Java was mainly bubonic, but cases with pneumonic features accounted for 6% - 8% of the total incidence of the disease and outbreaks of pneumonic plague, usually claiming 2-10 victims only, were met with (Wu Lien-teh, Wilcocks).

As summarized by Wilcocks, the brown Malayan house-rat, R. rattus diardi, R. concolor inland, and to a very small extent R. norvegicus were implicated in the plague outbreaks in Java, the first-mentioned rodent being considered as the chief culprit. X. cheopis was the principal vector.

(6) India

In a discussion on the endemicity of plague in India, Sharif postulated that the infection is at present entrenched in the following foci:

(a) Three endemic centres situated near the foot of the Himalayas which, though occasionally appearing to have been independent of one another, possibly form part of a big sub-Himalayan focus. These centres were responsible for plague outbreaks in east Punjab, the United Provinces, and districts of Bihar north of the Ganges.

(b) One focus in central India comprising the watersheds of the Vindhyas, Bhanrer, and Maikal ranges, and the Mahadeo hills.
Three centres in southern India situated respectively in the watersheds of the Western Ghats in Bombay State and Mysore; in watersheds located in the districts of Salem, Coimbatore, Nilgiri, and Madura; in the Hyderabad State. All three foci have been very active lately.

There can be no doubt that the endemic centres in southern India became established after the city of Bombay had become infected in 1896. As stated by Sharif, the first of them (Bombay and Mysore States) had been reached by plague in 1898. Nilgiri became involved in 1903 (George & Timothy). The infection spread in 1898 from the then Bombay Presidency into the adjacent parts of Hyderabad State, but the situation there became serious in 1911 only when Hyderabad city became affected (Rao).

It seems possible on the other hand that the endemic foci in the Himalayas are partly of long standing. Endemicity has been known to exist in the districts of Gharwal and Kumaon since 1823, but, as assumed by Gill, the infection persisting there was perhaps "a relic of the great pestilence in the 17th century". Though these areas, which are situated within the region involved at present, are known to have been responsible for plague outbreaks up to the year 1877 only, latent infection might have continued to persist in some part of the Himalayan foothills to become active again early in the present century.

The mortality from plague in India from 1898 to 1948 may be summarized as in table I:

<table>
<thead>
<tr>
<th>Period</th>
<th>Total deaths from plague</th>
<th>Annual average</th>
<th>Deaths during each period expressed as percentage of total deaths 1898-1948</th>
</tr>
</thead>
<tbody>
<tr>
<td>1898-1908</td>
<td>6,032,693</td>
<td>548,427</td>
<td>47.88</td>
</tr>
<tr>
<td>1909-1918</td>
<td>4,221,528</td>
<td>422,153</td>
<td>33.51</td>
</tr>
<tr>
<td>1919-1928</td>
<td>1,702,718</td>
<td>170,272</td>
<td>13.52</td>
</tr>
<tr>
<td>1929-1938</td>
<td>422,880</td>
<td>42,288</td>
<td>3.36</td>
</tr>
<tr>
<td>1939-1948</td>
<td>217,970</td>
<td>21,797</td>
<td>1.73</td>
</tr>
<tr>
<td>Total 1898-1948</td>
<td>12,597,789</td>
<td>247,015</td>
<td>100.00</td>
</tr>
</tbody>
</table>

*After Bhore, Kaule*
but afterwards became worse once more, the annual incidence-rates for 1945, 1946, and 1947 being increasingly in excess of that recorded in 1939. Kaul assumed that the war with Japan was at least partly responsible for this recrudescence because other health data available in India at the time also showed a turn for the worse. However, the acute food scarcity existing in 1946 and 1947 in certain parts of the country, which necessitated large-scale movements of grain from central collecting stations to various provinces, might have facilitated the dissemination of plague, and a similar influence was probably exerted by the movements of large groups of people in connexion with the partition of India in August 1947. Since these unfavourable conditions were of a temporary nature, it would be tempting to ascribe the considerably reduced incidence of the disease in 1948 and 1949 to an improvement of the general situation. It is, however, disquieting to note that the 1950 plague mortality in the Central Provinces (now Madhya Pradesh) was markedly in excess of the figures reported for the three previous years. Still, the plague mortality there, as well as in India in general, was considerably lower in 1951 and 1952.

### TABLE II. ANNUAL MORTALITY FROM PLAGUE IN INDIA DURING THE PERIOD 1939-52

<table>
<thead>
<tr>
<th>Year</th>
<th>Total plague mortality</th>
<th>Bihar</th>
<th>Bombay State</th>
<th>Central Provinces (Madhya Pradesh)</th>
<th>Madras</th>
<th>Punjab</th>
<th>United Provinces (Uttar Pradesh)</th>
<th>Other States</th>
<th>Hyderabad State</th>
<th>Mysore State</th>
</tr>
</thead>
<tbody>
<tr>
<td>1939</td>
<td>28,257</td>
<td>1,936</td>
<td>1,472</td>
<td>862</td>
<td>324</td>
<td></td>
<td>21,662</td>
<td>6,753</td>
<td>2,352</td>
<td></td>
</tr>
<tr>
<td>1940</td>
<td>19,799</td>
<td>1,040</td>
<td>5,573</td>
<td>283</td>
<td>1,169</td>
<td></td>
<td>11,726</td>
<td>7,503</td>
<td>2,593</td>
<td></td>
</tr>
<tr>
<td>1941</td>
<td>11,964</td>
<td>129</td>
<td>5,311</td>
<td>761</td>
<td>1,726</td>
<td></td>
<td>4,035</td>
<td>2,713</td>
<td>5,417</td>
<td></td>
</tr>
<tr>
<td>1942</td>
<td>10,577</td>
<td>108</td>
<td>680</td>
<td>129</td>
<td>701</td>
<td></td>
<td>8,953</td>
<td>857</td>
<td>3,775</td>
<td></td>
</tr>
<tr>
<td>1943</td>
<td>13,578</td>
<td>266</td>
<td>715</td>
<td>144</td>
<td>4,865</td>
<td>1</td>
<td>7,558</td>
<td>1,483</td>
<td>3,685</td>
<td></td>
</tr>
<tr>
<td>1944</td>
<td>21,525</td>
<td>834</td>
<td>2,514</td>
<td>910</td>
<td>1,736</td>
<td>61</td>
<td>15,454</td>
<td>5,253</td>
<td>5,527</td>
<td></td>
</tr>
<tr>
<td>1945</td>
<td>29,751</td>
<td>1,523</td>
<td>11,779</td>
<td>575</td>
<td>1,644</td>
<td>203</td>
<td>14,024</td>
<td>6,631</td>
<td>6,016</td>
<td></td>
</tr>
<tr>
<td>1946</td>
<td>32,967</td>
<td>8,869</td>
<td>3,405</td>
<td>189</td>
<td>2,254</td>
<td>245</td>
<td>18,206</td>
<td>4,056</td>
<td>2,694</td>
<td></td>
</tr>
<tr>
<td>1947</td>
<td>41,745</td>
<td>6,258</td>
<td>2,129</td>
<td>2,442</td>
<td>3,075</td>
<td>1,704</td>
<td>26,126</td>
<td>1,791</td>
<td>1,502</td>
<td></td>
</tr>
<tr>
<td>1948</td>
<td>9,757</td>
<td>902</td>
<td>555</td>
<td>2,426</td>
<td>978</td>
<td>196</td>
<td>4,364</td>
<td>311</td>
<td>1,128</td>
<td></td>
</tr>
<tr>
<td>1949</td>
<td>7,557</td>
<td>647</td>
<td>722</td>
<td>2,860</td>
<td>151</td>
<td>227</td>
<td>2,904</td>
<td>2,135</td>
<td>852</td>
<td></td>
</tr>
<tr>
<td>1950</td>
<td>8,881</td>
<td>557</td>
<td>95</td>
<td>3,204</td>
<td>42</td>
<td>35b</td>
<td>2,073</td>
<td>224</td>
<td>719</td>
<td></td>
</tr>
<tr>
<td>1951</td>
<td>12,411</td>
<td>5</td>
<td>7</td>
<td>475</td>
<td>60</td>
<td></td>
<td>1,275</td>
<td>93</td>
<td>542</td>
<td></td>
</tr>
<tr>
<td>1952</td>
<td>1,007</td>
<td>0</td>
<td>1</td>
<td>457</td>
<td>9</td>
<td></td>
<td>504</td>
<td>11</td>
<td>272</td>
<td></td>
</tr>
</tbody>
</table>

Totals: 235,286 22,894 38,261 13,407 18,759 2,640 128,582 443 40,559 38,572

a Not included in totals  
b East Punjab only
Generally speaking, in India as well as in China plague is now a rural rather than an urban problem. It should be noted in this connexion that Bombay city, where plague had been rampant up to 1923 and perennial manifestations had continued to exist until 1934, is now almost free. In Calcutta, which apparently became affected in 1895 when suspicious cases were noted among a group of soldiers recently arrived from Hong Kong (Rao), and where the infection persisted until 1925, considerable outbreaks, due possibly to an importation from Bihar (Greval), took place in 1948 and 1949. Only two autochthonous cases were recorded in 1950 but their number rose to 16 in 1951, and to 54 in 1952.

Dealing with the seasonal incidence of the disease in his classical study "Twenty years of plague in India", White maintained that "epidemics normally attain their maximum severity in Bombay in October; in the Central Provinces in February; in the United Provinces and Bihar in March; and in the Punjab in April. In the remaining provinces, taken together, March is the month of maximum mortality".

While the bubonic type of the disease is by far the most frequent in India, small outbreaks of pneumonic plague have been observed occasionally, recently by Seal and Seal & Prasad at Calcutta and Gaya (Bihar).

So far no evidence has been found that wild rodents played a role in the causation of plague outbreaks in India, the common species of commensal rats forming the usual reservoir of the infection. However, as will be discussed later, the bandicoots which are apt to come or even live near man, also deserve some attention. The "Indian" plague flea X. cheopis is the typical vector of the infection.

(7) Western Asia (Iran, Iraq, Turkey in Asia, and Syria)

A joint consideration of the plague regions in Western Asia seems justified in so far as according to Tholozan and Payne the mountains of Kurdistan were the chief endemic centre for a large area comprising Persian Kurdistan and adjacent parts of Persia, Turkish Kurdistan, and parts of Mesopotamia. Payne added that from this area plague extended "to Northern Persia on the shores of the Caspian (Resht) in 1877, to Baku on the western and Astrakhan on the northern shore of that sea; and up the Volga to the village of Vetlianka and its neighbourhood in 1877-79".

Presumably the persistence of the infection in the interior of western Asia was in part responsible for past plague invasions of Syria and Palestine, but it must be noted that these countries were open to inroads of the pest by the sea-route as well.

Findings made by Baltazard et al. after two limited outbreaks of pneumonic plague had been observed in the south of Iranian Kurdistan in October-November 1947 have added much to the scanty information hitherto available in regard to the western-Asiatic focus. Baltazard and
his colleagues were able to prove the existence of plague in three species of *Meriones* as well as in a few other rodents and in one weasel. The fleas involved belonged mainly to the genus *Xenopsylla* (*conformis* group) but partly also to that of *Nosopsyllus*. A species of mice (*Mus musculus bactrianus*) was met with but commensal rats appeared to be altogether absent. It is legitimate, therefore, to include Kurdistan among the plague areas where wild rodents form the reservoir of the infection.

In regard to Iraq, it was stated by Kaul 69 that plague was absent during the period 1937-44 though in 1938 plague-infected rats were still found in Baghdad. The disease reappeared in February 1945, when 46 cases were reported, mainly from Amara and vicinity (Stowman 142).

In Turkey, a plague epidemic in three villages on the Syrian border took place in March-April 1947. According to Erzin & Payzin 22 19 persons were affected of whom 14 had axillary buboes and 5 septicaemic features; 13 of these patients succumbed. A report 67 on this outbreak added that the infection, apparently present among the rats as well as among man, had been derived from Syria where plague used to occur in sporadic form among the desert nomads. Actually, after the termination of the outbreak on Turkish territory 6 cases of plague were notified in the Syrian village of Varta.

(8) *Western Arabia*

Though the early writers dealing with the nosogeography of plague were unanimous in stating that the mountainous area of Assyr, situated in the border regions of present-day Yemen and Saudi Arabia, was an endemic focus of long standing, the last outbreak known to have occurred there in the past took place in 1906. It is, therefore, of great interest to note that the presence of plague in these parts was re-affirmed in 1951 and 1952.

The 1951 outbreak took place in three villages of the province of Khawlan, situated in Yemen near the Saudi-Arabian border and, lasting from March until September, led to the occurrence of 12 bubonic cases and one instance of pneumonic plague. Anti-epidemic action, including vaccination and dusting with DDT was taken by a WHO team which had been dispatched after the presence of the disease had been reported at the end of July.

In June 1952, plague was found to be present in the village of Benibashar, situated in the Saudi-Arabian part of the Assyr area 50 miles from Abha, but as far as is known at present, only 4 cases with 3 deaths were observed. Adequate anti-epidemic action was taken, including arrangements to prevent passage of pilgrims from Yemen to Mecca through the affected region.

It would be of great importance to ascertain the species of rodents responsible for these outbreaks. There can be hardly any doubt that wild rodents rather than rats are involved. In fact, it seems probable that the Assyr area forms an outlying part of a vast enzootic area in Western Asia centring in Kurdistan.
(9) Palestine

While plague cases or even limited epidemics had been met with in Palestine after the British occupation at the end of the first World War, the disease appears to have been absent during the period 1925-40 (Stowman 142). However, the infection, presumably imported from the Suez Canal Zone, reappeared at Haifa in 1941. A considerable epizootic resulted which reduced the incidence of *R. rattus* from 63% to 2.7%, but only 10 human cases were notified.

Jaffa became involved in the winter of 1942-3 when a rat-caused outbreak led to 15 cases with 9 deaths. Only 1.5% *R. rattus* were found at the time and the incidence of *X. cheopis* (38%) seemed less than that at Haifa.

Plague persisted in both places and occasional instances of the disease were observed also at Tel Aviv. The total case incidence was 93 in 1944 and 38 in 1945 when 19 cases with 9 deaths were recorded at Jaffa from October until December. In 1946 there were only 13 cases, but the disease became epidemic at Haifa in 1947 when, including 3 cases in a secondarily involved village, 17 attacks were notified in June and July (Pollock, Haddad & Valero 45).

Europe

(1) Ajaccio (Corsica) and Taranto (Italy)

The essential features of the ephemeral outbreaks occurring soon after the second World War at Ajaccio and at Taranto are given in table III.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Dates of occurrence</th>
<th>Origin</th>
<th>Number of cases</th>
<th>Number of deaths</th>
<th>Rats and fleas involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajaccio</td>
<td>May-July 1945</td>
<td>Apparently imported from North Africa</td>
<td>13</td>
<td>10</td>
<td><em>R. norvegicus</em> (90%) <em>R. rattus</em> (10%) <em>X. cheopis</em></td>
</tr>
<tr>
<td>Taranto</td>
<td>September-November 1945</td>
<td>Possibly importation of rags by ship</td>
<td>29</td>
<td>15</td>
<td><em>R. norvegicus</em> <em>R. rattus</em> <em>X. cheopis</em> Nosopsyllus fasciatus</td>
</tr>
</tbody>
</table>

* Bernard et al., Martorana, Schulz

(2) Malta

After an absence of over a hundred years plague appeared in Malta in 1917 when a batch of 8 cases with 4 deaths occurred among dockyard
workers and their contacts. The first victim was said to have been "infected from a sick rat which he found in a box containing stores coming from Mesopotamia where the disease was epidemic" (Bernard 11).

A further outbreak, ascribed to the importation of straw and hay from North Africa, took place from April to November 1936, when 25 bubonic cases with 10 deaths were recorded. An epizootic, involving mainly R. norvegicus, was found to be responsible for this epidemic. Leptopsylla segnis was the most frequent rat flea (48.75%), followed by X. cheopis (37.5%); Nosopsyllus fasciatus was rarer (13.75%) (Bernard 11).

Plague again appeared in Malta in June 1945 when an epizootic which involved mainly the area of the commercial port began to manifest itself and led to an epidemic lasting until the end of the year. The 75 cases with 20 deaths recorded during this period were followed by 5 further cases with 2 deaths up to June 1946. Infected rodents (mainly R. norvegicus) were found up to February 1947 (Cauchi,14 Barnett 4).

(3) Portugal: Azores Islands

Plague outbreaks, often showing a high incidence of cases with pneumonic features, were recorded since 1908 in several of the Azores Islands (Wu Lien-teh 119) but within recent years only São Miguel Island seems to have been involved. An extensive epidemic (744 cases) had occurred there in 1922-3 (Stowman 142), but since then sporadic cases only, averaging 8 per year during the period 1942-8, have been recorded. Two cases were notified in 1949, since when plague seems to have been absent.

Recent plague outbreaks in the Azores, suspected to be due to epizootics among field-mice (mulots), were rural in character and appeared chiefly during the seasons when crops were harvested and grain was brought to the granaries.154

R. norvegicus predominated amongst the commensal rodents, but black rats (mainly R. rattus) and M. musculus were also found. Leptopsylla segnis was the most numerous flea of these rodents, followed by Nosopsyllus fasciatus, X. cheopis being only the third most frequent (Jorge 85).

North and North-west Africa

(1) Egypt

Fifty-five years after Egypt had become free from plague, the infection, possibly imported from Bombay, reappeared in 1899 at Alexandria. Port Said, the second big port of the country, became affected in 1900, Suez in 1904.

As soon as the infection was established in the two principal ports, it began to travel inland. Invading first the chief places of the provinces and
districts but soon spreading to other towns and villages, plague gradually reached most localities of the Nile valley from the coast to Aswān (Wakil, Makar). As shown by statistics compiled by Wakil for the period 1899-1930 (see table IV), the case incidence in Upper Egypt was considerably higher than that in the ports and Lower Egypt.

**TABLE IV. INCIDENCE OF PLAGUE IN EGYPT, 1899-1930**

<table>
<thead>
<tr>
<th>Area</th>
<th>Cases</th>
<th>Deaths</th>
<th>Mortality percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Ports and Western Frontier Province</td>
<td>3,797</td>
<td>19.6</td>
<td>2,146</td>
</tr>
<tr>
<td>Lower Egypt</td>
<td>3,201</td>
<td>16.5</td>
<td>1,245</td>
</tr>
<tr>
<td>Upper Egypt</td>
<td>12,388</td>
<td>63.9</td>
<td>6,881</td>
</tr>
<tr>
<td>Totals</td>
<td>19,386</td>
<td>100.0</td>
<td>10,272</td>
</tr>
</tbody>
</table>

While plague continued to be comparatively frequent in Upper Egypt where the province of Asyūt had become the chief focus, the situation in the country in general became increasingly favourable from 1935 onwards as shown by the following figures supplied by Makar:

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of cases in Egypt</th>
<th>Cases in Asyūt</th>
<th>Percentage of total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1931-5</td>
<td>941</td>
<td>389</td>
<td>41.3</td>
</tr>
<tr>
<td>1936-7</td>
<td>183</td>
<td>157</td>
<td>85.5</td>
</tr>
</tbody>
</table>

In 1938 only 11 cases were recorded in the country as a whole. However, while the ports remained free and Lower Egypt had an almost clear bill in 1939 and 1940, there were considerable outbreaks, involving 169 and 452 cases respectively, in Asyūt.

As summarized by Stowman, from 1941 onwards there was little evidence of plague even in Asyūt. Unfortunately, however, as in the first, so also during the second World War a serious situation developed in the Suez Canal Zone. Outbreaks commenced successively at Suez in November 1943, at Ismailia and in its district in March 1944, and in May of the same year at Port Said where sporadic cases had been noted since 1940. The total incidence of plague from November 1943 to September 1944 was 712 cases (Stowman). The disease reappeared in the Canal ports in February 1945, but the situation gradually improved and by August 1946 the infection had disappeared (Tomich).

Alexandria, which had had a clear record since 1935, reported 15 cases with 5 deaths in January 1947. Since then, however, plague seems to have been absent from Egypt.
The plague seasons in Egypt were, according to Wakil,¹³ three as follows:

<table>
<thead>
<tr>
<th>Zone</th>
<th>Onset</th>
<th>Peak</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Egypt</td>
<td>March</td>
<td>April</td>
<td>May</td>
</tr>
<tr>
<td>Middle Egypt</td>
<td>April</td>
<td>May</td>
<td>June</td>
</tr>
<tr>
<td>Nile Delta and Suez</td>
<td>April</td>
<td>June</td>
<td>July</td>
</tr>
<tr>
<td>Mediterranean ports</td>
<td>May</td>
<td>July</td>
<td>October</td>
</tr>
</tbody>
</table>

Thus, as in other plague-affected countries, particularly in China, the seasonal incidence of the disease in Egypt stood in correlation with the latitude in which the various foci were situated, being earliest in the south and latest in the north.

The frequency with which the different types of human plague were met with during the period 1904-28 was illustrated by Wakil (see table V).

TABLE V. FREQUENCY OF DIFFERENT TYPES OF HUMAN PLAGUE IN EGYPT, 1904-28

<table>
<thead>
<tr>
<th>Zone</th>
<th>Total plague cases</th>
<th>Bubonic and septicaemic cases</th>
<th>Pneumonic cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Ports and Western Frontier Province</td>
<td>3,011</td>
<td>2,925</td>
<td>85</td>
</tr>
<tr>
<td>Lower Egypt</td>
<td>2,650</td>
<td>2,595</td>
<td>85</td>
</tr>
<tr>
<td>Upper Egypt</td>
<td>11,933</td>
<td>10,389</td>
<td>1,592</td>
</tr>
<tr>
<td>Totals</td>
<td>17,659</td>
<td>15,909</td>
<td>1,750</td>
</tr>
</tbody>
</table>

As will be noted, the pneumonic type, while rare in the coastal regions and Lower Egypt, was comparatively far more frequent in the south of the country.

Dealing with the rodents in his classical study on plague in Egypt, Wakil enumerated "as the species of plague rats most commonly found associated with man" R. norvegicus, R. rattus, and Acomys cahirinus.

The Norway rats were very prevalent in the residential quarters of the port cities, R. rattus being restricted to the dock areas. Acomys cahirinus was rare in Alexandria and Port Said, more frequent in Suez.

R. norvegicus was scarce in Upper Egypt where, according to Petrie & Todd,¹¹ R. rattus formed 60.6% of the total rat population and Acomys cahirinus 38.3%.

X. cheopis was the most prevalent species of rat fleas throughout Egypt. Leptopsylla segnis, which came next in order, was more often encountered in the ports than in Upper Egypt. According to data supplied by Wakil, R. rattus was more heavily flea-infested than the Norway rats. In his
opinion this factor in conjunction with the frequency of the former species of rat was partly responsible for the higher plague incidence in Upper Egypt.

(2) Tripolitania

As can be gathered from a report received in May 1950 in answer to an inquiry on plague in Africa instituted by the WHO Expert Committee on Plague at its first session, a solitary plague outbreak, which was probably due to importation and led to 12 confirmed and 2 suspected cases, took place in May 1940, in a locality 12 kilometres outside Tripoli on the Tripoli/Garian road.

Since no infected rats could be found, Modica postulated that this outbreak was due to the importation of plague-infected "insects or acari" in grain supplies. However, no proof for this assumption was obtained.

(3) Tunisia

Plague, which had been absent from the coastal areas of North Africa west of Egypt since 1822, reappeared in the port of Tunis in 1907, imported, as Jorge believed, by ship via Marsilles, France. Apart from a pneumonic outbreak which claimed 65 victims in 1929-30, sporadic cases only were observed in Tunis. However, the disease, appearing usually in the bubonic but occasionally in the pneumonic form, was epidemic in the south of Tunisia in 1920-1 and fairly frequent during the period 1926-31 when a total of 1,095 cases was recorded, mainly in the districts of Kairwan and Sfax (Stowman). Only 37 cases were notified from 1932 until 1943, the years 1932-4 and 1942-3 being clear. However, from August 1944 until March 1945 an outbreak of bubonic plague (37 cases with 10 deaths) took place at Ferryville (Magrou) and some cases were also noted at Tunis and Bizerta. Tunisia seems to have been free from plague since 1946.

It was claimed that both the 1920-1 and the 1926 epidemics had been due to the immigration of infected wild rodents from Tripolitania. However, no definite proof exists that such animals played a role in the Tunisian outbreaks. Gobert claimed to have found suspicious signs in field-rats and gerbils but not much reliance can be placed upon his evidence (Wu Lien-teh). Ristorcelli, working in a region where plague was said to have been present in the past, could find no trace of recent epizootics. It is noteworthy, however, that some of the wild-rodent species in Tunisia, particularly the Psammomys which abound in the south, are highly susceptible to experimental infection.

Norway rats were by far the most frequent in Tunis, but black rats (R. rattus alexandrinus and R. rattus) and two species of mice were met with as well. The rodents found infected during the period from 1935 to 1940 and again in 1944 belonged to the following species:
Species | Number infected
--- | ---
*R. norvegicus* | 75
*R. r. alexandrinus* | 29
*R. r. rattus* | 13
*M. gentilis* | 9
*M. azoricus* | 3

Total 129

*X. cheopis* was the most preponderant species of rat fleas in Tunis but *Leptopsylla segnis* and *Nosopsyllus fasciatus* were also present.

(4) Algeria

The importation of plague into the Algerian port of Philippeville, occurring in 1899 already (Jorge 66), led at first to no serious consequences, the total incidence of the disease in the North African ports up to 1907 amounting to 82 cases.

Though plague manifestations became fairly frequent in Algeria from 1911 onwards, Grenouilleau 42 felt certain that they were due to repeated importation of the infection by maritime or caravan routes and not to endemicity. Up to 1935 major epidemics were observed twice only—in 1921, when 185 cases with 97 deaths were recorded at Aumale, and in 1931, when 86 cases of pneumonic plague were notified in the department of Constantine. The incidence of the disease from 1935 onwards may thus be summarized:

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of cases</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1935</td>
<td>11</td>
<td>10 of the cases were noted at Philippeville</td>
</tr>
<tr>
<td>1936</td>
<td>10</td>
<td>3 of the cases were notified at Algiers</td>
</tr>
<tr>
<td>1937</td>
<td>3</td>
<td>All at Algiers</td>
</tr>
<tr>
<td>1939</td>
<td>2</td>
<td>&quot;</td>
</tr>
<tr>
<td>1940</td>
<td>18</td>
<td>&quot;</td>
</tr>
<tr>
<td>1944</td>
<td>95</td>
<td>94 of the cases occurred at Algiers</td>
</tr>
<tr>
<td>1945</td>
<td>11</td>
<td>Small pneumonic outbreak (8 cases) at Oran (Roux &amp; Mercier 128, Gordon &amp; Knies 40)</td>
</tr>
<tr>
<td>1946</td>
<td>2</td>
<td>Both at Oran</td>
</tr>
<tr>
<td>1950</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>1935-50</td>
<td>158</td>
<td>Only 2 of the cases were notified from the hinterland</td>
</tr>
</tbody>
</table>

Throughout these years most of the plague cases were observed during the period from August to November.

*R. norvegicus* was the preponderant species at Algiers, Bône, and Oran, but as usually *R. rattus rattus* and *R. rattus alexandrinus* were considerably more frequent in the port area of Algiers than in the city proper (Meunier 99). To judge from not numerous observations, the two subspecies of *R. rattus* were more liable to contract plague than the Norway rats.

As reported by Grenouilleau & Carle 43 during the period 1937-44 the rats of Algiers yielded 73.6% of *X. cheopis* as against 19.1% *Leptopsylla segnis*, and 6.4% *Nosopsyllus fasciatus*. 
(5) Morocco

Plague in Morocco became first manifest within recent times in 1909-10 when, according to Jorge, a total of 25 cases was observed in military stations of the Casablanca district. A violent epidemic, claiming 8,000-10,000 victims, took place in 1911 in the district of the Doukkalla and adjacent areas of the hinterland. During the period 1912-9 outbreaks of varying severity continued to appear in these parts and the infection spread to the ports of Casablanca and Rabat where, however, not much havoc was caused.

Further epidemics occurred from 1922-4 and again during the period 1929-35 but were not on a considerable scale. However, the plague situation became quite serious in 1940 from when onwards the case incidence was as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940</td>
<td>1,099</td>
</tr>
<tr>
<td>1941</td>
<td>2,337</td>
</tr>
<tr>
<td>1942</td>
<td>583</td>
</tr>
<tr>
<td>1943</td>
<td>393</td>
</tr>
<tr>
<td>1944</td>
<td>227</td>
</tr>
<tr>
<td>1945</td>
<td>828</td>
</tr>
</tbody>
</table>

In 1940 the disease appeared first in April among tribes in the southern area of Agadir but spread in September to the Marrakech region. In 1942 Casablanca further north became affected, probably through grain transport from the southern foci. The case incidence decreased in 1942 to become fairly low in 1943 and 1944 but the infection spread during this period to Rabat, Port Lyautey, and once more to Marrakech. In the port of Casablanca, where plague had continued to exist since 1941, a minor outbreak took place in 1944, and a severe epidemic in 1945. The whole of Morocco was free in the following year (Stowman, Kaul).

Though plague in Morocco was prevalently bubonic in character, pneumatic cases, sometimes in groups, were seen during the 1911 outbreak and a few limited pneumatic epidemics were observed afterwards (Wu Lien-teh). The disease did not show a predilection for any particular season of the year (Sanguy).

Jorge was inclined to think that, though plague became first rampant in the interior of Morocco, "it was probably of maritime origin", inapparent infection of the commensal rats having become established in the ports and then spreading inland. Dealing with the rat population in the ports of Morocco, he came to the following interesting conclusion:

"Norvegicus is preponderant in the north, while in the south it is replaced by rattus species, especially alexandrinus;—a murine transition of a mediterranean regime to that of a central African one. Even in the north rattus tends to spread at the expense of its rival."  

b "Le norvegicus est prépondérant au Nord et cède dans le Sud la place au rattus, surtout à l'alexandrin;—c'est la transition murine du régime méditerranéen au régime de la Grande Afrique. Au Nord même le rattus tend à accroître son domaine aux dépens de son rival."
R. norvegicus were found in the hinterland together with Meriones which were sometimes more numerous.

X. cheopis was the preponderant rat flea. Nosopsyllus fasciatus and very rarely X. brasiliensis and X. astia were also found.

It is of great interest to note that, in the opinion of Blanc & Baltazard, human fleas and lice played an important, if not a preponderant role in the epidemic spread of plague in Morocco. The merits of this contention will be discussed later.

(6) French West Africa

Though a few plague cases had been noted in a southern port in 1912 (Jorge 66), it was in April 1914 that the infection, probably imported by ship from Casablanca, made its real entry into French West Africa in a manner characteristic of the Black Death rather than of modern African outbreaks. As often in the local manifestations of the 14th century, the initial outburst at Dakar was ushered in by a pneumonic phase and it was only 2½ months after the onset that an epizootic became manifest and the bubonic type became prevalent in man. A wide area even including the Cape Verde Islands was reached by the epidemic which, lasting until January 1915, claimed almost 9,000 victims (Wu Lien-teh 159).

Further plague manifestations in French West Africa became practically restricted to a triangular area in Western Senegal with its tip at Dakar in the west and its northern angle near St. Louis. The incidence of the disease rose after a quiescent period in 1915 and 1916 to reach a maximum of 7,999 cases in 1920 (Damez 17). The infection was also active from 1928 to 1930, during which period an epidemic at St. Louis described by Lefrou took place, and again in 1934. Then a marked decline lasting until 1942 set in. Numbers of cases of plague from that year onwards are given below (Kaul 69):

<table>
<thead>
<tr>
<th>Year</th>
<th>Dakar</th>
<th>Senegal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1943</td>
<td>32</td>
<td>266</td>
<td>298</td>
</tr>
<tr>
<td>1944</td>
<td>570</td>
<td>59</td>
<td>639</td>
</tr>
<tr>
<td>1945</td>
<td>4</td>
<td>54</td>
<td>58</td>
</tr>
<tr>
<td>1946-9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

It will be noted that in French West Africa as well as in several of the areas dealt with above the hitherto favourable plague situation deteriorated considerably during the second World War, when the spread of the infection was facilitated through "a certain amount of war destruction and inadequate storing facilities to meet the war emergency" (Stowman 142).

The plague season in French West Africa fell into the period from June to August (Sorel 189). Human plague was in general of the bubonic type but pneumonic cases were met with and were possibly frequent in some outbreaks (Wu Lien-teh 158).
As confirmed by recent statistics from Dakar, *R. rattus alexandrinus* was by far the most frequent among the commensal rodents, *R. rattus rattus* less common, and *R. norvegicus* not numerous. However, as pointed out by Cazanove (quoted by Jorge 66), the comparative frequency of these rodents was apt to vary in different locations: the Norway rats of Dakar frequented the port and sewers, *R. rattus rattus* was at home in European houses, *R. rattus alexandrinus* in the habitations of the indigenous population.

Plague-positive animals found at Dakar within recent years belonged to the following species:

<table>
<thead>
<tr>
<th>Year</th>
<th><em>R. rattus alexandrinus</em></th>
<th><em>R. rattus rattus</em></th>
<th><em>R. norvegicus</em></th>
<th><em>M. musculus</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1935</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>1944</td>
<td>21</td>
<td>33</td>
<td>7</td>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td>Totals</td>
<td>31</td>
<td>34</td>
<td>8</td>
<td>5</td>
<td>78</td>
</tr>
</tbody>
</table>

Various wild-rodent species have been found plague-infected in Senegal and it was suspected that one of them, the giant rat *Cricetomys gambianus*, played a causal role. It has to be noted in this connexion that recently all rural foci were situated on the railway line leading parallel to the coast from St. Louis to Dakar. It was assumed that the storage and transport of ground-nuts along this route proved attractive to the wild rodents.

Though *X. cheopis* was prevalent, in Senegal as in Morocco a possible role of other fleas in the spread of human plague was suspected. Attention was paid in this respect not only to *Pulex irritans* but also to *Synosternus pallidus* which, though rare on the rats, abounded in human habitations (Kartman 68). The relative importance of these three flea species in the spread of the infection will be discussed later.

**Central Africa**

(1) *Uganda*

When considering the plague situation in Central Africa, it is advisable to deal first with Uganda, where most probably the ancient African centre of the infection mentioned before was situated. It serves as a corollary for this assumption that, as soon as observations became possible in modern times, plague foci of long standing were detected in Uganda (Roberts 123).

Though there was little evidence for the continued existence of the disease in the Protectorate at the close of the last and quite early in the present century, there can be little doubt that the infection was inapparent rather than altogether absent. Moreover, it was claimed that during this period the construction of the Uganda Railway, lasting from 1896 to 1901, led to an importation of plague from India. Be this as it may, it is certain that from 1903 to 1908 most of the ports on Lake Victoria became involved
and that by 1906 the infection had become so firmly entrenched that from then until 1947 it was never absent from Uganda in any year (Hopkins). According to figures culled from the report of Hopkins, the mortality reported as due to plague in Uganda during the three decades from 1910 to 1939 was as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1910-9</td>
<td>31,305</td>
<td></td>
</tr>
<tr>
<td>1920-9</td>
<td>17,410</td>
<td></td>
</tr>
<tr>
<td>1930-9</td>
<td>11,387</td>
<td></td>
</tr>
<tr>
<td>1910-39</td>
<td>60,102</td>
<td></td>
</tr>
</tbody>
</table>

The situation which, as shown above, had gradually improved from 1920 onwards became quite favourable during the period from 1940 to 1949:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940</td>
<td>278</td>
<td>268</td>
</tr>
<tr>
<td>1941</td>
<td>218</td>
<td>213</td>
</tr>
<tr>
<td>1942</td>
<td>354</td>
<td>338</td>
</tr>
<tr>
<td>1943</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>1944</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>1945</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1946</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1947</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1948-9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1940-9</td>
<td>884</td>
<td>834</td>
</tr>
</tbody>
</table>

Note: According to a statement made in the March 1953 issue of the WHO Epidemiological and Vital Statistics Report, 14 plague cases with 8 deaths occurred in Uganda early in 1952.

As was emphasized by Hopkins, the endemic foci in Uganda from which the plague outbreaks originated were invariably situated in rural localities and not in townships.

To judge from statistics showing the monthly incidence of the disease from 1935 to 1947, cases occurred throughout the year without showing a regular predilection for any particular season. Though human plague was mainly bubonic in character, cases with pneumonic features were met with, recently by Hennessey.

It is interesting to note that, in significant contrast to the original foci in Central Asia, plague seems not to have become entrenched among the wild-rodent species in Uganda.

In recent times R. rattus formed the principal reservoir of the infection in Uganda; it was in many of the plague areas practically the only rat found in human habitations and endemicity never became established in regions where this species was absent. However, R. rattus did not seem to have invaded Uganda before the beginning of the present century so that it could not have been responsible for the plague outbreaks occurring before that time. Its place must then have been taken by R. natalensis (Mastomys coucha auctt.) which continues to be the most abundant hut-rat
in the parts of the Protectorate not invaded by the black rat (*R. rattus*), and which has a flea fauna similar to that of the latter. It is likely that *R. natalensis* continued to take a limited part in the causation of plague (Hopkins 56).

*Xenopsylla brasiliensis* was, in the opinion of Hopkins, "the normal initiator" of the plague outbreaks in Uganda which, as noted above, invariably started in rural localities. *X. cheopis*, nearly always confined in the plague areas to large townships, helped to carry on the epidemics when such places became invaded, but seemed in general not a vector of great importance in Uganda.

(2) **Kenya**

The earliest plague outbreak on record in Kenya, occurring in 1902 at Nairobi, though not necessarily autochthonous, was probably of local origin because, as pointed out by Roberts, 123 Mombasa, the only port through which the infection could have entered, had been free from the disease for years and remained so until 1912.

From 1906 onwards plague began to spread in Kenya but up to the present its incidence has remained usually below the level reached in Uganda (Roberts 124). As summarized by Kaul, 69 600-1,000 cases per year occurred in Kenya from 1926 to 1931, below 300 cases annually from 1932 to 1937, sporadic instances of infection only from 1938 to 1940. However, as shown by the following statistics, the situation became comparatively serious in 1941 and 1942:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1941</td>
<td>781</td>
<td>196</td>
</tr>
<tr>
<td>1942</td>
<td>754</td>
<td>333</td>
</tr>
<tr>
<td>1943</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>1944</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>1945</td>
<td>56</td>
<td>21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1946</td>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td>1947</td>
<td>55</td>
<td>16</td>
</tr>
<tr>
<td>1948</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>1949</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>1952</td>
<td>59</td>
<td>7</td>
</tr>
</tbody>
</table>

It should be noted that in Kenya as in Uganda the endemic foci of plague were situated in rural areas.

A comparatively high incidence of cases with pneumonic features was recorded in some outbreaks, recently by Plum, 114 but attention must be paid in this connexion to the following statement (Hunter 69):

"The disease is mostly bubonic in type: septicaemic and pneumonic cases are common, true inspiration pneumonia is rare."

In a study on the transmission of plague in Kenya published in 1950, Roberts 124 claimed that *R. rattus* was the only rodent involved and that *X. cheopis* and *X. brasiliensis* served as vectors. Human infection was intense in the *cheopis*-infested areas, probably because this flea which was associated with rats living underground was in far closer and more constant contact with man than *X. brasiliensis* which infested the roof-rats. It is,
However, important to note that Heisch, investigating the Rongai area of Kenya, where plague had been endemic for many years, was able to prove the presence of the infection not only in *R. natalensis* ("*Mastomys coucha*") but also in *Arvicanthis abyssinicus*, *Otomys angoniensis* and *Rhabdomys pumilio*. A pool of 21 fleas (*Dinopsyllus ellobius lopus*), collected from an *Arvicanthis* burrow, also proved positive for *P. pestis.*

According to Heisch there seemed little doubt "that *P. pestis* is firmly entrenched in the wild rodents round Rongai and that they are playing an important part in propagating and perpetuating the disease”.

(3) Tanganyika

Since the old trade route from Uganda to the coast led through Tanganyika (formerly German East Africa), it is not surprising to find evidence for the early existence of plague in the latter territory. Outbreaks in the Uhehe county of the southern province of Iringa seem to have taken place as early as 1886 and 1889, and the existence of endemic plague in that area was confirmed by the German observers of the 1903-4 outbreak at Iringa (Wu Lien-teh 159). Another endemic focus was detected in 1897 by Koch and Zupitza in Kisiba, a locality between the Kagera River and Lake Victoria (Wu Lien-teh 158).

Further and sometimes fairly extensive outbreaks continuing in the then German territory from 1906 to 1919 included epidemics in the Gasseni district on the eastern slopes of the Kilimanjaro in 1912 (Lurz 76), in the Mwanza district and eastern Lake Victoria area in 1913, and at Mwanza township and Dar el Salaam in 1914. After the British occupation, manifestations of the disease continued to be frequent in the central and Lake provinces up to 1928. Except in 1931, when 238 cases were recorded, plague was inconspicuous or even absent during the period from 1928 to 1936. The incidence of the disease from then onwards was as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
<th>Locality involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>1937</td>
<td>72</td>
<td>47</td>
<td>Lake province</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>17</td>
<td>Central province</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>Iringa</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>1938</td>
<td>3</td>
<td>0</td>
<td>Northern province</td>
</tr>
<tr>
<td>1941</td>
<td>2</td>
<td>2</td>
<td>&quot;</td>
</tr>
<tr>
<td>1948</td>
<td>311</td>
<td>174</td>
<td>Central province</td>
</tr>
<tr>
<td>1949</td>
<td>18</td>
<td>14</td>
<td>&quot;</td>
</tr>
<tr>
<td>1951</td>
<td>263</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>1952 *</td>
<td>575</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

* Incomplete

To judge from figures showing the monthly plague incidence from 1936 to 1949, epidemics in Tanganyika usually ran their course during the
period from February to July, reaching their peak in March and April. The bubonic type was prevalent but pneumonic manifestations were recorded by the German observers, particularly by Lurz.79

Black rats, recently identified as *R. rattus alexandrinus*, appear to be the only rodents playing a causative role in the Tanganyika outbreaks with *X. brasiliensis* as the probable vector.

(4) Belgian Congo

In compiling this summary on the plague situation in the Belgian Congo, advantage was taken not only of the voluminous published literature, but also of an excellently documented report received in July 1950, in answer to the inquiry instituted by the WHO Expert Committee on Plague, from Dr. A. Duren, of the Ministry for the Colonies, Brussels.

Plague exists in the Belgian Congo in two foci situated in the extreme north-east of the colony near the Uganda border—the Lake Albert focus and the Lake Edward focus.

Nosogeographically, the first of these foci forms part of the plague areas in Uganda, because Lake Albert, which marks the border between that territory and the affected region in the Belgian Congo, served as a means to convey the infection rather than as a bar to its progress. For this reason it seems probable that, though the presence of plague in the Lake Albert focus was detected in 1928 only, the disease had been of long standing.

Cases in the Lake Edward focus were first notified in 1938. The invasion of this area was evidently due to an importation of the infection from the Lake Albert region, possibly through plague fleas which had been carried by passengers or in goods arriving from there by motor-truck (van Riel & Mol 121).

The incidence of the disease in the two foci is shown in table VI.

| TABLE VI. INCIDENCE OF PLAGUE IN LAKE ALBERT AND LAKE EDWARD AREAS, BELGIAN CONGO, 1928-51 |
|---------------------------------|-----------------|-----------------|-----------------|
| Period                         | Lake Albert focus | Lake Edward focus |
|                                | Cases | Deaths | Cases | Deaths |
| 1928-37                        | 133   | —      | —    | —      |
| 1938-47                        | 249   | 226    | 107  | 93     |
| 1943-51                        | 70    | 68     | 38   | 34     |

It will be noted that thus far the plague morbidity in the Belgian Congo was low. The maximal case incidence observed in any year was 65 in
the Lake Albert focus (in 1939) and 29 in the Lake Edward focus (in 1941). The case fatality-rate on the other hand was high, amounting since 1938 to 91.5% in the former focus and to 87.6% in the latter. Commenting upon the severity of the infection, the report to WHO stated that instances of ambulatory plague seemed to be altogether absent in the Belgian Congo. Cases with lung features were observed and were apparently frequent in the Lake Albert focus in 1939 (van Hoof 55) when, as noted above, the incidence of the disease was higher than usual. Plague cases seem to be most frequent during the period from April or May to September or October.

FIG. 2. HOSPITAL FOR PLAGUE PATIENTS IN SPIETELAU, VIENNA, 1679

Several wild-rodent species, particularly *Arvicanthis abyssinicus*, have been found naturally infected in the Belgian Congo, but, since these animals had been obtained in the vicinity of villages where rat plague existed, they had apparently become secondarily involved.

In the Lake Albert focus *Rattus natalensis* ("*Mastomys coucha*”) was by far the most frequent rodent found in human habitations (98%). Among the few other rodents found *Arvicanthis abyssinicus* was comparatively the most conspicuous. It is interesting to note that quite recently an invasion of *R. rattus alexandrinus* has been noted in the Kasenyi region, but so far no instance of infection has been found in this species. *Rattus natalensis ugandae* seems therefore the sole reservoir of plague in the focus.
The rodents found in the huts of the Lake Edward focus were *R. rattus alexandrinus* (52%), *R. natalensis* (32%), and *Arvicanthis abyssinicus* (14%). So far the infection has not been detected in the last species, so that the two first-mentioned rodents appear to be responsible for the plague manifestations in the area.

Both *X. cheopis* and *X. brasiliensis* which are considered as the vectors of plague in the Belgian Congo occurred in the Lake Albert focus, the former forming a large majority of the rat fleas in the north and west of the area, the latter being almost exclusively present in the east and south.

In the Lake Edward focus *X. brasiliensis* was largely preponderant.

Thus far no natural infection has been found in the Belgian Congo in the wild-rodent flea *Dinopsyllus ellobius lypusus* (*D. lypusus* auctt.) but it has been proved to be capable of transmitting plague under experimental conditions; *Ctenocephalides felis strongylus*, which in the Belgian Congo infests man in place of *P. irritans*, was unable to do so.

**Madagascar**

For a study of the plague situation in Madagascar it was possible to consult, in addition to the voluminous literature, a most valuable summary prepared by the Health Services in Tananarive for the WHO Expert Committee on Plague and containing information up to July 1950.

Plague was introduced into Madagascar in November 1898, when a ship bringing a rice cargo from India served as the vehicle of the infection. In the following years outbreaks continued to appear in the major ports but never caused much havoc.

The infection was again introduced into the port of Tamatave in March 1921, leading to an epizootic and 107 bubonic cases with 71 deaths. In June of the same year pneumonic plague appeared at Tananarive on the high plateau of the island and claimed 46 victims in the course of three weeks. Four months afterwards foci of bubonic plague became manifest in adjacent localities and signs of rat infection began to be found. Since that time the disease has persisted on the high plateau, practically all parts of which became successively involved.

Occasional outbreaks were noted in the ports as well, but these never caused much harm and soon terminated. Further, while the outbreaks on the coast continued to be bubonic in nature, the infection showed on the high plateau a marked tendency to lead to secondary lung involvement and thus to produce foci of pneumonic plague. Though no major outbreaks of pneumonic plague occurred, the incidence of this form of the disease was invariably considerable, averaging according to Le Gall 29% during the period 1935-9. Occasionally, the incidence of pneumonic plague was higher, for instance amounting to 56.2% in 1946 (Favarel 27).
The plague incidence in Madagascar from 1933 to 1950 was as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1933</td>
<td>3,933</td>
<td>1942</td>
<td>181</td>
</tr>
<tr>
<td>1934</td>
<td>3,605</td>
<td>1943</td>
<td>234</td>
</tr>
<tr>
<td>1935</td>
<td>3,493</td>
<td>1944</td>
<td>184</td>
</tr>
<tr>
<td>1936</td>
<td>2,006</td>
<td>1945</td>
<td>185</td>
</tr>
<tr>
<td>1937</td>
<td>916</td>
<td>1946</td>
<td>278</td>
</tr>
<tr>
<td>1938</td>
<td>630</td>
<td>1947</td>
<td>274</td>
</tr>
<tr>
<td>1939</td>
<td>681</td>
<td>1948</td>
<td>240</td>
</tr>
<tr>
<td>1940</td>
<td>754</td>
<td>1949</td>
<td>143</td>
</tr>
<tr>
<td>1941</td>
<td>272</td>
<td>1950</td>
<td>153</td>
</tr>
</tbody>
</table>

It will be noted that from 1936 onwards the incidence of the disease decreased to reach a level of less than 300 cases per year in 1941. Large-scale inoculation campaigns with the live vaccine of Girard and Robic had been started in November 1935 (Robic 125) and were no doubt responsible for this decrease of the morbidity. However, as recently pointed out by Lepage,75 the incidence of the disease from 1941 to 1948, though moderate, had remained fairly uniform so that apparently a level had been reached which could not be lowered any more through inoculation campaigns alone. However, application of DDT was started and led to a further decrease in incidence as shown by the following figures:

<table>
<thead>
<tr>
<th>Period</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st quarter of 1948</td>
<td>135</td>
</tr>
<tr>
<td>, , , , 1949</td>
<td>61</td>
</tr>
<tr>
<td>, , , , 1950</td>
<td>34</td>
</tr>
</tbody>
</table>

Lepage also pointed out that during the early months of 1950 no plague case had been recorded in the Emyrne district where DDT had been used most systematically.

Unfortunately, further observations did not fully justify this optimistic attitude. As noted by Robic in the 1950 and 1951 reports of the Pasteur Institute, Tananarive,126, 127 statistics showing the plague incidence in Madagascar not during the calendar, but during the "epidemic" years (1 May to 30 April of the following year) from 1946 onwards were as follows:

<table>
<thead>
<tr>
<th>&quot;Epidemic&quot; year</th>
<th>Plague cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1946-47</td>
<td>312</td>
</tr>
<tr>
<td>1947-48</td>
<td>278</td>
</tr>
<tr>
<td>1948-49</td>
<td>158</td>
</tr>
<tr>
<td>1949-50</td>
<td>109</td>
</tr>
<tr>
<td>1950-51</td>
<td>241</td>
</tr>
<tr>
<td>1951-52</td>
<td>291</td>
</tr>
</tbody>
</table>

In explaining the recently increased plague incidence, Robic pointed out that (a) since 1949 the disease had been found to be present in forest regions situated beyond the confines of the formerly known endemic areas and that (b) in 1951-2 a considerable increase of the plague incidence was noted in the province of Emyrne (131 cases as against 89 in 1950-1),
where pneumonic cases, due evidently to the unrecognized existence of bubonic cases, preponderated. Summing up the situation, Robie stated that "plague is certainly diminishing in the towns and well administered villages everywhere where DDT application can be fully effective, e.g. in and round Tananarive town. It persists in the rural areas, where, it would seem, it is far from becoming extinct".

The incidence of bubonic plague on the Madagascar high plateau was maximal during the period from 1 December until 31 March, and at low ebb during June and July. Pneumonic cases on the contrary were comparatively more frequent from July to December and less numerous during the hot season from December to February (Le Gall 73).

*R. rattus alexandrinus* and *R. rattus frugivorus* are the species of commensal rats commonly met with on the coast as well as on the high plateau, but the former predominates. *R. rattus rattus* is preponderant in the forests; it occasionally enters store houses but is hardly ever found inside human habitations.

No evidence has been forthcoming thus far that wild rodents play a role in the causation of plague, but one species, *Brachytarsomys albicauda*, living mainly in the forests, has been found rather susceptible to experimental infection.

There can be no doubt that the black rats, in the first line *R. rattus alexandrinus*, form the plague reservoir in Madagascar. *X. cheopis* is the only flea recognized there as vector of the infection.

**South Africa**

(1) *Union of South Africa*

In compiling this summary, advantage has been taken not only of an ample literature but also of a valuable report on the plague situation in the Union of South Africa from January 1935 to December 1949, prepared by Davis for the WHO Expert Committee on Plague.

Plague gained a foothold in the Union of South Africa in 1900 when, on account of the Boer War, large amounts of forage had to be imported from infected South-American ports. As summarized by Mitchell, during the period from 1900 to 1902 serious epidemics took place at Cape Town, Port Elizabeth, East London, and other centres in Cape Province, as well as at Durban and Pietermaritzburg. Further outbreaks occurred in 1903 at King Williams Town, Queenstown, and elsewhere, followed in 1904 by a considerable epidemic with an initial pneumonic phase in Johannesburg and its vicinity. The infection persisted there as well as in Port Elizabeth and East London until 1905, but seemed to be altogether absent from the Union from 1906 to 1911. In 1912 an outbreak, due evidently to a re-importation of the infection from eastern ports, occurred.
HISTORY AND DISTRIBUTION

at Durban. As was afterwards realized, that epidemic marked the end of the initial plague period in the Union of South Africa which, because it was characterized by the occurrence of rat-caused outbreaks in urban areas, has been termed the "murine" phase (Davis 19).

From 1914 onwards outbreaks were noted in remote rural localities, first in Cape Province, from 1916 onwards also in the Orange Free State, whence the infection spread in 1917 to a neighbouring district of Transvaal. The total incidence from 1914 to 1918 (when two fatal cases were recorded in the Orange Free State) was 189 cases with 132 deaths. Though it was suspected that wild rodents might be responsible for these manifestations of the disease, proof for this assumption could be obtained only in 1921 (Mitchell 98).

It was formerly believed that a gradual propagation of plague to the rural areas had taken place, the striped mouse (Rhabdomys pumilio) contracting the infection in the outskirts of the infected urban centres and passing it on to other wild-rodent species (Thornton 145). Though such a spread took place occasionally (Mitchell 98), it was of main importance that a direct transport of infected rats and fleas by rail or other means of traffic led to the establishment of three primary distributing centres of wild-rodent plague which were situated in south-western Transvaal and north-western Orange Free State, in the Cape midlands, and in the Uitenhage district near Port Elizabeth respectively (Fourie, 29 Davis 19).

As the result of an expansion of the infection from these primary foci, a vast area, comprising more than half of South Africa, from the Cape to Barotseland in Northern Rhodesia became affected. It would seem that a spread beyond the limits thus reached is not to be expected (Davis 19).

The incidence of the disease from 1 July 1919 to the end of June 1949 was as follows:

<table>
<thead>
<tr>
<th>Period</th>
<th>Cases</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1919-25</td>
<td>556</td>
<td>Expansion of plague from the original foci due to a continued spread of plague among wild rodents.</td>
</tr>
<tr>
<td>1925-31</td>
<td>466</td>
<td>Outbreaks mainly in new areas, as a result of major extensions of the enzootic area in the karroo (Cape midlands) and northern Orange Free State. Spread also into South-West Africa and Bechuanaland.</td>
</tr>
<tr>
<td>1931-7</td>
<td>687</td>
<td>Epidemic in the Orange Free State. Extension and consolidation of the infection in other parts of the affected areas.</td>
</tr>
<tr>
<td>1937-49</td>
<td>646</td>
<td>Continuation of perennial or almost perennial outbreaks in hyperenzootic areas situated in the northern Orange Free State, a district on the Transkei border in the eastern Cape area, and in the Uitenhage Port Elizabeth districts. Sporadic manifestations in the other parts of the enzootic area (Davis 19).</td>
</tr>
</tbody>
</table>

To judge from the available information, the incidence of human plague during the period 1950-2 was low, amounting to 14 cases with 2 deaths in 1950, 14 cases with 6 deaths in 1951, and 2 fatal cases in 1952. 

---

"To judge from the available information, the incidence of human plague during the period 1950-2 was low, amounting to 14 cases with 2 deaths in 1950, 14 cases with 6 deaths in 1951, and 2 fatal cases in 1952."
The incidence of human plague in the Union of South Africa was found to be highest during a season lasting from December to April. However, the wild-rodent epizootics being unaffected by seasonal changes, epidemics have occurred in all months of the year (Davis 21). Bubonic manifestations preponderated, but pneumonic outbreaks have been recorded both during the "murine" phase and subsequently in the wild-rodent foci, recently by Gale 31 and Clark & Goldberg.15

Though, as stated by Davis,21 over 100 species and subspecies of rodents and other small animals are at risk of infection and some 60 species of fleas have to be regarded as actual or potential vectors, the main reservoir of wild-rodent plague in the Union of South Africa is formed by two gerbils, Tatera brantsi and Desmodillus auricularis, associated with Xenopsylla philoxera (X. eridos auctt., not of Jordan and Rothschild) and X. piriei respectively. Human infection was in most instances not directly derived from the wild rodents or through their fleas, the semi-domestic R. natalensis ("Mastomys coucha") and R. rattus acting as intermediaries between the primary gerbil reservoirs and man. R. natalensis carried a mixed fauna of wild and commensal rodent fleas, while R. rattus met with in rural areas was infested with X. brasiliensis, the flea considered as the main vector of human plague in the Union.

Though, as noted above, rodents with semi-domestic or domestic habits were apt to become involved, instances of a transition of the infection from the wild rodents to urban rat populations have been almost totally absent thus far. Only one outbreak at Port Elizabeth in 1938 is on record where a relatively short-lived focus was established amongst the commensal rats and gave rise to 28 cases. Positive rats were found also at Johannesburg in 1943, 1944, and again in 1951, but no general epizootics resulted (Innes 62). Plague in the Union of South Africa appeared thus within recent times almost invariably in rural localities, where as a rule a few cases only occurred at one and the same time. Under these circumstances it is not surprising to find that, according to Ferguson,28 1,005 individual outbreaks took place from 1920 to 1949. Since he recorded the occurrence of 2,361 cases during this period, the case incidence per outbreak averaged 2.3.

(2) Basutoland

The recent plague incidence in Basutoland, which nosogeographically forms a part of the enzootic areas in the Union of South Africa, was as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1935</td>
<td>December</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>1936</td>
<td>January-February</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>1937-41</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1942</td>
<td>February</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>1943</td>
<td>January, March, June</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>1944</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
(3) Bechuanaland

Though Ngamiland in the Bechuanaland Protectorate was reached in 1928 by a wave of wild-rodent plague spreading from the north-west Cape area and the enzootic then established culminated in epizootics in 1934-5 and possibly also in 1939-40, throughout this period only two instances of human plague were recorded—in April 1935. However, the reappearance of an epizootic in 1944 led to a major epidemic which, lasting from October to December, resulted in 304 cases with 156 deaths. Sporadic cases continued up to March 1945 (Davis 18). The further incidence of plague was as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1945</td>
<td>18</td>
</tr>
<tr>
<td>1946</td>
<td>68</td>
</tr>
<tr>
<td>1947</td>
<td>2</td>
</tr>
<tr>
<td>1948</td>
<td>0</td>
</tr>
<tr>
<td>1949</td>
<td>24</td>
</tr>
</tbody>
</table>

As stated in the Bechuanaland Protectorate Medical and Sanitary Report for 1949, signs of an active epizootic were noted during that year in several areas. The Gobabis district in South-West Africa, close to the Bechuanaland frontier, was also found to be affected and several fatal cases of human plague were observed there at the end of 1949.

Gerbil species (Tatera and Desmodillus) formed the permanent reservoir of the infection which, as will be perceived, was apt to flare up every fourth or fifth year. R. rattus and M. musculus appeared to be absent from Bechuanaland, but the multimammate mouse (R. natalensis) acted as the intermediary between the wild rodents and man.

The gerbils were found to be infested with X. eridos (X. philoxera) and its close relative X. hipponax. Both these species occurred in addition to X. brasiliensis on the multimammate mice, but the last-mentioned flea seemed to be responsible for the majority of human infections (Davis 18).

(4) Northern Rhodesia

Suspicious outbreaks were reported in the Luangwa Valley of Northern Rhodesia in 1917 and 1918, but their plague nature seems not to have been confirmed (Davis 21). More recently, however, the infection appeared in the Barotse Province which was evidently invaded by wild-rodent plague from the south through the Kalahari (Davis 29).
The incidence of the disease in that province since its appearance in 1937 was as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Cases</th>
<th>Year</th>
<th>Month</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1937</td>
<td>January</td>
<td>9</td>
<td>1944</td>
<td>February</td>
<td>2</td>
</tr>
<tr>
<td>1938-9</td>
<td>—</td>
<td>0</td>
<td>1945-6</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>1940</td>
<td>February</td>
<td>4</td>
<td>1947</td>
<td>December</td>
<td>1</td>
</tr>
<tr>
<td>1941</td>
<td>—</td>
<td>0</td>
<td>1948</td>
<td>January-February</td>
<td>7</td>
</tr>
<tr>
<td>1942</td>
<td>October, December</td>
<td>14</td>
<td>1949</td>
<td>March</td>
<td>2</td>
</tr>
<tr>
<td>1943</td>
<td>December</td>
<td>5</td>
<td>1950</td>
<td>March</td>
<td>2</td>
</tr>
</tbody>
</table>

It will be noted that the plague season fell into the period from October to February when during the midsummer rains day and night temperatures were high (Davis 20). Occasional pneumonic cases were noted.

Though so far no direct proof seems to have been obtained, it is most probable that in Northern Rhodesia, as elsewhere in South Africa, gerbils are the primary reservoir of plague, and that the multimammate mouse is instrumental in bringing the infection to man. Possibly swamp-rats (Otomys, Dasymys, Pelomys) form an accessory reservoir.

The gerbils were found to harbour X. philoxera, X. hipponax, and occasionally Dinopsyllus ellobius, which was the only flea infesting the swamp-rats. It is interesting that X. philoxera, a notorious vector, was with one exception found solely in those areas of Barotseland where plague was enzootic. However, since X. hipponax, which abounded in the regions free from philoxera, might be a vector, no undue stress ought to be laid upon the absence of the latter flea.

R. natalensis was infested by the wild-rodent fleas mentioned above and by X. brasiliensis which no doubt was of main importance in conveying the infection to man (Davis 20).

North America

(1) United States of America

The incidence of human plague in the USA from 1900 to 1950 may be summarized as follows:

(a) Rat-caused epidemics (Mohr 102)

<table>
<thead>
<tr>
<th>Locality</th>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Francisco, Calif.</td>
<td>1900-4</td>
<td>120</td>
<td>114</td>
<td>Plague existed probably before 1900 (see below).</td>
</tr>
<tr>
<td></td>
<td>1907-8</td>
<td>186</td>
<td>92</td>
<td>2 of these patients and probably also 3 suspected victims had pneumonic plague. Rat infection persisted until 1917 (Fricks 30).</td>
</tr>
<tr>
<td>Seattle, Wash.</td>
<td>1907</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
**HISTORY AND DISTRIBUTION**

<table>
<thead>
<tr>
<th>Locality</th>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Orleans, La.</td>
<td>1914-5</td>
<td>31</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1919-21</td>
<td>25</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Pensacola, Fla.</td>
<td>1920</td>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Galveston, Tex.</td>
<td>1920</td>
<td>18</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Beaumont, Tex.</td>
<td>1920</td>
<td>14</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Los Angeles, Calif.</td>
<td>1924</td>
<td>41</td>
<td>34</td>
<td>33 of the patients had lung features, but these were in part secondary in nature (Wu Lien-teh 159).</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>448</strong></td>
<td><strong>286</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) **Infections contracted from wild rodents or through their fleas** (after Link 76. 77)

<table>
<thead>
<tr>
<th>State</th>
<th>Year of detection of:</th>
<th>Wild-rodent plague</th>
<th>Human plague</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>1908</td>
<td>1908</td>
<td>80</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Oregon</td>
<td>1935</td>
<td>1934</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Utah</td>
<td>1936</td>
<td>1936</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nevada</td>
<td>1936</td>
<td>1937</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Idaho</td>
<td>1936</td>
<td>1940</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>New Mexico</td>
<td>1938</td>
<td>1949</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Arizona</td>
<td>1938</td>
<td>1950</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>1908-51</strong></td>
<td></td>
<td>91</td>
<td>57</td>
<td></td>
</tr>
</tbody>
</table>

*Remarks:* While most of these cases occurred singly, one pneumonic outbreak of wild-rodent origin took place at Oakland, California, in 1919 and claimed 13 victims.

Taken at their face value, these statistics suggest that in the USA, as well as in South Africa and some countries of South America, an initial "murine" phase of plague eventually led to wild-rodent infection. However, according to some observers, the appearance of the disease in North America was due not to its recent introduction by the sea-route, but to an early importation through wild rodents which, coming overland across the Bering Strait from Central Asia, brought *Pasteurella pestis* with them as a population regulator (Jacobsen, 64 Meyer 98). Meyer 98 pointed out in this connexion that it was difficult to believe in as rapid a spread of wild-rodent plague as ought to have taken place had the infection been recently introduced. Likewise he maintained that as a rule wild-rodent plague in North America

"remained confined to foci and no convincing evidence has been produced that some wide-ranging animal has started new epizootics ".

While it would be out of place to discuss this point at the present juncture, it should be noted here that it is difficult to believe in the fascinating hypothesis set forth above for the following reasons: First, should plague have come overland from Central Asia, it should have appeared in Canada earlier than in the USA. Actually, however, it appeared in that Dominion much later than in the USA and was no doubt imported from there. Further, as will be discussed in chapter 2, the plague strains isolated in the USA...
are biochemically different from those in Central Asia. Finally, it is by no means certain that the usually given date of entry of the infection into the USA was the actual one. Kinyoun \textsuperscript{71} felt sure that plague had been present in San Francisco in 1898 already and quoted a report claiming that “in all probability, the disease had existed on the Pacific Coast since 1896”. Seeing that plague had reached the coast of China as early as 1867, one might even wonder if it had not been carried to California long before 1896, so that there was far more time for a spread of the infection to the hinterland than is usually allowed for.

As will be gathered from the first of the statistics inserted above, rat-caused plague, though repeatedly appearing in the USA, has never shown marked tendencies to spread or persist. This is all the more remarkable because, with the exception of Washington State, the commensal rats in the plague-affected localities showed a considerable infestation with \textit{X. cheopis}. \textit{R. norvegicus} was the most preponderant rat species.

Wild-rodent plague was present not only in the States where it led to human infection, but also in others, as shown by the following (Link \textsuperscript{76}) :

<table>
<thead>
<tr>
<th>State</th>
<th>Year of detection</th>
<th>State</th>
<th>Year of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>1908 *</td>
<td>Arizona</td>
<td>1938</td>
</tr>
<tr>
<td>Montana</td>
<td>1935</td>
<td>New Mexico</td>
<td>1938</td>
</tr>
<tr>
<td>Oregon</td>
<td>1935</td>
<td>Colorado</td>
<td>1941</td>
</tr>
<tr>
<td>Idaho</td>
<td>1936</td>
<td>North Dakota</td>
<td>1941</td>
</tr>
<tr>
<td>Nevada</td>
<td>1936</td>
<td>Oklahoma</td>
<td>1944</td>
</tr>
<tr>
<td>Utah</td>
<td>1936</td>
<td>Kansas</td>
<td>1945</td>
</tr>
<tr>
<td>Wyoming</td>
<td>1936</td>
<td>Texas</td>
<td>1946</td>
</tr>
<tr>
<td>Washington</td>
<td>1937</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Wild-rodent plague had evidently existed in the Contra Costa county of California since 1903 at least.

The low incidence of human infections derived directly from the wild rodents or through their fleas is in striking contrast to the large area, comprising 131 counties in 15 States, where evidence of plague among these animals has been found. Discussing this problem, Meyer \textsuperscript{95} stated that the disease

“occurs among wild rodents in wooded or rural districts uninhabited or only sparsely inhabited by man, but human contact with the infective agent probably is established in exceptional instances”.

Eskey \& Haas,\textsuperscript{25} in their classical study on “Plague in the western part of the United States”, came to the conclusion that at least three groups of rodents constituted the great primary reservoirs of the infection—the ground-squirrels, which were widely involved in the coastal regions and in the northern part of the Intermountain Plateau; the wood-rats, which formed the plague reservoir in the southern deserts; and finally the prairie-dogs, which harboured the infection in the plateau region of Arizona and New Mexico. Mohr \textsuperscript{102} endorsed this opinion but also incriminated sage-brush voles and certain meadow mice.
FIG. 3. GEOGRAPHICAL DISTRIBUTION OF CASES OF HUMAN PLAGUE REPORTED FROM 1948 TO 1952

Map prepared by WHO on the basis of official data made available by governments.
While, as noted above, the danger of a spread of plague to man through direct contact with wild rodents or through their fleas is slight, secondary involvement of the rats or other rodent species living near man might greatly enhance the chances for human infection.

The possibility of such a transition had been claimed by the advocates of an overland introduction of the disease from Central Asia who pointed out that up to 1908 large numbers of ground-squirrels had been imported into San Francisco for culinary purposes (Meyer 94). It was also suspected that an infection recently derived from wild rodents had been responsible for the epizootic causing the 1924 Los Angeles epidemic (Vu Lien-teh 159), and that the presence of plague among the rats of Tacoma (Washington State) in 1942 and 1943 had been due to an importation of the infection from the hinterland through grain transports (Hundley & Nasi 30). Wild-rodent fleas had been found upon several occasions on commensal rats and isolated instances of rat plague had been detected in urban centres as well as in rural areas of California (Meyer & Holdenried, 97 Moehr 102).

Working recently on a Californian ranch round which wild-rodent plague was present, Meyer & Holdenried 97 were able to prove the presence of the infection in rats (R. rattus rattus and R. norvegicus) which were in part infested with ground-squirrel fleas. L. segnis was the preponderant specific parasite on these rats, while Nosopsyllus fasciatus was rare and X. cheopis absent. However, in the opinion of the authors, the wild-rodent fleas might have been able to maintain the infection which they had introduced. Meyer & Holdenried came to the important conclusion that:

"on numerous occasions, rural plague has swept with destructive force through native rodent populations with little if any danger to human beings, but when domestic rats (Rattus) or mice (Peromyscus, Microtus, or Mus), which may live in man's immediate environment and the fleas of which will attack and thus infect him, are involved, a much more serious problem arises".

Fortunately, this potential danger is fully realized, due attention being paid to rodent and flea control in and round human settlements.

(2) United States of America — Hawaii

Plague became manifest in Honolulu in November 1899, a few months after patients suffering from the disease had been found on two ships arriving from Hong Kong. The infection not only persisted for about 10 years in Honolulu, but spread to adjacent districts as well as to other islands of the archipelago:

<table>
<thead>
<tr>
<th>Locality</th>
<th>Period</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oahu Island:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honolulu</td>
<td>1899-1910</td>
<td>187</td>
</tr>
<tr>
<td>Other places</td>
<td>1902-7</td>
<td>41</td>
</tr>
<tr>
<td>Kauai</td>
<td>1901-6</td>
<td>15</td>
</tr>
<tr>
<td>Maui</td>
<td>1900</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1930-2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1938</td>
<td>1</td>
</tr>
</tbody>
</table>
As will be noted, within recent years human plague was present in the Hamakua district of Hawaii Island only, where after a clear interval a case was recorded in 1949 and where rodent infection persists to date. Positive results were also obtained in 1949 with two batches of pooled tissues from Maui rats.

The district of Hamakua contained many sugar plantations and, as stated by Eskey, human-plague infection was contracted in the cane-fields rather than in the houses. *R. rattus alexandrinus* formed half of the rat population in the district, but *R. norvegicus* and *R. hawaiiensis*, a small mouse-like animal rarely entering houses, were also met with. *X. cheopis* constituted 70% of the rat fleas.

The mortality from human plague was high since, as stated by Hampton, 360 of the 397 patients seen from 1899-1933 and all 17 observed from then up to 1944 died.

(3) Canada

An importation of plague from the USA being anticipated, a large-scale wild-rodent survey was made in Western Canada in 1938. No positive results were obtained (Gibbons 34).

However, in 1939 the prevalence of an epizootic among the ground-squirrels of Alberta was noted, and bacteriological findings proving the presence of plague were made in one of these animals taken at Stanmore, a locality about 180 miles (290 km) north of the boundary separating Canada from Montana, USA. Since, as noted above, wild-rodent plague had been found to exist in that State since 1935, there can be no doubt that the infection had spread from there into Canada.

Further evidence of plague in the Alberta ground-squirrels was found in 1939-42, and again in 1945. Spread of the infection from Alberta to an adjacent region of Saskatchewan was noted in 1946 (Humphreys & Campbell 58). Pools of ground-squirrel fleas were found positive for plague in 1947 both there and in Alberta.

The ground-squirrels involved in Canada belonged to the species *Citellus richardsoni richardsoni*. They harboured *Opisocrostis labis, O. tuber-

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* Besides in the Hawaii archipelago, plague has been reported within recent years in another Pacific island, New Caledonia, where early in 1941 a village near the seaport of Goro, situated about 30 miles east of Noumea, the capital, became affected. Up to March 1, nine cases with six deaths were recorded, eight of which were stated to have occurred among pupils of a native school. Prior to this epidemic, which was bacteriologically confirmed, an unusual mortality among the “brush or coconut-tree” rats in the Goro region had been noted. It was also stated that plague was “latently endemic in small areas of the colony”.

The entire population of the plague-affected area was vaccinated. Plague serum and sulfathiazole were used for treatment.
culatus, and Oropsylla rupestris, three species of fleas found able by Eskey & Haas 25 to convey plague under experimental conditions.

It is reassuring that according to Humphreys & Campbell 58 commensal rats seemed not to have colonized to any extent in Alberta. However, they infested the large municipalities of Saskatchewan.

Thus far the existence of human plague has not been proven in Canada, but it is possible that one fatal case occurred in Alberta before the existence of wild-rodent infection had been established. The man in question was breeding minks which he fed with ground-squirrels from a locality later found to be plague-affected. Several of the minks died and their breeder got scratched whilst skinning one of these animals (Gibbons & Humphreys 35).

South America

(1) Venezuela

Plague, believed to have been imported from Trinidad, appeared in 1908 in the port of La Guaira and soon spread from there to Caracas, the capital of Venezuela, where it continued to occur until 1919, leading to a total of 204 cases with 99 deaths.

Even before this “urban” phase had come to an end, the disease had spread in 1910 to rural areas of Miranda State, in one district of which it continues to exist. An adjacent region of Aragua State, reached by the infection in 1939, also remained involved. As stated by Isaac Riaz,68 the two affected districts cover an area of 1,000 km² (386 square miles)—1% of Venezuelan territory.

The plague incidence in the two affected regions from 1910 to 1951 was:

**Miranda State**

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1910</td>
<td>35</td>
<td>1928</td>
<td>10</td>
</tr>
<tr>
<td>1911</td>
<td>18</td>
<td>1932</td>
<td>10</td>
</tr>
<tr>
<td>1914</td>
<td>16</td>
<td>1933</td>
<td>7</td>
</tr>
<tr>
<td>1919</td>
<td>110</td>
<td>1950</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1951</td>
<td>7</td>
</tr>
</tbody>
</table>

Total cases 1910-51 = 218

**Aragua State**

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1939</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>1943</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>1948</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>1949</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

1939-49 37 17
At Caracas *R. norvegicus* formed 98% of the rat population and *X. cheopis* 95% of the rat fleas as against 3.4% *X. brasiliensis* (Hecht 48).

The commensal species (*R. norvegicus, R. rattus rattus* and *rattus alexandrinus*) were also present in and around the settlements of the plague-affected rural areas and were no doubt responsible for the appearance of plague in man, *X. cheopis* and *X. brasiliensis* serving as vectors. However, Isaac Riaz 63 postulated with much reason the existence of wild-rodent foci where the infection was carried over during the often prolonged periods of its absence from human settlements. Actually, instances of natural plague were found in two wild-rodent species, *Heteromys anomalus* and *Sigmodon hirsutus*. Isaac Riaz claimed in this connexion that the fleas of the genus *Polygenis*, which formed the bulk of the fauna on the wild rodents, were particularly apt to produce smouldering enzootics because they did not become blocked when ingesting plague bacilli. It is, however, of interest that sometimes a high percentage of *Xenopsyllae*, particularly *X. brasiliensis*, was found on the wild rodents (Hecht, 49 Isaac Riaz 63). An increased incidence of the latter flea was noted in June 1943, one month before the appearance of the outbreak in Aragua State. The presence of 52.2% *X. brasiliensis* and 20.3% *X. cheopis* in May 1942 did not lead to an epidemic, but one might venture to suggest that a high prevalence of *Xenopsyllae* on the wild rodents was of importance only when at the same time plague was active amongst these animals. The necessity for a coincidence of these two factors might explain why in the rural areas of Venezuela years with manifestations of human plague are separated by long periods of quiescence.

(2) *Brazil*

It is generally accepted that plague, imported by the sea-route, first appeared in Brazil in 1899 when Santos and a few months afterwards São Paulo, lying inland from that port, became infected. From then onwards up to 1906 Rio de Janeiro, Fortaleza in the State of Ceará, Pernambuco, Rio Grande do Sul, and other ports became successively involved. From 1907 onwards the infection began to spread to inland cities and towns but disappeared since 1934 rapidly from these centres while persisting in rural areas which remain in part involved to date (Barreto & Castro 8). The total incidence of the disease from 1899 to 30 June 1949 is shown by the following approximate figures (Moll & O'Leary; 103 A. Castro, communication to WHO Expert Committee on Plague, 1950):

<table>
<thead>
<tr>
<th>Period</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1899-1929</td>
<td>5,638</td>
</tr>
<tr>
<td>1930-4</td>
<td>535</td>
</tr>
<tr>
<td>1899-1934</td>
<td>6,173</td>
</tr>
</tbody>
</table>


Details of the plague incidence in Brazil from 1935 to 1949 are given in table VII (A. Castro, communication to WHO Expert Committee on Plague, 1950).

<table>
<thead>
<tr>
<th>Year</th>
<th>São Paulo</th>
<th>Rio de Janeiro</th>
<th>Minas Gerais</th>
<th>North-East Brazil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>Deaths</td>
<td>Cases</td>
<td>Deaths</td>
<td>Cases</td>
</tr>
<tr>
<td>1935</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1936</td>
<td>21</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1937</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1938</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1939</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1940</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1941</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1942</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1943</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1944</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1945</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1946</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1947</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1948</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1949</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1935-49</td>
<td>38</td>
<td>24</td>
<td>19</td>
<td>12</td>
</tr>
</tbody>
</table>

Total cases 1899-1949 = 9,248

It will be noted that except for one outbreak in Minas Gerais State in 1946-7 which has been dealt with by Macchiavello & Martins de Almeida, from 1943 onwards north-eastern Brazil alone was involved. The situation there is given in table VIII.
As shown by table VIII, the situation was serious in four of the six States concerned, particularly in Pernambuco.

### TABLE VIII. CASES OF AND DEATHS FROM PLAGUE IN THE STATES OF NORTH-EASTERN BRAZIL, 1935-49

<table>
<thead>
<tr>
<th>Period</th>
<th>Alagoas</th>
<th>Bahia</th>
<th>Ceará</th>
<th>Paraíba</th>
<th>Pernambuco</th>
<th>Piauí</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1935-9</td>
<td>74</td>
<td>23</td>
<td>79</td>
<td>31</td>
<td>317</td>
<td>95</td>
<td>16</td>
</tr>
<tr>
<td>1940-4</td>
<td>256</td>
<td>36</td>
<td>126</td>
<td>39</td>
<td>108</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>1945-9</td>
<td>53</td>
<td>8</td>
<td>319</td>
<td>48</td>
<td>249</td>
<td>44</td>
<td>3</td>
</tr>
<tr>
<td>1935-49</td>
<td>383</td>
<td>67</td>
<td>524</td>
<td>118</td>
<td>674</td>
<td>160</td>
<td>9</td>
</tr>
</tbody>
</table>

*a Until 30 June

As was to be expected, the seasonal incidence of plague in São Paulo in the south of Brazil was different from that farther north. Most of the cases of bubonic plague in São Paulo occurred during the sultry days of summer with the peak in January (Oliveira, Moll & O'Leary). In marked contrast to this a pneumonic plague outbreak at São Paulo in 1936 took place during the winter in July (de Moura Albuquerque), and the same held true of an earlier pneumonic plague epidemic observed at Santa Maria in the State of Rio Grande do Sul in 1912 (Wu Lien-teh).

The period of maximal plague incidence in Rio de Janeiro was from September to January, commencing thus earlier than in São Paulo (Rangel, quoted by Moll & O'Leary).

As shown by monthly figures supplied to the WHO Expert Committee on Plague by Castro for the period from 1935 to 1949, the seasonal plague incidence in the north-east of Brazil was not as clear-cut as in the south of the country:

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>233</td>
</tr>
<tr>
<td>February</td>
<td>194</td>
</tr>
<tr>
<td>March</td>
<td>235</td>
</tr>
<tr>
<td>April</td>
<td>190</td>
</tr>
<tr>
<td>May</td>
<td>103</td>
</tr>
<tr>
<td>June</td>
<td>75</td>
</tr>
<tr>
<td>July</td>
<td>403</td>
</tr>
<tr>
<td>August</td>
<td>312</td>
</tr>
<tr>
<td>September</td>
<td>372</td>
</tr>
<tr>
<td>October</td>
<td>364</td>
</tr>
<tr>
<td>November</td>
<td>250</td>
</tr>
<tr>
<td>December</td>
<td>242</td>
</tr>
</tbody>
</table>
Still, the incidence of plague in north-eastern Brazil during these years was comparatively highest from July to October, thus roughly corresponding to the seasonal period mentioned by Barreto for Pernambuco. He claimed an earlier onset for Ceará which is situated farther north (May-June) and stated that in both States the period of maximal plague incidence coincided with the harvest season.

While, as noted above, occasional pneumatic epidemics occurred, bubonic plague was largely preponderant, 95.7% of the cases observed in Brazil from 1936 to 1945 being of this type as against 2.16% pneumatic and 1.7% septicaemic cases (Barreto & Castro). The plague mortality was generally low and, as will be discussed in a subsequent chapter of this monograph, atypically mild cases were frequent.

Though, as will be recorded later, natural plague has been found in a whole series of Brazilian wild rodents, it was held by Macchiavello and other observers that these animals did not form a primary reservoir of the infection but were secondarily or even accidentally involved.

While *R. rattus* was preponderant in the rural areas of the north-east, Norway rats were most common in some of the ports and in São Paulo State. In Rio de Janeiro this species and *M. musculus* were more frequent than *R. rattus* and *rattus alexandrinus*. Though *X. brasiliensis* occurred everywhere side by side with *X. cheopis*, the comparative frequency with which the former flea was present on the rats increased pari passu with the latitude of the regions in question (Barreto & Castro). *X. cheopis* was considered as the plague vector in Brazil (Macchiavello).

(3) *Argentina*

Apparently by-passing Buenos Aires and the Argentinian ports on the Paraná River, plague became manifest first in South America at Asunción, situated far inland in Paraguay, which had been reached in April 1899 by an infected steamer. From this original focus the disease was soon carried back to Rosario and other river ports in Argentina, at the end of the year also to Buenos Aires.

The initial period of the infection thus established was followed first by a stage during which plague was carried inland by rail, then by further progress of the pest to remote regions in the interior where wild-rat foci were created. A wide area, extending from the provinces of Jujuy and Salta on the Bolivian border in the north to Patagonia in the south, thus became gradually involved (Sussini).

As pointed out by Barrera, generally speaking, the inland foci of plague in Argentina fell into two groups:

(a) Those in the central part of the country, particularly in the provinces of Rio Negro, La Pampa, Mendoza, and San Juan, with a sparse population and no agriculture. Since grain stores which might have attracted the
rodents to the settlements were absent, contact with infected animals was restricted to chance meetings in the fields, and the incidence of human plague was consequently low.

(b) Those in the north, especially in Santiago del Estero, Tucuman, and Salta, where accumulation of agricultural products attracted the rodents to the settlements and houses, and the incidence of human cases was accordingly higher.

The number of plague cases in Argentina from 1899 to 1930 amounted to about 6,200 (Moll & O'Leary 103). The recent incidence of the disease was as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1931</td>
<td>37</td>
<td>24</td>
</tr>
<tr>
<td>1932</td>
<td>61</td>
<td>19</td>
</tr>
<tr>
<td>1933</td>
<td>63</td>
<td>25</td>
</tr>
<tr>
<td>1934</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>1935</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>1936</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>1937</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>1938</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>1939</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>1940</td>
<td>228</td>
<td>192</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1941</td>
<td>56</td>
<td>28</td>
</tr>
<tr>
<td>1942</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td>1943</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1944</td>
<td>107</td>
<td>63</td>
</tr>
<tr>
<td>1945</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1946</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>1947</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1948</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>1949</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1951</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Note*: In 1940 the provinces of Santiago del Estero, Cordoba, and Tucuman were mainly involved, in 1944 Salta and Jujuy Provinces.

Though these figures show the trend of the infection rather than the exact incidence of the disease, which was probably higher, they indicate that on the whole the plague situation in Argentina was favourable within recent years. The ports were free after 1931 with the exception of a small outbreak (8 cases with 3 deaths) in November 1946 at Buenos Aires (Moll & O'Leary 103). Considering the large extent of the areas involved, the morbidity in the rural districts was as rule a rather low and, moreover, as is characteristic of the manifestations caused by wild rodents, human cases were usually not grouped together but appeared in numerous foci which were independent of one another (Outes, Villafañe Lastra et al. 152). It must be noted, however, that pneumonic cases were comparatively frequent and that repeated though usually limited pneumonic plague epidemics were observed. Miyara et al. 100 recorded 27 such outbreaks with a total of 222 deaths and one instance of recovery for the period 1913-38.

Villafañe Lastra et al. 152 noted the interesting fact that within recent years there was a notable increase of outbreaks taking place in autumn and winter whereas formerly the plague incidence had been highest in summer.
Among the commensal rodents of Argentina, Norway rats were predominant in the towns as well as in the rural areas. *R. rattus rattus* was less common, *R. rattus alexandrinus* least frequent, except in the coastal provinces. *X. cheopis* was the prevalent flea species on these animals (Moll & O'Leary).

As summarized by Macchiavello, natural plague was found in the following wild-rodent species of Argentina:

- *Cavia pamparum*
- *Hesperomys murillus cordovensis*
- *Galea musteloides* (2 subspecies)
- *Lepus europaeus*
- *Caviella (Microcavia) australis* (2 subspecies)
- *Sylvilagus brasiliensis gibsoni*
- *Graomys griseoflavus* (2 subspecies)

Two of the fleas found on these rodents, *Delosrichus* (formerly *Parapsyllus*) talis (on *Caviella australis*) and *Polygenis* (formerly *Rhopalopsyllus*) platensis cisandinus (on *Gr. griseoflavus*) have been found capable of transmitting plague by their bites and attacking man (Barrera).

The relative importance of the commensal rats and of the wild rodents, as well as the question of a transition of plague from the former to the latter or vice versa have been the subjects of much debate. No doubt can exist that originally the infection spread from the commensal rats (probably from *R. norvegicus*) to the wild-rodent species (Villafane Lastra et al.), and it is also certain that foci have become established where wild rodents alone are responsible for the causation of human plague as well as for the perpetuation of the infection. However, in other localities a spread of the disease from the wild to the commensal rats was observed and the latter then played a subsidiary or even a preponderant role in the causation of human attacks. Barrera maintained that opportunities for contact between the wild and the commensal rodents were present, particularly in the agricultural areas of North Argentina where both were attracted by the grain stores, and a study of the available literature confirmed that involvement of the commensal rats in the chain of infection was far more frequently noted there than in the central provinces.

(4) *Bolivia*

Though the presence of plague in southern Bolivia was bacteriologically confirmed in 1928 only, there can be little doubt that outbreaks, due primarily to an importation of the infection from North Argentina, have occurred since 1921. The somewhat incomplete information on these manifestations of the disease, culled from Moll & O'Leary's compilation as well as from other sources (Veintemillas, Mealla, Prado Barrientos, Siles, Cors, Benavides), may thus be summarized:

---

*Footnote:* Some further species of wild rodents occasionally found naturally infected in Argentina are enumerated in Annex 1 (page 623).
<table>
<thead>
<tr>
<th>Area</th>
<th>Year</th>
<th>Season</th>
<th>Cases</th>
<th>Deaths</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarija Department</td>
<td>1921</td>
<td>January-July</td>
<td>1,525</td>
<td>642</td>
<td>Several localities involved</td>
</tr>
<tr>
<td></td>
<td>1921-2</td>
<td>December-May</td>
<td>375</td>
<td>300</td>
<td>Several localities involved; 87 of the cases were pneumonic</td>
</tr>
<tr>
<td></td>
<td>1937-8</td>
<td>December-March</td>
<td>90</td>
<td>18</td>
<td>Preceded by rat epizootic</td>
</tr>
<tr>
<td></td>
<td>1943</td>
<td>June</td>
<td>10</td>
<td></td>
<td>Outbreak at Moreta</td>
</tr>
<tr>
<td></td>
<td>1944</td>
<td></td>
<td>12</td>
<td></td>
<td>Infected rats still found in 1946</td>
</tr>
<tr>
<td>Vallegrande Province</td>
<td>1928</td>
<td>June-July</td>
<td>300</td>
<td>88</td>
<td>Bacteriologically confirmed</td>
</tr>
<tr>
<td></td>
<td>1929-34</td>
<td></td>
<td></td>
<td></td>
<td>Suspicious outbreaks</td>
</tr>
<tr>
<td></td>
<td>1935</td>
<td>July</td>
<td>12</td>
<td>9</td>
<td>Two foci</td>
</tr>
<tr>
<td>Tomina Province</td>
<td>1933</td>
<td>January</td>
<td>800</td>
<td></td>
<td>In the Department of Chuquisaca</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>Over 100</td>
<td></td>
<td>Yearly outbreaks</td>
</tr>
<tr>
<td></td>
<td>1934-7</td>
<td>December-February</td>
<td>140</td>
<td>81</td>
<td>Rat epizootics present</td>
</tr>
<tr>
<td></td>
<td>1938-40</td>
<td></td>
<td>5</td>
<td></td>
<td>In the Department of Chuquisaca</td>
</tr>
<tr>
<td></td>
<td>1944</td>
<td></td>
<td>1</td>
<td></td>
<td>In the Department of Chuquisaca</td>
</tr>
<tr>
<td></td>
<td>1946</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>1938</td>
<td>August-October</td>
<td>150</td>
<td>62</td>
<td>At Choreti and Camiri</td>
</tr>
<tr>
<td></td>
<td>1943</td>
<td></td>
<td>10</td>
<td></td>
<td>At Gutierrez</td>
</tr>
<tr>
<td></td>
<td>1944</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1945</td>
<td></td>
<td>79</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1946</td>
<td></td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>El Palmar (Chaco Boliviano)</td>
<td>1938</td>
<td>April-July</td>
<td>90</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

While, as noted above, a rat epizootic was responsible for the 1937-8 outbreak in the Tarija Department and the same held true for at least some of the subsequent epidemics, the cause of the earlier plague manifestations in Bolivia has not been elucidated. Signs of rat epizootics seemed then to be absent and, though in later years a few wild rodents were found to have succumbed to plague (Alvarado,1 Macchiavello 88), there was no evidence to prove that the infection had become entrenched amongst them. It was postulated therefore that human parasites might have been responsible for the early spread of the disease, the more so because the people used to crowd together to hold prolonged wakes over the dead. Since similar claims have been made elsewhere, it is proposed to deal later in a general manner with the role of human parasites in the spread of plague.

(5) Peru

As Moll & O’Leary 103 state:

"Peru has the unhappy distinction of having had more plague than any other American country, and no plague-free years since its introduction."
FIG. 4. INCIDENCE OF HUMAN PLAGUE IN 1952

Map prepared by WHO on the basis of official data made available by governments.
Following its importation by the sea-route into the port of Callao in 1903, the disease spread along the coast of Peru, most of the principal ports becoming infected within two years, and eventually invaded 10 of Peru's 20 departments, as well as the three special provinces of Tumbes, Callao, and Moquegua. The situation became worst in the coastal departments of Lambayeque, Libertad, and Lima, as well as inland in Cajamarca (Eskey,\textsuperscript{23} Moll & O’Leary \textsuperscript{103}). The former three areas remained infected to date while Cajamarca reported cases in 1948.

The total incidence of plague in Peru up to 1952 may be summarized thus:

<table>
<thead>
<tr>
<th>Period</th>
<th>Cases</th>
<th>Annual average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1903-12</td>
<td>8,865</td>
<td>886</td>
</tr>
<tr>
<td>1913-22</td>
<td>6,922</td>
<td>692</td>
</tr>
<tr>
<td>1923-32</td>
<td>4,642</td>
<td>464</td>
</tr>
<tr>
<td>1933-42</td>
<td>1,087</td>
<td>109</td>
</tr>
<tr>
<td>1943-52</td>
<td>738</td>
<td>74</td>
</tr>
<tr>
<td>1903-52</td>
<td>22,254</td>
<td>445</td>
</tr>
</tbody>
</table>

Note: The maximal yearly incidence was in 1908 (1,691 cases). A second peak (1,200 cases) was reached in 1926.

As shown by the classical study of Eskey,\textsuperscript{23} the central part of Peru, situated between the 7th and 13th degrees of latitude and specially the areas between the 7\textsuperscript{o} and 9\textsuperscript{o} with an average annual mean temperature between 69\textdegree{}F (20.5\textdegree{}C) and 71\textdegree{}F (21.7\textdegree{}C), suffered most from plague. There the infection spread rapidly to rural as well as urban communities, produced more cases than elsewhere in the country, and showed little tendency to disappear spontaneously. However, the degree of rat infestation of the houses was also of importance. Thus the ports of Paita and Mollendo, though situated well away from the zone where the climate favoured the spread of plague, suffered heavily because their wooden buildings were attractive to the rats. Lima, on the contrary, though open to inroads of the infection as far as the climatic conditions were concerned, had a lesser morbidity than these two ports because of having better-class houses.

Generally speaking, the annual plague epidemics in Peru tended to reach their peak during the summer months. However, there as elsewhere the plague seasons fell into an earlier period in areas where the winter months were warm than in localities with a colder climate (Eskey \textsuperscript{23}).

Human plague in Peru was mostly bubonic. Pneumonic plague was rare in general but one outbreak, claiming 21 victims in the department of Junin, was mentioned by Moll & O’Leary.\textsuperscript{103}

As stated by Eskey,\textsuperscript{23} \textit{R. norvegicus}, \textit{R. rattus}, and \textit{R. rattus alexandrinus} were common in the towns of northern Peru, while in the central and southern coastal areas as a rule Norway rats greatly exceeded the other two species. In the rural districts near Lima \textit{R. norvegicus} and \textit{R. rattus alexandrinus} were found. \textit{X. cheopis} was the most common rat flea and, according to Eskey, the only important plague vector.
Ramos Díaz, investigating a plague outbreak in the mountainous region of Lambayeque, found that the epidemic was preceded by an epizootic among the guinea-pigs kept in the house of the first patient. The rats \((R. \text{ rattus})\) in this locality lived in the fields but visited the houses at night, thus coming in contact with the guinea-pigs. The latter were infested with \(P. \text{ irritans}\) as well as \(X. \text{ cheopis}\). Ramos Díaz was of the opinion that the former flea conveyed the infection to man and also believed that transport of \(P. \text{ irritans}\) in garments might lead to sporadic human infections without the intervention of rats.

Though formerly wild rodents had been found naturally plague-affected in Peru, the infection was apparently not entrenched among such species. Recently, however, Macchiavello, was able to prove the existence of wild-rodent foci

(a) on the Peruvian-Ecuador border where squirrels \((\text{Sciurus stramineus nebouxi})\) and some other species were found to be involved and the flea of the former rodent \(\text{Polygenis litargus}\), was found to transmit the infection;

(b) in the Andean region of Huancabamba, where an \(\text{Akodon}\) and an \(\text{Oryzomys}\) formed the primary reservoir of the infection and a \(\text{Pleochaetis}\) species appeared to be the principal vector.

(6) Ecuador

After having reached Guayaquil by the sea-route in 1908, plague not only persisted in that port until 1930 but soon began to invade both coastal and inland areas of Central Ecuador. The province of Loja in the south also became involved eventually, but this was due to repeated importation of the disease from adjacent endemic areas in Peru.

The progress of plague in Ecuador up to 1940 has been well illustrated by a table compiled by Moll & O'Leary:

<table>
<thead>
<tr>
<th>Locality</th>
<th>Period</th>
<th>Cases</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal Zone:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guayaquil</td>
<td>1908-39</td>
<td>7,921</td>
<td>After an absence from 1931 to 1934 plague was reintroduced in 1935 to last until 1939.</td>
</tr>
<tr>
<td>Provinces of Los Ríos</td>
<td>1909-39</td>
<td>416</td>
<td>According to Sáenz Vera, two small outbreaks, due to importation from Peru, took place in El Oro in 1940 and 1944; cases were once more reported there in 1950.</td>
</tr>
<tr>
<td>El Oro and Guayas (except Guayaquil)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manabi Province</td>
<td>1913-37</td>
<td>337</td>
<td></td>
</tr>
<tr>
<td>Central Zone:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chimborazo Province</td>
<td>1909-40</td>
<td>1,335</td>
<td>Infection continues to exist up to the present</td>
</tr>
<tr>
<td>Tungarahuana Province</td>
<td>1916-29</td>
<td>187</td>
<td></td>
</tr>
<tr>
<td>León Province</td>
<td>1926, 1929</td>
<td>68</td>
<td>Plague had been imported from Peru (Sáenz Vera)</td>
</tr>
<tr>
<td>Canar</td>
<td>1933</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>
Localities **Period** | **Cases** | **Remarks**
---|---|---
**Southern Zone:**
Loja Province | 1921-40 | 1,411 | Plague, which has probably been present since about 1919, continues to occur to date

Having become exclusively rural in character, the infection persists in Ecuador, mainly in the mountainous areas of Chimborazo and Loja Provinces, as shown by the following data:

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
<th>Year</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1941</td>
<td>39</td>
<td>14</td>
<td>1947</td>
<td>21</td>
<td>—</td>
</tr>
<tr>
<td>1942</td>
<td>5</td>
<td>2</td>
<td>1948</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>1943</td>
<td>15</td>
<td>9</td>
<td>1949</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>1944</td>
<td>36</td>
<td>11</td>
<td>1950</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>1945</td>
<td>38</td>
<td>16</td>
<td>1951</td>
<td>35</td>
<td>19</td>
</tr>
<tr>
<td>1946</td>
<td>45</td>
<td>19</td>
<td>1952</td>
<td>38</td>
<td>*</td>
</tr>
</tbody>
</table>

* Information incomplete

In general, plague in Ecuador was most rampant in December, of low incidence in June. However, in Loja Province outbreaks occurred chiefly in the dry season from May to December (Moll & O’Leary 108).

While, generally, bubonic cases were most common, the incidence of pneumonic plague was comparatively high in the mountainous areas. Sáenz Vera 129 noted in this connexion in 1941 that, while only 43 out of the 8,000 odd cases observed in the coastal areas since 1908 were pneumonic, there were 194 instances of pneumonic plague (22.2%) among the 874 cases recorded in Chimborazo Province from 1913 onwards, invariably in rural localities. According to Murdock 105 three pneumonic epidemics took place in Ecuador during 1939:

<table>
<thead>
<tr>
<th>Period</th>
<th>Province</th>
<th>Locality</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>January-February</td>
<td>Chimborazo</td>
<td>Riobamba</td>
<td>17</td>
</tr>
<tr>
<td>April</td>
<td></td>
<td>Columbe</td>
<td>14</td>
</tr>
<tr>
<td>September</td>
<td>Loja</td>
<td>Cofradía-Loja</td>
<td>At least 7</td>
</tr>
</tbody>
</table>

Except in the case of pneumonic plague, the plague mortality in Ecuador was as a rule low. Two peculiar forms of the disease, angina pestosa (tonsillar plague) and viruela pestosa (plague-pox) were met with there.

While *R. norvegicus* was preponderant in Guayaquil, *R. rattus alexandrinus* and *rattus* were more frequent than the first-mentioned rat in the sierra towns which had become infested only after the construction of the railway (Martinez 90). As maintained by Macchiavello, 85 commensal rats (*R. rattus rattus* and *rattus alexandrinus*) were also prevalent in the rural areas of the interior. He considered that these rodents were of prime importance in the causation of the plague outbreaks there, the guinea-pigs, amply bred in the houses for culinary purposes, playing merely an auxiliary role. While in the mountains as well as elsewhere in Ecuador *X. cheopis*
was the common vector of the infection, Macchiavello came to the interesting conclusion that at altitudes over 9,100 feet (2,770 m), where conditions were unfavourable for that flea, *Nosopsyllus londiniensis* took an important part. Since its vector capacity was not high, human cases tended to be sporadic.

Apart from the above-mentioned focus comprising adjacent parts of Ecuador and Peru, wild rodents played no role in the causation of plague in the former country.

* * *

As will be gathered from the account rendered above, in many of the countries dealt with the incidence of plague has markedly decreased within recent years and in some the disease has ceased to be manifest for the present. However, even apart from the fact that some areas remain seriously involved, it is not yet possible to be complacent about the plague situation in the world.

As its history teaches, plague has often shown a decline even if left alone but was apt to flare up again in due course. Hence, while appreciating the strenuous efforts now often made to combat the scourge, one should be careful not to ascribe to the measures implemented what might really be the outcome of a periodicity of the infection.

While human plague has become a quite preventable as well as an almost always curable disease, it is disappointing to see that on account of administrative or financial difficulties it is still often impossible to take full advantage of the modern methods of treatment and prevention.

Moreover, even universal success in these directions will not eradicate the infection which will continue to lurk among the rodents unless effective action against these pests can be combined with the therapeutic and prophylactic work.

There is hope that, in addition to the campaigns undertaken for the specific purpose of plague control, general progress in health and wealth will gradually prove inimical to the commensal rats. It is to be feared, however, that the vast primary reservoirs of the infection among the wild rodents will remain unassailable for a long time to come.

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Chapter 2

THE PLAGUE BACILLUS

CLASSIFICATION

Following a proposal made by Rahn in 1937, the plague bacillus, Pasteurella pestis, together with P. pseudotuberculosis, P. multocida, P. haemolytica, and P. tularensis, was classed in the family of Parvobacteriaceae and in the genus Pasteurella Trevisan, in the 5th and 6th editions of Bergey's Manual of determinative bacteriology.

As van Loghem pointed out recently, with much reason, this classification is rather unfortunate because it places micro-organisms such as the plague and pseudotuberculosis bacilli, which possess normal dimensions, among the Parvobacteriaceae, characterized by their exceptionally small size. Van Loghem proposed to class these two species in a new genus, called Yersinia in honour of the discoverer of the plague bacillus.

No doubt this recommendation deserves serious consideration because it was always doubtful whether or not the plague and pseudotuberculosis bacilli which, while closely related to each other, stand apart from the pasteurellae in general, should be grouped among them. In view of the fact, however, that so far the change in nomenclature advocated by van Loghem seems not to have attracted much attention, it did not appear advisable to adopt it for the purposes of this monograph.

MORPHOLOGICAL CHARACTERISTICS

Normal and involution forms

P. pestis, which belongs to the class of Gram-negative micro-organisms, appears typically as a short, ovoid bacillus showing bipolar staining but is actually characterized by great variability in shape (see fig. 5-9). Variations in length are frequent so that both short, coccoid forms and longer rods ("Kokkentypus" and "Stäbchentypus" of the German
authors) are found. Thinner and thicker filaments, sometimes branching, have been described by different observers. More important still are the well-known involution forms (fig. 8 and 9), such as mould- and yeast-like formations, and feebly-staining bladder and ring forms (Petrie 129).

As a rule these deviations from the typical form do not indicate that the bacilli in question have undergone profound changes in other respects. Usually they may be restored to normal morphology through subculture or, failing that, through animal inoculation. Although old avirulent strains may show an atypical morphology, becoming similar in appearance to involution forms (Burgess 26), the reverse by no means holds true, atypically shaped bacilli often proving fully virulent.

That even marked morphological changes represent as a rule merely a response to unsuitable extrinsic conditions is proved by their comparative frequency in older infections or in the primary bubo of acutely affected rodents or human beings as well as in decomposed carcasses or dead bodies. In fact, Sata 154 was able to observe the gradual transition from normal to involution forms in the carcasses of decomposing animals killed by plague. Moreover, we know of a number of factors which can bring about such a transition in vitro.

The fact that marked involution forms are produced on agar containing 3% of salt (Hankin & Leumann 83) will be discussed later.

Albrecht & Ghon 2 and others noted the appearance of polymorphous or filamentous forms on media containing glycerol or sugars. The latter were studied in detail by Wade 187 who found that the production of involution forms was dependent upon acidification, and did not occur in non-fermented media. This confirmed the observation of Westenrijk 192 that a more acid reaction of the media was apt to produce longer forms.

The investigations of Westenrijk also showed a marked influence of the abundance, scantiness, or absence of oxygen. Thus, on the surface of fluid cultures well-staining coccoid forms were found, whereas below the surface longer forms with less-marked or no bipolar-staining were present. De Smidt 169 also found that the upper part of bouillon cultures contained typical bacilli actively multiplying in chains (fig. 7), while at the bottom the organisms passed through remarkable involutionary changes before autolysis.

Cacace 27 established that addition of potassium chromate (0.01%-0.05%) to bouillon cultures led to filament formation; addition of alcohol favoured chain formation; and addition of carboxyl acid, the appearance of coccoid forms. Won 193 recently produced giant involution forms when growing plague bacilli on blood-agar to which 25-50 mg of crystalline camphor had been added.

In the experience of Bouffard & Girard, 24 incubation at lower temperatures was apt to produce longer forms.

The appearance of atypical forms under bacteriophage action was recently described by Korobkova, 99 Favarissova, 52 and P'an, Tchan & Pochon. 126, 127

Though not much attention seems to have been paid to the influence exerted in this direction by treatment with sulphonamides, it could be shown that this also is apt to lead to the appearance of involution forms (see fig. 9). Wayson & McMahon 190 noted in this connexion that the few microorganisms found in guinea-pigs succumbing to plague under sulfadiazine
PLAGUE BACILLUS

FIG. 5. LYMPH-NODE FLUID IN HUMAN BUBONIC PLAGUE \((\times 1,500)\)

FIG. 6. LUNG SMEAR IN HUMAN PNEUMONIC PLAGUE \((\times 1,500)\)

FIG. 7. 24-HOUR CULTURE IN BROTH OF VIRULENT STRAIN (195/P) \((\times 1,500)\)

FIG. 8. EARLY STAGES OF INVOLUTION FORMS ON HORMONE AGAR \((\times 1,500)\)

FIG. 9. INVOLUTION FORMS IN LYMPH-NODE OF SULFADIAZINE-TREATED MOUSE \((\times 1,500)\)
treatment showed signs of involution, while smears from the control animals displayed a normal morphology of the bacilli. Roux & Mercier stated that the sputum smears of pneumonic-plague patients treated with sulfathiazole showed alteration of the structure of the micro-organisms, and similarly Pollitzer (unpublished observation) could see in the sputum of such a patient recovering under sulfadiazine treatment a gradual replacement of typical plague bacilli by involution forms.

An interesting observation, suggesting that atypical bacillary forms may be prevalent at the initial stage of plague manifestations, was made by Magrou & Brisou in the course of an epizootic of rat origin among guinea-pigs kept in the laboratory.

At first no characteristic gross lesions could be detected in the carcasses of these animals, and smears from their organs showed only small, round, Gram-negative corpuscles which seemed in part vacuolated. Cultures made at that stage of the epizootic remained sterile or growth was tardy. Later on, typical forms were found in the smears and normal growth was obtained. Magrou & Brisou were afterwards able to detect similar atypical forms in smears from the bubo punctate of patients who had been inoculated or suffered from pestis minor.

Haffkine (quoted by Dieudonné & Otto) had pointed out that growth on dry agar promoted the appearance of involution forms. This matter was further studied by Zammit & Alcock who found that in drier media not infrequently coccoid and very short bacillary forms (generally associated with some oval bipolar-staining forms) were present, while in smears from moist agar, especially from the condensation water, more-typical forms were seen. When investigating this problem in a more general manner, Epstein came to the conclusion that bipolar staining was not peculiar to the Pasteurella group but could be demonstrated to some degree in other kinds of bacteria as well if they were cultivated in media rich in water.

These observations seemed to suggest that phenomena of osmotic pressure have an important bearing upon the morphology and staining properties of P. pestis (Petrie). Interesting observations in this direction were made by Dudchenko when studying a plague-like capsulated microorganism. This bacillus formed under unfavourable conditions a firm capsule, and intracellular pressure consequently became high. Dudchenko assumed that the latter factor was responsible, on the one hand, for the displacement of the endoplasm to the poles of the bacterial cell and, on the other hand, for the formation of various misshapen involution forms. However, as will be noted below, some recent workers have tried to explain the presence of bipolar staining in another manner.

Capsule and "envelope"

Though Kitasato and many other observers were of the opinion that the plague bacillus was a capsulated organism, Rowland came to the conclusion that a capsule was present under certain conditions only—at the site of inoculation in experimentally infected rats and in bacilli which
FIG. 10. FIRST DESCRIPTION OF THE PLAGUE BACILLUS BY YERSIN

Les morts, avant d'être enterrés au cimetière, sont disposés pendant une heure ou deux dans une sorte de cave. Ils sont déjà dans leurs cercueils et recouverts de chaux. On ouvre un de ces cercueils, j'élève un peu la chaux pour découvrir la région susjacente. Le bubon est bien net, je l'élève en moins d'une minute et je monte à mon laboratoire. Je fais rapidement une préparation, et la mets sous le microscope. Au premier coup d'œil, je reconnais une véritable purée de microbes, tous semblables. Ce sont de très petits bâtonnets, trapus, à extrémités arrondies, et aux mal colorés (Bleu de Loeffler).
had been grown in media containing serum. Rowland claimed on the other hand that the plague bacilli possessed a slimy "envelope" which was not easily visible in unstained preparations but could be demonstrated in indian-ink preparations and differed from the capsule in having no definite outer limit.

The presence of such an "envelope" was again postulated by Schütze in 1939, by Bhatnagar in 1940 and recently by Seal in 1951. However, Sokhey, who published in 1940 the results of a systematic study of this matter, came to the conclusion that while the plague bacillus possessed a capsule under all circumstances, the envelope seemed to be only "an unstained capsule plus a halo which is produced by a peculiar settling down of the fine particles of India ink at a distance from the capsule due to the operation of physical forces". Amies, while categorically refuting this explanation, confirmed through studies of wet Indian ink preparations with the aid of dark-field examination that "the so-called envelope of *P. pestis* is nothing more than a particularly well-developed bacterial capsule".

The presence of a capsule (membrane cellulaire) was also confirmed by P'an, Tchan & Pochon when studying the cytology of the plague bacillus in preparations coloured according to Robinow's method with crystal violet. The cellular membrane was seen to disintegrate and finally to disappear under the influence of a specific bacteriophage.

It is of great interest to note that, generally speaking, a capsule, though typical for *P. pestis*, is not present under all circumstances. Chertnik stated in this connexion that the organism was provided with a capsule as long as only sufficiently moist media were used for cultivation. Most favourable conditions for the development of capsules were created when the plague bacilli were cultivated at a temperature of 36°-37°C in an atmosphere containing 25%-30% carbon dioxide—preferably on Martin's peptone agar. Chertnik also claimed that addition of rabbit blood greatly favoured capsule formation, but it is noteworthy that according to Amies this was apt to favour the production of non-capsulated variants.

Confirming the findings of earlier workers, Chertnik maintained that the pseudotuberculosis bacillus showed no definite (ausgesprochene) capsule and was consequently devoid of the "membrane antigen" possessed by the plague bacillus—observations supported by the postulations of Schütze, Bhatnagar and Seal that the pseudotuberculosis bacilli had no "envelope".

Amies, who worked with the avirulent Tjiwidej strain used by Otten as a plague prophylactic, was able to produce uncapsulated ("naked") forms uncovered by any extracellular substance either through repeated subcultivation or by treating the growths with aqueous solvents such as potassium thiocyanate, depriving in both ways the organisms of their main immunogenic antigen. He noted that the change to the non-capsulated type produced through subcultivation was heralded by the appearance of
large ovoid cells with a strong affinity for fuchsin, which were first in minority but rapidly became predominant on further subculture.

**Motility**

Though a few observers have claimed that the plague bacillus shows motility, the overwhelming majority of experts are agreed that it is an immotile organism. Since it has been proved on the other hand that the pseudotuberculosis bacillus is typically motile, it would seem at first glance that this difference would greatly facilitate the otherwise rather difficult differentiation between these two species. Unfortunately, however, considerable practical difficulties are encountered in this direction. On the one hand, plague strains may show such marked Brownian movement that great experience may be necessary to decide whether a given bacillus is immotile or not. On the other hand, the pseudotuberculosis bacillus, although it is usually motile when grown at temperatures below 26° C, does not as a rule show this property when incubated at 37° C (Kossel & Overbeck; 101 Arkwright 4). Moreover, since it has been demonstrated that the motility of this micro-organism is due to the presence of flagella, one must expect that immotile O forms occur (Schütze 159).

Favarissova, 51 who tested the motility of 17 pseudotuberculosis strains cultivated at 14°-16° C with the aid of hanging-drop preparations or of Levinthal’s method, found that cultures kept for a long time in the laboratory were apt to be immotile but that motility could be restored by frequent subculture at suitably low temperatures, especially if bouillon containing serum or semi-solid agar were used for this purpose. Favarissova came to the conclusion that motility tests were of value for the differentiation of plague and pseudotuberculosis bacilli.

As stated by Seal, 162 the difficulties confronting microscopical examinations for motility may be obviated by the use of stab-cultures in semi-solid agar at an incubation temperature of 25° C or a little less. He claimed that, if tested in this manner, pseudotuberculosis strains showed a characteristic tree-like growth as an evidence of motility, whereas the development of *P. pestis* remained restricted to the immediate vicinity of the stab. It must be noted, however, that prolonged growth of this organism in stab-cultures is apt to lead to the formation of tuft-like outgrowths (Pollitzer 135).

**Granules and nuclei**

As summarized by Pollitzer 135 in 1936, the presence of granules or nucleus-like formations in *P. pestis* had been noted by a few workers:

Schultz 157 observed granules in plague cultures kept for four years without subcultivation and considered them as a means of providing for the survival of the organisms. Subcultures showed typical growth and the granules did not stand heating at 50° C for 1 1/2 hours so that they were certainly not in the nature of spores.

Fusco & Patane 35, 50 stated that granules may be demonstrated in smears from plague cultures or even sometimes in those directly taken from a plague bubo if an ammoniacal
solution of methylene blue be used for staining. Results were best if cultivation was made on beef serum solidified with 15% glucose. Similar granules were found in certain allied organisms, including the pseudotuberculosis bacillus, if these were treated in an identical manner.

The existence of nucleus-like formations in the plague bacillus has been claimed by some workers such as Pokrovskaya,¹³³,¹³¹ Lugovaya & Lebedeva,¹⁰⁷ and Tshernobaev¹⁸¹ who all stated they had confirmed the nature of these bodies with the reaction of Feulgen and Rossenbeck. It should be added that more recently Korobkova⁹⁹ reported the occurrence of giant forms with a well-differentiated nucleus in the transparent smooth colonies produced in plague cultures through bacteriophage action.

Studying the cell division of P. pestis with the aid of Robinow's method of acid hydrolysis and Giemsa staining, Wei, Tchan & Pochon¹⁹¹ noted the presence of a chromatic corpuscle near each pole of the resting bacterial cells. In the course of division these two corpuscles first became joined together in the form of a dumb-bell and then separated into four granules. As a result, each daughter cell contained two chromatic corpuscles which again became situated near the poles of the micro-organisms. Wei and his co-workers came to the conclusion that the bipolar appearance of the plague and allied bacilli was due not to protoplasmic condensations near the poles, as had been assumed by most previous workers, but to the presence of the chromatic corpuscles described above. They admitted, however, that reserve materials serving for the nutrition of the bacterial cells might be accumulated round these bodies.

As shown by further investigations by P'an, Tchan & Pochon,¹²⁶,¹²⁷ the action of a specific bacteriophage interfered with the normal cytological evolution of the plague bacillus, the chromatic granules at first becoming swollen and fused together, then transformed into filaments, and finally disintegrating. Using tannin as a mordant and Robinow's method of coloration with crystal violet, the same workers showed that bacteriophage action, besides preventing further cell divisions, led not only to a gradual disintegration and final disappearance of the cell membrane but also to the appearance of granules coloured with crystal violet. P'an and his co-workers assumed that these bodies were abnormal constituents of the bacterial cell, their appearance being accelerated by action of the bacteriophage.

It should be noted that Shabaiev & Pletnikova¹⁸⁵ had previously described the presence of metachromatic granules in plague bacilli grown on peptone agar which had been sterilized at 125⁰-130⁰ C or on media containing phosphate compounds.

Modes of reproduction

The modes of reproduction of P. pestis were recently studied by Scanga¹⁵⁵ with the aid of the electronic microscope. He found that this organism, which is characterized by a marked pleomorphism, also showed reproduction modes different from that of simple binary fission in the transverse diameter, such as atypical fission or multiplication through the formation of small coccoid bodies, which appeared either at the pole of
the bacterial cells or laterally. Scanga postulated, therefore, that *P. pestis* was capable of multiplying through a process of gemmation.

**CULTURAL CHARACTERISTICS**

*Growth limits and requirements*

It is gratifying to note that the rather incomplete knowledge formerly available in regard to the environmental conditions and substrates suitable for the growth of the plague bacillus has been supplemented through recent systematic studies.

Working on the basis of previous observations by Sokhey, Sokhey & Habbu determined the optimum and limiting temperatures for the growth of the plague bacillus in broth. Cultivation was possible within a temperature range of from −2°C to +45°C, with an optimum at 27°-28°C when the yield was about five times larger than that at 37°C. It should be noted in this connexion that, as previously established by Sokhey, a temperature of 37.5°C was optimal for the growth of sparsely seeded plague bacilli on blood-agar.

Sokhey & Habbu further established that, at an incubation temperature of 28°C, plague bacilli developed in broth within the pH range 5.0-9.6. Growth was fairly satisfactory when the pH of the medium was between 6.6 and 8.0 and was maximal within the range 7.2-7.6.

Studying the number of viable plague organisms in broth cultures, Sokhey reached the important conclusion that the growth resulting after an incubation of 48 hours bore no relation to the total quantity of the medium or to its surface area, but was directly proportional to the circumference of the latter. In order to obtain identical results in successive examinations it was, therefore, essential to use invariably tubes of the same internal diameter and to keep them during incubation invariably in the same position and in an undisturbed state free from shocks.

Interesting results were obtained by Sokhey & Habbu when studying the rate of growth of the plague bacillus in broth. Two stationary phases were noted which lasted from 0 to 1 hour, and from 21 to 24 hours, respectively. After each of these phases, there was a logarithmic growth phase.

This phenomenon seemed to be due to the fact that at first growth took place in the body of the medium only where the oxygen tension was 0.72 ml-0.75 ml per 100 ml of the medium. The initial growth phase came to an end in about 21 hours when the oxygen content had fallen to 0.04 ml per 100 ml. However, a second growth phase on the surface of the medium commenced about three hours afterwards.

In a further study Sokhey recorded the interesting observation that a lag phase was absent when *P. pestis* were implanted into the filtrate of

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*Sokhey* stated in a later paper (1952): "We have since found that, although this observation is not strictly correct, it still does hold because the growth in the body of the medium which we had failed to note is so very small as compared with the growth at the circumference."
a broth culture previously used to grow the same organisms for about six hours—obviously because during this period of preliminary growth "the organism, through lysis, had added to the medium some essential nutrient material which permitted of fresh growth in geometric progression from the very start." Sokhey concluded, therefore,

"that the lag phase was caused by the lack of some essential nutritive materials in the culture medium, and that this lack was made good by the lysis of the organism itself. The lag phase represented the period during which the lysis was going on, and continued until a sufficient amount of the nutrient had been added to permit of growth in geometric progression ".

Studying the nutritional requirements of the plague bacillus, Rao found three amino-acids—proline, phenylalanine, and cystine—to be essential for its growth, while glycine, though not indispensable, exerted an important stimulating action. As established by Rao and confirmed by all subsequent observers, accessory growth factors ("bacterial vitamins") were not required for the cultivation of the plague bacillus.

As shown by further investigations of Rao, besides the above-mentioned amino-acids, serine, alanine, glutamic acid, tyrosine, and methionine were of service as nitrogen and energy sources for the growth of the plague bacillus and were therefore suitable for enrichment of the media. Glucose and lactic acid were found to be the best and, at the same time, the least expensive carbon sources that could be used to supplement the media.

Rao found two chemically defined protein-free media—hydrolysed gelatin + cystine, and proline + phenylalanine + cystine + glycine—suitable for cultivating plague bacilli, but pointed out that in general "hydrolysates of simple proteins (such as gelatin or casein) in which the presence of essential amino-acids is ensured will prove to be the most useful ".

In fact, as recently described by Sokhey, Habbu & Bharucha, casein hydrolysate proved an excellent substrate in the preparation of plague vaccine.

In order to study the influence of haematin and certain other compounds upon the growth of the plague bacillus, Rao used a basic medium containing 12 amino-acids. He found haematin highly active in speeding up growth, while cozymase, thiamine, and nicotinic acid exerted a similar but less marked influence. The combination of haematin, thiamine and nicotinic acid had the greatest effect. On the basis of these investigations, Rao suggested that

"the four growth stimulants may be essential components of the [bacterial] cell, being synthesized in the course of growth, and that their occurrence and ready availability in the environment will greatly facilitate the rapidity of growth and invasion of the organism ".

This postulation is supported by the above-mentioned observations of Sokhey.
Results similar to those of Rao were recorded by Yaoi et al. who studied the nutritional requirements of the plague bacillus with the aid of Mueller & Johnson's hydrolysed casein medium to which cystine had been added.

Further studies on the growth requirements of *P. pestis* supplementing the work of Rao may be summarized as follows.

Doudoroff succeeded in cultivating plague strains in an ammonia-salts-glucose medium to which cystine and phenyl-alanine had been added. Berkman was able to grow plague and pseudotuberculosis bacilli in a medium containing 15 amino-acids, inorganic salts, and glucose. "Haemorrhagic septicaemia" pasteurellae (pasteurellae sensu stricto), on the other hand, failed to develop in this medium or in hydrolysed gelatin unless nicotinamide and pantothentic acid had been added.

A chemically defined fluid medium, which duplicated the amino-acid composition of casein, was recommended for the cultivation of *P. pestis* by Rockenmacher et al. Tested with 17 virulent and 10 avirulent strains, this medium, probably because it met the requirement for an amino-acid balance, proved fully satisfactory even when small inocula were used. However, it was necessary to add haematin or blood, or to resort to inoculation under reduced atmospheric pressure to obtain success with an identically prepared agar medium.

Studying the nutrition of three virulent and three avirulent strains on a basal medium containing glucose, ammonium sulfate and other inorganic salts, Hills & Spurr established the interesting fact that the growth requirements of *P. pestis* varied in accordance with the temperature of incubation. At temperatures of 32°C or below, a medium containing phenylalanine, valine, isoleucine, cysteine, methionine and haemin proved optimal. The medium best suited for growth at 36°C had to contain in addition alanine, leucine, serine, threonine, biotin and pantothenate. A mixture of twenty amino-acids was required when biotin and pantothenate were omitted.

The most likely explanation of this discrepancy seemed to be that "at higher temperatures, katabolic processes leading to the death of the organism are accelerated relative to the synthetic processes and the organism becomes dependent on a wider range of preformed nutrients ".

Englesberg, working with one avirulent plague strain, found that this required as nitrogen and sulfur sources for growth DL-phenylalanine, DL-valine, DL-isoleucine, DL-methionine and thiosulfate. In the presence of DL-methionine, thiosulfate was found to be replaceable only by either L-cysteine, sulfide, or sulfate, and in the presence of thiosulfate, DL-methionine was replaceable only by DL-homocysteine or L-cystathionine. *P. pestis* had, therefore, a dual sulfur requirement which seemed due to its inability to synthesize cysteine and methionine from inorganic sulfate.
Though the practical aspects of plague laboratory work, including methods of cultivation, will be dealt with later in chapter 5, it is necessary to discuss here the general features of the growth of *P. pestis* on or in plain media: agar, gelatin, and broth.

(a) Growth on agar. The youngest forms of plague colonies becoming visible on agar plates about the end of the first day of growth were described by Markl as being extremely delicate, translucent, colourless, and coarsely granulated with strongly scalloped edges. After 48 hours, two types of colonies could be distinguished—type I colonies which were roundish, sharply defined, and raised with steep edges, grey-white in reflected light and bluish in transmitted light; and type II colonies which were larger, with a central nucleus, and a more or less broad, delicate peripheral zone with wavy edges; the central part of these type II colonies was sometimes poorly developed, so that they appeared uniformly flat and delicate (Albrecht & Ghon quoted by Petrie).

Markl maintained that these two types originated from the early colonies described above, either directly or through intermediate stages, and were due to an adaptation of the bacilli to adverse conditions. Thus on plates to which plague toxin had previously been added the two types were conspicuous whereas on control dishes colonies of the early type grew, which only gradually changed over.

From these statements, though the descriptions given do not tally in all details, it appears likely that the two above-mentioned colony types of the plague bacillus correspond to the smooth and rough variants described by recent workers. Colonies of these variants are shown in fig. 11 and 12.

**FIG. 11. NORMAL COLONY OF P. PESTIS ON AGAR (S TYPE)**

**FIG. 12. ATYPICAL P. PESTIS COLONY (R TYPE) INDUCED BY PHAGE ACTION**
As first noted by Yersin in 1894, especially in cultures directly implanted with material from the animal or human body, different rates of growth may be conspicuous, leading to considerable differences in the size of the colonies, sometimes to the development of veritable giant colonies (usually of type II) beside minute dwarf colonies. These differences were not, however, of a stable character, so that by subcultivation of either type again large and small colonies were obtained (Frosch).

Cultivating under suitable conditions on agar slants one obtains a delicate, greyish-white layer of growth which, when touched with a platinum needle, displays a slimy consistency considered by MacConkey to be characteristic for the plague bacillus in contrast to the pseudotuberculosis bacillus.

(b) Growth on gelatin. After incubation at 20°-22° C for 2-3 days, plague colonies develop on gelatin dishes in the form of fine, semi-transparent dots with a protruding, coarsely granulated centre and a delicate, transparent peripheral zone with wavy edges. Impression films from these colonies show a characteristic microscopic appearance owing to the presence of loops and filaments. Kossel & Overbeck, who first noted this feature, ascribed differential-diagnostic importance to it, but this claim was not confirmed by other observers who found loop formation sometimes absent in plague colonies and present in those of the pseudotuberculosis bacillus. It should also be noted that on gelatin, as on agar, further development of the plague bacillus leads to marked variations in the appearance of the colonies. A further drawback is that, in the case of contaminated material, liquefaction of the gelatin by some of the concomitant micro-organisms may interfere with continued observation of the cultures. Nevertheless, some workers reported success with contaminated material when incubating the gelatin dishes at low temperatures. Zlatogorov even recommended freezing of the inoculated plates for a period of 2-3 days.

(c) Growth in nutrient broth. According to the descriptions usually given, growth of the plague bacillus in broth starts with the appearance of floccules on the bottom and at the side of the culture tubes or flasks, followed by the formation of a surface film from which threadlike strands hang down. As shown by Haffkine, this growth from the surface may be facilitated by the addition to the medium of a little oil or other fatty fluid which forms a superficial film and thus facilitates the formation of the hanging strands or stalactites towards which stalagmites grow up from the bottom of the tube or flask. One may, however, obtain such growth without any addition if one carefully avoids vibration of the cultures.

The question of the extent to which this peculiar growth in broth is characteristic for the plague bacillus has been the subject of much debate. Some observers ascribed differential-diagnostic importance to this feature, maintaining that the plague bacillus invariably produces such sedimentary
growth in bouillon whereas the pseudotuberculosis bacillus renders this medium uniformly turbid. However, as first shown by Schütze,\textsuperscript{158} this holds true for the smooth form of the pseudotuberculosis bacillus only, whereas its rough form produces a flocculent growth in bouillon. More important still, the recent observations of Wats & Puduval,\textsuperscript{189} recorded below, have proved that, similarly, the smooth variant of the plague bacillus produces uniform turbidity in bouillon. Since, however, the persistence of the plague bacillus in a purely smooth form seems to be an exception
rather than the rule, it is not surprising to find that usually this microorganism produces a sedimentary type of growth in nutrient bouillon.

(d) Growth from small inocula. Though, as discussed above, plain media may be used for the cultivation of the plague bacillus, it is as a rule impossible to initiate growth on ordinary agar or in nutrient broth unless large inocula are implanted. The following observations, proving this curious point, were made by Pollitzer \textsuperscript{135} in \textit{Plague : A manual for medical and public health workers}.

Drennan & Teague \textsuperscript{44} found that when agar plates were sown sparsely with plague bacilli, no growth occurred. They ascribed this phenomenon to the presence of inhibitory
substances formed during preparation of the media and found that addition of sodium sulfite neutralized this supposed effect. Noting that growth of plague bacilli also took place in the vicinity of a variety of micro-organisms developing on agar plates, the assumed that these bacteria exerted a similar neutralizing action.

The above observations were confirmed by Meyer & Batchelder 118 and also by Bokalo et al. 22, 23 who found that different sarcinae stimulated the growth of plague bacilli plated out from diluted suspensions.

Schütze & Hassanein 161 could induce growth from small inocula not only by adding sodium sulfite to the media, but also by adding blood or sterilized broth cultures of a variety of bacteria, or by keeping the plates under anaerobic conditions. Believing that the difficulty of initiating growth of plague bacilli lying widely scattered on agar plates was due to an oxygen sensitivity of the micro-organisms, the two authors suggested that sodium sulfite and blood acted as reducing agents. The action of sterilized broth cultures was, in their opinion, due to the presence of substances of an enzymic nature.

Whereas in the experience of Schütze & Hassanein certain other pasteurellae showed a growth inhibition similar to that of the plague bacillus, pseudotuberculosis bacilli grew well even if small inocula were used.

Wright 195 believed that plague bacilli failed to grow from small inocula in broth because the constituents of this medium had become oxidized. To overcome this difficulty he recommended adding the peptone to the broth at an early stage of preparation. He confirmed that plague bacilli were readily destroyed by exposure in thin layers on an agar surface to the action of air, particularly at 37°C. This could be prevented by (a) reducing the oxygen content of the atmosphere to some point between 0.5% and 1.5%, and (b) adding to the media blood (0.1%), serum (10%), or sodium sulfite (0.05% or even less).

Observations regarding this subject made since 1936 may be summarized thus:

Schütze, 160 working with virulent and avirulent, rough and smooth plague strains, found practically all these variants equally unable to develop when thinly seeded on plain agar; only one smooth avirulent strain from Java grew well under these circumstances. The plague bacilli thus behaved differently from other pasteurellae such as P. aviseptica and lepiseptica, the rough variants of which developed well on plain agar even if small inocula were implanted.

Sokhey 171 recorded experiments confirming the observation that the addition of defibrinated blood, serum, copper sulfate, or sodium sulfite promoted the growth of sparsely seeded plague bacilli on agar surfaces, but stated it as his opinion that a possible reduction of the oxygen tension by the addition of these reducing agents was not the sole explanation of the increased growth. He considered blood-agar as the medium of choice for the cultivation of sparsely seeded plague bacilli on solid media (see fig. 13 and 14) and, as has been noted already, found an incubation temperature of 37.5°C optimal for this purpose.

Rao, 143 discussing the findings of Schütze & Hassanein, suggested that the growth-stimulating factor present in sterile culture filtrates was catalase, while the factor preventing growth in the presence of molecular oxygen was probably hydrogen peroxide.
Further investigations in this direction were recently recorded by Herbert.\textsuperscript{66} This worker was unable to cultivate three virulent and two avirulent plague strains with the aid of the synthetic media successfully used by Rao and by Doudoroff. Anaerobically his strains grew in a mixture of 20 amino-acids and glucose, but to obtain aerobic growth from small inocula it was necessary to add whole blood or a peptic digest of blood to the media. Herbert found that the factor in peptonized blood responsible for this effect was haematin but that growth from small inocula was also obtainable if pure recrystallized haemin was added to plain agar.

While admitting that intrinsic differences between the strains used by him, and by Rao and Doudoroff, respectively, might have played a role, Herbert laid stress upon the fact that large inocula had been used by Rao and initially also by Doudoroff.

Herbert, like Sokhey,\textsuperscript{171} doubted that the failure of initiating cultivation with small inocula was mainly a problem of oxygen tension as had been assumed by some previous observers. Sharing the opinion of Rao,\textsuperscript{133} he postulated that the growth inhibition was due to a production of hydrogen peroxide by aerobically-growing plague bacilli and ascribed the effect of blood and blood-substances to their utilization by the organisms for the synthesis of catalase which decomposes hydrogen peroxide.

The fact that the media could also be made suitable for the growth of small inocula through the addition of substances such as sodium sulfite, thiolacetate, or animal charcoal could be explained as well by this hypothesis. For, as stated by Herbert, the effects of these reducing substances could be ascribed either to a direct chemical reaction with the hydrogen peroxide or to their reaction with oxygen in the medium which would prevent the formation of $H_2O_2$. Animal charcoal, on the other hand, catalytically decomposes hydrogen peroxide.

The following procedures were recently recommended to promote growth from small inocula:

Samsonov\textsuperscript{153} reported success when organ lysates, prepared from the spleen or liver of laboratory animals, were added to the media. Bistrenin et al.\textsuperscript{21} used pepsin-trypsin digests of albuminous substances for the same purpose while Jastchouk\textsuperscript{88} obtained favourable results with Fieldes' peptic blood-broth. It should be kept in mind, however, that, as recently emphasized by Girard,\textsuperscript{67} ordinary blood-agar is as suitable as these more elaborate media for obtaining satisfactory cultures of the plague bacillus, regardless of the size of the inoculum.

As noted above, one of the methods recommended by Schütze & Hassanein\textsuperscript{161} for promoting the growth from small inocula was cultivation under anaerobic conditions. Good results with this procedure were recently reported by Girard & Neel.\textsuperscript{76} Using an agar medium which had been pre-
pared with a special brand of peptone (Uclaf No. 17) and to which glucose had been added for stab cultures, these two authors were able to start growth from small inocula in instances where surface cultivation had given negative or more tardy results. Repeated subcultivation under anaerobic conditions seemed to exert an unfavourable influence upon the virulence of the strains.

An elaborate method of promoting the growth of plague bacilli devised by Devignat 32 was to bubble air through broth cultures in a special apparatus. An abundant yield was obtained quickly and uniform suspensions suitable for agglutination tests were obtained. It should be noted in this connexion that the plague bacilli growing in aerated broth cultures did not become linked together in chains, as is the case in ordinary bouillon cultures, but remained isolated.

Devignat & Schoetter 40 noted that repeated passage of plague bacilli through aerated media was apt to lead to the appearance of involution forms and resulted in a loss of virulence of the strains. However, Devignat 33 was able to establish that their virulence remained unimpaired if quicklime instead of potassium hydroxide was used for the removal of water and carbon dioxide from the air to be passed through the culture tubes. He was of the opinion that radioactivity conferred upon the air by the potassium hydroxide had been responsible for the loss of virulence.

Although he failed to initiate growth when implanting single plague bacilli directly upon nutrient media, Tehan 178 was able to cultivate such organisms, isolated with the aid of a micromanipulator, when using a preliminary enrichment method described by him in the following terms:

"We then deposited a few droplets containing the single cell on a cover-slip in a hanging drop, under oil immersion. After a few hours during which a number of cell divisions took place, we injected a little peptone water by means of a micro-pipette into the droplet. From ten separate single cells we thus obtained three cultures in the oil immersion." b

Tehan pointed out that the proportion of positive results obtained with the aid of this method was in accord with the observations of Otten, 125 who found in cultures of his avirulent plague strains about one-third viable and two-thirds dead micro-organisms.

It is curious to add that Yii 199 obtained quick and abundant growths of plague bacilli when using media prepared with rat meat or rat liver and heart infusions. He claimed that the time required for cultivation of the micro-organisms on these substrates was about half that necessary in the case of beef-extract media and that the yield obtained with the new method was considerably higher.

b "Nous avons alors déposé sur la lamelle à l'intérieur de la chambre à huile des gouttelettes contenant la cellule isolée. Après quelques heures au cours desquelles se produisent plusieurs divisions cellulaires, nous injectons, toujours à la micropipette et à l'intérieur de la gouttelette, un peu d'eau peptonée. L'opération est renouvelée 3 ou 4 fois. Ainsi nous avons obtenu, sur 10 isolements, 3 cultures apparentes dans la chambre à huile."
**Dissociation**

Discussing the problem of the dissociation of the plague bacillus in 1936, Pollitzer\(^{135}\) pointed out the curious fact that the question of whether this micro-organism occurred normally in the smooth (S) or the rough (R) form had been answered in diametrically opposite ways by different observers. Several of them, particularly Bezsonova & Lenskaia,\(^{16,17}\) considered the rough as the usual and virulent type of the plague bacillus and the same has been maintained by some recent authors such as Bezsonova\(^ {14}\) and Korobkova.\(^{98}\)

Pirie,\(^{132}\) on the other hand, distinguished between a "normal" smooth and a "mutant" rough type as follows:

<table>
<thead>
<tr>
<th>Normal S type</th>
<th>Mutant R type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bipolar-staining, round-ended, plump bacilli</td>
<td>Long, thin bacilli, often thready, bipolar staining indistinct</td>
</tr>
<tr>
<td>Agar colonies small, smooth, regular, convex; colourless or faintly bluish</td>
<td>Agar colonies larger, often granular, rugose; yellowish or reddish-brown</td>
</tr>
<tr>
<td>Growth sticky</td>
<td>Growth not sticky</td>
</tr>
<tr>
<td>Homogeneous clouding in broth</td>
<td>Agglutinative sedimentation in broth</td>
</tr>
<tr>
<td>Virulent</td>
<td>Non-virulent</td>
</tr>
</tbody>
</table>

Observations made by Pirie regarding the behaviour of his strains in salt solutions gave no clear-cut results. However, Rachinski,\(^{140}\) who on the whole confirmed Pirie’s findings when carefully studying the dissociation of a plague strain, noted that his S variant produced a homogeneous suspension in normal saline while the R variant failed to do so.

It is of great interest that, following a suggestion made by Pollitzer\(^ {135}\) in 1936, Wats & Puduval\(^ {159}\) recently made observations similar to those described above, not only on the virulent and avirulent variants of a laboratory strain, but also upon primary cultures isolated during a plague epidemic from the blood and buboes of patients. The results of their investigations may be summarized thus:

<table>
<thead>
<tr>
<th>Laboratory strain: 120/5H</th>
<th>Virulent form</th>
<th>Avirulent form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organisms showing normal ovoid form</td>
<td>Organisms long and thin, exhibiting pleomorphism</td>
<td></td>
</tr>
<tr>
<td>Majority of agar colonies small, smooth, transparent, convex, glistening, with regular margins</td>
<td>Majority of agar colonies fairly large, with pin-pricked or rugose surface, flat, opaque, with serrated or broken peripheries</td>
<td></td>
</tr>
<tr>
<td>Uniformly homogeneous growth in broth</td>
<td>Granular growth in broth with deposit at bottom and sides and fairly clear fluid</td>
<td></td>
</tr>
<tr>
<td>Stable in 0.85% saline</td>
<td>Salt-sensitive</td>
<td></td>
</tr>
</tbody>
</table>

The two colony types described above were also present in the bubo and blood cultures from plague patients, but in the blood cultures an
intermediate form was found in addition, consisting of medium-sized colonies with irregular margins and a ruffled surface, sometimes showing a nipping in the centre.

Wats & Puduval added that

"In colonial characteristics, morphology of the organisms, nature of growth in broth, salt stability and other characteristics the small 'smooth' colonies resembled those obtained from the virulent cultures of 120/5H, and the large whitish 'rough' colonies were similar to the ones obtained from the avirulent strain. The intermediate type microscopically showed normal type of ovoid organisms as well as marked pleomorphism and, as its name implies, seemed to be a middle stage between the two colonies ."

Observations on the percentage of S and R forms, made by the two workers when subculturing single colonies of virulent and avirulent stock strains on blood-agar at room temperature (27°-29° C), and 37° C, respectively, are summarized in table IX.

**TABLE IX. PERCENTAGE OF SMOOTH AND ROUGH COLONIES FROM VIRULENT AND AVIRULENT STRAINS OF PLAGUE BACILLI INCUBATED AT 27°-29° C AND 37° C**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Percentage of colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27°-29° C</td>
</tr>
<tr>
<td></td>
<td>Smooth</td>
</tr>
<tr>
<td><strong>Virulent:</strong></td>
<td></td>
</tr>
<tr>
<td>120/5H</td>
<td>85</td>
</tr>
<tr>
<td>Virinabai</td>
<td>27</td>
</tr>
<tr>
<td>34/B</td>
<td>30</td>
</tr>
<tr>
<td>35/B</td>
<td>60</td>
</tr>
<tr>
<td>36/H</td>
<td>58</td>
</tr>
<tr>
<td><strong>Avirulent:</strong></td>
<td></td>
</tr>
<tr>
<td>120/5H</td>
<td>2</td>
</tr>
<tr>
<td>P</td>
<td>10</td>
</tr>
<tr>
<td>Q</td>
<td>6</td>
</tr>
<tr>
<td>55/H</td>
<td>75</td>
</tr>
</tbody>
</table>

It will be noted that generally speaking smooth colonies vastly preponderated in the cultures of virulent plague strains kept at body temperature, but were less numerous in the case of room-temperature incubation. With one noteworthy exception, the avirulent strains yielded an overwhelming majority of rough colonies regardless of differences in the incubation temperature.

The findings of Wats & Puduval, which are in accord with those made by earlier observers such as Pirie and Rachinski, not only render it likely that the smooth type is characteristic for naturally occurring or
freshly isolated plague strains, but also go a long way towards explaining why so many observers had come to a divergent opinion. For it may be maintained that, in the laboratory at least, the plague bacillus is to some extent invariably in a process of dissociation and therefore apt to respond quickly to any unfavourable environmental influence with reactions suggestive of a transition from the normally smooth to the rough state. However, changes in extrinsic features, such as the morphological aspect, character of growth on solid media or in broth, and behaviour in salt solutions, are by no means necessarily accompanied by changes in essential intrinsic properties, such as virulence and immunogenic power. Hence, as has been justly claimed by such observers as Otten, Girard, and Macchiavello, it would be wrong to apply the definitions of dissociation found appropriate in the case of other bacterial genera too rigidly to the case of the plague bacillus, which shows in some respects a behaviour of its own.

Levine & Garber found addition of triphenyl tetrazolium chloride to tryptose agar in a final concentration of 0.005% useful for a differentiation of smooth and rough forms of *P. pestis*. The smooth colonies were about 2 mm in diameter and round, and had a sharply defined carmine-red centre. The rough colonies were of an irregular shape and diffusely pink in colour.

As described by Garber et al. in a subsequent paper, identical morphological differences between smooth and rough colonies could be detected when *P. pestis* cultures grown on tryptose agar, to which 0.2% glucose and 0.8% yeast extract had been added, were examined in obliquely transmitted light.

**Pigment formation**

Instances of pigment formation in plague cultures, or, as Devignat called it, of chromogenic dissociation, have been described by a few observers—more recently, Bezsonova (development of yellow pigmentation in a strain of Indian origin which changed to a brown or pink coloration in subcultures), Korobkova (formation of yellow pigment), and Devignat who, in the course of the preparation of live avirulent plague vaccine, observed the sudden appearance of a yellowish coloration in his EV strain which dissociated at the same time into rough and smooth variants.

A further instance of pigment formation by *P. pestis* was recorded in 1952 by Girard, who found a reddish-pink colony consisting of Gram-negative bacilli morphologically identical with *P. pestis* on an agar plate seeded about three weeks previously with material from a culture of the avirulent EV strain, which had been kept for ten years without subculture. Transfer of this colony in bulk after admixture of a drop of plague bacteriophage resulted in pigmented growth, the bacilli in question having
apparently lost the antigenic and immunizing properties of the parent strain. However, non-pigmented secondary growth, showing all the characteristics of the original strain, eventually became manifest on the surface of one of the peptone water-tubes used for subcultivation.

Crystal formation

As stated by Girard\textsuperscript{74} occasional appearance of crystal needles had been observed by him and by Robic on agar slants seeded with the avirulent EV plague strain. It was found afterwards that such crystal formation took place regularly if a 2\% agar medium (pH 7), made from fresh beef infusion and containing 20 g "Uclaf" peptone besides 5 g NaCl per litre, was used for cultivation of this strain. The crystals in question, appearing after a cultivation of about two weeks amidst the plague bacilli, had usually a white, but sometimes a yellowish colour. Their appearance seemed to enhance rather than to impair the vitality of the growths in question. Subcultures made from these were free from crystals. Two other avirulent plague strains as well as a pseudotuberculosis strain grown on the same medium showed no crystal formation.

Mutation

As summarized by Pollitzer\textsuperscript{135} up to 1936 no convincing observation of a mutation of the plague bacillus had been recorded, the atypical strains described by a few workers appearing to be variants due to dissociation rather than true mutants. The same seems to hold true for some of the more recent observations. For instance, Macchiavello\textsuperscript{108} claimed to have found two plague strains which in his opinion had undergone true and stable mutation, but it would seem that these aberrant growths differed from typical plague strains in the degree of their reactions rather than fundamentally.

It deserves attention, however, that Bezsonova et al.\textsuperscript{18,19} claimed to have observed five instances of a spontaneous transmutation of plague into pseudotuberculosis bacilli and that Korobkova\textsuperscript{99} and Tumansky\textsuperscript{182} maintained that they had produced such a transition through bacteriophage action. Since, as has been mentioned already and will be discussed more fully in chapter 5, opinions as to what tests are valid for a differentiation between plague and pseudotuberculosis bacilli vary considerably, great caution has to be exerted in evaluating these claims.

Most unusual findings were recently made by Reynes\textsuperscript{145} when dealing with a strain which had been isolated from a patient suffering from an atypical form of plague described by Alain & Reynes.\textsuperscript{1} Soon after isolation this strain not only acidified fluid glycerol and rhamnose media, but also proved pathogenic for chickens. However, when re-examined five years
afterwards, the strain failed to produce acidity in the above-mentioned media and, as confirmed by Girard, displayed in general typical features of plague.

To ascribe the changes in the behaviour of this culture to mutation is impossible, for whereas a plague strain might cause different biochemical reactions at different times, it could never prove pathogenic for chickens. Great attention must be paid, therefore, to the suggestion of Girard, that probably besides the plague bacillus another micro-organism had been present in the original cultures which was responsible for the aberrant reactions but which eventually disappeared. Quite possibly such instances of a mixed infection, which might be responsible for unusual clinical and laboratory findings, are less rare than is usually assumed.

As Girard admitted himself, pending further studies it is impossible to gauge the significance of the experience made with the above-mentioned pigment-forming EV substrain and that made with another substrain which produced no pigment but also showed evidence suggestive of mutation under the influence of plague bacteriophage.

**BIOCHEMICAL PROPERTIES**

*Alkali production in broth cultures*

Notwithstanding some early statements to the contrary, it is by now generally agreed that growth of the plague bacillus in broth results in the production of alkali consisting, according to Mayr, partly of ammonia. It should be noted, however, that according to some authorities, such as Korobkova, passing acidity may precede alkali formation.

The following details may be mentioned in this connexion:

Bannerman found a regular increase of alkalinity in broth cultures week by week which reached its maximum, corresponding to 1.5%-2% N/NaOH, in 6-8 weeks. While further growth ceased at this time, the bacilli were apt to survive in such alkaline media for at least 18 months. Moreover, neutralization resulted in further growth until once more the maximum of alkalinity had been reached, so that the stoppage of growth could not have been due to an "exhaustion" of the media as was originally assumed by Haffkine. Bannerman thought it best to use slightly acid broth for plague work.

Naidu & Jung, repeating such investigations on a larger scale, found that the maximum of alkalinity, followed by a series of small rises and falls, was reached between 5 and 11 weeks of growth in broth. Even at the end of the cultivation period of 176 days abundant growth was obtained on agar subcultures.
Petrie\textsuperscript{129} reported in 1929 on similar work; his data may be summarized as follows:

<table>
<thead>
<tr>
<th>Growth media</th>
<th>Number of strains</th>
<th>Cultivation period (weeks)</th>
<th>Cultivation temperature (°C)</th>
<th>pH initial</th>
<th>pH final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary broth</td>
<td>5</td>
<td>9</td>
<td>6-12</td>
<td>7.3</td>
<td>7.8</td>
</tr>
<tr>
<td>Broth with 1% horse serum</td>
<td>53</td>
<td>7</td>
<td>10</td>
<td>7.4</td>
<td>8.4</td>
</tr>
<tr>
<td>Ordinary broth</td>
<td>6</td>
<td>8</td>
<td>11-15</td>
<td>7.6</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Otaka\textsuperscript{124}, testing different media, found the hydrogen-ion concentration of the medium increased when meat juice was used for cultivation of plague bacilli and diminished when peptone water was inoculated; growth in ordinary bouillon seemed to represent a balance between these opposite reactions. He recommended, for carbohydrate tests particularly, a medium the pH of which did not change during cultivation.

Nikanoroff\textsuperscript{122} making comparative tests with sugar-free broth, found the pseudotuberculosis bacillus a more rapid and energetic alkali-producer than the plague bacillus and, as will be discussed later, recommended special media to demonstrate this difference between the two micro-organisms.

**Action on milk**

Most workers maintain that plague bacilli do not curdle milk and that they, as well as the pasteurellae sensu stricto, fail to produce alkalinity in litmus milk. In view of the fact that, generally speaking, the pseudotuberculosis bacilli turn litmus milk alkaline, it was recommended to use this medium for differential-diagnostic purposes. However, as pointed out by Nikanoroff\textsuperscript{122} the value of this and other tests based upon changes in reaction of the media is not absolute because occasionally poor alkali-producers are encountered among the pseudotuberculosis strains. Aberrant reactions have been observed in case of the plague bacillus as well.

**Hydrogen sulfide and indole production**

General agreement exists that both plague and pseudotuberculosis bacilli fail to produce hydrogen sulfide in the course of cultivation, while the pasteurellae sensu stricto do so.

The difference displayed by these micro-organisms in regard to the production of indole is not so absolute, for whereas plague and pseudotuberculosis bacilli invariably prove negative in this respect and most of the other pasteurellae positive, Schütze\textsuperscript{159} stated that a few of the latter also fail to produce indole.

**Reduction of nitrates**

Observations regarding the ability of the above-mentioned micro-organisms to reduce the nitrates contained in the media to nitrites,
eventually producing nitrous acid, gave divergent results. Schütze \(^{159}\) dealing with the pasteurellae sensu stricto, stated that “nitrates are reduced to nitrites”, but Henriques \(^{85}\) found that the strains examined by him did not produce this reaction. Further, although the pseudotuberculosis bacilli usually prove negative in this respect, some exceptions to this rule, noted by Girard \(^{64}\) and by Konovalova, \(^{96}\) will be discussed later.

Testing plague cultures for the presence of nitrites, Gore \(^{78}\) invariably got positive results. His experiences were confirmed by Micheletti \(^{119}\) who noted that even in ordinary bouillon a certain amount of nitrous acid was produced by the plague bacillus, and more recently by Petragnani, \(^{128}\) who considered tests for the presence of nitrous acid, made with cultures grown in liver bouillon for 24-48 hours, as one of the cardinal methods for differentiation between plague and pseudotuberculosis bacilli. Devignat & Chevalier, \(^{39}\) on account of systematic studies carried out with media which were practically or even chemically free from nitrates, fully confirmed that strains of \(P. \text{pestis}\) were capable in part of producing nitrites from proteins present in these media.

Petragnani’s claim that apart from the plague bacillus only cholera vibrios gave positive reactions when tested in this manner was not confirmed by Henriques, \(^{85}\) who obtained identical results with \textit{Shigella} and \textit{Alcaligenes faecalis} (\textit{Bacillus faecalis alcaligenes}) strains. Devignat \(^{37}\) thus listed the microorganisms which could produce nitrites in nitrate-free broth media:

<table>
<thead>
<tr>
<th>Strongly positive</th>
<th>Slightly positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Pasteurella pestis}</td>
<td>\textit{Aerobacter aerogenes}</td>
</tr>
<tr>
<td>\textit{Alcaligenes faecalis}</td>
<td>\textit{Hemophilus pertussis}</td>
</tr>
<tr>
<td>\textit{Corynebacterium diphtheriae}</td>
<td>\textit{Klebsiella pneumoniae}</td>
</tr>
<tr>
<td>\textit{Hemophilus influenzae}</td>
<td>\textit{Moraxella bovii}</td>
</tr>
<tr>
<td>\textit{Moraxella duplicata}</td>
<td>\textit{Mycobacterium paratuberculosis}</td>
</tr>
<tr>
<td>\textit{Vibrio comma}</td>
<td>\textit{Proteus morganii}</td>
</tr>
<tr>
<td>\textit{Vibrio fetus}</td>
<td>\textit{Proteus rettgeri}</td>
</tr>
</tbody>
</table>

\textit{Note:} In contrast to Henriques, \(^{85}\) Devignat did not obtain positive results with \textit{Shigella dysenteriae} and allied species.

The validity of the nitrite reaction for differentiating plague and pseudotuberculosis bacilli was doubted by Fusco \(^{57}\) and by Girard. \(^{64}\) The former maintained that not only plague but also pseudotuberculosis bacilli gave a positive reaction for nitrites when 24-hour broth cultures (or the condensation water of agar cultures) were tested with the reagent of Griess. Girard found that one out of eight pseudotuberculosis strains caused a slight reduction from nitrates to nitrites. Out of his 103 plague strains, practically all of which seem to have been isolated in Madagascar, 90 gave a positive reaction with the reagent of Griess which, however, varied considerably in intensity. Four strains, which appeared to be
negative at first, also showed the presence of nitrites when re-examined three months later, but nine strains (five of which were virulent) proved constantly negative. Girard concluded, therefore, that tests for nitrites were not as valuable for the differentiation of plague and pseudotuberculosis bacilli as those with rhamnose.

It deserves great attention, however, that, as shown by the experiences of Konovalova and of Devignat, racial differences exist among the plague strains isolated in different areas.

Konovalova tested a large number of plague strains as well as some pseudotuberculosis strains for nitrites, with the following results:

<table>
<thead>
<tr>
<th>Media used</th>
<th>Plague strains</th>
<th>Pseudotuberculosis strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate-free ordinary broth</td>
<td>6, 140</td>
<td>2, 8</td>
</tr>
<tr>
<td>Same broth with 0.1% potassium nitrate</td>
<td>9, 137</td>
<td>10</td>
</tr>
</tbody>
</table>

Commenting upon these findings, Konovalova stated that all Indian, Italian, and Transbaikalian plague strains at her disposal gave a positive result when grown in broth to which potassium nitrate had been added, while, with two doubtful exceptions, all the strains from south-east Russia proved negative. In nitrate-free bouillon also positive reactions were given only by strains received from abroad, including all three Indian strains.

Since the classification of the plague bacillus into races postulated by Devignat is based upon fermentation tests as well as upon such tests for the presence of nitrites, his findings will have to be discussed later.

**Enzyme activity**

(a) Catalase and ribonuclease. Herbert found that whenever the plague strains tested by him could be induced to grow in his amino-acids-glucose medium or on agar plates they produced catalase, even if no haemin had been added to the media. Considering catalase to be a haematin-protein, Herbert concluded, therefore, that the plague bacillus was able to synthesize haematin.

Investigating the relation between catalase activity and virulence of the plague bacillus, Rockenmacher found 14 virulent strains significantly more active in decomposing hydrogen peroxide than 11 avirulent strains and suggested, therefore, that tests measuring the catalase activity might be used for screening the virulence of plague strains in vitro.

The ribonuclease activity of *P. pestis* was studied by Woodward who, with the aid of hydrochloric acid precipitation and uranium fractionation, was able to show that the ribonucleic acid of yeast was enzymatically decomposed by living plague bacilli as well as by those killed with phenylmercuric acid and by preparations free from bacterial cells.
(b) *Fibrinolysins.* Carrying out tests with serum-free fibrin clots, Madison\textsuperscript{112} found that the plague bacillus showed marked fibrinolytic activity, particularly for rat and guinea-pig fibrin.

(c) *Coagulase.* Jawetz & Meyer,\textsuperscript{89} while unable to confirm that *P. pestis* had fibrinolytic powers, established with the aid of Fisk’s method that both virulent and avirulent plague strains showed a coagulase activity. However, only rabbit and guinea-pig plasma was coagulated, and not that of other species, including man.

(d) *Urease.* It may conveniently be added that, as recently established by Fauconnier,\textsuperscript{50} 24 pseudotuberculosis strains of human or mammalian origin, as well as 7 out of 9 such strains of avian origin, produced rapid hydrolysis of urea on the synthetic medium of Ferguson or on Roland-Bourbon’s urea-tryptophane medium. With the exception of the above-mentioned atypical, probably impure, strain of Reynes,\textsuperscript{145} the 35 plague strains tested showed no urease activity. Fauconnier therefore recommended this method for differentiation of plague and pseudotuberculosis bacilli.

*Action on carbohydrate substances.*

The action exerted by the plague bacillus on sugars or allied carbohydrate substances has been studied by numerous investigators with the result that the micro-organism was found capable of producing acidity but no gas in media containing certain of these substances, leaving others unchanged. Details of these findings as well as results obtained with the pseudotuberculosis bacillus and the pasteurellae sensu stricto are set forth in table X, which is based upon a summary of the literature available up to 1936 by Pollitzer\textsuperscript{135} as well as upon recent records, especially those of Devignat & Boivin,\textsuperscript{38} Francis,\textsuperscript{54} \textsuperscript{55} Henriques,\textsuperscript{55} Matumoto,\textsuperscript{115} Prado,\textsuperscript{135, 139} and Russo.\textsuperscript{151, 152}

*Differential diagnosis.* Though a considerable amount of early work had been done with carbohydrate-containing media, it was only in 1926 that Colas-Belcour\textsuperscript{30} made a definite proposal to use them for differential-diagnostic purposes. He then recommended glycerol-litmus agar to distinguish between plague and pseudotuberculosis bacilli, supposing that the former produced no change in this medium while pseudotuberculosis strains invariably acidified it. However, as suggested by some observations made before 1926 and confirmed by ample further investigations, glycerol-containing media are unsuitable for this purpose in view of the frequent occurrence of plague strains which, like the pseudotuberculosis bacillus, produce an acid reaction in such substrates.

A recommendation to use media containing rhamnose instead of glycerol media for the differentiation of the two organisms was made by Bezsonova.\textsuperscript{11, 12} It has to be noted in this connexion that, without any well documented exception, pseudotuberculosis bacilli rapidly acidify rhamnose-
**TABLE X. ACTION OF VARIOUS PASTEURELLAE ON CARBOHYDRATE SUBSTANCES**

<table>
<thead>
<tr>
<th>Carbohydrate substance</th>
<th>P. <em>pestis</em></th>
<th>P. <em>pseudo-tuberculosis</em></th>
<th>Pasteurellae sensu stricto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adonitol</td>
<td>±</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>-</td>
<td>±</td>
<td>-a</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+a</td>
<td>±</td>
<td>-a</td>
</tr>
<tr>
<td>Dextrin</td>
<td>±</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>±d</td>
<td>-a</td>
<td>-a</td>
</tr>
<tr>
<td>Erythritol</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Galactose</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycerol</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycogen</td>
<td>+a</td>
<td>-b</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>-a</td>
<td>-a</td>
<td>-</td>
</tr>
<tr>
<td>Inulin</td>
<td>-b</td>
<td>-a</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+a</td>
</tr>
<tr>
<td>Laevulose</td>
<td>±</td>
<td>+</td>
<td>-a</td>
</tr>
<tr>
<td>Maltose</td>
<td>+c</td>
<td>+</td>
<td>+a</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>±e</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saccharose</td>
<td>±b</td>
<td>±</td>
<td>+a</td>
</tr>
<tr>
<td>Salicin</td>
<td>±</td>
<td>+a</td>
<td>-a</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>±e</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>±</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Trehalose</td>
<td>±d</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Xylose</td>
<td>±</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>

+ = acidification; — = no acidification; ± = positive or negative.

a Some exceptions were noted.
b Late acidification was observed in some cases.
c Pons found only two-thirds of his freshly isolated strains positive.
d Negative reactions were more common than positive ones.
e One slightly positive reaction noted by Devignat & Boivin.

containing media while plague bacilli almost invariably fail to do so, though they sometimes produce late acidification. Therefore the value of this method has been endorsed by several observers, quite recently and emphatically by Girard.

Russo and Prado drew attention to two other substances—melibiose, which in their opinion was specifically attacked by the *pseudo-tuberculosis* bacillus, and glycogen, the acidification of which they considered as almost characteristic for the plague bacillus. Matumoto, who
repeated tests with glycogen only, found that the plague bacillus gave inconstant reactions in media containing it. It would be most desirable not only to follow up this matter and to make further tests with melibiose, but also to re-examine in general, and in a thorough manner, the behaviour of the plague bacillus and the micro-organisms more or less resembling it in media containing sugars and allied carbohydrate substances.

Though a few claims to the contrary have been made, the bulk of the available evidence shows that generally speaking the reactions produced by individual strains of the plague and pseudotuberculosis bacillus in carbohydrate-containing media were stable in character. This contention is well illustrated by the observations of Francis, who found that a plague strain, when re-examined after 20 years and again 5 years later, behaved in such media as it had done originally.

While, in agreement with the general rule set forth above, no convincing evidence exists that the reactions produced by plague bacilli in media containing glycerol are subject to changes (Chen), the problem of the behaviour of these micro-organisms in rhamnose media is far less unequivocal.

Bezsonova et al. stated they had found five plague strains which, though at first inert in rhamnose media, became capable of acidifying them after having been kept in the laboratory for years. However, as noted above, Bezsonova and her co-workers ascribed this change to a transmutation of the strains into pseudotuberculosis bacilli.

Korobkova claimed to have obtained rhamnose-positive variants of *P. pestis* through bacteriophage action. Similarly it was reported by Berlin & Borzenkov, and also by Tumansky, that plague strains kept in liquid media containing rhamnose were apt to dissociate into two variants, one of which was capable of producing acidity when grown in the presence of rhamnose. Krainova claimed in this connexion that only glycerol-positive strains from Manchuria, Mongolia, and south-east Russia could acquire this property, the glycerol-negative cultures obtained from India and Italy, as well as Girard & Robic's EV strain, remaining unaltered when subjected to passage through rhamnose media. She assumed, therefore, that the Eurasian strains were more closely related to the pseudotuberculosis bacillus than those obtained from other parts of the world.

**Action on glycerol for race differentiation**

Though, as stated above, tests with glycerol-containing media are not useful for differential-diagnostic work, they deserve, as first established by Bezsonova, great attention for another reason. Noting that the plague strains isolated in south-east Russia, Transbaikalia, and Turkestan invariably acidified glycerol media whereas some strains from India and one from Italy failed to do so, she considered it likely that glycerol-positive and glycerol-negative strains did not exist side by side, only one kind of strain occurring in any given area.
Amplifying this idea, Kurauchi and Berlin & Borzenkov distinguished between two races of plague bacilli—a glycerol-positive continental race characteristic, according to Berlin & Borzenkov, in the first line of the central Asiatic plateau and adjacent territories, and an oceanic race, found in the peninsulas and islands of the tropics into which plague had been introduced by the sea-route. Berlin & Borzenkov also assumed the existence of transitional areas where both races might occur. Chen and Matumoto expressed agreement with these views.

As shown by table XI, which embodies the results obtained by the above-mentioned as well as other observers, no doubt can exist that the plague strains isolated in Manchuria, Mongolia, south-east Russia, Transbaikalia, and Turkestan produced always, or practically always, an acid reaction in glycerol media. Less ample evidence tends to show that a second area where glycerol-positive strains prevail, or are even present singly, exists in the Belgian Congo, Uganda, and part of Kenya. Thus it would seem that the presence of glycerol-positive strains is characteristic for territories where plague has existed since time immemorial—as in Central Asia and the adjacent areas—or is at least of very long standing, as we have much reason to assume is the case in Central Africa. At the same time the prevalence of glycerol-acidifying strains in the latter area goes a long way towards confirming Payne's hypothesis mentioned in chapter 1 that "the African was a colony of the Asiatic plague".

It appears on the other hand that, as postulated by Berlin & Borzenkov, glycerol-negative strains prevail or are even found singly in the countries which were infected—mainly by the sea-route—during the present pandemic. The fact that the strains isolated in California fall into this category speaks against the assumption that the origin of plague there is connected with an ancient immigration of wild rodents from Asia.

That one of the "transitional" areas presupposed by Berlin & Borzenkov might be located in the Union of South Africa is rendered likely by the observations of Pirie, who found in one isolated focus of Cape Province four glycerol-positive strains, while the 19 strains from other local foci examined by him did not alter the reaction of glycerol media.

The occurrence of both glycerol-positive and glycerol-negative strains was also noted in Japan and in the Philippines. Details furnished for the former country by Matumoto may be summarized thus:

<table>
<thead>
<tr>
<th>Locality</th>
<th>Year</th>
<th>Number of strains examined</th>
<th>Glycerol negative</th>
<th>Glycerol positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yokohama</td>
<td>1913</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Tokyo</td>
<td>1914</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Osaka</td>
<td>1922</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Yokohama</td>
<td>1926</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Osaka</td>
<td>1929</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
### TABLE XI. BEHAVIOR OF PASTEURELLA PESTIS IN GLYCEROL MEDIA

<table>
<thead>
<tr>
<th>Locality</th>
<th>Number of strains examined</th>
<th>Acidification of glycerol</th>
<th>Bibliographical reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Arabia</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Argentina</td>
<td>50</td>
<td>45</td>
<td>2</td>
</tr>
<tr>
<td>Brazil</td>
<td>45</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>California</td>
<td>65</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>Ceylon</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>China (Fukien)</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Belgian Congo</td>
<td>43</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Egypt</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>England</td>
<td>32</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Formosa</td>
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<tr>
<td>France</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>French West Africa</td>
<td>4</td>
<td>4</td>
<td>0</td>
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<tr>
<td>Germany (Hamburg)</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Greece</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hawaii</td>
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<td>10</td>
<td>0</td>
</tr>
<tr>
<td>India</td>
<td>20 b</td>
<td>20</td>
<td>0</td>
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<td>Indochina</td>
<td>2</td>
<td>1</td>
<td>1 c</td>
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<tr>
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<td>2</td>
<td>0</td>
</tr>
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<td>Japan</td>
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<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Java</td>
<td>8</td>
<td>7</td>
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<tr>
<td>Kenya</td>
<td>69</td>
<td>53</td>
<td>16</td>
</tr>
<tr>
<td>Madagascar</td>
<td>8</td>
<td>6</td>
<td>0 e</td>
</tr>
<tr>
<td>Manchuria</td>
<td>84 f</td>
<td>23</td>
<td>1 f</td>
</tr>
<tr>
<td>Mongolia</td>
<td>20</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Palestine</td>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Philippines</td>
<td>11</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>South Africa</td>
<td>25</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>South-east Russia</td>
<td>157</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>Southern USA</td>
<td>23</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Sumatra</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Thailand</td>
<td>0</td>
<td>0</td>
<td>1 g</td>
</tr>
<tr>
<td>Transbaikalia</td>
<td>6</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Turkestan</td>
<td>22</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Turkey</td>
<td>±</td>
<td>3</td>
<td>1 i</td>
</tr>
</tbody>
</table>

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*According to Devignat, the plague strains isolated in Uganda also invariably acidified glycerol media.

*It is of great interest that out of the 12 Indian strains recently examined by Wagle & Habbu, four had been isolated in North India—one in Uttar Pradesh and three in Bettiah, Bihar, not far from the Nepalese border.

*As stated in the text (page 97), the glycerol-positive strain of Reynes was probably impure.

*The Java strain found positive by Chen was stated to be a subculture of Otten's avirulent Tjiwidej strain, which usually does not acidify glycerol media.

*Girard repeatedly stated that all plague strains isolated in Madagascar proved negative on glycerol and rhamnose media.

*Included are 13 strains from Vladivostok and neighbouring places which had become secondarily invaded during the 1921 pneumonic-plague epidemic.

*This was a strain in the series of D'Aunoy said to have come from Manchuria.

*Francis was not certain that the glycerol-positive strain forwarded to Washington from Thailand had actually been isolated in that country.

*This glycerol-acidifying strain had been isolated during the 1947 epidemic at Akcakale on the Syrian border, whereas the three other strains examined in Turkey came from ports.
Matumoto drew attention to the fact that strains isolated during the same outbreak all showed a uniform behaviour in glycerol media and also suggested, with much reason, that Japan had been reached by two waves of the infection which seemed to have been imported first from the north, possibly from Manchuria, and then from the south.

As suggested by Devignat, a similar state of affairs might have existed in Kenya where, in contrast to the findings made in the Belgian Congo and Uganda, de Smidt recorded the occurrence of glycerol-negative strains. Since, however, plague had been imported into Kenya by the sea-route before these findings were made, Devignat considered it likely that the manifestations of the disease, during which the strains in question had been isolated, resulted from this sea-borne infection. This assumption has been fully confirmed by the recent observations of Heisch, which showed that all plague strains isolated thus far in the Rongai area of Kenya acidified glycerol media.

As will be gathered from the observations discussed above, a striking contrast exists between the glycerol reactions of the plague strains isolated in the original foci of the infection in Central Asia and those found in most of the secondarily invaded areas throughout the world. No doubt a change in the behaviour of the strains towards glycerol must have taken place during the spread of the infection from Central Asia, but it is difficult to determine where and how this transition took place. Owing to the lack of actual observations, it can be merely inferred that the plague strains in Burma are glycerol-negative. It is nevertheless, in the opinion of the present writer, most likely that the change took place when the infection, passing from the wild-rodent foci in Central Asia to Burma, became entrenched among Rattus rattus, which has its original home in that country and in Assam.

This assumption is not contradicted by the fact that the plague strains in Uganda and in the Congo did not alter their behaviour towards glycerol because, as noted before, in these areas the infection at first became entrenched not among the usual species of commensal rats, but among R. natalensis (Mastomys coucha).

Although, as pointed out by Berlin & Borzenkov, glycerol-negative plague strains belonging to the oceanic race were responsible for the manifestations of the disease due to recent importations of the infection by the sea-route, there can be no doubt that the outbreaks taking place during the "Black Death" were caused by glycerol-positive strains and the same probably held true of the pandemic in Justinian's time, which seems to have originated in Central Africa.

\[\text{It is interesting to note in this connexion the belief expressed by Girard that, regardless of geographical distribution, acidification of glycerol media was peculiar to plague strains from wild rodents, while growths of rat or rat-flea origin produced no acidity. However, as pointed out by Chen, this assumption could not be regarded as generally valid because, for instance, the plague strains isolated in California, even if of direct wild-rodent origin, proved negative in glycerol media.}\]
Bearing in mind the fact that pneumonic plague was rampant during the “Black Death” and also that the devastating pneumonic-plague outbreaks in Manchuria (1910-11, 1920-1) as well as that in Shansi (1917-18) were caused by glycerol-positive strains, it would be tempting to assume that the continental race of the plague bacillus might be particularly apt to produce lung involvement. It must be noted, however, that pneumonic features were by no means invariably conspicuous in outbreaks caused by this race of *P. pestis* and that, on the other hand, the incidence of pneumonic plague was conspicuous in areas where only glycerol-negative strains were present, for instance in Madagascar. Generally speaking, although the differentiation of the plague bacilli with the aid of glycerol tests is valuable for the solution of nosogeographical problems, thus far no convincing evidence exists of any fundamental difference between glycerol-positive and glycerol-negative strains.

Considering the results of tests not only in glycerol media but also those obtained with the aid of rhamnose, Kawashima came to the conclusion that the plague bacilli might be divided into three groups: strains acidifying neither substance; those producing acid in both; and finally, those positive in glycerol media only. Since, according to the observations of the Russian workers quoted above, tests with rhamnose might yield less stable results than those with glycerol, it does not seem advisable to use the former for purposes of classification. It should be noted, moreover, that Chen found two plague strains not falling into Kawashima’s scheme because they produced late acidification in rhamnose media though no change in those containing glycerol.

It is, however, of great interest that Devignat recently proposed to classify the plague bacilli as follows:

<table>
<thead>
<tr>
<th>Proposed subgroups</th>
<th>Tests with glycerol</th>
<th>Production of nitrous acid</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. pestis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. <em>orientalis</em></td>
<td>−</td>
<td>+</td>
<td>Corresponding to Berlin &amp; Borzenkov’s oceanic race</td>
</tr>
<tr>
<td>var. <em>antiqua</em></td>
<td>−</td>
<td>−</td>
<td>Found in Central and North Asia and in Central Africa</td>
</tr>
<tr>
<td>var. <em>mediaevalis</em></td>
<td>+</td>
<td>−</td>
<td>Found in south-east Russia</td>
</tr>
</tbody>
</table>

Devignat was of the opinion that the “var. *mediaevalis*” of the plague bacillus was capable of becoming transmuted into pseudotuberculosis bacilli which also acidified glycerol media but did not produce nitrous acid.

Seeing that Girard expressed serious misgivings regarding the reliability of tests showing a reduction of nitrates to nitrites or production of nitrous acid, further studies would seem necessary before Devignat’s classification could be accepted.
VITAL RESISTANCE

It is significant that the bulk of the enormous literature dealing with the behaviour of the plague bacillus outside the living organism dates back to the first years after the discovery of this bacillus, when the time-honoured idea that contaminated objects, the so-called "fomites", were of great epidemiological importance, still loomed large in the minds of workers. Further research established that the role of contaminated objects in the propagation of plague was rather limited, infection as a rule being insect-borne, especially flea-borne, or—in the case of the pneumonic type—spreading directly from man to man. In many ways, therefore, the earlier studies on the vital resistance of the plague bacillus may now be said to possess academic rather than practical interest. Further, numerous though observations of this kind are, their results are but rarely comparable or generally applicable. This is due not only to the different extrinsic conditions, such as light, temperature, and humidity, under which the work was done, but also to differences in the objects examined. It is probable that when pure cultures were tested, those of long standing, adapted to a life on artificial media, were more resistant than freshly isolated growths. On the other hand, no doubt can exist that in its natural environment the plague bacillus is often better protected than in plain fluids or culture media and is hence less amenable to harmful influences. Ample evidence for this is furnished by tests with material such as pneumonic-plague sputum, and by observations upon the prolonged survival of the bacilli in dead bodies and carcasses of infected animals, as well as by comparative tests in vitro (Schiavone & Trerotoli; Galeotti; Wright). Wright ascribed the rapid destruction of plague bacilli unprotected by the addition of substances such as blood-serum or gelatin in watery solutions to features suggestive of plasmolysis and thought that this adverse effect might be counteracted by adjusting the ion balance of the fluids.

In fact, Girard found that at temperatures not exceeding 25° C plague bacilli could be kept alive and virulent in normal saline for periods up to one week. At low temperatures (2°-4° C) the bacilli could survive in normal saline for up to two years, remaining pathogenic for intracutaneously infected guinea-pigs for periods up to 220 days and for intraperitoneally infected mice up to 685 days.

Resistance to physical and chemical agents

Data of practical importance concerning the vital resistance of the plague bacillus to various physical and chemical agents may be summarized thus:

(a) Sunlight. While generally speaking the plague bacillus is easily killed by sunlight, the length of time required for this varies within quite
considerable limits according to the kind of vehicle and the thickness of the
layer used, the kind of material upon which the infected matter is placed,
and the temperature.

Thus, as summarized by Pollitzer, one hour's exposure to the sun was sufficient
to kill plague bacilli spread in thin layers on coverglasses, but they survived for up to
4 hours when exposed in the same manner in thick layers. The bacilli contained in thin
layers of pneumonic-plague sputum were killed by sunlight in 2-5 hours, but at tem­
peratures below 12° C it took up to 12 hours' exposure to sunlight to sterilize pneumonic­
plague sputum expectorated into sterile dishes. According to Toyoda & Yasuda, this period was prolonged to 14 hours if hempen material instead of glass was used as substratum.

(b) Dry and moist heat. Dry heat was stated to be not very effective
against the plague bacillus, for exposure at 100° C was found to be necessary
for periods up to one hour to ensure killing of the organisms. Lower
temperatures administered for the same interval of time often proved
ineffective.

While it is agreed that plague bacilli on instruments or on other inanimate
objects are almost immediately killed if the latter are immersed in boiling
water, opinions as to the length of time necessary to ensure sterility at
lower temperatures under the conditions used for vaccine preparation vary
considerably. Whereas it is now the standard practice of the Haffkine
Institute to sterilize the brews through an exposure at 55° C for 15 minutes,
earlier workers recommended keeping the vaccine flasks in the water-bath
for at least one hour at 65° C. Presumably, however, insufficient care was
taken by the earlier observers to ensure an adequate temperature inside
the flasks.

(c) Cold. As confirmed by numerous observations, the plague bacillus
is not sensitive to the action of low temperatures. Slow growth continues
to take place when plague cultures are kept in the refrigerator at a tempe­
ration of 4° ± 2° C (Sokhey); and Kasanski even found that agar
cultures kept for 5 1/2 months at -31° C remained viable though showing
some loss of virulence. Indeed, it may be maintained that low temperatures
are beneficial for the development of the plague bacillus because they
inhibit the growth of concomitant bacteria which is often inimical to it.

It should be noted in this connexion that Shurupoff recovered virulent
plague bacilli from the dead bodies of 6 out of 17 pneumonic-plague victims
who had been interred in a soil rich in salt for periods up to one year, having
become frozen during the winter. Francis, keeping the whole spleen of a
plague-infected guinea-pig at -15°C, obtained virulent growth from it
after 7 years.

(d) Desiccation. Though, generally speaking, desiccation, particularly
if taking place rapidly at high temperatures, is harmful to the plague bacillus,
this rule does not hold good under all circumstances.
Experience has shown that plague bacilli in sputum or similar vehicles where they are protected by mucus or proteins, may withstand exsiccation for considerable periods, especially if placed on substrates like cotton-wool and similar materials which in their turn offer some protection to the bacilli.

It is interesting to note in this connexion that Pollitzer \(^{135}\) was able to obtain positive cultures from plague sputum which seemed to have become quite dry and that Nikanoroff \(^{121}\) found that such dried sputum remained virulent for periods up to 165 days.

Uriarte & Morales Villazón \(^{186}\) established that the viability and virulence of plague strains could be maintained without subculturing for more than two years if the original growths were kept in vacuo over phosphorus pentoxide. Reitano \(^{144}\) found that cultures thus dried and kept remained viable for 30 months, but noted that they had become avirulent and non-immunogenic. However, as established by recent experiences, rapid drying from the frozen state is a suitable means of preserving plague cultures for laboratory work. Seal & Habbu \(^{163}\) obtained satisfactory results with this method by adding 5% of gum acacia to broth cultures, 50% of the organisms surviving and remaining virulent for periods of at least 7 months.

The question of how long cereals remain apt to harbour plague bacilli has been studied by a few investigators, more recently by Ori \(^{123}\) and Semikoz & Achourova.\(^{164}\)

Ori established that plague bacilli, when sheltered from light, could remain virulent on maize for 54 days. However, contaminated maize placed in the centre of a large quantity of the grain in the hold of a ship proved virulent for only 14 days. Effective sterilization could be carried out by keeping the maize in a drying-chamber for 10 minutes at 70°C.

According to Semikoz & Achourova, grain contaminated slightly with organs, blood, and faeces of plague-infected mice became innocuous when kept for 1-8 days under conditions corresponding to those in elevators. If the grain was more heavily contaminated and humid faeces were admixed, storage in the elevator for 27 days seemed necessary.

(e) Disinfectants. While plague bacilli, when tested in pure culture, show little resistance to disinfectants in the usual concentrations, they are apt to be less vulnerable when contained in substrates which may retard the action of the disinfectants or neutralize their effect.

This point is well illustrated by a series of tests made during the 1920-1 pneumonic epidemic when the following concentrations were found necessary to ensure sterilization of sputum samples (room temperature of 17°C):
Plague sputum was exposed in open Petri dishes in rooms fumigated with sulfur or formalin. Negative bacteriological results were invariably obtained after 12 hours' exposure to sulfur fumes. In three experiments the plague bacilli survived 24 hours' exposure to formalin.

It has to be added that calcium chloride, though found to be fairly effective in the case of plague cultures, failed to sterilize pneumonic-plague sputum even if left to act for 24 hours in a concentration of 20% (Breining 25).

It would be of interest to test plague sputum with modern disinfectants, some of which displayed a remarkably strong action upon plague cultures. This holds particularly true of the mercury-phenol compounds tested by Caius and co-workers, quoted by Pollitzer. Graham 9 reported in this connexion that p-chloromercurophenol destroyed in vitro plague bacilli in a dilution of 1:400,000,000 in 24 hours. It should also be noted that Skorodumoff & Mitchurina 167 found gentian violet (pyoktanin) and ethoxydiamino-acridine lactate (rivanol) bactericidal when tested with plague cultures in dilutions of 0.01% and 0.02%.

(f) Fixation and staining. Considering the statements made above it is not surprising to find that the methods usually employed for the fixation and staining of smears prepared from plague material are by no means invariably apt to ensure the killing of the micro-organisms (Jettmar, Berdnikov, Tinker & Rudnev 179). It is noteworthy that alcohol fixation which, as will be discussed later, is in general preferable for the preparation of plague smears, gave markedly better results than the usual method of fixation over a flame.

Vitality of cultures

Whereas constant incubation at 37° C is inimical to prolonged life of plague growths, such cultures may remain alive for months if kept at temperatures below 20° C, and even for many years if stored in the refrigerator. Most remarkable findings made in this respect by Francis, when keeping numerous first agar subcultures of a ground-squirrel strain at 5-10° C, may be summarized thus:

<table>
<thead>
<tr>
<th>Period of storage</th>
<th>Survival</th>
<th>Virulence</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 years</td>
<td>33 of the 48 originally preserved slants gave growth on subculture</td>
<td>22 of the 33 subcultures proved virulent, killing subcutaneously infected guinea-pigs within a week</td>
</tr>
<tr>
<td>25 years</td>
<td>25 of the 33 originally preserved slants found viable 5 years previously gave growth on subculture</td>
<td>All 25 subcultures caused acute illness in subcutaneously infected guinea-pigs but only three of them died of plague within 8 days</td>
</tr>
</tbody>
</table>
That most of the slants tested after 20 years still proved virulent, and that virulence still existed to some extent even after 25 years, was no doubt due to the fact that the originally preserved growths had been kept without subcultivation which exerts so unfavourable an influence in this respect that it can be used as a means of producing avirulent strains.

A classical observation showing the unfavourable influence of subcultivation upon virulence was recorded by Francis: a sealed agar culture of a strain of ground-squirrel origin kept untouched for 9 years at 10°C proved viable and fully virulent while the same strain when subcultivated every three months throughout this period became avirulent.

**Influence of symbionts**

Though, as has been noted before, a few bacterial species facilitate the growth of the plague bacillus from small inocula, in general its development is much hampered by the presence of other micro-organisms.

Faddeeva & Tshernobaev, who made a systematic study of this matter, distinguished between bacteria antagonizing the growth of the plague bacillus and those permitting or even favouring it as follows:

<table>
<thead>
<tr>
<th>Antagonistic</th>
<th>Symbiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus mucosus</em></td>
<td><em>Sarcina</em></td>
</tr>
<tr>
<td><em>Pneumococcus</em></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Coliform-typhoid group</td>
<td><em>Vibrio parancholerae</em></td>
</tr>
<tr>
<td><em>Alcaligenes faecalis</em></td>
<td><em>Klebsiella (Bacillus) ozaenae</em></td>
</tr>
<tr>
<td><em>(Bacillus faecalis alcaligenes)</em></td>
<td><em>Bacillus aromaticus</em></td>
</tr>
<tr>
<td><em>Chromobacterium prodigiosum</em></td>
<td><em>Bacillus subtilis</em></td>
</tr>
<tr>
<td><em>Pseudomonas pyocyanea</em></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus mesentericus</em></td>
<td></td>
</tr>
</tbody>
</table>

Dysentery bacilli were partly antagonistic, partly symbiotic

While it must be admitted that the bacteria enumerated in the first column above, and also some others such as *B. proteus vulgaris* (Tumansky), hamper the growth of the plague bacillus, it is an open question to what extent this phenomenon is due to a specific antagonism of the micro-organisms in question or of their products of metabolism and not merely to the comparatively slow and non-luxuriant growth of *P. pestis* which, as it were, is crowded out of existence or at least of demonstrability. It is noteworthy in this connexion that, as shown by Jettmar, co-existence of plague and tuberculosis bacilli was not harmful to either of the two organisms in vitro or experimentally. On the other hand, it is important to note that, as recently recorded by Girard, rats infected with *Mycobacterium lepraemurium* (Stefansky's "Bazillus der Rattenlepra") showed a marked resistance to experimental infection with *P. pestis*. Girard also adduced evidence suggesting that human sufferers from advanced leprosy were markedly resistant to plague.
Though, as shown above, the plague bacillus may, under especially favourable conditions, persist for even longer periods of time outside the living organism, such observations do not invalidate the general rule that this bacillus has feeble powers of resistance to adverse extrinsic influences and is, therefore, not well adapted to a saprophytic life (Petrie129). Moreover, it must be emphasized that, though inanimate objects may become and remain for some time infected, the chances of their proving infective, leading to a spread of the disease, are usually remote.

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Chapter 3

PROBLEMS IN IMMUNOLOGY

VIRULENCE OF THE PLAGUE BACILLUS

Since immunological terms like "virulence", "virulent" or "avirulent" are often used rather indiscriminately with reference to bacilli, it is necessary to begin the present disquisition by defining in what sense they are applied here. In doing so, the apt distinction drawn by Jawetz & Meyer between virulent and avirulent plague bacilli may be quoted:

By a virulent plague bacillus, we mean one that will multiply greatly and finally bring about the death of a given susceptible host animal when introduced in numbers not large enough to be toxic without multiplication. An avirulent plague bacillus may then be defined as one not being able to cause the death of the same susceptible host animal unless introduced in numbers sufficient to produce toxic death without multiplication.

Thus, as these authors point out, mere invasiveness cannot be made the criterion of virulence, this term indicating the capacity of the organisms to multiply in the tissues of the host to a dangerous extent. At the same time a sharp distinction has to be made between virulence and toxicity. Since, as justly maintained by Jawetz & Meyer, "death from plague in final analysis is always due to a toxaemia produced by decomposition products of the plague bacillus", one might consider the virulence of Pasteurella pestis as the means of bringing about a fatal termination of the disease which, however, is ultimately due to the toxicity of the organisms.

Generally speaking, the virulence of the plague bacillus is high. In a classical experiment Barber was able to show that six out of nine guinea-pigs and two out of twelve monkeys which had been inoculated with single plague bacilli succumbed to the infection. Identical results have been obtained with guinea-pigs by Otten while, as will be discussed below, infective doses consisting of not more than six to twelve plague bacilli have been found suitable for standard virulence tests with mice.

It may be further maintained that on the whole the virulence of P. pestis is remarkably stable. Petrie, summarizing the data of several workers,
found that out of 152 laboratory strains of diverse origin, some of them many years old, 12 only showed marked impairment or absence of virulence while the remaining 92% were virulent. The interesting experiments of Francis with a 20-year-old plague strain, referred to previously (see page 108), serve as a corollary for these observations.

The fact remains, however, that not only may the virulence of plague cultures be abated by artificial means but the cultures may become avirulent spontaneously, sometimes even without being kept in the laboratory for prolonged periods (Revenstorf 161).

It has been claimed also by a few observers that plague strains of low virulence may become prevalent under natural conditions. The evidence brought forward in this respect in connexion with the supposed existence of “chronic” rat plague will be discussed in a later chapter; but it may be noted here that Pirie 140 referred in 1936 to a perceptible loss of virulence shown by plague strains recently isolated in the Union of South Africa from human as well as rodent sources. Likewise, Macchiavello 108 maintained that the plague cultures obtained in north-east Brazil were often less virulent than those met with in other countries and were apt to undergo a total loss of virulence when exposed to adverse conditions in the laboratory. Meyer et al., 119 studying an instance of chronic plague meningitis in California, had the impression that the recent cases of plague in the western states merely reflected the behaviour of an agent which caused mild infections with a tendency to latency.

Measurement of Virulence of Strains

The following methods used in the past for measuring the virulence of plague strains, though now of historical interest rather than of actual importance, may be mentioned:

1) The procedure recommended by Kolle & Martini 84 consisted of rubbing an appropriate test dose into a carefully-shaved area of standard size on a guinea-pig’s abdomen. The same procedure was also used for some time in the Haffkine Institute where, however, rats instead of guinea-pigs served as test animals (Sokhey 179).

2) Kolle & Krumbein 93 employed a syringe needle of standard calibre which was dipped into a bacterial suspension of definite concentration and then introduced under the skin near the root of a rat’s tail.

3) Rowland, 156 who also used rats as test animals, worked with a syringe so arranged that one turn of a screw caused delivery of 0.1 ml of the culture to be examined.

4) An alternative method adopted in the Haffkine Institute consisted of the subcutaneous administration to rats of an infective dose containing 0.003 mg of the spleen of a rat that had died of acute plague. As was to be expected, it gave grossly inconsistent results (Sokhey 179).

An exact method of determining the virulence of plague strains was introduced by Sokhey, 179 who had previously devised a method of counting...
the number of viable plague bacilli present in broth cultures. The principle of this procedure was to make progressive dilutions, each one-tenth the strength of the preceding one, from the growths to be tested and to spread 0.05 ml of the diluted fluids on blood-agar slants which were then incubated for 48 hours. Using adequate dilutions, it was thus possible to obtain growth of suitably low numbers of colonies.

Sokhey had also established that the strain of white mice inbred in the Haffkine Institute was highly susceptible to plague, ten or even less highly virulent organisms being sufficient to cause a 100% mortality.

To determine the virulence of a plague strain, 0.5 ml of a broth subculture was implanted into a tube containing 9.5 ml of nutrient broth and this second subculture was incubated at 25°-27°C for 48 hours, care being taken to keep the tube in a vertical position and free from jolts or jars.

At the termination of the incubation period progressive tenth dilutions of the growth were made and used on the one hand for subcutaneous infection of mice, and on the other for determination of the number of viable plague bacilli with the aid of the method mentioned above. Experience had shown that, in the case of highly virulent strains, 0.2 ml of the 10⁻⁷ dilution contained the minimal lethal dose (m.l.d.) consisting of 6-12 organisms. This and the 10⁻⁶ and the 10⁻⁸ dilutions were used, therefore, for injecting batches of five to ten mice with doses of 0.2 ml per animal.

Results were expressed as the smallest number of organisms of a given strain of *P. pestis* which, when administered subcutaneously, killed approximately 100% of the animals within three to eleven days.

The superiority of Sokhey's new test over the method of rat infection formerly used in the Haffkine Institute is shown as follows:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Old test</th>
<th>New test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of animals infected</td>
<td>number of organisms given</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>2 (4.2)</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>5</td>
</tr>
</tbody>
</table>

* The figures in brackets indicate the average number of days elapsing between infection and death.

As will be noted, the two strains appeared to be equally virulent when the old method was used, but showed a marked difference in their virulence when tested with the aid of the procedure recommended by Sokhey.

Gokhale, studying the oxygen uptake of three virulent and four avirulent plague strains with the aid of Warburg's manometric technique, came to the conclusion that the enzymatic properties of both types were identical. However, as noted in chapter 2, Rockenmacher found the catalase activity of virulent strains to be greater than that of avirulent growths and suggested that tests measuring this activity might be used
for screening the virulence of plague cultures in vitro. So far no practical advantage seems to have been taken of this recommendation.

**Maintenance of Virulence of Strains**

The problem of maintaining the virulence of plague cultures was exhaustively dealt with by Sokhey.179

*Passage through susceptible animals*

Sokhey noted that the method of passage through susceptible animals, which had been almost universally used hitherto for this purpose, possessed serious drawbacks. While tests with highly susceptible animals gave satisfactory results, the virulence of the strains was likely to be impaired if the passage animals happened to be resistant to plague. Yet no method was available to determine beforehand their receptivity to the infection. Worse still, the use of massive doses for the infection of the passage animals was apt to give a wrong impression of the virulence of the strains used, as is shown thus:

<table>
<thead>
<tr>
<th>Number of organisms per house-rat (subcutaneously)</th>
<th>Number of rats inoculated</th>
<th>Number of deaths</th>
<th>Number of days between inoculation and death</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>24</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>64,500</td>
<td>25</td>
<td>1</td>
<td>17.40</td>
</tr>
<tr>
<td>132,250,000</td>
<td>25</td>
<td>25</td>
<td>3.4 (average)</td>
</tr>
</tbody>
</table>

As will be seen, the strain in question, though actually of very low virulence, would have appeared fully satisfactory, had reliance been placed upon tests with massive inocula.

*Storage at low temperatures*

Bearing in mind that storage at low temperatures was helpful for preserving the virulence of plague cultures, Sokhey recommended replacing the method of animal passage by the following procedure:

Primary cultures from human cases with severe septicaemia were obtained by plating venous blood on agar slopes. After four days' growth at room temperature (26°-32°C), they were tested for purity with the aid of the cultural and biochemical methods recommended by Gore (Taylor 197). After a culture had been found pure, its virulence was measured quantitatively; if found highly virulent, that is, if 6 to 12 plague bacilli per Haffkine-Institute-inbred mouse killed not less than 80% of the animals used in an average period of about seven days, large numbers of subcultures on 5% rabbit-blood agar slopes were made from the primary culture, and the tubes were sealed on the flame and stored in a refrigerator at 4° ± 2°C. A tube was removed from time to time to measure the virulence. It was found that cultures stored in this manner retained their virulence unimpaired for at least three years.
Drying from the frozen state

Still better results could be obtained by drying from the frozen state; by this method "virulence is maintained much longer, probably indefinitely" as was stated by Sokhey\textsuperscript{182} in 1947. According to Sokhey (personal communication), the following procedure is actually used in the Haffkine Institute for freeze-drying plague cultures:

The strains in question are grown for 48 hours on blood-agar slants at 28°C. Three parts of a suspension prepared from such growths are then mixed with one part of a 20% solution of gum acacia which had been adjusted to pH 7.0–7.2 and, after distribution in tubes, had been sterilized for half an hour at 120°C.

The mixture of the culture suspensions and the gum acacia solution is well emulsified, distributed in suitable containers and mechanically shaken to ensure an even distribution of the bacilli in the menstruum. After distribution in quantities of 0.1 ml in tubes of a suitable size (115 mm × 8 mm) the dispensed emulsions are rapidly frozen at -30° to -50°C. The tubes are then attached to the manifolds of a cryochem apparatus and the vacuum is brought up to 200 in five minutes. To avoid the possibility of thawing, the tubes are kept immersed in methyl acetone at -30°C for about 30 minutes. The process of desiccation is continued for 24 hours; the growths are then sealed under vacuum and tested with a vacuum tester. The dried growths are stored at a temperature ranging from 0° to +1°C.

For regeneration, a small quantity of broth (about 5 ml per tube of dried culture) is added; the mass is emulsified, transferred to a sterile tube, and incubated at 37°C overnight. The material is then planted on agar slopes.

As has been shown by comparative tests, gum acacia solution, among all the emulsifiers tried, yielded the highest survival rate (51.0\%) of \textit{P. pestis}. The virulence of plague cultures preserved as described above was recently found to be unabated after five years.

Mitigation of Virulence of Strains

In order to evaluate the methods used for attenuating the virulence of plague strains, it is necessary to determine first in what manner the transition from the virulent to the avirulent state takes place. Theoretically, there are two possibilities—either that all bacterial cells composing a virulent plague culture and possessing an equal degree of virulence undergo some change which renders them uniformly avirulent or that, virulent and avirulent bacilli being both pre-existent in the original growth, the loss of virulence is due to a process of dissociation by which the avirulent elements become preponderant or even solely present. Actually, there is no reason to doubt the validity of the latter assumption which, as pointed out by Jawetz & Meyer,\textsuperscript{82} is strongly supported by the fact that Otten\textsuperscript{134} was able to obtain
avirulent subcultures by single-colony picking from virulent growths of *P. pestis*.

Discussing the various methods for attenuating the virulence of plague strains, Jawetz & Meyer aptly distinguished between (a) the above-mentioned procedure of Otten which took advantage of a process of "natural" dissociation and (b) "enforced" dissociation effected by exposing the strains to adverse environmental conditions.

Most important among the latter procedures were:

(1) Repeated subcultivation at weekly intervals, a method used successfully by many workers, more recently by Girard & Robic, Pirie & Grasset, Sokhey, Macchiavello, and Hsue.

(2) Prolonged cultivation of virulent plague strains in broth to which alcohol had been added. Hetsch, when introducing this method, recommended the use of gradually increased alcohol concentrations (0.5%-5%) and incubation at high temperatures (41°C-43°C). Donskov & Lochov claimed to have obtained avirulent variants within a month through cultivation in broth containing 10% alcohol, and within a few days when using an alcohol concentration of 15%. Jawetz & Meyer started with hormone broth containing 0.5% alcohol, incubating the growths for three weeks at 32°C. Subcultures were then made in 3% alcohol broth and kept for three weeks at 32°C, and for a further four weeks at 40-60°C.

A third procedure, originally used by Burgess, was to attenuate the virulence of plague strains with the aid of passage through immune animals. To judge from the experiments made by Burgess himself and by Jawetz & Meyer, it was apparently far more difficult to obtain permanent results with this method than with those mentioned above.

Russian workers (Pokrovskaya; Korobkova) were able to obtain avirulent variants of *P. pestis* through bacteriophage action. Otten, while confirming that avirulent strains of high antigenic value could be produced with the aid of this method, found it rather laborious and time-consuming.

As has been noted previously (see page 88), bubbling of air through broth cultures in the apparatus devised by Devignat led to an impairment of the virulence of the strains. Devignat took practical advantage of this procedure to render three virulent plague strains avirulent and also to abate a slight increase in virulence of the EV strain (initials of the child from whom it was obtained) used by him for inoculation.

Describing the methods for the laboratory diagnosis of rat plague, Petragnani mentioned, without giving details, that the virulence of plague strains could be attenuated through cultivation on bile media or media prepared with "vegetable" materials.
IMMUNOLOGY

TOXIN OF THE PLAGUE BACILLUS

Nature of Toxin

Petrie,\textsuperscript{138} analysing problems of plague immunity in 1929, pointed out that workers on this subject somewhat loosely applied the terms antigen, endotoxin, immunizing substance, and vaccine, almost as if they were interchangeable. He himself, though certain that \textit{P. pestis} possessed a specific endotoxin which was associated with the soluble protein of the bacillus and which was set free in broth cultures by disintegration of the bacilli after their death or in bacillary suspensions by extraction methods, stated it as his belief that no principal difference existed between the antigenic substances present in (a) old autolysed broth cultures, (b) the purer products obtained by extracting the bacillary bodies, and (c) “whole” vaccines, whether prepared by Haffkine’s or other methods. Petrie emphasized in particular that all preparations used for active immunization against plague contained toxin, toxoid, or a mixture of these antigens.

Although a few workers such as Markl,\textsuperscript{112, 113, 114} Kossel \& Overbeck,\textsuperscript{100} and Dieudonné \& Otto\textsuperscript{34} suggested that the toxin of \textit{P. pestis} might represent a mixture of metabolic products of the living bacteria and of endotoxic substances set free after their disintegration, most experts are convinced that the plague bacillus has an endotoxin only, as originally postulated by Rowland\textsuperscript{156} and Besredka (quoted by Girard \& Sandor\textsuperscript{65}).

However, Girard and some other French workers stressed that the “endotoxin” of \textit{P. pestis} differed in some ways from the typical endotoxins of other bacterial species and resembled in other respects the exotoxins. Thus Girard\textsuperscript{55} and Girard \& Sandor\textsuperscript{65} noted that the plague bacillus did not possess the glucidolipoid complex found in the endotoxins of numerous other Gram-negative micro-organisms. On the other hand, as summarized by Ramon, Girard \& Richou,\textsuperscript{150} the plague endotoxin was similar to the exotoxins insofar as it consisted, like these, of proteins, could be easily transformed into toxoid, and was rather thermodabile.

Hence, although there is no reason to revise the concept that the plague toxin is an endotoxin, it will be noted that in this as in many other respects \textit{P. pestis} shows peculiar features.

Preparation of Toxin

The methods used by earlier workers for obtaining plague toxin consisted either of the filtration of old broth cultures containing autolysed plague bacilli or of extraction processes. The following procedures deserve mention:

\textit{Filtration}

Petrie,\textsuperscript{138} modifying a method devised by Markl,\textsuperscript{112, 113, 114} prepared toxic extracts thus: a medium of ordinary peptone broth (pH 7.0–7.3),
with or without the addition of 1% normal horse serum, was distributed into flasks in such a manner as to ensure good access of air to the cultures which were kept at room temperature (10°-15°C) for about two months. At the end of this time toluene was added to each flask and the flasks were put aside for a few days to allow the toluene to act. Seitz filtration was then used.

**Extraction**

Nucleoprotein extracts were first prepared in 1897 by Lustig & Galeotti\textsuperscript{107} who, treating suspensions of *P. pestis* with 1% potassium hydroxide solution and then slightly overneutralizing with 0.5% acetic acid, obtained the nucleoprotein in the form of a white flocculent precipitate. This substance conferred immunity against plague on rats if used in doses of 0.36 mg.

Rowland\textsuperscript{156} prepared toxic nucleoproteins by treating agar-grown plague cultures, which had been killed with chloroform, with anhydrous sodium sulfate. The extracts thus obtained were fatal to rats within 18 hours when administered in doses of 0.05–0.1 mg; doses of 0.001–0.01 mg afforded substantial protection against plague infection. Simple digestion of chloroform-killed plague cultures in saline yielded solutions which possessed the same chemical properties as the extracts obtained with sodium sulfate and could be rendered toxic and immunogenic if care was taken to remove the chloroform which formed a loose combination with the nucleoproteins (Rowland\textsuperscript{157}).

Recently Girard\textsuperscript{52, 54} obtained toxic extracts by three times freezing and thawing suspensions of plague bacilli according to the method of Grasset & Gory,\textsuperscript{71} then centrifuging and filtering. The filtrate, if administered in doses of 0.05–0.2 ml, killed mice within 6 to 36 hours.

Jawetz & Meyer\textsuperscript{82} prepared toxic extracts in the following manner: plague bacilli grown at 37°C were

"suspended in buffered saline (pH 7.4) and adjusted to a density of 20 billion [20 milliard] organisms per ml. These suspensions were incubated for 48 hours at 37°C and then left for 24 hours at 4°–6°C in the refrigerator. The bulk of the cellular material was then centrifuged off (2,000 r.p.m. for 90 minutes), and the supernatant fluid filtered through W Berkefeld candles and frozen in small pyrex bottles at −76°C. At this temperature no deterioration of the toxic power took place over a period of at least three months."

As will be mentioned later (page 125), Baker et al.\textsuperscript{6} were able to obtain from acetone-dried plague bacilli, through extraction with neutral salt solutions, a water-soluble and a water-insoluble antigenic fraction. The former was shown to contain at least three antigenic compounds including a toxic fraction (fraction II), soluble in ammonium sulfate at 0.33 saturation and at pH 7.0–7.5, and almost completely precipitated when the ammonium sulfate concentration was raised to 0.55–0.67 saturation.
Baker and co-workers pointed out that it had not been possible to isolate this toxin fraction in a state approaching chemical or immunological purity, all preparations being “contaminated” with the immunogenic fraction I in sufficient quantities to produce antibodies to the latter in rabbits. However, Baker and his colleagues claimed to have succeeded in preparing toxins free of fraction I by serological techniques. They observed that plague bacilli grown at room temperature were fully toxic, but extracts of such bacilli contained very little fraction I. It proved possible to remove the residual fraction I by absorption with either fraction IA or IB antisera. The resulting absorbed extract showed no decrease in toxicity, and produced antiserum in rabbits capable of neutralizing plague toxin. However, this antiserum was devoid of protective value for mice, did not agglutinate antigenically-complete plague bacilli to significant titre, and did not react with either fraction IA or IB except to a very slight degree in sensitive ring-tests. Its value in inducing immunity in mice and guinea-pigs has not as yet been investigated.

**Bacteriophage action**

Girard reported recently that he could obtain toxic lysates by adding bacteriophage to three-day-old cultures of his EV strain and a virulent plague strain and filtering after seven hours when incomplete lysis of the bacilli had taken place. Mice infected intraperitoneally with 0.2 ml, or subcutaneously with 0.5 ml, of these lysates succumbed 10–18 hours later.

Girard was unable to obtain toxic filtrates from broth cultures of *P. pestis* incubated at 28°C before the 6th–9th day; bacteriophage action seemed thus greatly to accelerate the normal process of bacteriolysis.

It is of interest to note in this connexion that, although almost without exception pseudotuberculosis strains do not yield toxic filtrates or extracts, Lazarus & Nozova were able to demonstrate the presence of toxic lysates when subjecting 8-hour-old cultures of two *P. pseudotuberculosis* strains of American origin to bacteriophage action. Girard, who confirmed this result, ascribed the much more rapid appearance of toxic substances in the lysates from these two strains than in those obtained from plague bacilli to the considerably quicker growth of *P. pseudotuberculosis*.

**Plague toxoid**

The transformation of the plague toxin into toxoid (anatoxin) is easily effected by adding 3.4% formalin to plague cultures and incubating at 37°C for a few days. As maintained by Girard, the toxoid thus formed is not capable of protecting mice or rats against the plague toxin. It is interesting to add that, according to MacConkey, the toxin in old autolysed solutions of plague nucleoprotein had undergone a partial change into toxoid, but that this comparatively atoxic substance stimulated the production of plague antitoxin in the horse.
Heat Resistance of Toxin

The problem of heat resistance of the plague toxin is rather involved. As pointed out by Petrie, the time factor was of marked influence as well as the temperature so that storage under a comparatively low temperature for prolonged periods had the same effect as short exposure (half an hour to one hour) to temperatures within the limits of 37°-70°C. Petrie emphasized that according to his experience toxin solutions or extracts, in order to retain their toxicity, had to be kept constantly at temperatures not higher than 3°C.

Some recent observations on the upper limits of the heat resistance of plague toxin showed that it became inactivated by exposure for four hours to 55°C (Meyer, quoted by Girard), for one hour to 65°C, or for half an hour to 80°C (Gheltenkoff).

Susceptibility of Experimental Animals

Valuable data on the susceptibility of laboratory animals to plague endotoxin assembled by Petrie may be summarized as follows:

Mice

Mice are highly susceptible to the toxin, subcutaneous or intraperitoneal administration usually killing the animals within 18 hours. By measuring the toxicity of broth toxins from a large number of plague strains by injecting graduated doses intravenously into mice, Petrie obtained the following results:

<table>
<thead>
<tr>
<th>Number of toxins tested</th>
<th>Average m.l.d. * (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>0.013 (1/76)</td>
</tr>
<tr>
<td>26</td>
<td>0.0095 (1/105)</td>
</tr>
</tbody>
</table>

* The average m.l.d. of 17 broth toxins prepared from Shiga-dysentery strains was 0.0053 (1/190) ml.

White rats

White rats usually died after 48 hours when the toxin had been given subcutaneously, and 24 hours after intraperitoneal administration. The lethal dose of broth toxins was 0.05-0.5 ml per 100 g of body-weight, smaller amounts being needed for intraperitoneal than for subcutaneous administration. Rowland’s nucleoprotein killed the animals in doses of 0.1 mg.

Guinea-pigs and rabbits

These animals withstood large doses of the toxin, a dose of 27 mg of plague nucleoprotein (that is, a quantity 270 times the m.l.d. for white rats) failing to cause death in guinea-pigs. However, some of the animals, though not showing acute symptoms, developed a condition of marasmus some weeks later.
Goats and horses

These animals were found susceptible to the toxin and died from toxaemia if given excessive doses.

Jawetz & Meyer, while finding mice highly sensitive to plague toxin, noted that guinea-pigs could withstand at least 5,000 lethal mouse-toxin doses. Tame rats and ground-squirrels (Citellus beecheyi beecheyi) combined, in the experience of these workers, a relatively low susceptibility to plague infection with a high resistance to the endotoxin.

ANTIGENIC STRUCTURE OF THE PLAGUE BACILLUS

The antigenic structure of P. pestis has been studied by chemical as well as serological methods. Moreover, some workers determined the antigenic make-up of this micro-organism by noting differences in the immunogenic qualities of its antigens for different rodent species. According to the methods of investigation used, the different findings concerning the antigenic structure of P. pestis are here grouped under the following subheadings:

Water-Soluble and Water-Insoluble Fractions

In the opinion of Rowland, which was shared by subsequent observers such as Morison et al, the plague bacillus appeared to be built up by two varieties of protein, one soluble in distilled water or saline and containing immunizing and toxic substances, the other insoluble and possessing no antigenic or toxic properties. Noting that, according to the observations of Rowland, the plague bacilli retained their shape after extraction of the soluble protein, though appearing fragile and easily breaking up, Petrie drew the inference that the cell membrane was composed of the insoluble fraction.

Baker et al. were able to obtain from buffered suspensions of a virulent plague strain, grown for three days at 37°C with the aid of acetone precipitation at -70°C, repeated washing with acetone, and drying in vacuo, a bacterial powder of high antigenicity and toxicity (see page 122). Extraction of this substance with neutral salt solutions yielded a water-soluble and a water-insoluble antigenic component, the former being toxic and immunogenic for mice and rats but of little activity for guinea-pigs, and the latter, while inert as far as mice and rats were concerned, proving of high immunogenic value for guinea-pigs when tested as an alum precipitate.

Soluble fraction

As shown by saturation or precipitation with ammonium sulfate at various concentrations, the water-soluble fraction contained at least three
antigenic compounds, called fraction IA, IB, and II by the authors who thus described the properties of the former two substances: 

Both fractions IA and IB gave rise in rabbits to potent antisera which apparently agglutinated all plague strains tested, with the exception of one avirulent growth thought to be devoid of an "envelope" by Schütze, but were incapable of neutralizing plague toxin. Both fractions induced immunity in mice as well as in white rats and monkeys (Meyer) but not in guinea-pigs.

The relationship between fractions IA and IB could not be exactly defined. They were almost identical serologically, but fraction IB, which could be obtained in crystalline form, possessed a slightly lower immunogenic value. Baker and his colleagues suggested that the normal antigen present in the bacterial cells might be fraction IA which contained a carbohydrate component besides protein, while the carbohydrate-free fraction IB was an artefact formed during the death and treatment of the bacilli.

Again referring to this postulation in an article published in 1952, Baker et al. stated that "since cultures of antigenically complete plague bacilli produce viscous colonies on suitable media, it may be that the surface antigen of intact plague bacilli is the viscous, carbohydrate-containing Fraction IA and that crystalline Fraction IB is formed from Fraction IA during extraction, through loss of the carbohydrate moiety ".

As shown by the serological studies of Baker et al., fraction I (IA and IB) was formed by all virulent strains tested as well as by a salt-stable avirulent strain whereas salt-instable avirulent growths produced only traces. However, incubation at 37°C seemed necessary to produce these antigens in quantity, plague bacilli grown at room temperature yielding only relatively small amounts.

The observation that fraction I was practically absent in salt-instable avirulent growths, is of great interest in view of the claim of Bhatnagar that "it is the envelope substance which bestows salt stability on a suspension of the plague bacillus ". It is also important to note in this connexion that, as established by Amies through examination of wet Indian ink preparations under dark-field illumination (see chapter 2, p. 76), plague bacilli

"which have been stripped of their envelopes begin to flocculate into small clumps to which the ink particles soon become adherent. This is presumably due to the fact that the normal electric charge on the bacterial cell is removed simultaneously with the envelope. In consequence the potential falls below the critical value and spontaneous agglutination is the result. Removal of a surface antigen is well known to produce a decrease of suspension stability in weak electrolytes, hence the salt sensitivity of rough variants ".

Electron microphotographs of avirulent and virulent \textit{P. pestis} strains are shown in fig. 15 and 16.

Drawing attention to the observations of other workers, it should be noted that Shrivastava, in the course of an earlier study on the antigenic

\textit{a} The properties of the toxic fraction II have already been dealt with on page 122.
FIG. 15.
AVIRULENT P. PESTIS
(×9,300)

FIG. 16.
VIRULENT P. PESTIS
(×8,600)
structure of *P. pestis*, had been able to isolate the following fractions from the supernatant fluid of Haffkine vaccine:

(a) an active protein fraction by saturating the fluid with sodium sulfate;

(b) a nucleoprotein fraction by precipitation in the cold with dilute hydrochloric acid;

(c) an active polysaccharide fraction with the aid of alcohol precipitation in the presence of sodium acetate.

Further work in this direction was done by Seal & Mukherji, who were able to isolate, by precipitation with sodium sulfate in different concentrations, a specific soluble substance of the plague bacillus which proved highly active serologically.

Continuing these investigations with the aid of filtrates of casein hydrolysate broth media in which virulent and avirulent plague bacilli and pseudotuberculosis bacilli respectively had been grown at 28°C for 24 days, Seal claimed to have isolated five different protein fractions by precipitating the filtrates at different saturations of sodium sulfate. Similar protein fractions could be isolated also from the water-soluble extracts of virulent plague bacilli grown on casein hydrolysate agar at 37°C for 72 hours. Seal maintained that the antigen “A”, obtained at one-third saturation with Na₂SO₄ from virulent as well as from avirulent protective plague strains, was the specific antigen of *P. pestis*, corresponding to the “envelope” antigen of Schütte; he stated that this fraction was practically absent in avirulent non-protective plague strains, and completely lacking in pseudotuberculosis strains.

In a further communication Seal stated that:

“A polysaccharide yielding osazone resembling that of Arabinose with a melting point of 166-168°C has been isolated from the supernatant of Haffkine plague vaccine as well as from the specific soluble protein and, to a less extent, from the bacterial debris of both virulent and avirulent protective plague strains. It is absent in non-protective avirulent plague and pseudotuberculosis organisms. The protective substance of the plague bacillus is probably a polysaccharide-protein complex”.

As shortly referred to in chapter 2, Amies was able to remove the capsular substance of *P. pestis* with the aid of aqueous solvents such as potassium thiocyanate and thus to obtain the specific antigen of this organism in soluble form. This substance, which after purification by ethanol fractionation appeared to be a simple protein, was strongly antigenic, a few micrograms being sufficient to induce a high level of immunity in experimental animals.

The question of what relationship exists between (a) the protein and polysaccharide antigens obtained by Seal, (b) the immunogenic substance isolated by Amies, and (c) the fraction I of Baker et al. is of great interest and importance.
Baker et al. expressed the opinion that Seal's protein fractions were not sufficiently well characterized to permit comparisons. Probably they were mixtures of fractions IA and IB together with other materials. According to Baker and his co-workers, Seal's polysaccharide possibly represented the carbohydrate moiety of fraction IA.

Amies maintained that his soluble surface antigen differed from the fraction IA of Baker et al. insofar as it was apparently a simple protein, whereas the latter was a protein-carbohydrate complex. Amies added, however that:

"At this stage... it would be unwise to attach much importance to this apparent discrepancy. In view of the approximately equal potency of the two antigens it seems improbable that they differ radically in the chemical constitution of the active constituent. The presence of slight impurities in one or both could account for the differences in chemical behaviour."

In the opinion of Baker et al. "the 'envelope' protein described by Amies may be Fraction IB. However, further work will be necessary to establish this identity.

Insoluble fraction

As recorded by Baker et al. in 1947, only small amounts of protein could be extracted with the aid of mild alkalis from the water-insoluble residue of the bacterial powder obtained from alcohol suspensions of \textit{P. pestis} through acetone precipitation. Anhydrous phenol, liquified with 10-15% acetone, dissolved about 25% of the water-insoluble fraction but both the phenol-soluble fraction and the insoluble residue contained the antigen protecting guinea-pigs. In contrast to fraction I, the water-insoluble antigen did not produce specific protective antibodies in the serum of monkeys or man (Meyer 118).

Further discussing the water-insoluble fraction in 1952, Baker et al. stated that "this component of \textit{P. pestis} has not been studied in detail, but some fraction of it is undoubtedly the 'somatic' antigen of Schütze."

"Envelope" and Somatic Antigens

Working with serological methods, Schütze reached the conclusion that the plague bacillus possessed two antigens, one contained in its "envelope" and the other in its somatic portion. This concept of the existence of an "envelope" and a somatic antigen has been accepted by practically all subsequent workers but, as discussed below, some of them assumed that in addition to these two a third antigenic factor was present in virulent plague bacilli. Since the belief in the existence of an "envelope" is not shared by all observers, the term "envelope antigen" is not very felicitous. Apart from the fact, however, that it has been widely accepted, it would be difficult to replace it. To speak of a "capsular" antigen or, following
Chertnik,\textsuperscript{45} of a "membrane" antigen, seems not permissible in view of the findings of Rowland\textsuperscript{156} recorded above.

Schütze\textsuperscript{163} stated that the "envelope" antigen was thermolabile, becoming haptenized (incapable of giving rise to antibodies) after exposure to 100°C for 15 minutes, and was destroyed after one hour at that temperature. According to further experiments of this author,\textsuperscript{164} heating for 15 minutes at 80°C was sufficient for haptenizing the "envelope" antigen. The somatic antigen was more heat-stable, still provoking antibody formation in the rabbit after one hour at 100°C and reacting in vitro even after three hours' exposure to the same temperature.

The conclusions reached by Schütze\textsuperscript{163} when comparing the antigenic structure of the plague and pseudotuberculosis bacillus may be summarized as follows:

\begin{table}[h]
\centering
\begin{tabular}{llll}
\hline
\textbf{Species} & \textbf{Antigen} & \textbf{Common} \\
\textit{P. pseudotuberculosis} & Flagellar Smooth somatic & rough somatic \\
\textit{P. pestis} & "Envelope" - & \\
\hline
\end{tabular}
\end{table}

Bhatnagar\textsuperscript{13} postulated that the pseudotuberculosis bacillus possessed both group- and type-specific somatic antigens.

Studying the antigenic structure of the plague bacillus with the aid of chemical procedures, Kurauchi & Homma\textsuperscript{101} were in agreement with Schütze that this micro-organism possessed two independent antigens—a somatic antigen which shared immunogenic characteristics with the pseudotuberculosis bacillus, and a capsular antigen. Considering that the latter alone was of importance in conferring immunity against plague infection, they called it the "specific immunizing fraction".

The two workers extracted this substance by a procedure not specified in their report, purified it by the "acid-acetone" method, and concentrated it. They found it contained 1.84\% nitrogen, 8\% ash, and 40\% reducing substances.

Experimenting with this substance, Kurauchi & Homma found it to confer a stronger immunity when administered twice to guinea-pigs and rats than ordinary plague vaccine. Three monkeys which received two injections also withstood a challenge infection with virulent plague bacilli but a fourth, which had received one dose only, succumbed. It was found, however, that after absorption of the specific soluble substance by metallic salts satisfactory results could be obtained with a single injection.

\textbf{Serological Properties and Virulence}

Schütze,\textsuperscript{165} as well as Wats et al.\textsuperscript{202} and Jawetz & Meyer,\textsuperscript{82, 83} emphasized that it was impossible to distinguish with the aid of serological tests between virulent and avirulent, smooth or rough plague bacilli. Jawetz & Meyer\textsuperscript{84} also stated that a factor which enhanced spreading and capillary per-
meability had been found in extracts of both virulent and avirulent *P. pestis*.

Russian observers (Fadeeva; 36 Gheltenkoff & Khvorostukina; 47 Korobkova 88) postulated that virulent plague bacilli possessed an antigen similar to the Vi antigen in the salmonellae, but Jawetz & Meyer 82 were unable to confirm this claim. Before dealing with the explanation they gave for the antigenic difference between virulent and avirulent plague strains, it is necessary to set forth the opinion held by Otten regarding the antigenic structure of *P. pestis*.

Otten, 131 comparing the immunizing power of his avirulent "Tjiwidej" strain with the potency of other avirulent plague strains, came to the conclusion that

"most of these strains possess an antigen especially active in guinea-pigs but producing less or even slight effect in rats. The strain ‘Tjiwidej’ on the other hand, is the best antigen for rats but must be put behind others as regards guinea-pigs. This difference is of a qualitative nature, as it cannot be overcome by increasing the dose”.

In a subsequent paper 134 Otten maintained that this difference remained manifest when tests were made with heat-stable (somatic) antigens of these various strains after the heat-labile ("envelope") antigens had been rendered inactive through heating. Otten postulated, therefore, that the somatic antigen was composed of a “guinea-pig” antigen and a “rat” antigen, present in different proportions in the various strains.

Jawetz & Meyer, 82 while considering Otten’s hypothesis as insufficient, were also disinclined to believe that different immunity mechanisms, due to variations in the resistance to plague toxin, might be at work. They assumed that some antigenic constituent, chemical group, or property, present in most live vaccines, but often absent in “killed” ones, might explain the divergent results obtained in different animal species. Mice might not be in need of this factor, thus becoming easily immunized with killed vaccines. It was, however, essential for guinea-pigs which, therefore, could be protected by specially-prepared killed vaccines only—for instance, the sugar vaccine of Minervin et al.122 and Haffkine vaccine (Sokhey; 180 Sokhey & Maurice 190).

Generally speaking Jawetz & Meyer assumed that there was

"little doubt of the presence of some property or chemical group responsible for virulence in the plague bacillus, since some natural dissociants seem to be identical with virulent organisms in all respects except for a complete loss of virulence”.

They stated in a later paper 53 that the agent at work might be of an enzymic nature. Rockenmacher’s 154 claim that the catalase activity of virulent strains is greater than that of avirulent growths is of great interest in this connexion.

It deserves attention, however, that in the course of their recent work, Baker et al.7 found no evidence “for the existence of a definite entity responsible for virulence, as Jawetz & Meyer suggested.”
NATURAL RESISTANCE TO PLAGUE

Although, as will be discussed later, most animals other than rodents are insusceptible to plague, the immunological aspects of their resistance seem to have been fully studied only in the case of birds.

Birds

The natural resistance of birds to plague cannot be considered as absolute. Thus Giaxa & Gosio (quoted by Dieudonné & Otto\textsuperscript{34}) found starving pigeons and sparrows susceptible and, as observed by Albrecht & Ghon\textsuperscript{2} and others, intravenous or intraperitoneal administration of large doses of highly virulent plague bacilli to normal birds occasionally produced fatal infections. However, these exceptions do not invalidate the rule that under ordinary circumstances birds are resistant to plague.

London\textsuperscript{106} ascribed this resistance to the presence of specific humoral antibodies in normal birds, but both Flu\textsuperscript{39} and Hoessli (quoted by Meyer\textsuperscript{118}) failed to confirm this claim. As maintained by Meyer\textsuperscript{118}, no credence should be given to the assumption that the insusceptibility of chickens or other birds was due to their high body-temperature (42°C or 107.6°F). Thus, in the opinion of this author, “phagocytic destruction of plague bacilli by fixed macrophages in all probability constitutes an important, if not the determining, factor in the natural resistance of birds to plague infection”.

It is interesting to note that this insusceptibility to plague is not shared by chick embryos (Buddingh & Womack;\textsuperscript{19} Jawetz & Meyer\textsuperscript{83}). Jawetz & Meyer observed in this connexion that, while virulent plague bacilli introduced in small numbers multiplied rapidly and caused the death of chick embryos within four to eight days, small numbers of avirulent bacilli failed to proliferate freely. Another interesting point was that a potent plague serum was unable to protect the embryos against \textit{P. pestis} because, as these workers assumed, a cellular defence mechanism, without which the serum could not exert its protective action, was still absent.

Rodents

Although plague is primarily a disease of the rodents, even these animals are by no means uniformly susceptible to the infection. Not a few of the numerous species composing the order \textit{Rodentia} have been found to be rather, or even completely resistant to experimental infection with \textit{P. pestis}. More important still, marked differences in plague susceptibility, due either to the existence of differently receptive races or to seasonal influences, have been found even in the species most concerned in the perpetuation of the infection.
While the mechanism underlying this racial or seasonal resistance seems not yet to have been fully elucidated, all available evidence tends to show that, as in the case of the birds, so also in naturally plague-resistant rodents a principal role is played by cellular defence factors.

Proof for this contention seems to have been furnished by Bhatnagar & Shrivastava \(^\text{14}\) who worked with Bombay rats (\textit{Rattus rattus}) so resistant to the infection that 91\% of the animals survived subcutaneous injection with \(3-4 \times 10^3\) virulent plague bacilli. Through a continued study of the blood picture of the infected animals, the two observers were able to show that during the first 48 hours after infection the polymorphonuclear leucocytes reacted against the invaders. Then an increasing number of clastocytes appeared in the blood and obviously continued the fight until on the seventh day, simultaneously with the disappearance of these cells and the appearance of young polymorphonuclears, the condition of the animals improved.

Similarly Meyer \(^\text{118}\) found that in the comparatively resistant cotton rat (\textit{Sigmodon hispidus hispidus}) the reticulo-endothelial cells of the liver were instrumental in removing \textit{P. pestis}. It is also noteworthy that, as shown by Larson (quoted by Meyer \(^\text{118}\)), the fatal termination of plague infection in monkeys was preceded by a drop in lymphocytes and monocytess.

An interesting observation on the seasonal changes in plague susceptibility was that according to Meyer \(^\text{115}\) guinea-pigs surviving from litters, other members of which had been fully amenable to infection during the summer, became quite resistant during the winter. Washed peritoneal-exudate cells of such animals were able to destroy in vitro several hundred plague bacilli within 3–24 hours.

Plague in hibernating wild rodents will be dealt with in a later study, but it should be mentioned here that according to Gaiski, \(^\text{43}\) who devoted much effort to a study of this problem, the resistance of such animals during hibernation was due not to immunological factors but to bacteriophage action.

\textbf{Man}

No convincing evidence is available to show that a natural immunity to insect-borne plague exists in man. In particular there is no secure foundation for the belief that certain races are less susceptible to this type of the infection than others, the differences observed in this respect being, in all probability, due to greater or lessened chances for infection and not to racial differences in susceptibility. The existence of so-called healthy carriers of bubonic plague, postulated by a few observers, must not necessarily invalidate the rule that no natural resistance to insect-borne plague exists in man, because such individuals, having probably passed through an unnoticed attack of pestis minor, were presumably convalescent and not healthy carriers.
It cannot be claimed, however, that the same explanation holds true in the case of the carriers of pneumonic plague observed upon several occasions or, more generally speaking, of persons who did not contract this highly infectious form of the disease in spite of taking no precautions when in prolonged close contact with pneumonic-plague patients, for instance, in wards (Wu Lien-teh 204). While it is possible, therefore, that instances of a resistance to pneumonic-plague infection exist, they are of such rare occurrence as to be of no practical importance.

MECHANISM OF ACTIVE IMMUNITY TO PLAGUE

Earlier views on the mechanism of active immunity to plague may thus be summarized:

Infecting white rats by the intraperitoneal route, Markl 115 found that lysis and phagocytosis of the bacilli competed with the rate of multiplication of those remaining free in the exudate.

Similar results were obtained by Rowland 158 with intraperitoneally- and subcutaneously-infected rats. Finding that virulent bacilli multiplied slowly in the body of vaccinated rats, and that avirulent bacilli did likewise even in normal rats while virulent infection progressed rapidly in the latter, he reached the conclusion that the essential factor in plague immunity was that which affected the multiplication of the bacillus.

Commenting upon these findings of Markl and Rowland, Petrie 138 stated that the part taken by phagocytosis in the defence mechanism could be explained on the basis of an opsonic action. He noted in this connexion that Douglas (quoted by Brooks 18) had found a good opsonic response in man after inoculation with Haffkine vaccine while Brooks 18 had the same result when injecting white rats with the soluble protein of P. pestis. However, the bacteriolytic part of the defence mechanism could not be easily explained, since most workers failed to demonstrate a bactericidal element in immune serum.

Malone et al., 111 estimating the bactericidal power of rat-blood and afterwards infecting these animals with a standard dose of plague bacilli, found that the results obtained by these two methods did not tally, artificially immunized rats having a better chance for surviving after the challenge infection than the moderately-resistant Bombay rats or the fully-susceptible Madras rats, regardless of their haemobactericidal power. Malone and his co-workers assumed, therefore, either that the blood of immune animals possessed in vivo some antibacterial quality which could not be demonstrated in the laboratory, or that part of their immunity was derived from sources outside the blood stream ("tissue" or "antiblastic" immunity).
Role of Phagocytosis

Dealing with the behaviour of *P. pestis* in normal and immune experimental animals (guinea-pigs and mice), Jawetz & Meyer found that if smaller inocula were given to immune animals, the bacilli were frequently fixed at the site of infection and slowly destroyed there. Administration of large numbers of virulent bacilli led to their rapid distribution in actively-immunized as well as in normal animals, but in the case of the former this was followed in a few days by the disappearance of the micro-organisms first from the blood, then from the liver and spleen, and afterwards from the lymph-nodes and site of infection.

Continuing their studies, Jawetz & Meyer were able to confirm the above-mentioned opinion of Petrie as well as the results of recent work by Joukov-Verejnikov & Fadeeva by showing that the serum of animals immune to plague was unable to destroy or lyse *P. pestis* either in vitro or in vivo in the absence of phagocytic cells. Whole blood of plague-immune animals could destroy a much greater number of plague bacilli than blood of normal animals, this activity being found to be primarily inherent in the plasma which probably either made the bacilli more easily digestible for the phagocytes or conferred specific qualities upon the latter.

Assessing their results as a whole, Jawetz & Meyer found that the problem of plague immunity was rather complex, and that "in all probability different mechanisms may be responsible for a greater or lesser share in the total picture of immunity in the various laboratory animals".

In a recent comprehensive survey of the problems of plague immunity, Meyer pointed out that, as shown by the investigations of Burroughs, the intact skin was capable of barring the entrance of plague bacilli to a considerable degree. Since, however, infection through flea-bites led to a direct invasion of the capillaries, a further defence mechanism, furnished by the leucocytes and auxiliary elements, had to come into play.

In order to study the factors involved, Meyer and his co-workers made ample use of cytograms, a method originally devised by Rowland and also used by Pokrovskaya & Kaganova, to whose work reference is made below. Experimenting with different animals, particularly with monkeys and mice, Meyer and colleagues used the following technique:

"An area of the ear or sternum is inoculated with plague bacilli and a fine needle is inserted into the area. Samples of the tissue fluid are aspirated at regular intervals and suitably-stained smears are made for cytological examinations."

As stated by Meyer, during the first two hours after infection, when in normal as well as in moderately-immune animals the injected area became markedly oedematous, the films prepared from either normal or immune animals did not differ strikingly. Then, however, marked differences began to appear, as shown by the following summary of Meyer's principal findings:

1. Between two and eight hours after injection the plague bacilli became agglutinated in immune but not in normal animals. Many single organisms were swollen and indis-
tinct in the immune animals and, in contrast to the non-immune, the number of the bacilli was definitely reduced.

(2) With the arrival of polymorphonuclear leucocytes between four and six hours after, phagocytosis became pronounced in the immune animals while only occasional cells of the non-immune ingested a few bacilli. By the 12th hour phagocytosis dominated the microscopic picture in the immune animals while in the non-immune animals not more than 10% of the leucocytes were able to ingest plague bacilli in the early stages of the infection. Thus, while in the exudate of the immune host phagocytosis competed successfully with the rate of multiplication of the plague bacilli, in the non-immune animals the bacilli multiplied extracellularly without hindrance and phagocytosis practically ceased by the 24th hour.

(3) Within 24–36 hours the serous tissue fluid in the immune animals became converted into a dense cellular exudate. Simultaneously the leucocytes clumped and the exudate condensed, thus aiding fixation of the bacilli. The few micro-organisms which did not become phagocytized were ultimately taken up by polyblasts of the macrophage series.

Summing up the problem of plague immunity in general, Meyer considered that

"phagocytosis is the most important mechanism which animals and man use in guarding against and disposing of a plague infection. The mesenchymal tissue cells are responsible not only for cellular immunity, but for humoral defense as well. Their cytoplasm, modified by effective contact with antigen, develops adaptive enzymes and thus circulating antibodies... which in turn remove the antiphagocytic property of the slimy 'envelope' of the plague bacillus. ... The effectiveness of the mechanism and consequently the fate of the host are determined by the balance between bacterial multiplication and the efficacy of the clearing mechanism".

"Organ" and Local Immunity

Some evidence exists that under certain circumstances even in immune animals the lungs are less resistant to plague infection than organs like the liver and spleen.

It should be noted in this connexion that, as established by Batzaroff and confirmed by other workers such as Girard and Jawetz & Meyer, animals partly immune to plague, and therefore succumbing to a challenge infection after prolonged illness, regularly showed marked lung involvement in the absence of liver and spleen lesions.

Discussing these findings, Jawetz & Meyer drew attention to the observation of Sprunt & Camalier that the resistance of the lung to bacterial infections was lowered through circulating toxins. Experimental animals like guinea-pigs, monkeys, and also mice, if partially immune, could withstand the original impact of the infection but at the same time plague bacilli persisted and slowly multiplied at the site of the infection and in the regional lymph-nodes, and disintegration of part of the micro-organisms led to the liberation and circulation of endotoxins. The latter led to the establishment of a locus minoris resistentiae (site of least resistance) in the lungs so that pneumonic foci could become established even as
the result of a minor bacterial invasion. That no foci of the infection developed in the liver and spleen was probably due to an enhanced cellular activity which not only prevented multiplication of the plague bacilli but also resulted in an increasing toxin resistance.

The explanation given by Pokrovskaya & Kaganova for the low resistance of the lungs to plague infection was that the cells of this organ were lacking in phagocytic power. Discussing the pathogenesis of two human cases of chronic meningitis and encephalitis observed in California, Jawetz & Meyer similarly assumed that the bacilli reaching the brain early in the disease could persist and slowly multiply there because they were protected from phagocytic action.

Petragnani claimed that a local immunity could be created through instillation of avirulent plague bacilli into one eye of guinea-pigs. Such animals survived if some weeks later virulent bacilli were instilled into the same eye but succumbed to generalized plague if the unprotected eye were infected.

Anti-infectious and Antitoxic Immunity

A distinction between an anti-infectious and an antitoxic immunity was made by some observers.

In the experience of Jawetz & Meyer, a high antitoxic resistance did not by itself protect against plague infection whilst a high anti-infectious immunity was invariably accompanied by a certain degree of toxin resistance. This was in agreement with the results previously obtained by Markl, but not with those of Rowland who claimed that anti-infectious immunity was conferred by the administration of plague toxin or toxoid.

In the opinion of Otten, antitoxic immunity could be more easily produced than anti-infectious immunity but was of little value under natural conditions where the invasive power of the plague bacilli was the decisive factor. Jawetz & Meyer, while admitting that in anti-infectious immunity the defence was primarily directed against bacterial multiplication and not against liberated endotoxin, considered it as likely

"that active or passive antitoxic immunity can temporarily ward off the effects of products of bacterial disintegration and thereby permit the development of an efficient defense mechanism directed against the multiplying bacilli".

As established by these workers, fairly good antitoxic immunity could be produced in mice by either live avirulent or formalin-killed vaccines, provided a restimulating injection was administered in addition to the initial dose.

ACTIVE IMMUNITY TO PLAGUE

It is certain that human beings as well as rodents, if surviving a plague attack, are apt to be resistant to the infection. However, owing to the fact that until recently instances of recovery from severe forms of the
disease were infrequent in man, little information is available on the solidity and duration of this naturally-produced state of immunity.

As pointed out by Dieudonné & Otto, the fact that people who had recovered from plague were unlikely to contract this infection again, had long been known. For instance, attempts were sometimes made in the past to employ preferably persons who had survived an attack of the disease (the so-called "mortis") as attendants in the plague wards. Dieudonné & Otto also noted that this empirical knowledge had been confirmed in recent times by the demonstration of specific antibodies in the serum of convalescents. At the same time, however, they were careful to point to some instances of re-infection of individuals who had previously had bacteriologically-confirmed attacks of plague and maintained in general that the immunity to this disease, acquired under natural conditions, was evidently relative in degree and limited in duration. Meyer has recently endorsed this opinion.

Attempts to protect human beings against plague, in the same way as variolation was practised in the case of smallpox, were made at an early date.

The Hungarian physician Weszpremi, who seems to have been the first to use this method (1755), was followed by Samoilovski in Russia who, having become infected with pus from a bubo in 1781, contracted the disease in a slight form and recommended, therefore, protection against plague by means of a tampon moistened with such pus which was applied to the arm with the aid of a bandage. As was to be expected, the use of this method led sometimes to disastrous results so that it soon came into discredit (Dieudonné & Otto).

The modern history of plague vaccination may be said to have begun in 1895 when Yersin, Calmette & Borrel showed that rabbits could be immunized against this infection by repeated inoculation with suspensions from agar cultures killed by exposure to 58°C for one hour. About a year later Haffkine obtained identical results when using broth cultures of P. pestis sterilized by heating.

Since that time large-scale advantage has been taken of anti-plague inoculation not only with killed vaccines grown either in broth or on solid media but, recently, also with live avirulent bacilli. The possibility of using extracts prepared from P. pestis by chemical methods for the same purpose has also received attention.

Killed Plague Vaccines

Evaluation of potency

Although, as testified by an enormous literature, numerous methods or modifications of methods have been recommended for the preparation of killed vaccines, until recently the value of these procedures could not
be properly assessed. Almost invariably it was impossible to arrange for actual tests in the field on a sufficiently large scale and, moreover, most difficult adequately to interpret the effects of these methods in man. It would seem at first glance that tests made in the laboratory ought to have given less doubtful results, but no exact methods were available for this purpose and great uncertainty existed regarding the proper choice of test animals.

Discussing the latter problem, Taylor considered both the lower monkey species used in plague work and the rabbit as unsuitable for testing vaccines on account of the variability of their response to challenge doses.

Guinea-pigs also appeared unsuitable for vaccine work, both on account of their resistance to toxins and because they did not respond well to immunization.

Trapped wild rats (*R. rattus*), which were largely used in the Haffkine Institute, were by no means ideal. In this connexion Taylor pointed out the necessity of transporting susceptible rats from Madras to Bombay, which resulted in a considerable mortality. Moreover, the animals proved very susceptible to the toxic action of the vaccine, frequently 20% to 30% succumbing when the usual test dose of 0.5 ml was administered. The average immunity of the rats, on the other hand, was only in the vicinity of 30% to 40%. Therefore wild rats, though useful for tests on broad lines, were unsuitable for biological standardization or the elucidation of finer differences between the vaccines tested.

Laboratory-bred albino rats were found less susceptible than the wild *R. rattus* and irregular in their response to infection with various doses.

However, as announced by Taylor, Sokhey had recently established that the strain of white mice inbred in the Haffkine Institute had proved excellent. These animals were as a rule fully protected when receiving 0.03 ml of Haffkine vaccine while 10 times this amount was required to produce toxin deaths. At the same time their susceptibility to the challenge infection was very regular.

The great value of the white mouse for testing plague vaccines has been recently endorsed by Meyer et al. who reached the conclusions that

1. the lyophilic envelope fraction IB antigen is probably essential in the maximum protection of man;

2. since this antigen is equally indispensable in the protection of mice against plague, it is deemed advisable to use mice rather than guinea-pigs to measure the immunogenic potency of a plague vaccine.

The method of biological standardization of plague vaccines introduced by Sokhey to replace the former unsatisfactory procedures was based upon the use of the method for measuring the virulence described earlier (page 116); it consisted in determining the minimum quantity of a plague vaccine that would save a white mouse challenged seven days after administration of
the second dose of the vaccine under test. As described by Sokhey & Maurice, the procedure was as follows:

The quantity of the vaccine to be administered was divided in two equal doses and given subcutaneously with a seven-day interval. Five mice were used for each quantity of vaccine and four to five quantities were used at a time. The minimum quantity that saved at least three out of five mice during a 25-day period of observation, commencing with the day on which they had been given the challenge dose, was taken as the protective dose of that particular vaccine. The results for all the vaccine quantities used had to be consistent among themselves and the result of duplicate tests had to tally. The mortality of the control animals had to be 100%.

Again, discussing the method of biological standardization, Sokhey & Maurice stated that they had obtained good results with Haffkine vaccine in guinea-pigs as well as in white mice, as shown thus:

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Total vaccine dose (ml)</th>
<th>Challenge dose (number of organisms)</th>
<th>Number of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.80</td>
<td>1 million</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1.25</td>
<td>1 million</td>
<td>0</td>
</tr>
<tr>
<td>5 (controls)</td>
<td>—</td>
<td>1 million</td>
<td>5</td>
</tr>
</tbody>
</table>

Given in two equal doses at seven-day intervals.

It should be noted that satisfactory results with guinea-pigs have been recently reported also by Wayson et al. and by Meyer who worked with vaccines treated with alum or other synergists.

Comparing the efficacy of killed vaccines for white mice (body-weight 25–30 g) and white rats (200–270 g), Sokhey & Habbu found that, contrary to the statements made by some other observers, the latter animals could be equally-well protected by Haffkine broth vaccine grown at 28°C and agar vaccine prepared from cultures incubated at 37°C:

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Mouse-protective dose (ml)</th>
<th>Rat-protective dose (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haffkine vaccine (28°C), four week’s growth</td>
<td>0.004</td>
<td>0.030</td>
</tr>
<tr>
<td>Agar vaccine (37°C), 1,000 million organisms per ml</td>
<td>0.004</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Relation of virulence to potency

Summarizing the literature available up to 1936, Pollitzer stated that the necessity of using the most virulent strains possible for the preparation of plague vaccines had been stressed by pioneers in this field such as Haffkine and the German Plague Commission as well as by all subsequent workers. However, some recent observations have thrown doubt upon the absolute validity of this claim. Thus Schütze, experimenting with Haffkine-type vaccines prepared from virulent and avirulent plague strains, came to the conclusion that “virulent cultures do not result in more potent vaccines for either rats or mice than do avirulent ones”. Likewise Sokhey & Habbu obtained the following results when comparing two heat-
killed vaccines (A and B) prepared from strains showing marked differences in virulence:

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Mouse m.l.d. (number of organisms)</th>
<th>Mouse-protective dose (number of organisms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>6,500,000</td>
</tr>
<tr>
<td>B</td>
<td>300,000,000</td>
<td>5,300,000</td>
</tr>
</tbody>
</table>

Sokhey & Habbu concluded that these experiments had not been carried to a logical end, but the observations so far made showed that even considerable differences in the virulence of strains did not make any difference to the protective power of a heat-killed vaccine.

Assessing the comparative value of virulent and avirulent plague strains for the manufacture of killed vaccines, it must be kept in mind, however, that while the antigenic value of the former is and remains equal under proper conditions of storage, avirulent growths show great variations in potency (Sokhey) and, as will be discussed later (page 153), even the antigenic value of one and the same strain may deteriorate.

Relation of toxicity to potency

The relation of the toxicity to the potency of the vaccines was thus summarized by Pollitzer in 1936:

Haffkine was convinced that a definite degree of toxic reaction was desirable and necessary for effective immunization; in fact the dosage of his prophylactic fluid for man was originally determined on the basis of toxicity. Rowland, though pointing out that the toxicity of a vaccine could be abated without interfering with the antigenic properties, was in accord with Haffkine's views. Taylor, summarizing the subsequent experiences of the Haffkine Institute, admitted some influence of storage on the toxicity as against the potency, but also insisted upon a close relationship between these two properties not only in the case of Haffkine's fluid but in that of all effective plague vaccines. Petrie, discussing the theoretical aspects of this problem, stated that there was no clear proof of the existence of an independent antigen apart from the toxin and the opsonic antigen which conferred an active immunity against the living culture. The rapid induction of immunity conferred by the nucleoprotein seemed, in Petrie's opinion, to bear a relation to the rapid absorption of plague toxin or the rapid opsonic response rather than to the slower action of a bacterial antigen of the thermostable type.

Considering this evidence, Pollitzer stressed the fact that, as shown by some of the above-mentioned and also by other observers, the toxicity of plague vaccines could be abated without any considerable loss in potency. Thus, as shown by Schütze and confirmed by Sokhey & Maurice, incubation of broth vaccines at 37°C instead of 27°C reduced their toxicity without interfering with their antigenic properties.

Measuring the toxicity of broth- and agar-vaccines by determining the doses which killed 50% of the white mice used (body-weight 26–27 g), Sokhey & Habbu obtained the following results:

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Toxid doses (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haffkine vaccine (28°C), four weeks' growth</td>
<td>0.2</td>
</tr>
<tr>
<td>Agar vaccine (37°C), 1,000 million organisms per ml</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Sokhey & Habbu concluded from this and confirmatory tests that agar vaccine was much less toxic than broth vaccine. At the same time, however, they recorded encouraging results when using formalin instead of heat to sterilize the vaccine obtained from four-week growths in casein hydrolysate:

<table>
<thead>
<tr>
<th>Method of sterilization</th>
<th>Toxic dose for mice (LD₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat (54°C) + phenol (0.5%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Formalin (0.05%) + phenylmercuric nitrate</td>
<td>0.4</td>
</tr>
<tr>
<td>(1 mg per 100 ml)</td>
<td></td>
</tr>
<tr>
<td>Formalin (0.075%) + phenylmercuric nitrate</td>
<td>0.5</td>
</tr>
<tr>
<td>(1 mg per 100 ml)</td>
<td></td>
</tr>
</tbody>
</table>

Since, as will be discussed later (see page 144), the new casein-hydrolysate vaccine was an excellent prophylactic, these observations support the view that it is possible to reduce the toxicity of plague vaccines without biasing their antigenic value.

**Comparative potency of supernatant fluid and solid portion of vaccines**

While some early observers such as the German Plague Commission were of the opinion that the antigenic potency of Haffkine vaccine was vested in the solid portion containing the body-substances of the bacilli, most workers were agreed that since the antigenic fraction of *P. pestis* responsible for the production of immunity apparently passes into solution, the whole value of this vaccine resides in the fluid portion (Taylor).

Dealing again with this problem recently, Sokhey & Habbu confirmed that, in the case of broth vaccines, the total protective power resided in the supernatant fluid, regardless of whether incubation at 27°C or 37°C had been used. They likewise observed that when 48- or 72-hour agar growths incubated at 37°C were suspended in water and the bacilli were left in this for three to seven days, the total immunogenic power was contained in the clear fluid separated from the solid part of the suspension. However, in the case of growths incubated at 27°C, the clear fluid obtained in the same manner had very poor protective power.

Sokhey & Habbu suggested that it might be advantageous to use only the supernatant fluid of broth vaccines grown at 27°C or of suspensions of agar cultures incubated at 37°C for inoculation because such vaccines would be less toxic.

**Methods of killing vaccines**

*Heat.* The pioneers of antiplague inoculation resorted to heat to render their vaccines innocuous and this practice has been followed until recently by most workers.

Haffkine first sterilized his broth-vaccine brews by exposing them for one hour to 70°C but soon reduced the temperature to 50°–55°C and the
period of heating to 15 minutes. While this period remained the standard in the Haffkine Institute, temperatures of 60°–64°C were used for the preparation of broth vaccine after the departure of Haffkine in 1905 and until Sokhey demonstrated that they exerted a most deleterious effect on the potency of the brews. The method of applying a temperature of 55°C for 15 minutes (Taylor; Sokhey) was therefore once more adopted.

**Antiseptics.** While sterilization by heat remained until recently the standard method for the preparation of Haffkine vaccine, other workers began to use antiseptics for the same purpose. Thus mere phenolization at room temperature was employed for the manufacture of the agar-grown Lister Institute vaccine and was also utilized by Burgess. Row and Nikanorov (quoted by Pollitzer) worked with glycerol but their products, which were destined for the treatment of plague patients rather than for prophylaxis, had the drawback of requiring prolonged storage before use.

Formalin, which had previously been used by Batchelder to prepare plague antigens for laboratory purposes, was actually used for the manufacture of plague vaccines by French workers as well as in the USA (Meyer) during the second World War, and has also been adopted for the production of the new casein-hydrolysate vaccine in the Haffkine Institute (Sokhey & Habbu).

Comparing different methods of killing vaccines, Sokhey & Habbu recorded the following results:

<table>
<thead>
<tr>
<th>Method</th>
<th>Moose protective dose (ag. vaccine)</th>
<th>Moose protective dose (broth vaccine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating at 54°C</td>
<td>0.0065</td>
<td>0.006</td>
</tr>
<tr>
<td>Merthiolate (1/500,000)</td>
<td>0.0068</td>
<td>0.006</td>
</tr>
<tr>
<td>Sulfathiazole (1%)</td>
<td>0.0070</td>
<td>—</td>
</tr>
<tr>
<td>Alcohol (Felix)</td>
<td>0.0160</td>
<td>—</td>
</tr>
</tbody>
</table>

**Types of killed vaccines**

(1) *Haffkine's vaccine.* Bearing in mind that, owing to the introduction of the new casein-hydrolysate vaccine, the methods of manufacturing the classical Haffkine fluid have become a matter of historical rather than of actual interest, it is proposed to deal here only with those principles involved in its preparation which remain important for future work.

(a) **Incubation temperature:** The incubation temperature of about 27°C originally recommended by Haffkine has been found best for the preparation of fluid plague vaccines by most subsequent workers. As confirmed by the exact tests of Sokhey, broth vaccines grown at about 27°C were more potent than those incubated at 37°C because growth of *P. pestis* was maximal at or near the former temperature. Further, as stated by Taylor, incubation at 27°C was also beneficial insofar as it restrained the development of many other micro-organisms including the pasteurellae tested.
It was likewise a convenience that during the greater part of the year the average room-temperature at Bombay was about 27°C.

The lesser toxicity of broth vaccines grown at 37°C noted before is a point deserving great attention but, as has been found by Sokhey & Habbu, the use of formalin instead of heat for manufacture of such vaccines seems to go a long way towards restoring a balance in favour of incubation at 27–28°C.

(b) Period of incubation: Though Haffkine had recommended incubating his vaccine for six weeks, this practice was not strictly adhered to by his successors and finally an incubation period of four weeks was made the standard.

(c) Storage: It is generally agreed that storage of the Haffkine vaccine, while reducing its toxicity (Stevenson & Kapadia) did not exert an unfavourable influence on its potency. Taylor noted in this connexion that after a slight drop in potency which occurred during the first month, the vaccine, even if stored at room temperature, retained a high immunizing value for a year and lost but little of its potency during the following six months. Sokhey & Habbu even found that a batch of the vaccine which had been lying at room temperature for about 10 years still showed a very high protective power. They also made the following observations comparing the potency of two lots of broth vaccines, grown for four weeks at 28°C and heat-killed, with that of two lots of heat-killed agar vaccines, grown at 37°C and stored at different temperatures:

<table>
<thead>
<tr>
<th>Media</th>
<th>Storage temperature (°C)</th>
<th>Storage period (weeks)</th>
<th>Potency (mouse-protective dose) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth</td>
<td>0</td>
<td></td>
<td>0.0052</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>8</td>
<td>0.0040</td>
</tr>
<tr>
<td>Agar</td>
<td>0</td>
<td></td>
<td>0.0035</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>6</td>
<td>0.0060</td>
</tr>
</tbody>
</table>

It will be noted that, in contrast to agar vaccine, broth vaccine kept well even if stored at 45°C.

(2) Casein-hydrolysate direct vaccine. The preparation and properties of the new casein-hydrolysate plague vaccine were thus described by Sokhey & Habbu (see also Sokhey, Habbu & Bharucha):

A very much improved plague vaccine with a consistently high protective value can be prepared by the use of Mueller & Johnson’s casein hydrolysate. The medium is so prepared that controlled digestion produces a fluid which is entirely free of proteins and is perfectly limpid with a light-yellow tinge. It is adjusted to contain 270 mg of nitrogen per 100 ml.

The medium is distributed in quantities of 1 litre in modified Haffkine flasks of three-litre capacity and is seeded with specially selected strains
of *P. pestis* preserved by drying from the frozen state. The growths are incubated at 28°C for two weeks. Killing is done by the addition of 0.1% formalin, and phenylmercuric nitrate is used as the preservative.

The mouse-protective dose of this vaccine is 0.004 ml. It is much less toxic than the previous vaccine, since 0.6 ml, as opposed to 0.2 ml, is needed to kill a mouse. The keeping qualities of the new vaccine are very good; stored at 37°C for 18 months, it does not show any loss of protective power.

(3) *Agar-grown vaccines.* The technique originally adopted by the German Plague Commission for the manufacture of agar-grown plague vaccine consisted in washing off the growth of two-day-old virulent cultures with broth or saline, heating the collected washings for one to two hours at 65°C and afterwards adding carbolic acid so as to obtain a concentration of 0.5%.

The modifications of this procedure introduced by subsequent workers may be summarized thus:

(a) *Media:* While most of the earlier workers did not specify the composition of the solid media they used, Burgess recommended a "whole meat" agar as being simple to prepare and yielding abundant growth. Special media preferred by recent workers were glycerol agar (Barreto), hormone or hormone-sulfite agar, used for the preparation of the US "Army vaccine" during the second World War, and blood tryptose beef-heart agar, used by Wayson et al. for obtaining vaccines for experimental purposes.

(b) *Temperature and period of incubation:* The earlier workers do not seem to have been particular as to the temperature of incubation, apparently using temperatures ranging from 27°C to 30°C. Schütze seems to have been the first who insisted on incubation at 37°C because he assumed that in this way growths rich in "envelope" antigen resulted. Sokhey also found that agar vaccines grown at 37°C were far more potent than those obtained at 27°C, when the mouse-protective dose was 0.1 ml as against 0.002–0.004 ml in the case of the former. However, he did not ascribe this difference to the presence or absence of an "envelope" but pointed out that the plague bacilli grown at 37°C were bigger and that, consequently, the actual bulk of agar vaccine obtained at this temperature was greater than that of 27°C vaccine containing the same number of organisms. Sokhey assumed, however, that this was probably not the only factor involved.

An incubation temperature of 37°C was also found preferable by other recent workers such as Gracian. The US Army vaccine seems to have been grown at 30°C but Wayson et al. incubated the vaccines they prepared for comparison with it at 39°C.
The periods of incubation adopted by the various workers ranged from 40 to 72 hours.

(c) Standardization: Besides the usual methods of standardization (actual counting or opacity tests) some special procedures were utilized. Burgess,\textsuperscript{20} who formerly adjusted his plague vaccine to contain 3,000 million bacilli per ml (the standard previously adopted for the Lister Institute vaccine), afterwards used a standard of 1.5 mg of dried bacterial substance per ml and considered both these strengths as equivalent.

A more expedient method originally devised by Japanese workers was to weigh the sediment obtained by centrifuging the vaccine and then to dilute the latter so that each ml contained 6 mg of the sediment (Pollitzer \textsuperscript{147}). Sokhey\textsuperscript{181} used agar vaccines adjusted to contain 1,500 million plague bacilli per ml so as to give them the potency possessed by broth vaccine incubated at 27\textdegree C for four weeks. A lower standard (1,000 million) was adopted by Barreto,\textsuperscript{9} a higher for the Army vaccine (2,000 million per ml).

Comparing agar- and broth-grown plague vaccines, it has to be noted that the former can be manufactured more easily and expeditiously than the latter. This difference is of great importance in emergencies, when it will be possible quickly to prepare agar-grown vaccines from freshly-isolated local strains. In areas where plague occurs perennially, however, stocks of broth-grown vaccine, which in contrast to agar-grown vaccine possesses excellent keeping qualities, can be prepared beforehand.

Sokhey & Habbu,\textsuperscript{185} while considering the protective power of Haffkine vaccine grown for four weeks at 28\textdegree C roughly to equal that of agar vaccines obtained through cultivation at 37\textdegree C, admitted that the former was more toxic. It was on account of this drawback that new research work was started which led to the introduction of the casein-hydrolysate vaccine. As has been noted, this combines high potency with low toxicity.

(4) \textit{Pseudotuberculosis} vaccine. As a result of previous work by Rowland and others (see Pollitzer \textsuperscript{147}), a vaccine prepared from formalin-killed pseudotuberculosis bacilli (containing 4,000 million per ml) was introduced for human antiplague inoculation in Madagascar (Boye \textsuperscript{16, 17}). According to Boyé this product protected about 51\% of domestic mice against infection with a tenfold m.l.d. of plague bacilli.

(5) \textit{Lipo-vaccine}. A lipo-vaccine, also introduced by French workers, was prepared according to Boyé,\textsuperscript{16, 17} in the following manner:

Plague bacilli were grown for 36 hours at a temperature of 34\textdegree C on agar of pH 7.4, then washed off with saline containing 5 ml of formalin per litre. After storage at 37\textdegree C for ten days, 12.5 g of centrifuged and dried bacilli were suspended by a special process in an oily excipient.

Pons & Advier,\textsuperscript{149} challenging grey mice with 200 virulent \textit{P. pestis} ten days after prophylactic inoculation, found the lipo-vaccine to give a 70\%
survival as compared with 51.5% in animals protected with aqueous vaccine and 20% in the controls. Almost equally good results were obtained when both plague and pseudotuberculosis bacilli (6,000 million of each species per ml) were used for preparation of the lipo-vaccine.

(6) Sugar vaccine. As summarized by Pollitzer, Minervin et al. obtained remarkably good results when protecting sisels with heat-killed suspensions of avirulent plague bacilli in a saccharose solution. Korobkova et al. worked with a similar vaccine which they called AD vaccine (vaccin "adénature"). However, in a subsequent report, Korobkova recommended using virulent instead of avirulent plague bacilli for the preparation of this vaccine and also stressed the necessity of sterilizing it without the application of heat so as not to damage the heat-labile Vi antigen of *P. pestis* supposed by her to exist. Consequently, she added to a concentrated suspension of virulent plague bacilli grown at 37°C a double volume of 80% saccharose solution and let the mixture stand at room temperature for 20-25 days to effect sterilization.

Korobkova found that two injections of this vaccine protected 82-100% of white mice against a challenge infection with highly virulent plague bacilli and that protection was also afforded to 50-85% of guinea-pigs which had been given three vaccine doses. A sugar vaccine prepared with the avirulent EV strain was less potent. The addition of 0.05% agar to the sugar vaccines increased their protective power.

(7) Precipitated vaccines. The procedures adopted by Wayson et al. for the preparation of plague vaccines with the aid of precipitation methods were as follows:

(a) Alcohol-precipitated vaccine: Two volumes of 95% ethanol were added to the phenolized saline suspensions of virulent plague bacilli grown on blood tryptose beef-heart agar for 40 hours at 39°C, and the mixtures were centrifuged after having been held at 5°C overnight. The supernatant was discarded, and the sediment was washed in saline, centrifuged, and resuspended in saline and merthiolate (1/7,500) to make up the volume of the original phenolized saline suspension, the bacterial content of which had been standardized.

(b) Alcohol- and alum-precipitated vaccine: Two volumes of 95% ethanol were added to the phenolized saline suspension as above and held overnight at 5°C; 2.7 ml of a 10% sodium bicarbonate solution and 25 ml of a 4% potassium alum solution were added per 100 ml of volume, and the mixtures were allowed to stand at 5°C for five hours. The supernatant fluid was removed by centrifuging and the sediment resuspended in saline and held at 5°C for 40 hours. Final treatment was the same as in case of the alcohol-precipitated vaccine.

Results in guinea-pigs immunized with these vaccines and three weeks later either exposed from time to time during a month to the bites of infected
fleas or challenged by subcutaneous infection with a large dose of virulent plague bacilli were as follows:

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Flea-infected guinea-pigs</th>
<th>Subcutaneously infected guinea-pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tested</td>
<td>clinical</td>
</tr>
<tr>
<td>Alcohol-precipitated</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Alcohol-alum-precipitated (divided dose)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol-alum-precipitated (single dose)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>&quot;Army&quot;</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Typhoid</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

Wayson and his collaborators expressed the opinion that alcohol- and alum-precipitated vaccines were not superior to those obtained through alcohol precipitation alone.

Meyer obtained the following results when challenging guinea-pigs which had been immunized with ordinary or precipitated vaccines by various routes:

<table>
<thead>
<tr>
<th>Agent used for killing organisms</th>
<th>Adjuvant</th>
<th>Number infected</th>
<th>Survival number</th>
<th>Survival percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>None</td>
<td>29</td>
<td>5</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>Alum</td>
<td>30</td>
<td>13</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>&quot;Falba&quot;</td>
<td>28</td>
<td>16</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alcohol</td>
<td>None</td>
<td>30</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>Alum</td>
<td>30</td>
<td>17</td>
<td>56.7</td>
</tr>
<tr>
<td></td>
<td>&quot;Falba&quot;</td>
<td>30</td>
<td>24</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Formalin</td>
<td>None</td>
<td>40</td>
<td>10</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Alum</td>
<td>37</td>
<td>20</td>
<td>54.0</td>
</tr>
<tr>
<td></td>
<td>&quot;Falba&quot;</td>
<td>37</td>
<td>33</td>
<td>89.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* "Falba" is a mixture of oxycholesterin and lanolin
b Control animals

It will be noted that remarkably good results were obtained with formalin-killed and Falba-precipitated vaccines and to a slightly lesser degree also with those killed by alcohol and precipitated with the aid of Falba.

(8) Chemically-prepared extracts of *P. pestis*. As has been noted before (page 130), Kurauchi & Homma were able to immunize guinea-pigs, rats, and monkeys satisfactorily against plague infection with their "specific soluble substance", particularly if this had been absorbed by metallic salts.

Meyer et al. recorded the results (given in table XII) which they obtained with the various fractions of *P. pestis* separated by them with the aid of chemical procedures.
TABLE XII. RESULTS OF DOSING DIFFERENT ANIMALS WITH VARIOUS FRACTIONS OF PASTEURIELLA PESTIS

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Guinea-pigs</th>
<th>White rats</th>
<th>Cotton rats</th>
<th>Monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dosage (mg)</td>
<td>Result a</td>
<td>Dosage (%)</td>
<td>Result a</td>
</tr>
<tr>
<td>Fraction 1A</td>
<td>1.5</td>
<td>0/20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>&quot;</td>
<td>1.5</td>
<td>0/20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fraction 1B</td>
<td>1.5</td>
<td>0/19</td>
<td>3.07</td>
<td>14.19</td>
</tr>
<tr>
<td>&quot;</td>
<td>1.5</td>
<td>1/20</td>
<td>3.07</td>
<td>18.18</td>
</tr>
<tr>
<td>Insoluble residue</td>
<td>2.5</td>
<td>10/20</td>
<td>0.35</td>
<td>7.20</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.5</td>
<td>16/20</td>
<td>0.35</td>
<td>3.78</td>
</tr>
<tr>
<td>Insoluble residue</td>
<td>12.5</td>
<td>13/20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>&quot;</td>
<td>12.5</td>
<td>19/20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Formalin-killed suspension</td>
<td>1.5</td>
<td>2/10</td>
<td>0.35</td>
<td>17.19</td>
</tr>
<tr>
<td>&quot;</td>
<td>1.5</td>
<td>8/10</td>
<td>0.35</td>
<td>18/18</td>
</tr>
</tbody>
</table>

*a* The first figure in these columns indicates the number of survivors after challenge infection, the second the number of animals used.

It will be noted that the best results in guinea-pigs were obtained with alum-precipitated preparations of the water-insoluble residue but that a formalin-killed and alum-precipitated vaccine also proved quite satisfactory. In white rats, on the other hand, good success was obtained with fraction 1B as well as with formalin-killed suspensions of *P. pestis* while the insoluble residue gave indifferent results.

The vaccines obtained with the aid of bacteriophage will be dealt with later on.

**Live Avirulent Vaccines**

Although some of the pioneers, particularly Kolle & Otto,

95 had shown that experimental animals, including guinea-pigs, could be rendered immune to virulent plague infection through prophylactic administration of *P. pestis* strains, the virulence of which had been lost spontaneously or had been abolished by artificial means, Strong 19 in Manila seems to have been the first modern worker who took practical advantage of live avirulent plague bacilli to immunize 900 human beings. Since the disease was not present in Manila at the time, the actual value of the method could not be assessed, but it was significant that the serum of those inoculated was found to contain agglutinins and was also apt to protect experimental animals against challenge infection with *P. pestis*.
However, the history of inoculation with live avirulent plague vaccine may be said to have really begun in 1934 when Girard & Robic and Otten paved the way for the large-scale use of this prophylactic in Madagascar and Java respectively. The great success obtained in these two areas led to the introduction of this type of plague vaccination into other countries, such as Argentina (Savino), the Belgian Congo (Devgnat), Brazil (Goobar), French West Africa (Rotman), Tunisia (Magrou), and the Union of South Africa (Grasset).

Girard & Robic's EV strain

The properties of the strain described by Girard & Robic, and much used in Madagascar and elsewhere for human inoculation, are as follows:

The EV strain had become rather avirulent while being subjected for five years to monthly agar subculture at 16°-20°C, after that time proving innocuous to guinea-pigs and rabbits when administered percutaneously, subcutaneously, conjunctivally, or by feeding. Subcutaneous injection of guinea-pigs was apt to produce a local induration which disappeared in about a week without involving the regional lymph-nodes (Girard & Robic). Intraperitoneal injection of one-third of a slant, or more, of this strain was apt to produce in guinea-pigs or rabbits a fatal peritonitis terminating in septicaemia, but the bacilli isolated from the blood were avirulent. If lesser, though still considerable, doses were given intraperitoneally, and the animals were sacrificed from the 5th to the 15th day after injection, nodules were found to be present in the spleen, and more rarely in the liver, which did not contain the bacilli. This process was accompanied by spleen hypertrophy which disappeared about the 20th day after injection.

Girard & Robic laid great stress upon these reactions because they assumed that the antigenic potency of their strain was due to preservation of some of its virulence and toxicity. In a later publication, Girard & Radaody-Ralarosy also recorded that, in contrast to non-immunogenic avirulent strains, the EV strain possessed invasive powers, subcutaneous administration of a dose of 1,000 million leading to the appearance of the bacilli after 40 hours in the blood, towards the 4th day in the spleen, and somewhat later in the liver. The bacilli disappeared in the same order within two days after injection from the blood, and within 11 and 13 days, respectively, from the spleen and liver.

At the same time, however, prophylactic administration of the EV strain protected guinea-pigs against challenge with enormous doses of virulent plague bacilli by various routes, including the bite of infected fleas and intratracheal infection.

While R. rattus behaved in much the same way as guinea-pigs, mice and white rats, being toxin-sensitive, could not tolerate large doses of the EV strain. They could, however, be satisfactorily immunized with smaller doses.
Otten's avirulent strains

For his early work Otten used the Tjiwidej strain, named after the place where it had been isolated from a rat in 1929. This strain had lost its virulence spontaneously after having been kept in serum-agar stab cultures at 5°C for four months.

Subcutaneous administration of even 5 ml. of a broth culture of the Tjiwidej strain or of suspensions prepared from whole agar slants proved harmless for guinea-pigs and R. rattus diardii. At the same time, the strain even in single doses proved highly immunogenic for these rodent species which Otten considered most susceptible to plague.

While fresh suspensions of the Tjiwidej strain seemed to possess little toxicity if administered subcutaneously, rats inoculated intraperitoneally were apt to succumb to toxaemia, apparently because this mode of administration led to a rapid liberation of endotoxin. For the same reason, 35% of rats which had been injected subcutaneously with suspensions of the Tjiwidej strain heated to 60°C succumbed within 48 hours to toxaemia. Similar results were obtained by Anchezar when heating suspensions of the EV strain for half an hour at 58°C.

The Tjiwidej, like the EV strain, produced a nodule at the site of subcutaneous injection (Otten; Savino & Anchezar). Although, according to the latter authors, no macroscopically visible reaction was produced in the spleen, Girard & Robic could prove its presence through histological examination. The Tjiwidej strain possessed invasive powers, Otten finding live bacilli present for periods of up to one week at the site of subcutaneous injection as well as in the organs, particularly in the spleen.

As has been mentioned before, Otten experimenting with a number of other plague strains, was able to isolate avirulent variants through single-colony picking. He emphasized most appropriately that in this way alone it was possible to produce an irreversible loss of virulence.

One of the above-mentioned strains, which had been originally isolated during the 1920-1 pneumonic epidemic at Harbin, yielded an avirulent variant possessing a higher immunogenic power for guinea-pigs than the Tjiwidej strain, which in its turn protected rats particularly well. Consequently mixtures of these two strains were prepared for human inoculation but, as the Harbin strain was rather toxic, it had to be used in a smaller dosage. When inoculation work was resumed after the second World War, it was decided at the Netherlands meeting on tropical hygiene, 1948 (Nederlandsche Vereeniging voor tropische Geneeskunde) to dispense with this strain and use the Tjiwidej strain alone.

Comparing the latter with their own strain, Girard & Robic thought the Tjiwidej strain to be more attenuated. They agreed with Otten that it protected rats better than guinea-pigs, while the EV strain had reverse properties.
Strains used in the Union of South Africa

As stated by Grasset, the live plague vaccine at first used in the Union of South Africa was so prepared as to contain equal proportions of the EV and Tjiwidej strains with a total concentration of 1,000 million organisms per ml. Later, however, in place of the Tjiwidej strain an old strain of Rowland's, labelled K/120, was utilized in conjunction with the EV strain. The strain K/120 was highly immunogenic for *R. natalensis* as well as for guinea-pigs and rats, while the EV strain did not protect the latter or multimammatite mice quite so well if virulent South African strains were used for challenge infection (Grasset).

Strains used in other countries

While the EV strain alone was used in Argentina, the Belgian Congo, Brazil, French West Africa, and Tunisia, Korobkova established that guinea-pigs were immunized particularly well by a combination of this and her strain 46-S, obtained from a virulent plague growth through bacteriophage action. A single injection of a mixture of both strains or an initial inoculation with 46-S followed one week later by EV inoculation protected guinea-pigs against challenge infection eight months later. Only 78% of the animals inoculated with EV vaccine alone survived, however, if challenged after the same interval of time.

Views on immunity mechanism

Strong considered the process of immunization produced by the administration of live avirulent plague bacilli as a true vaccination, the organisms multiplying in the tissues and their successive generations stimulating the production of corresponding groups of antibodies (Otten). As has been stated before, in the opinion of Girard & Robic, the EV strain was immunogenic because it had not altogether lost its virulence and toxicity, possessing, in contrast to non-immunogenic avirulent strains, invasive powers. In fact, in a later communication Girard maintained that "the 'avirulent' and vaccinating strains are strains whose virulence is only weakened (and not attenuated which would imply that they are definitely fixed in that state)."

According to Girard, virulence had to be defined in relation to the animals, the mode of inoculation, and the dosage used. Thus 10,000 EV organisms, while innocuous if introduced subcutaneously or intraperitoneally, killed a guinea-pig when given intracerebrally. Girard postulated that the immunity engendered by inoculation with live avirulent plague strains was of a cellular rather than a humoral category, and maintained that this assumption was supported by the work of Pokrovskaya & Kaganova who claimed to have enhanced the defence mechanism of the lung against...
plague infection through the administration of live avirulent vaccines by
inhalation. As stated by Girard, the antigenic properties with which the
immunizing power of the avirulent strains was associated could become
degraded; the factors known to bring about this process were frequent
subcultivation at 37°C and temperature variations to which the strains
were subjected during transport or storage. Robic had shown that
the virulence of the strains could be restored to a certain degree in the
laboratory, but in the opinion of Girard this had never been observed
under natural conditions.

In connexion with this last-mentioned claim, attention should be drawn
to Devignat’s recent observation of a slight and temporary increase in
virulence of the EV strain he used for vaccine preparation. He states that:

(a) A guinea-pig inoculated subcutaneously with 2 ml of a batch of
EV vaccine died five days later with typical autopsy findings, yielding
cultures of the strain from its organs.

(b) The vaccine batches available at the time produced more marked
local and general reactions in man than previous lots. As noted before
(page 120), Devignat successfully used his method of bubbling air through
broth cultures of the strain to suppress this slight increase in virulence.

Otten, refuting the view that the immunizing action of live avirulent
plague strains was associated with their invasive powers, ascribed the
superiority of live over killed vaccines to differences in the antigenic pro-
properties which, while deteriorating during preparation of the latter vaccines,
remained intact in the former. Jawetz & Meyer also maintained that
the immunogenic activity of avirulent plague strains was a function of their
antigenic make-up, and not of their invasive or pathogenic power.

Finding a close correlation to exist between a high fraction IB content
of avirulent plague strains and their power in small doses of protecting mice
against challenge infection as well as of stimulating the formation of anti-
bodies in monkeys and man, Meyer et al. concluded that the immunogenic
activity of avirulent plague bacilli depended in part upon their antigenic
make-up. At the same time, however, these workers, sharing the views
of Strong, maintained that

"there is every reason to believe that the high degree of immunity is dependent upon
persistence of the antigenic agent in susceptible cells of the body, and not merely on
the intactness of the antigenic components".

Recent work by Walker et al. confirmed the view that avirulent
plague strains may engender immunity in two different ways. As sum-
marized by these observers:

"Those strains which are effective in stimulating development of immunity in mice
may be the ones that, even though incapable of extensive multiplication, are rich in
soluble envelope antigen or may be the ones that, although less productive of envelope
antigen, have the capacity to multiply and to attain numbers adequate to provide neces-
sary quantities of envelope antigen".
Preservation of antigenic potency

To preserve the antigenic potency of avirulent plague strains destined for vaccine manufacture, Girard stressed the necessity of keeping them constantly at a temperature of 2°-4°C and of subcultivating them not more frequently than once a year. He added that it had been possible in this way to keep the EV strain intact for 14 years.

Storage

As maintained by Otten and Girard, live avirulent plague vaccines cannot be stored for prolonged periods but must be used within a maximum of one month.

Otten noted in this connexion that, if kept at 5°C, his vaccine remained potent for several weeks. Even at room temperature (23°C) the potency decreased only slightly within this time but the number of bacteria diminished and simultaneously the toxicity of the vaccine increased. Tumansky even maintained that live avirulent plague vaccines did not deteriorate for 16 months if kept at -13°C.

Grasset, while in agreement with these views, stated that lyophilization of live avirulent plague vaccines considerably increased their keeping properties. Experimenting with multimammate mice, he found that lyophilized samples, even if kept in the refrigerator for two years, lost little of their potency. Cultures from these samples showed some delay in growth, but subcultures developed well.

Experiences recorded by Girard in 1939 and again in 1948 regarding the lyophilization of live avirulent vaccines were unsatisfactory. He found that lysis of the avirulent plague bacilli in normal saline occurred very slowly so that even after two years viable organisms could be found in suspensions with an original bacterial content of 1,000 million per ml. Nevertheless, Girard considered it essential to use the EV vaccine rapidly so as to administer a maximum of viable avirulent bacilli. However, this situation has now become altered in so far as, to judge from a report published in 1952, satisfactory laboratory experiences have been made with further samples of lyophilized EV vaccine prepared by Girard.

Comparative Potency of Killed and Live Avirulent Vaccines

The laboratory evidence available in regard to the comparative value of killed and live avirulent vaccines, with which alone we are concerned at the present juncture, has been the subject of much, and sometimes even acrimonious, debate.

To compare the results obtained by numerous investigators with either killed or live vaccines would be most difficult, not only because they worked with differently susceptible species or races of animals and challenged
them with differently sized infective doses, but mainly because they used methods of different accuracy to standardize the dose.

It would be expected that the results of comparative tests made by the same worker at the same time would be of great value for assessing the merits of killed and live vaccines respectively. Unfortunately, however, the results obtained by such comparative tests are quite often not really comparable: the workers interested in killed vaccines matched these against live avirulent preparations, the potency of which was not as high as that of the live vaccines actually used in areas like Java and Madagascar; while the advocates of live vaccination tested their products against killed ones less carefully manufactured and standardized than the vaccines now issued by, for instance, the Haffkine Institute.

For these reasons it seems advisable, instead of comparing the experiments made by individual workers, to weigh on the one hand the total evidence available in regard to the killed vaccines, and on the other that brought forward in favour of the live avirulent vaccines. If this is done, no doubt can exist in an impartial mind that, if carefully prepared and fully potent products are used in proper dosages and adequate challenge tests are made, equally good laboratory results can be obtained with both categories of plague vaccines. It follows that the question as to which of them should be used for man cannot be decided through experiments with laboratory animals but must be settled according to observations made in the prophylaxis of human plague—a problem which will receive full attention in a later chapter.

**Onset and Duration of Active Immunity to Plague**

Rowland \(^{158}\) established that immunity produced in white rats by plague nucleoprotein evolved rapidly, being distinctly evident after 24 hours and reaching its maximum on the third day. Stevenson \(^{191}\) (see also Stevenson & Kapadia \(^{195}\)), inoculating a large number of Madras rats with Haffkine's vaccine, found that immunity began to develop within a few hours and rose till the second or third day. On the other hand it is interesting to note that according to the German Plague Commission \(^{41}\) immunity in monkeys appeared much later, being slight on the fifth and reaching its maximum on the seventh day. As pointed out by Petric,\(^{138}\) these results were consistent with the observations of Brooks on the development of opsonins: in the white rat the maximum response occurred on the first or second day, and in man on the fifth or sixth day.

In the case of the live avirulent plague vaccines, immunity became apparent, according to Girard & Robic,\(^{64}\) five to ten days after inoculation; Otten,\(^{134}\) however, states that it appeared after five to seven days and became maximal after two to three weeks.
The statements made by a few observers that, in experimental animals as well as in man, plague inoculation was followed by a "negative phase" during which the inoculated individuals were particularly susceptible to the infection, will be dealt with in connexion with the problems of plague prophylaxis. As will be shown, fears of this kind are unwarranted.

Rowland found, in the course of the above-mentioned experiments, that the immunity conferred by his nucleoprotein was unimpaired at the end of three months and still appreciable at the end of five months. Dieudonné & Otto, dealing with this problem in a general manner, stated that no exact information existed as to the duration of the immunity produced in animals by inoculation with killed cultures, but took it to last for months; thus, according to Kolle, rats protected by a single injection still showed after five months a marked immunity, even when infected intraperitoneally.

These observations have been corroborated by experiments made with live avirulent plague vaccines. Otten stated that the immunity produced in guinea-pigs by administration of the Tjiwidej strain was still fairly satisfactory after six months but had markedly decreased after the ninth month. The EV vaccine was found to produce in these animals an immunity lasting for about a year or even longer (Girard).

PASSIVE IMMUNITY

Even since Yersin, Calmette & Borrel had demonstrated in 1895 that the serum of rabbits treated with killed \textit{P. pestis} cultures was apt to prevent or even cure infection in normal animals, numerous workers have endeavoured to produce immune sera suitable for the treatment and prophylaxis of human plague. The results of these labours will be appreciated in a later chapter devoted to the clinical aspects of the disease (see page 409). It is appropriate here, however, to describe the methods of producing the sera and of assessing their value, and to define their properties.

Production of Immune Sera

\textit{Animals used}

While horses were the usual, they were by no means the only, animals chosen for the production of plague immune sera, several workers preferring other smaller or larger domestic animals such as goats, sheep, calves, mules, bullocks, and buffaloes.

Attention was also paid to the possibility of using the rabbit which, as noted above, was the animal chosen by Yersin and his co-workers for their original experiments, and which was continually used to produce immune sera for laboratory needs. Naidu et al., though obtaining excellent experimental results with rabbit sera, considered the yield from this animal
insufficient for actual serum production. However, the use of rabbits for this purpose was again recommended by Korobkova and by Jawetz & Meyer. In view of the fully-satisfactory experimental results of the latter, a concentrated rabbit immune serum for the treatment of human plague was actually produced in the USA (Meyer).

Although the rabbit, in contrast to the horse, has the advantage, experimentally, of being susceptible to infection with *P. pestis*, in view of the great general suitability of the latter animal for serum manufacture it is not surprising to find that most workers continue to use it for the large-scale production of plague immune sera. Particularly noteworthy in this connexion is the new horse-produced plague serum of the Haffkine Institute, the efficacy of which is shown by the following comparative results obtained by Sokhey:

<table>
<thead>
<tr>
<th>Types of sera</th>
<th>Mouse-protection dose (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Haffkine</td>
<td>0.05</td>
</tr>
<tr>
<td>Buffalo (Naidu et al.)</td>
<td>0.30</td>
</tr>
<tr>
<td>Pasteur Institute</td>
<td>0.50</td>
</tr>
<tr>
<td>Lister Institute</td>
<td>0.50</td>
</tr>
<tr>
<td>Commercial product</td>
<td>No protection with 0.5</td>
</tr>
</tbody>
</table>

**Antigens used**

A whole series of antigens or combinations of different antigens has been used by the various workers bent on the production of potent plague immune sera.

Generally speaking, the pioneers in this field used either killed plague cultures alone, or first these and then live virulent bacilli; some, however, completed the process of immunization by administering also toxic filtrates of plague cultures or similar products to their animals. Some workers relied on the latter alone; for instance, nucleoproteins were used by Lustig and others, broth filtrates by Markl and Dean (Pollitzer). Recently, live avirulent plague bacilli were used in place of the above-mentioned antigens (Girard; Robic; Pirie & Grasset; Schütze; Savino & Anchezar; Jawetz & Meyer; Korobkova).

Using a combination of the above-mentioned antigens, Sokhey prepared the new Haffkine serum by immunizing horses first with living avirulent plague cultures, then with live virulent growths, and finally with filtrates of broth cultures which had been incubated for three weeks at 27°C.

The following special methods of immunization also deserve mention:

(a) Joukov-Verejnjkov et al. reported good results in guinea-pigs with sera obtained by immunization of horses with lysates of "envelope" cultures of *P. pestis* or with isolated "envelope" substances.

(b) Girard & Sandor found that the serum of horses which had been hyperimmunized with plague anatoxin (toxoid) gave experimental
results identical with those obtained using sera which had been prepared with the aid of live avirulent but toxic plague bacilli.

(c) Meyer\textsuperscript{117} obtained potent antiserum by the immunization of rabbits and monkeys with the crystalline fraction IB plague antigen.

\textit{Concentration of sera}

As reported by Russell\textsuperscript{159}, fractionation of the sera prepared by Naidu and his colleagues with different concentrations of sodium sulfate had shown that the protective power was vested in the globulin fractions and not in the albumin fraction. While it would seem that no large-scale practical advantage was taken of these findings, actual use has been made of concentrated plague immune sera introduced by Pirie & Grasset.\textsuperscript{141, 142} Their original procedure consisted of two fractional precipitations by means of sodium sulfate and isolation of the pseudoglobulin fraction which, in their opinion, contained the majority of the plague antibodies. However, they afterwards used a one-process method to obtain the "euglobulin-pseudoglobulin constituents" which gave more-satisfactory results.

Meyer,\textsuperscript{117, 118} experimenting with rabbit immune sera, found that the antibodies were mainly contained in the gamma-globulins which were consequently isolated for the preparation of concentrated serum.

\textbf{Methods for Assessing the Potency of Immune Sera}

Although, as summarized by Jawetz & Meyer,\textsuperscript{84} tests for measuring the potency of plague immune sera had been proposed by numerous investigators, most of the earlier methods were unsatisfactory because, at best, they permitted merely a rough evaluation of the results.

An accurate and fully-reproducible biological test introduced by Sokhey\textsuperscript{177, 181} was based upon the use of the highly and uniformly susceptible Haffkine-Institute-inbred white mouse and of the standard infective dose as used also for the evaluation of plague vaccines. The principle of the test was to determine the minimum of a given serum which would protect half the number of mice used against the standard infective dose given simultaneously. Details of the test were as follows (Sokhey\textsuperscript{181}) : for any given serum five graduated doses were decided upon, after a preliminary test, so that the 50\% end-point protective dose fell about the middle of the selected series. For each dose a batch of five mice was used. Both the serum and the standard test infective dose were given at the same time subcutaneously, but in different parts of the abdominal wall.

Any mouse dying during an observation period of 30 days was examined for evidence of plague infection by smear and cultural examination. At the end of the observation period all survivors were killed and likewise
examined. The same held good of the ten controls used for each test which, given the infective dose only, were usually dead by the ninth day.

To avoid inaccurate results, each determination was made twice. From the results of these two determinations the 50% end-point was calculated according to the method of Reed & Muench or another suitable method. A potent antiplague serum was required to have a minimum mouse-protective dose of not more than 0.05 ml.

The principle of a "curative test" introduced by Sokhey at the same time was to determine the minimum quantity of a given serum which would save a plague-infected mouse when serum administration was begun after the development of bacteraemia (which with the standard infective dose used became apparent within 72 hours). Essential features of the test were:

For any given serum five graded doses were decided upon so that the 50% end-point curative dose fell about the middle of the selected series. For each dose a batch of five or six mice was used. The animals were first given the standard test infective dose and 72 hours afterwards the serum in four equal portions, the first being administered intravenously and the remaining three subcutaneously at 24-hour intervals. This determination was done twice to avoid inaccurate results and the mice were observed as described above. On the results of the two determinations, the 50% end-point was calculated. A suitable plague serum was required to possess a minimum mouse curative dose of 0.4 ml or less.

Following up earlier work by MacConkey and others, Jawetz & Meyer proposed a mouse-protective test based upon the use of a standard serum. They defined as the provisional standard unit the amount of serum which would protect 50% of mice from death when 1,000 average lethal doses (a.l.d.)—about 2,000-5,000 plague bacilli—were injected intra-abdominally 60 minutes after administration of 0.5 ml of serum dilutions by the same route. The potency-determination of the unknown plague immune sera was carried out similarly to the assay of antipneumococcal and antipertussis sera.

It should be noted that Meyer & Foster utilized a similar mouse-protection test for the measurement of protective serum antibodies in human volunteers inoculated with plague prophylactics.

Together with their mouse-protective test, Jawetz & Meyer recommended a technique for a toxin-antitoxin neutralization test, using for this purpose the toxin prepared according to the method described earlier (see page 122). For performance of the test saline dilutions of the sera in quantities of 0.5 ml were injected intra-abdominally into white mice, followed 30 minutes later by 0.3 ml of the toxic filtrates diluted in saline solutions. For purposes of comparison an arbitrary scale was chosen, indicating the relative capacity of a given serum to protect mice against a standard dilution of the test batch of toxin. It was found most advantageous to test three twofold dilutions (1:4, 1:8, and 1:16) of serum (ten
mice each) against a filtrate dilution of 1:5 (approximately 10 a.l.d.). The mice were observed for 48 hours and the deaths recorded. The results were expressed as the number of mice surviving over the total number of mice used per serum.

**Properties of Immune Sera**

Schütze, Bhatnagar, and Gheltenkoff concluded from their serological studies that plague immune sera contained two kinds of antibodies, corresponding to the "envelope" and somatic antigens of *P. pestis*.

In order to study the antibody content of plague sera by immunochemical procedures, Girard & Sandor used a method of serum fractionation devised by Sandor, with the aid of which it was possible to distinguish between the bacterial antibodies contained mainly in the euglobulins, and the antitoxins present in the pseudoglobulins.

Investigating two sera, prepared with toxic live avirulent plague bacilli and with plague toxoid respectively, Girard & Sandor were able to separate three fractions, namely: (a) euglobulin I, which showed no antitoxic activity, but possessed antibacterial, agglutinating, and protecting properties; (b) euglobulin II, a comparatively inert lipo-protein fraction; and (c) pseudoglobulins, representing the antitoxin and endowed with but feeble precipitating, agglutinating, and protective properties.

Studying this matter further, Sandor et al. found that the sera obtained from horses, which had been immunized intravenously with either live plague bacilli or toxoid, contained large amounts of euglobulin I. However, in sera produced through subcutaneous administration of plague toxoid, this euglobulin was largely replaced by another fraction, called euglobulin IIA by the authors. In contrast to the above-mentioned sera, those produced by the subcutaneous route possessed no flocculating or agglutinating properties. While the protective action exerted by all the sera in mice experiments appeared to be vested in the pseudoglobulins as well as in the euglobulins, euglobulin I, if present in considerable quantities, was definitely more active than the pseudoglobulins.

The classification of plague immune sera has been the subject of much debate. As was mentioned on page 135, the serum of plague-immune animals is unable to destroy *P. pestis* in vitro or in vivo in the absence of phagocytic cells. It must likewise be noted that plague immune sera, even if obtained by administration of plague toxins (broth filtrates or the like) or by additional immunization with such toxins, possess only moderate antitoxic value. Petrie maintained in this connexion that, for no obvious reason, plague antitoxin in high concentration is not easily produced in horses. Gheltenkoff, however, believed that this was true of some of the horses only, while others produced sera of a satisfactory antitoxic titre. A careful selection of suitable animals was therefore essential.
Be this as it might, it is generally agreed that plague immune serum, fitting into neither the class of bactericidal nor that of antitoxic sera, belongs, like the anthrax and rinderpest sera, to the group of "anti-infectious" sera. Meyer, while stating that it is not exactly known to what the anti-infectious properties of plague immune serum are due, drew attention to the hypothesis of Petrie and of Jawetz & Meyer that it probably produces significant opsonization.

**SERODIAGNOSTIC METHODS**

**Agglutination**

*Preparation of agglutinating sera*

According to the usual procedure for the preparation of agglutinating sera, most plague workers used rabbits for this purpose and as a rule immunized them by the intravenous route.

Of the different antigens recommended by the various workers in this field, the following deserve mention:

To obtain an agglutinating serum Batchelder used a normal saline solution containing 0.25% formalin to wash off the growth of plague bacilli on hormone agar containing 0.025% of 10% sodium sulfate. The resulting suspension, which became sterile when kept for seven to eight hours at room temperature, was well tolerated by rabbits even if large doses were given intravenously and produced, when administered twice, a serum with a titre ranging from 1:1280 to 1:2560.

To produce sera against virulent *P. pestis* and also against pseudo-tuberculosis bacilli for his serological studies, Bhatnagar resorted to the use of silver nitrate as employed by Rainsford for the manufacture of TAB vaccine.

As noted already, Baker et al. obtained immune sera with good agglutinating properties when administering their fraction IA and IB antigens to rabbits. The use of a serum produced with the aid of the water-extractable protein of *P. pestis* was also recommended by Seal, who stated that "it may be possible to prepare this antiserum directly with the water-extractable protein solution of the virulent plague strain freed from bacterial debris by filtration, i.e. without preparing it at one-third saturation with sodium-sulphate".

When preparing agglutinating sera with live plague bacilli, most workers preferred strains which had lost their virulence spontaneously or which had been rendered avirulent artificially. It would seem, however, that Wats et al. used virulent strains to complete the immunization of rabbits which had been previously inoculated subcutaneously and then intravenously with graduated doses of plague cultures heat-killed by exposure to 55°C for 30 minutes. Greval & Dalal reported satisfactory results
when making agglutination tests with the therapeutic sera produced in the Haffkine Institute with virulent plague cultures.

**Preparation of suspensions**

The main difficulty in carrying out agglutination tests with plague bacilli is to obtain suitably uniform suspensions of the organisms. Though numerous procedures have been devised to overcome this impasse (see summary by Pollitzer, 147 and also Ciantini 26), Wats et al., 202 again studying this problem, found none of them fully satisfactory and therefore recommended the following method:

Roux bottles, containing meat-digest agar (pH 6.8) were sown with a thick suspension of plague bacilli and incubated for four days at 27°C or 37°C according to the nature of the agglutinable antigen wanted. The growth was then washed off with 20–30 ml of distilled water containing 1% carbolic acid. The washings were poured into a sterile test-tube containing glass beads and kept at room temperature (27°–29°C) for six hours with an initial shaking by hand for five minutes and an occasional shaking during the first two hours. The supernatant homogeneous layer was then pipetted off and left in the refrigerator overnight, so as to allow coarse particles to settle down. The stable portion obtained from the suspension was centrifuged and after the deposit had been washed in saline twice, it was re-suspended in normal saline containing 0.25% carbolic acid; 0.05% formalin was added if the suspension was produced in bulk for stock purposes.

It would seem that the simpler procedures recommended by Bhatnagar 13 and Seal 167 are not as reliable as the method described above.

As stated previously (see page 88), Devignat 30 had found that bubbling of air through broth cultures produced a rapid and homogeneous growth of *P. pestis*. Recently studying the problem of agglutination, 32 he used suspensions obtained with the aid of this method, stabilizing them by adding one drop of formalin per 20 ml of the growths.

**Rapid agglutination tests**

In addition to tube tests carried out in the classical manner, several workers recommended slide agglutination tests for the rapid diagnosis of human plague. Thus Panja & Gupta 135, 136 used the sera of patients in dilutions of 1:3 or 1:4. A mixture of plague bacilli obtained from a few different agar or blood-agar cultures incubated for one to two days at 37°C was distributed into drops of the serum dilutions and the results were read after one minute. Positive results were obtained in 15 out of 17 cases with a positive blood culture, usually on the seventh day after onset of the disease, sometimes earlier. The two negative cases had been tested on the third and fourth day respectively. In 11 bacteriologically negative cases, agglutination proved positive within 4 to 14 days after onset; 75 normal sera yielded negative results.
It should also be noted that recently Tumansky recommended Noble’s rapid method of tube agglutination (see Pollitzer) for the identification of plague cultures and for testing the sera of plague-suspect rodents with the aid of known strains of *P. pestis*.

A method recommended by Menezes for the diagnosis of rat plague likewise deserves mention: 250 mg of the spleen or liver of the suspect rats were suspended in 20 ml of normal saline and mixed with equal amounts of 1:10, 1:20, and 1:40 dilutions of immune sera raised against 37°C growths of *P. pestis*. The tubes were then incubated in a water-bath at 40°C and readings were taken after two to three hours. A positive reaction was manifested by a heavy deposit consisting of agglutinated plague bacilli.

Menezes admitted that the test was negative when plague bacilli were scanty in the organs of the rats, but stressed on the other hand that plague livers or spleens which had become putrid or had been kept for a month or even longer in a desiccated state or in glycerol, still gave a positive result.

**Types of agglutination**

While the existence of serologically-different races of *P. pestis* has been unanimously denied, attention has been drawn to the occurrence of two distinct types of agglutination. Wats et al. maintained that these differences depended “on the agglutinable antigen and not on the type of antisera employed”. Bhatnagar, on the other hand, ascribed them to the presence of two antibodies in plague immune sera.

Carrying out agglutination tests with plague bacilli cultivated at room temperature (27°-29°C) and at 37°C respectively, Wats and his co-workers obtained different results, as follows:

<table>
<thead>
<tr>
<th>Room-temperature growths</th>
<th>37°C growths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglutination slow</td>
<td>Agglutination rapid</td>
</tr>
<tr>
<td>Flakes small and uniform</td>
<td>Flakes larger and of varying size</td>
</tr>
<tr>
<td>Sediment compact</td>
<td>Sediment voluminous</td>
</tr>
<tr>
<td>Sediment not easily dislodged, small flakes seen in a clear fluid on shaking; clumps take time to reform and settle.</td>
<td>Sediment easily dislodged, uniformly distributed on shaking; clumps reform quickly (10 minutes) and settle in large masses.</td>
</tr>
</tbody>
</table>

Wats and his colleagues made the following interesting findings when carrying out comparative absorption tests:

(a) 37°C growths were capable of absorbing all agglutinins from the sera obtained by immunizing animals with either 37°C or room-temperature growths.

(b) Room-temperature growths, on the contrary, were capable of removing all antibodies only from sera raised against them and not from sera prepared with the aid of 37°C growths.

(c) 37°C growths, when heated at 100°C for one hour, reacted more or less like room-temperature growths.
Obviously, therefore, the 37°C growth of \textit{P. pestis} possessed an additional antigen which was destroyed by heat and was absent in cultures incubated at room temperature.

Bhatnagar,\textsuperscript{13} studying the agglutination of plague bacilli possessing an "envelope" and of plague and pseudotuberculosis strains which were devoid of an "envelope", with the aid of sera raised against virulent, avirulent immunogenic, and avirulent non-immunogenic plague strains, also noted the presence of two types of agglutination, as follows:

<table>
<thead>
<tr>
<th>&quot;Envelope&quot; agglutination</th>
<th>Somatic agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forms slowly</td>
<td>Forms slowly</td>
</tr>
<tr>
<td>Settles slowly</td>
<td>Settles slowly</td>
</tr>
<tr>
<td>Supernatant remains clear</td>
<td>Supernatant remains clear</td>
</tr>
<tr>
<td>Sediment voluminous, flakes large, woolly in character and varying in size</td>
<td>Sediment scanty, flakes small, uniform and gritty in character</td>
</tr>
<tr>
<td>In lower dilutions sediment easily dislodged and producing a shimmer in the serum-suspension mixture: in higher dilutions definite woolly particles visible.</td>
<td>Sediment easily dislodged and becoming similar to saline control on shaking in all the dilutions of serum.</td>
</tr>
</tbody>
</table>

It will be noted that there was a great deal of resemblance between Bhatnagar's somatic agglutination and that of room-temperature growths observed by Wats and co-workers on the one hand, and between the "envelope" agglutination and that of 37°C growths on the other.

\textit{Specificity of agglutination tests}

Summarizing the evidence available up to 1936, Pollitzer\textsuperscript{147} reached the conclusion that agglutination tests with plague and pseudotuberculosis immune sera as practised up to then formed no exact means of differentiating between these two bacterial species. He added, however, "that by a judicious application of the newer knowledge upon the antigenic structure of these two germs a satisfactory method might be evolved".

Bhatnagar\textsuperscript{13} reporting in 1940 on investigations of this problem, stated that (a) pseudotuberculosis immune sera did not react with plague bacilli, and (b) pure "envelope" sera, obtained through absorption of plague immune sera with \textit{P. pseudotuberculosis} strains, no longer agglutinated pseudotuberculosis bacilli. However, as will be gathered from table XIII, which summarizes observations recently recorded by Seal,\textsuperscript{169} this worker was not in full agreement with Bhatnagar's first claim.

To judge from these findings, a serological distinction between \textit{P. pseudotuberculosis} and virulent or avirulent protective plague strains can be made with most of the above-mentioned methods of examination (numbers 2 to 6), but only a pseudotuberculosis serum absorbed with \textit{P. pestis} is satisfactory for a distinction between pseudotuberculosis bacilli and avirulent protective plague strains.
TABLE XIII. SEROLOGICAL RELATIONSHIP BETWEEN P. PESTIS AND P. PSEUDOTUBERCULOSIS *

<table>
<thead>
<tr>
<th>Sera produced with</th>
<th>P. pestis</th>
<th>P. pseudotuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virulent and avirulent protective</td>
<td>Avirulent non-protective</td>
</tr>
<tr>
<td>(1) Virulent <em>P. pestis</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(2) Virulent <em>P. pestis</em> and absorbed with <em>P. pseudotuberculosis</em></td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>(3) <em>P. pestis</em> boiled for 1½ hour</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>(4) <em>P. pseudotuberculosis</em></td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>(5) Water-extractable protein of <em>P. pestis</em></td>
<td>+</td>
<td>c</td>
</tr>
<tr>
<td>(6) <em>P. pseudotuberculosis</em> and absorbed with <em>P. pestis</em> or <em>P. pestis</em> boiled</td>
<td>0</td>
<td>c</td>
</tr>
</tbody>
</table>

* According to Seal. 109

rulent non-protective plague strains as well. In actual practice one is not likely, however, to meet with the latter strains.

Vargues recently recorded that the plague hyper-immune serum prepared in the Pasteur Institute at Paris had the property of agglutinating suspensions of *Salmonella, Brucella* and *Proteus* in the cold. However, the agglutination titre was not higher than 1,320, so that this phenomenon could be easily distinguished from the agglutination of *P. pestis*, taking place at high titre at 37°C.

In the opinion of Vargues, the presence of euglobulin I in the immune serum was responsible for this unspecific agglutination in the cold. He stated in this connexion that, while the euglobulin I is easily soluble in normal saline at ordinary temperatures, it precipitates after the solutions are cooled to +4°C. The euglobulin is then apt to affix itself to inert corpuscles such as bacteria or horse erythrocytes, and to agglomerate them.

Scope of agglutination tests

Advantage may be taken of agglutination to test unknown strains with a plague serum of established potency and to examine the sera of suspects with the aid of known cultures. As has been noted, Panja & Gupta 135, 136 obtained good results with rapid slide tests and the value of agglutination for the diagnosis or retrospective diagnosis of human plague has also been endorsed by other recent observers, particularly by Favarel. 37, 38 This worker came to the opinion that, except in the case of recently inoculated individuals, agglutination even at low titres was
significant. Agglutinins appeared as a rule about the seventh day of illness. Two persons who had been cured showed negative reactions after nine and ten months respectively.

It is particularly noteworthy that, as recorded by Favarel and also by Huang et al. and Greval, treatment with sulfonamides or streptomycin, though apt to lead to negative bacteriological results, did not adversely affect those of agglutination tests.

**Haemagglutination**

Keogh et al. and some other workers recently recommended an indirect method of agglutination, based upon the observation that some micro-organisms have a component which can be adsorbed to erythrocytes and that erythrocytes thus sensitized are agglutinated by immune sera specific to the micro-organisms in question.

The possibility of using this method of haemagglutination in the case of the plague bacillus has been studied by Amies and by Chen.

Amies thus described the technique he used for this test:

"Both rabbit and horse anti-plague sera were employed, and the red cells were obtained from human, sheep and guinea-pig blood. The standard procedure was to use sheep cells. The preliminary absorption [to remove anti-species antibodies potentially detrimental to the erythrocytes from the sera] was carried out by adding one volume of packed cells to 10 volumes of inactivated serum and centrifuging one hour later. A 0.1 per cent solution of the purified envelope antigen was added to another portion of the erythrocytes in the proportion of one volume of packed cells to 10 volumes of antigen. After one hour’s contact at room temperature the cells were collected by centrifugation and washed 3 times in physiological saline. The agglutination test was then performed by adding 0.1 ml. of the packed sensitized cells to 1.0 ml. of serum in a dilution series from 1 in 10 to 1 in 10,000 or higher. The tubes were held in a water-bath at 37° for one hour and then placed overnight in the refrigerator, the results being read on the following morning."

In the opinion of Amies the haemagglutination test was unlikely to be of much value as a diagnostic procedure because of the slow development of immunity in plague, but was possibly useful for confirming retrospectively a diagnosis made on clinical grounds only besides being of unquestionable value for following the development of antibodies in animals undergoing immunization.

Chen established the interesting fact that the antigen responsible for haemagglutination, though present in old broth cultures of *P. pestis* as well as in extracts of killed and dried plague bacilli and in the alcohol-precipitated ‘envelope’ antigens, was absent in the protein fractions obtained with the aid of ammonium sulfate. The evidence accumulated by Chen indicated that this antigen, which was probably of a polysaccharide nature, was of no importance as a protective agent in plague infection.
Precipitation

While the opinions held by earlier workers (see Pollitzer\textsuperscript{147} for summary) regarding the value of precipitin tests for the diagnosis and differential diagnosis of plague had been divergent, this method was recently recommended by Cambosu\textsuperscript{22} and by Larson et al. (see chapter 5, p. 243) for the rapid recognition of rat plague.

The precipitation test with protein fractions of plague and pseudotuberculosis bacilli devised by Seal\textsuperscript{170} may be said to be of theoretical rather than of practical interest, the more so because the reactions obtained with the aid of this method closely tallied with those produced by agglutination tests.

Flocculation

Girard\textsuperscript{54} established that, especially in the case of patients with suppurating buboes of long standing, good diagnostic results could be obtained by mixing five drops of the patient's serum with 1 ml of plague endotoxin (filtrates of broth cultures or preferably extracts obtained by repeated freezing and thawing); flocculation took place in positive cases. Readings were taken after 30 minutes, 3 hours, and 24 hours.

At the same time Girard was unable to confirm the claim of Gheltenkoff\textsuperscript{46} that flocculation tests were useful for standardizing plague immune sera.

Haemolysins

The evidence available on the reactions produced by \textit{P. pestis} in blood-containing media is not only somewhat scanty but most contradictory.

Korobkova\textsuperscript{98} stated in this connexion that both plague and pseudotuberculosis bacilli were endowed with haemolytic properties, a clear zone appearing within 48-72 hours round colonies on agar plates which contained 5\% of defibrinated rabbit, guinea-pig, or horse blood. The same phenomenon was observed on plates containing sheep blood but, in contrast to the above-mentioned media, the sheep erythrocytes remained intact. In liquid media containing 2\% of blood, haemolysis became complete in five days.

As noted by Korobkova, filtrates of 12-day-old plague cultures caused feeble lysis of sheep erythrocytes. The haemolytic filtrates caused the death of white mice but not of rabbits. The haemolysins present in plague cultures were not neutralized by plague immune or antihaemolytic sera and possessed no immunogenic properties.

In contrast to the opinion of Bielonowski,\textsuperscript{15} Korobkova maintained that there was no significant relation between the haemolytic power and the virulence of plague strains. She found, however, that smooth avirulent plague strains produced haemolysis more slowly than virulent growths,
while rough avirulent strains reacted like *P. pseudotuberculosis*, lysing rabbit, guinea-pig, and horse erythrocytes even more rapidly than virulent plague strains.

In marked variance with these statements, Colichon maintained that pseudotuberculosis strains as well as the pasteurellae sensu stricto failed to produce haemolysis on blood-agar plates. Although admixture of human blood was most suitable to show up this difference between *P. pestis* and the above-mentioned micro-organisms, Colichon stated that it was permissible to use instead horse, rabbit, guinea-pig or grey-rat blood.

Wagle & Habbu (unpublished observations), cultivating five laboratory strains of *P. pestis* and two pseudotuberculosis strains at 28°C and 37°C respectively on blood-agar slopes prepared with human, guinea-pig, horse, and rabbit blood, were unable to confirm the findings of Colichon, haemolysis being absent in the case of both micro-organisms. Repeated passage of the plague strains through susceptible experimental animals (*Gunomys kok*) did not alter their behaviour on the blood-agar slopes.

The recommendation made by the WHO Expert Committee on Plague at its second session (1952) that this matter should be further studied deserves all the more attention because, as maintained by a few observers such as Stephan and Seal, *P. pestis* is capable of producing haemodigestion on blood-agar plates.

**Complement Fixation**

Summarizing the literature available up to 1936, Pollitzer stated that the use of complement-fixation tests for the purposes of plague laboratory work had been recommended by several investigators.

Thus Damperoff tested plague immune sera with the aid of this method, using either bacillary suspensions or extracts as antigens. He found fairly constant and reliable results but could not establish a correlation between the complement-fixing titre and the curative value of the sera in question.

Moses found in 25 out of 38 plague patients complement-fixing antibodies and twice demonstrated plague antigens; in most of these instances the blood had been taken on the fifth day of illness. In the experience of Shchastny, however, antibodies were present not earlier than in the second week.

The method of complement fixation for the diagnosis of human plague was again recommended by Joltrain and Simard. The former reported positive results in clinically uncertain cases, in some of which agglutination tests had been negative. No false positives were seen. Dickie confirmed the usefulness of the method but thought it of greater positive than negative value.
Grysez & Wagon (quoted by Dieudonné & Otto 34) testing old plague-infected tissues, stated that complement was specifically deviated even when there was advanced putrefaction. However, Piras 139 could not confirm this claim for he found the reaction to be positive up to the sixth day only and therefore considered it inferior to animal experiments.

The usefulness of complement-fixation tests for the diagnosis of human plague was again noted by Joltrain 87 in 1936—particularly in the case of convalescents or of patients with pestis minor.

Mitin, 123 trying out 40 different antigens, found it most suitable to suspend plague bacilli grown on agar in distilled water and to shake the suspension for two to four days in the dark. After centrifugation, the supernatant fluid was pipetted off and phenol was added so as to obtain a concentration of 0.5%.

Using his 40 antigens to make complement-fixation tests with the sera of immunized and non-immunized animals as well as with those of inoculated and non-inoculated human beings, Mitin obtained satisfactory results. He reached the conclusion that the method could not be used to diagnose sise plague because the sera of these rodents had anticomplementary properties. However, Kuznetsova & Dobrokhtova 102 claimed afterwards that the reaction was useful to assess the incidence of plague among the sises.

A new procedure for complement fixation tests with plague fraction I as standard antigen and specific high-titre plague immune serum, preferably such produced with fraction I, as standard antigen, was described by Chen et al. 24 who gave the following examples for the usefulness of this method:

(a) Determination of fraction I plague antigen in the bacilli or in tissue extracts of animals succumbed to experimental plague;

(b) Detection of antibodies to fraction I in the sera of human convalescents and immunized animals.

Chen and his co-workers maintained that besides being of value for the retrospective diagnosis of human plague, their method of complement fixation was apt to be of importance for the field diagnosis of wild rodent plague, specially “when the isolation of P. pestis or the interpretation of the pathological lesions at autopsy is rendered impossible by contamination or decomposition”.

The question as to whether complement-fixation tests are useful for differentiation between plague and pseudotuberculosis bacilli has been answered in different ways by different observers. To judge from the investigations of Zlatogorov & Mogilevskaya as well as from those of Boquet & Dujardin-Beaumetz (quoted by Pollitzer 147), complement-fixation tests were of value in distinguishing between plague and pseudotuberculosis strains; it should also be noted that Damperoff 26 obtained
negative results when making such tests with pseudotuberculosis bacilli and plague sera. However, Shchastny, Mitin, and recently Haas found that pseudotuberculosis bacilli merely gave less marked reactions than *P. pestis* when tested with plague sera, and the extensive studies of Greval & Dalal led to the same result.

As far as the diagnosis of human infections is concerned, it is of no vital importance that complement-fixation tests are unreliable, or at least not fully reliable, for differentiating between plague and pseudotuberculosis, because cases of the latter disease in man are rare, invariably solitary, and usually show peculiar clinical features distinct from those of plague.

**BACTERIOPHAGE INVESTIGATIONS**

As summarized by Harvey in a comprehensive survey of the bacteriophage problem with particular reference to plague and cholera, and confirmed by further investigations, in the case of plague, phages have been isolated from rather varied sources, such as rat faeces and the stools of convalescents; rat lymph-nodes and serum; buboes or blood from plague patients or convalescents; sewage; and canal water. It is of special interest that positive findings were not invariably restricted to localities where the infection was present.

Thus Girard detected an active lytic principle to *P. pestis* in rats from a former plague focus in Antananarivo, Madagascar, and postulated that a causal connexion might exist between the presence of phages in the rodents and the disappearance of the infection.

Lépine & Bilfinger, examining rats at Athens at a time when an enzootic seemed to be absent, found the serum of two out of 217 animals to be lytic for *P. pestis*.

Flu & Flu repeatedly isolated from sewage and canal water at Leyden, Netherlands, phages which were active against plague and pseudotuberculosis bacilli as well as against *Escherichia coli* and shigellae. Flu & Flu assumed that the phage strains had originated from rat intestines where they had been adapted to *E. coli*.

Although, as in the case of other infectious diseases, far-reaching claims have been made regarding the role played by bacteriophages in the development of a natural immunity against plague, it seems altogether unlikely that the influence they might exert in this direction is of general importance. Discussing this problem, Harvey stressed that Avari in India had been unable to find phages active against *P. pestis* in either Bombay or Madras rats.

The question of to what extent the action exerted by bacteriophages on plague growths is specific in nature and how far, consequently, observations made with the aid of these lytic agents are of differential-diagnostic importance, has been the subject of much discussion.
The early workers, though sometimes finding that the action of their phages was restricted to those plague strains to which they were adapted, admitted the existence of polyvalent strains possessing lytic powers for all plague growths. However, they were unanimously of the opinion that the action of plague bacteriophages was specific as far as other bacterial species, in particular *P. pseudotuberculosis*, were concerned. Advier established in this connexion that an agar surface treated with a plague phage ceased to be suitable for the growth of *P. pestis* but continued to be favourable for the development of other micro-organisms, and recommended this method for the purposes of differential diagnosis.

Bezsonova et al., working with two bacteriophages of wild-rodent origin, found them to lyse all 214 plague strains tested but to exert no action on 21 pseudotuberculosis strains. They considered, therefore, the use of suitable plague phages as a subsidiary means for the differentiation of the two organisms.

Girard noted that plague phages were able to lyse pseudo-tuberculosis strains to the same titre as pseudotuberculosis phages and later also confirmed the observation of Flu & Flu that both these phages were active against dysentery bacilli, while vice versa certain shigella phages lysed plague and pseudotuberculosis bacilli. Nevertheless he maintained the differential-diagnostic value of bacteriophage examinations made with
the aid of broth media, pointing out that the different appearances of
growth shown by plague and dysentery bacilli in the control tubes were
so striking as to preclude diagnostic errors.

In a paper published in 1947, Lazarus & Gunnison reached the con­
clusion that bacteriophage tests did not constitute a means of clearly
distinguishing between plague and pseudotuberculosis bacilli. Further
studies by Gunnison et al. showed, however, that it was possible to make
such a distinction with the aid of bacteriophage tests carried out at 20°C.
The technique used by Gunnison and his co-workers was as follows:

Advier’s phage strain was adapted on the one hand to an avirulent
plague strain (P phage), and on the other to a pseudotuberculosis strain
(PTB phage). The plague strains to be tested were first grown for 18-24
hours at a temperature of 18°-20°C in broth. Dry agar plates were then
implanted with the growths, each of which was spread over a circular
area, 2 cm in diameter, so that one dish could accommodate ten different
growths. After these had become dry, they were touched with a suspension
of one of the two phages. The plates were then incubated in an inverted
position for 48 hours at 20°C and 37°C respectively. Before carrying out
the tests proper, each phage was titrated against the culture to which it
had become adapted by testing serial tenfold dilutions of the phage in
question in the manner described above. The plates used for the titration
of P phage were held at room temperature, those of PTB phage were
incubated at 37°C. The highest dilutions giving confluent lysis were recorded
and for the final tests ten times this amount, the "critical test dilution",
was used.

The results obtained with 45 pseudotuberculosis strains and 52 plague
strains (35 of which were virulent) were as follows:

<table>
<thead>
<tr>
<th>Type of phage</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTB phage</td>
<td>Of no diagnostic value, lysing most plague cultures at 20°C or 37°C and not lysing most of the pseudo-tuberculosis cultures at 20°C.</td>
</tr>
<tr>
<td>P phage at 37°C</td>
<td>Lysed many pseudotuberculosis strains and failed to lyse a few plague strains.</td>
</tr>
<tr>
<td>P phage at 20°C</td>
<td>Showed a specific action for plague strains.</td>
</tr>
</tbody>
</table>

Attempts to cure plague-infected experimental animals with specific
phages gave negative or even most disappointing results as a rule, the
reated animals often succumbing more quickly to plague than the con­
trols. As convincingly shown by Pons in the case of guinea-pigs, this
phenomenon was due to the liberation of plague endotoxin by the bacteri­
ophage, which thus became a pathogenic instead of a therapeutic factor.

Joukov-Verejnikov & Favarissova, administering bacteriophage simulta­
aneously with plague infection to guinea-pigs, found that the animals so
treated died at the same time as the controls. The two workers maintained that the bacteriophage and the plague bacilli could co-exist in the animal body without influencing each other and, noting that in vitro addition of normal serum to a mixture of these two agents prevented lysis, expressed the opinion that the absence of phage action in vivo might have been due to phenomena of a colloidal nature.

Though occasional successes have been recorded, the results obtained when treating plague patients with specific phages were as a rule as disappointing as those in experimental animals. Moreover, this mode of plague treatment has become altogether obsolete in view of the availability of new therapeutic substances, the action of which is by far more constant as well as most markedly more effective.

Experimenting with different animals (grey and white mice, white rats, and guinea-pigs), Advier found that injection of a mixture of plague bacilli and bacteriophage, if tolerated by the animals, produced a solid immunity against subsequent plague infection. Compton, who had obtained identical results, maintained that the immunity thus produced did not result directly from the action of the bacteriophages but was due to the liberation of immunogenic substances from the bacilli.

To test this hypothesis, Compton used phage preparations treated with formaldehyde. The filtrate obtained by passing the lysates through Chamberland candles was incubated for five days to ensure sterility. Then formalin was added in the proportion of 0.4% (0.16% formaldehyde) and the mixture was heated in a vaccine bath at 60°C for one hour. It was then kept in the incubator for 18 days before use.

Compton claimed that better protection in experimental animals resulted from subcutaneous administration of this specific phage-lysed vaccine than from three inoculations with untreated bacteriophage.

Flu, who dealt in numerous publications with the same problem, described in 1933 the preparation of lysate as follows: A suspension of a 24-hour-old broth culture of virulent plague bacilli containing 5,000 million organisms per ml was mixed with 2% active phage and incubated at 37°C for 36 hours. The partially-lysed suspension was filtered through cotton-wool, shaken up with an excess of chloroform, and again left for 12 hours at 37°C. The fluid was then carefully decanted from the chloroform, carbolized to 0.5%, allowed to stand for five days at room temperature, and then tested for sterility. Before use the fluid, which was not quite clear, had to be shaken.

Flu stated that even one injection with the vaccine lysate protected 66% of white rats against infection with 10,000 m.i.d. of plague bacilli, whereas three inoculations protected 91% of the animals. He emphasized the necessity of using concentrated suspensions of virulent bacilli for preparation of the lysates, alleging that the failures reported by other investigators were due to the fact that they had worked with products poor in dissolved bacterial substances though rich in phages. Flu’s lysate remained
potent when kept for four months at room temperature. Lazarus & Gunnison confirmed that phage lysates of *P. pestis* were effective in protecting mice against plague infection, adding that the use of such preparations “offers a number of interesting possibilities for human vaccination”.

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Chapter 4

PATHOLOGY

BUBONIC PLAGUE IN EXPERIMENTAL ANIMALS

Guinea-pigs

Methods of infection

The highly susceptible guinea-pig can be easily infected with plague by different routes; indeed, as first shown by Albrecht & Ghon,\(^1\) mere rubbing in of infectious material into the shaven, depilated, or even quite intact skin is ordinarily sufficient to produce a fatal infection.

A few workers, such as Wayson & McMahon\(^{11}\) preferred intracutaneous administration of plague material to the above-described procedure of cutaneous infection.

Subcutaneous infection of guinea-pigs was effected not only in the usual manner with the aid of a syringe, but also by introducing a needle dipped into the infectious material under the skin or by putting the material to be examined into a skin-pouch (Hauttasche) (Maassen\(^{65}\)).

Two other procedures of practical importance are intraperitoneal and intra-ocular infection, the latter being performed either through instillation of the infective material into the conjunctival sac or, according to Cornil et al.,\(^{20,21}\) through subconjunctival injection.

In place of these artificial procedures, some workers used infected fleas to produce plague in their test animals.

Choice of methods

A choice between the different methods enumerated above depends upon the nature of the experimental work to be performed. It is obvious that only those among them which permit of the introduction of exactly measured doses with the aid of a syringe will be of real value for standardization tests. Subcutaneous infection is the procedure usually resorted to for work of this kind.
For diagnostic purposes Albrecht & Ghon’s method of cutaneous infection is of outstanding value because, as established by these two workers, positive results may be obtained with its aid when dealing with material so contaminated that cultures fail altogether. Other routes of infection in guinea-pigs are far less useful in such circumstances because the concomitant bacteria may rapidly kill the animals before plague infection develops or may lead to a mixed infection so that, as in the case of the original material, it may be impossible to demonstrate and isolate the plague bacillus.

It must be realized, on the other hand, that the method of cutaneous inoculation, which does not usually cause the death of the experimental animals until after 4-5 days, is less expedient than infection by the subcutaneous and particularly by the intraperitoneal route. However, since buboes become apparent in cutaneously infected guinea-pigs within 24-48 hours after inoculation, an early diagnosis may be made by using the fluid obtained by puncturing these incipient buboes with a syringe for bacteriological examination and intraperitoneal infection of rats (Martini 74).

A further point deserving attention is that, contrary to beliefs sometimes expressed, cutaneous inoculation of guinea-pigs, though serving as a barrier against most bacteria other than Pasteurella pestis, is apt to fail in case of the species most resembling it. Thus, as recently established by Haas,46 a pseudotuberculosis strain isolated from a rat could be as easily passed through guinea-pigs by the cutaneous as by the subcutaneous route. Also, although some workers considered cutaneous inoculation of guinea-pigs as an infallible means of differentiating between plague bacilli and the pasteurellae sensu stricto, occasionally organisms in the latter group proved pathogenic when administered to these rodents cutaneously (Kister & Schmidt 56).

In marked contrast to these findings, Haas 46 and Blanc & Baltazard 13 have shown that it was impossible to transmit pseudotuberculosis through the bite of Xenopsylla cheopis even though P. pseudotuberculosis could persist in the fleas for periods up to 35 days (Blanc & Baltazard 13). The pasteurellae sensu stricto were even rapidly destroyed in the fleas.

Subcutaneous inoculation of guinea-pigs is now much practised because increasingly ample use is being made of plague diagnosis with the aid of pooled organs of suspect rodents or their pooled fleas. As noted above, the method yields more rapid results than cutaneous inoculation, guinea-pigs injected with fully virulent material often dying within two to three days.

Intraperitoneal inoculation of these rodents leads to even more rapid death which may take place as early as 24-36 hours after infection. It is, however, essential not only to work with non-contaminated materials but also to take care not to administer an overdose of the infective material because rapid death from toxaemia may then take place and only a few bacilli may be present in the internal organs, yielding unsatisfactory growths
or none at all. In properly inoculated guinea-pigs an exudative peritonitis followed by bacteraemia develops.

Intraperitoneal inoculation is indicated in cases where a speedy diagnosis is required. For instance, Silva (quoted in the Journal of the American Medical Association 53) and Barreto & Castro 9 recommended direct intraperitoneal infection of guinea-pigs with the blood of patients as a convenient means to confirm the diagnosis of human plague.

As was first shown by Gotschlich,44 particularly rapid results may be obtained by taking material for microscopic and cultural examination from the peritoneal cavity of the living animals. He was thus able to find even 12 hours before death, when the guinea-pigs still appeared quite normal, numerous plague bacilli in the punctate. Ohoto 87 practised cardiac puncture and obtained, with 0.6 ml of blood, positive cultures 24 hours after intraperitoneal infection of guinea-pigs as against 34 hours and 72 hours in subcutaneous and cutaneous inoculation respectively. Gotschlich 44 found the intraperitoneal method also most useful when dealing with materials scanty in plague bacilli. Though death was often delayed in such cases and few or even no plague bacilli were found in the exudate, continued passage by the intraperitoneal route was apt to lead to positive results.

Conjunctival infection of guinea-pigs which leads to a purulent conjunctivitis is, for various reasons, not generally suitable. It is less safe than the other available procedures and, as maintained by Kolle,57 at the same time gives less constant results. Further, as claimed by Devignat et al.,25 contrary to usual beliefs this method is not fully specific. Devignat and co-workers were able on several occasions to isolate from the organs of conjunctivally infected guinea-pigs a salmonella resembling S. typhimurium (B. aertrycke).

**Autopsy findings**

Before dealing with the findings made when dissecting experimentally infected guinea-pigs, mention ought to be made of the changes observed during life at the site of infection.

As has been noted above, a primary exudative peritonitis develops in guinea-pigs inoculated intraperitoneally with adequate doses, a purulent conjunctivitis when virulent material is instilled into the eye of such animals. The records of early observers like Kolle 57 and the Plague Research Commission 59-97 indicate that cutaneous inoculation of guinea-pigs with *P. pestis* led typically to the appearance of small vesicles surrounded by a hyperaemic zone at the site of infection. Although these signs were apt to be manifest as early as 12 hours after inoculation, it was often possible to detect plague bacilli in the contents of the vesicles. Swelling of the regional lymph-glands followed within 24 to 48 hours. An inflammatory
process continued in typical cases at the site of inoculation where ulcers often formed.

A process developing more slowly and in a different manner in guinea-pigs infected by flea-bites was thus described by Wayson et al. \textsuperscript{118}

The immediate or almost immediate appearance of a red areola, 3-5 mm in diameter, round the bite-wound was followed within 24 to 72 hours by the development of a red papule and subsequently by the enlargement of contiguous lymph-nodes. If death was delayed, the papules were apt to become ulcerated. As stated by Wayson & McMahon,\textsuperscript{117} such papules formed also in guinea-pigs infected intracutaneously. It should be noted that as a rule external skin lesions remain absent at the site of subcutaneous infection; usually, however, subcutaneous infiltrations develop which in due course may become purulent and/or necrotic.

The task of dealing in a summary manner with the features found at autopsy not only in experimentally infected guinea-pigs but generally in plague-infected animals as well as human victims is most difficult because variations in the virulence of the causative strains, and also to a greater or lesser extent varying receptivity of the hosts, produce a whole gamut of morbid appearances. To describe findings which are "typical" under all circumstances is, therefore, impossible. Nevertheless, although transitory forms may cause some difficulty, distinction can be drawn between types of morbid appearance produced by plague infection in susceptible animals or man.

Attempting such a classification through an analysis of the postmortem records of more than 600 guinea-pigs experimentally inoculated with plague by various routes, Devignat et al.\textsuperscript{25} distinguished between three types of morbid appearances as follows:

\textit{Type 1}, found particularly in animals which had succumbed rapidly after inoculation with highly virulent strains or considerable doses of less-virulent plague bacilli.

In cutaneously infected animals belonging to this group a haemorrhagic infiltration adherent to the skin was present at the site of inoculation, while in subcutaneously injected animals a large infiltration, occasionally showing abscess formation, was located under the skin. In both cases one or several of the regional lymph-nodes had become transformed into a bubo showing a haemorrhagic aspect on cut section. The swollen gland was embedded in an oedematous mass of gelatinous appearance and consistency, which extended over a large part of the subcutaneous abdominal tissues. Spleen and liver were more or less enlarged but were smooth or at most finely granulated. The lungs were congested but free from consolidations (see fig. 18).

\textit{Type 2}, found particularly in animals inoculated with small numbers of highly virulent \textit{P. pestis} in the course of standardization tests.
Purulent reaction at the site of infection — a large groin bubo — subcutaneous congestion — congestion of the lungs — swelling and early fatty degeneration of the liver.
FIG. 19. PATHOGENOMIC SIGNS OF ACUTE PLAGUE IN A WHITE RAT

Left axillary bubo (-----) — subcutaneous congestion — pleural effusion of the lungs — enlargement and dome-like appearance of the liver.
While in animals belonging to this group the appearances at the site of inoculation did not differ from those described above, the massive gelatinous periadenitis, typical for type 1, was not invariably, though frequently, present. Spleen and liver were voluminous and friable, and the former organ in particular was studded with whitish granules of pinhead size which, when examined histologically, proved to be foci of necrosis usually surrounded by a belt of reticular cells. The lungs were congested as in type 1 but in some of the animals belonging to the second group disseminated foci of bronchopneumonia were present as well.

*Type 3*, found in guinea-pigs which, having been partially immunized with vaccines or serum, had survived for more prolonged periods.

While voluminous infiltrations, sometimes showing abscess formation, were present at the site of infection, no signs of a gelatinous periadenitis could be found in this type round the buboes. The spleen and liver were much enlarged and showed the presence of large whitish nodes. Similar nodes were also seen in the lungs. In contrast to the other types, particularly type 1, smears made from the organs of type 3 guinea-pigs showed few or no plague bacilli.

Devignat and his co-workers considered the massive gelatinous periadenitis met with in experimentally plague-infected guinea-pigs as pathognomonic. It has to be noted, however, that Haas observed this sign in 2 out of 39 guinea-pigs infected cutaneously or subcutaneously with a pseudotuberculosis strain isolated from a rat. He laid stress on the constant presence of liver nodules in his animals, claiming that nodules of an identical aspect were seldom encountered in plague-infected guinea-pigs.

The observations made by Cornil et al. on the morbid anatomy and histology of plague in guinea-pigs and white rats which had been inoculated with varying dosages of the same *P. pestis* strain, are of great interest and value even though they are based on an examination of small series of these animals.

Cornil and co-workers distinguished between the following three types of plague in their experimental animals which had been inoculated subcutaneously, intraperitoneally or subconjunctivally:

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Experimental animals</th>
<th>Period between infection and death (days)</th>
<th>Local reaction and bubo</th>
<th>Changes in internal organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperacute (forme sur-aiguë)</td>
<td>Guinea-pigs</td>
<td>3</td>
<td></td>
<td>Congestion of spleen; signs of parenchymatous degeneration—marked in liver and adrenals, less prominent in kidneys and myocardium; congestion of brain with small haemorrhages; little sign of bacterial embolism.</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>2-2½</td>
<td>Slight changes</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Experimental animals</th>
<th>Period between infection and death (days)</th>
<th>Local reaction and bubo</th>
<th>Changes in internal organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderately acute</td>
<td>Guinea-pigs</td>
<td>6-7</td>
<td>Local : necrosis and suppuration (suppuration nécrotique)</td>
<td>In addition to the above-described process of degeneration, necrotic foci due to bacterial embolism were present not only in the spleen, but also in the liver, lungs and adrenals, i.e., in organs possessing, in contrast to the kidneys, myocardium, and brain, an ample supply of venous blood.</td>
</tr>
<tr>
<td>(forme aigue moyenne)</td>
<td>Rats</td>
<td>3½</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subacute</td>
<td>Guinea-pigs</td>
<td>12</td>
<td>Bubo : suppurative adenitis in subcutaneously infected animals</td>
<td></td>
</tr>
<tr>
<td>(forme aigue prolongée)</td>
<td>Rats</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Considering these findings, Cornil and his colleagues formed the opinion that plague in their animals evolved in three stages: (a) a phase of local reaction quickly followed by (b) a stage of toxaemia (septico-toxémie diffuse) which led to degenerative changes in the internal organs, and (c) a final stage, characterized by septico-pyæmic metastases produced by bacterial emboli in some of the internal organs, which was reached only in animals surviving for comparatively longer periods.

A peculiar type of plague in guinea-pigs experimentally infected with plague strains from the Andes region of Ecuador was described by Macchiavello & Uriguen,72 the animals in question showing, in addition to regional buboes, marked involvement of the pelvic-aortic lymph-nodes as well as conspicuous, often necrotic, lung lesions. Macchiavello & Uriguen ascribed the appearance of these features to peculiarities of the plague strains, which seemed to be endowed with marked invasive power but little toxicity. They suggested that the strains might have been antigenically different from typical plague bacilli met with elsewhere.

**White Rats**

*Methods of infection*

While some workers considered cutaneous inoculation of white rats a suitable procedure for plague laboratory work, most observers reached the conclusion that this method did not give quite as constant results with these animals as with guinea-pigs. A much recommended procedure is, however, that of pricking the root of the tail of white rats with an injection needle which has been dipped into the material to be tested. One advantage of this procedure is that it may be easily and safely carried out in the glass jars in which the rats are kept before and after infection: the ordinary cover is temporarily replaced by a wooden one with a hole in the centre ("Impfdackel"); with the aid of a long forceps or clamp, the tail alone is drawn out and the inoculation is made (Kister, quoted by Dieudonné & Otto 23).
Kolle & Otto (quoted by Dieudonné & Otto 26) found this method useful when dealing with contaminated materials and also claimed that, with its aid, negative results were obtained with certain rat-pathogenic bacteria which proved fatal when injected subcutaneously.

Intraperitoneal infection of white rats, though as a rule producing no marked gross changes in the peritoneal cavity, is a useful method because, unless an overdose of the infective material has led to rapid death from toxaemia, numerous plague bacilli may usually be found in the scanty and serous peritoneal exudate, as well as at other sites, especially in the spleen.

In the past great attention has been paid to feeding experiments with laboratory rats because it was believed that the free-living rats usually contracted infection per os. Several investigators showed that rats may become infected in the laboratory when fed with plague cultures or carcasses and may develop mesenterial buboes. However, these findings were in strict contrast to those made under natural conditions. Moreover, as shown by Kolle & Otto (quoted by Dieudonné & Otto 26), even in the laboratory, infection by feeding deserved but little attention on account of the somewhat inconstant results.

Kolle & Otto expressed the same opinion in regard to conjunctival infection of white rats. However, this method was recommended by the German Plague Commission 32 even for little virulent and contaminated material.

Autopsy findings

As confirmed by the above-mentioned observations of Cornil et al., 20, 21 the autopsy findings in experimentally plague-infected white rats (see fig. 19) on the whole rather resemble those met with in guinea-pigs. Eastwood & Griffith 27 considered general redness and engorgement throughout the ventral subcutaneous tissues as features constantly and conspicuously present in white rats which succumbed to subcutaneous plague infection.

Susceptibility to pseudotuberculosis infection

A point of great importance is the reaction of white rats to infection with *P. pseudotuberculosis*. Since in the experience of most workers these animals proved resistant to such inoculations, non-pathogenicity for white rats was considered as one of the cardinal features distinguishing pseudotuberculosis from plague bacilli.

The recent observations of Haas 46 were on the whole in agreement with this contention. Though his pseudotuberculosis strain had been isolated originally from a *Rattus norvegicus*, it showed little pathogenicity for laboratory rats: four out of five such animals inoculated with it remained healthy; two of them had apparently become resistant to subsequent plague infection. The fifth rat, which had been injected sub-
cutaneously, was killed two weeks after infection. It showed a small ulcer at the site of inoculation and some nodules in the spleen. Smears from this organ proved negative, but a guinea-pig infected with material from it succumbed to pseudotuberculosis 27 days after subcutaneous inoculation.

However, since a few observers found pseudotuberculosis strains definitely pathogenic for white rats (Bezsonova 11), it would not be wise to classify otherwise dubious strains as plague solely because they are able to kill such animals. Certainly, however, a suspect strain which is pathogenic for guinea-pigs, yet spares white rats, is not one of plague.

**White Mice**

While in the past it was generally maintained that white mice were rather resistant to experimental infection with *P. pestis*, it has now been established that certain inbred races of this species are eminently suitable for plague laboratory work. Sufficient evidence regarding this point has already been brought forward (see page 117).

White mice which have been inoculated with plague material show, as a rule, no features pathognomonic for this infection at autopsy. Spleen enlargement and other non-specific signs of a generalized infection may, however, be met with in such animals. It is important to note that they are susceptible to pseudotuberculosis infection (Poppe 99).

**Rabbits**

Although, as recorded by Taylor, 113 certain races of rabbits may prove fully satisfactory, generally speaking these animals are not uniformly susceptible to plague, not uncommonly showing a resistance to virulent infection. As a rule, therefore, the use of rabbits for plague-diagnostic work is not to be recommended; the less so as they are fully susceptible to pseudotuberculosis infection. As has been noted, however, rabbits are indispensable for serological work and are at the same time excellent for the production of plague immune sera.

**Free-living Rodents**

As has been stated before, free-living rats have been used on a large scale for plague laboratory work in the Haffkine Institute. In south-east Russia ample advantage was taken for the same purpose of local sisle species, and in Africa of multimammate mice. These examples suffice to show that the use of such animals for the various needs of plague laboratory work is legitimate provided that the following precautions are taken:

(1) If the rodents in question have been received from a region where plague is endemic, due care must be exerted to ascertain that they are
free from the infection. It may become necessary to quarantine the animals for some time but this may rather bias their usefulness since some of the species concerned, particularly commensal rats, are apt to show a considerable mortality in captivity even when caught healthy. They also tend to mutilate or even partly to devour the carcasses of their dead mates, which may render postmortem examinations difficult.

(2) At the same time it must be ascertained whether the free-living rodents to be used for experimental purposes are fully susceptible to plague. It has been noted in this connexion that the Haffkine Institute had to send for its rats across India to Madras, a plague-free city with a rat population not resistant to the infection.

(3) It is imperative to free the animals, as soon as they are received, from fleas and other external parasites. The method of dipping the rodents into a pulicidal fluid like kerosene is effective but apt to lead to a considerable mortality. Webster 119 therefore recommend the picking-off of fleas and other parasites with a forceps or by hand, after some suitable repellant, such as spirits of camphor, has been applied with a cotton swab, but this tedious method is effective only when practised by a trained worker. Application of DDT would be far less time-consuming and fully effective, but, as will be noted later, this insecticide is to some extent toxic for the rodents as well as deleterious to their fleas.

Since it is usually difficult to fulfil these conditions, it is certainly preferable to use guinea-pigs, white mice, or white rats for plague laboratory work whenever possible.

Monkeys

Though numerous plague experiments with monkeys belonging to different species have been undertaken, for various reasons these animals have proved unsuitable for routine work with \( P. pestis \). As has been noted before, even in monkey species generally amenable to the infection, marked differences in the susceptibility of individual animals have been found to exist. In laboratories situated away from their natural habitats, monkeys are difficult to keep, while in countries like India, where they are at home, they may suffer from natural plague.

It is interesting that, in contrast to these experiences, Robic 101 found some species of lemurs (locally called "makis") were easily kept in the laboratory and were eminently suitable for plague research. When infected subcutaneously, these animals succumbed to an acute septicaemic form of the disease after three days, i.e., before the guinea-pigs and rats infected with identical doses of plague bacilli. The "makis" could also be effectively immunized with the aid of the EV strain and were apt to contract pneumonic plague if infected intranasally.
BUBONIC PLAGUE WITH SECONDARY SEPTICAEMIA IN MONKEYS

FIG. 20. LYMPH-NODE
P. Pestis parenchyma (Giemsa)  
(×225)

FIG. 21. LUNG
Alveoli and blood-vessels dilated; numerous  
P. pestis  
(×225)

FIG. 22. LIVER
Congestion of sinusoids and central cells; clusters of P. pestis in Kupffer’s cells and blood-vessels  
(×450)

FIG. 23. SPLEEN
Marked congestion of sinusoids and areas of hemorrhages; P. pestis throughout (Giemsa)  
(×450)
Bats

Leger & Baury\textsuperscript{64} found the African bat *Nyctinomus pumilus* so susceptible to plague infection that they recommended its use as a test animal. It should be noted in this connexion that the European species of the bat *Vesperugo noctula* was also found to contract the disease rapidly when inoculated subcutaneously (Gosio\textsuperscript{49}) and that Barrera\textsuperscript{8} could infect an Argentinian species by scarification. The Japanese bat, *Vespertilio abramus*, on the contrary, proved in the experience of Ohwada (quoted by Dieudonné & Otto\textsuperscript{26}) rather resistant to plague infection.

Birds

Domestic birds, like the pigeon or the chicken, being resistant to the usual methods of plague infection, are of some importance for differential diagnostic purposes; Kossel & Overbeck,\textsuperscript{59} for instance, recommended inoculation of pigeons as an easy means of distinguishing between plague and fowl-cholera bacilli. It should be noted in this connexion that, although some exceptions have been recorded, birds are generally resistant to infection with pseudotuberculosis as well as with plague bacilli (Poppe\textsuperscript{89}).

PNEUMONIC PLAGUE IN EXPERIMENTAL ANIMALS

Animals Used

As summarized by Wu Lien-teh\textsuperscript{124} and Pollitzer,\textsuperscript{88} earlier workers used the following animals when trying to produce pneumonic plague experimentally: camels, cats, dogs, commensal mice, guinea-pigs, monkeys, rabbits, rats, sisels, and tarabagans (Siberian marmots).

Recent workers, such as Bablet & Girard,\textsuperscript{4} Girard,\textsuperscript{35, 36} Favarel,\textsuperscript{29} and Neel,\textsuperscript{83} relied mainly on guinea-pigs, but white mice were used as well by Herbert\textsuperscript{19} and Meyer et al.,\textsuperscript{80} the latter also experimenting with cotton rats. Robic\textsuperscript{101} and Favarel\textsuperscript{28} found the "maki" fully suitable to produce primary lung infection with *P. pestis*.

Methods of Infection

Besides inhalation, various other methods have been utilized by different workers with the aim of producing experimental infections comparable to primary pneumonic plague in man. Most important among these procedures were:

(a) *Intratracheal infection*, originally used by Wyssokowitz & Zabolotny\textsuperscript{129} and later by workers such as Bablet & Girard,\textsuperscript{4} Girard,\textsuperscript{35, 36} Favarel,\textsuperscript{29} and Neel,\textsuperscript{83}
(b) *Intranasal infection*, first employed by Batzaroff\(^\text{10}\) and recently by Meyer and co-workers\(^\text{80}\) and—in the case of the “maki”—by Robic\(^\text{101}\)

(c) *Direct intrapulmonary injection* of plague bacilli as recommended by Shibayama.\(^\text{104}\) Advantage of this procedure seems to have been taken by only a few workers, e.g., Wu Lien-teh & Jettmar\(^\text{126}\) and, recently, Favarel\(^\text{28}\) when infecting “makis”.

**Evaluation of Results**

It has been much debated not only how easily a process identical to primary pneumonic plague in man could be produced in experimental animals, but whether it was at all possible to produce such a syndrome in the latter, particularly in guinea-pigs and rabbits.

It should be noted in this connexion that the claim of Wyssokowitz & Zabolotny\(^\text{129}\) to have produced primary pneumonic plague in anaesthetized monkeys was not confirmed by Nattan-Larrier or by Broquet (quoted by Bablet & Girard\(^\text{4}\)) who had access to the specimens of the two Russian workers.

General agreement exists that Batzaroff\(^\text{10}\) had been successful in producing this form of plague in guinea-pigs, rabbits, and monkeys by intranasal infection and had occasionally obtained the same result in rats and mice as well. However, Bandi,\(^\text{6}\) repeating such tests with guinea-pigs and also performing inhalation experiments with this rodent species and with rats, considered the resulting lung changes to be secondary in nature, plague infection reaching the lungs through the lymphatic channels.

Strong & Teague,\(^\text{111}\) who were also unable to produce primary pneumonic plague in guinea-pigs through inhalation, ascribed this failure to the shallow type of respiration in these animals or to the small size of their larynx. Shibayama\(^\text{104}\) incriminated the thick cluster of hair present in the nostrils of this species and maintained that the only method of causing primary pneumonic plague in guinea-pigs was to inject the infective material directly into the lungs.

The evidence regarding the rabbit was also contradictory, Martini asserting, in contrast to other observers, that septicaemic and not pneumonic infection resulted when plague bacilli were administered to these animals by inhalation (Wu Lien-teh\(^\text{124}\)). Attention was also drawn to the fact that pneumonia-like processes could be produced in test animals through the action of plague toxins, Signorelli,\(^\text{106}\) for instance, having been able to produce such a process by injecting Lustig’s nucleoprotein into the lungs of dogs.

However, without denying that some investigators were unable to produce primary lung involvement in experimental animals, it would be altogether wrong to consider their negative results as generally valid or even to assume, as some observers did, that pneumonic plague in animals
as well as in man was invariably secondary in nature, the infection entering through superficial mucous membranes instead of directly reaching the deeper respiratory tract. For irrefutable proof of the existence of primary pneumonic plague in adequately infected animals and also—as will be shown later—in man has been brought forward by several workers.

The following findings recorded in this connexion deserve attention here:

Strong & Teague,\textsuperscript{111} found that monkeys infected by inhalation practically always developed primary lung involvement, showing only slight and obviously secondary changes in the faucial and cervical tissues. Tonsillar infection on the other hand resulted in plague septicaemia with or without involvement of the cervical lymph-nodes, but never led to pneumonic changes.

Wu Lien-teh & Jettmar,\textsuperscript{126} performing inhalation experiments with tarabagans and sisels, definitely showed through histological examinations that the primary focus of infection was invariably situated in the deep portion of the respiratory tract.

Nattan-Larrier & Richard\textsuperscript{82} insisted upon the profound difference of the histological findings in experimental animals with primary and secondary pneumonic plague respectively. In the former a bacillary alveolitis was in the foreground, while the latter showed an abundance of the bacilli in the lymphatics of the lungs.

Bablet and Girard,\textsuperscript{4} also relying on careful histological examinations, obtained full proof that, in intratracheally infected guinea-pigs, bacteraemia was secondary to changes in the deep respiratory tract.

Recently Meyer and colleagues,\textsuperscript{80} infecting white mice, guinea-pigs, and cotton rats intranasally, and Neel,\textsuperscript{53} practising intratracheal infection of guinea-pigs, also definitely established that in their animals bacteraemia was secondary to the process developing in the lungs.

\textbf{Evolution of Primary Pneumonic Plague in Animals}

Describing the evolution of the morbid process in guinea-pigs infected with plague by the intratracheal route, Bablet & Girard\textsuperscript{4} distinguished between three successive phases:

\textit{(a)} A "broncholymphatic" stage characterized not only by an inflammatory reaction involving the mucosa and adjoining lymph spaces of the trachea and main bronchi but—as early as eight hours after infection—also by circumscribed alveolar lesions (bronchio-alvéolite pesteuse) round some intralobular bronchioli.

\textit{(b)} A pneumonic phase, becoming manifest 24-30 hours after infection and characterized by numerous lobular foci which were often situated around bronchioli. In due course extension and multiplication of these foci led to the formation of "plague abscesses" rich in plague bacilli and leucocytes which showed a tendency to break through into the bronchi.
(c) A short septicaemic stage demonstrable not earlier than 60 hours after infection through blood cultures. At the same time the pneumonic foci became confluent and plague bacilli abounded in the lumen of the bronchi and the trachea.

Examining mice and guinea-pigs killed at intervals of 6 to 12 hours after nasal instillation with plague material, Meyer et al. noted the evolution of a process similar to that mentioned above. These workers described the gross findings in mice which had succumbed to the infection (usually after 72-96 hours) in the following terms:

"The gross anatomical lesions in the mice vary slightly. In rapidly fatal infections a small amount of fluid is present in the chest cavities; all the lobes of the lung are dark red, in a state of diffuse exudative hemorrhagic edema, or there is lobular red consolidation in the left or right anterior or diaphragmatic lobe. Edematous fluid oozes from the trachea and occasionally from the nostrils. Parenchymatous changes in the liver and kidneys as well as fatty hemorrhages in the adrenal complete the picture of a toxemia."

The macroscopic features in intranasally plague-infected guinea-pigs, which as a rule were not visibly ill for the first two days but suddenly died between the 3rd and 5th day after infection, were thus characterized by Meyer and co-workers:

"Since, in contrast to mice, guinea-pigs are much more resistant to plague toxin, the gross lesions, aside from a generalized subcutaneous capillary injection, show little or no fluid in the pleural cavities. The lungs as a whole are voluminous, and portions of the apical or principal lobes are in different stages of red and gray hepatization... The pleural covering of these areas is rarely covered with fibrin. The bronchi and trachea are injected and covered with blood-streaked mucus. Bronchial and mediastinal lymph nodes are always enlarged, edematous and occasionally studded with early necroses; the regional lymph nodes of the neck and submaxillary space, as a rule, are not involved and the spleen is not enlarged. The infection is definitely confined to the respiratory tract."

**NATURAL PLAGUE IN ANIMALS**

**Commensal Rats**

For different reasons the autopsy findings in rats which had contracted plague under natural conditions may show considerable variations.

Even during epizootics much depends on the way in which the material for dissection is collected. Examining exclusively the animals found dead, one may except to come across a high percentage of specimens showing, in addition to frankly positive bacteriological findings, marked gross lesions. Even then, however, an appreciable number of rats may be found which, having apparently succumbed rapidly to an overwhelming infection, show little or even no macroscopic evidence of this although their organs teem with plague bacilli.
The percentage of rats showing marked gross signs of plague decreases in relation to the proportion, among the dead rats examined, of animals killed by the population of the plague-threatened area. Nevertheless, during epizootics one may hope to find even among the killed rats a number of specimens showing marked macroscopic evidence of infection—apparently animals which had been less able than their healthy mates to evade their pursuers.

Results of macroscopic examination are, however, apt to be rather disappointing if trapping methods are used to obtain rats for dissection. Even during epizootics, though carcasses showing incipient plague or, occasionally, displaying signs of having recovered from it may be encountered, animals with fully developed macroscopic features will be few and far between because, as aptly pointed out by Hundley & Nasi, rats suffering from plague generally prefer to stay in their nests. It is true that delirium and/or the desire for fresh air may ultimately drive them out, but, although prone to be killed while staggering about, such moribund animals will not be attracted by the baits offered in the traps.

As shown by Shih & Pollitzer, who had opportunities to examine many hundreds of rats, which included animals found dead and animals killed both during and between epizootics, the frequency of specimens showing marked pathognomonic signs at autopsy was definitely related to the stages of the epizootics. The number of such specimens started to increase at the onset of the plague seasons, reached a maximum at their peak, and then gradually declined to become minimal during the off-seasons. The frequency of grossly affected rats, and correspondingly the number of animals showing abundant bacteriological evidence of the infection, were thus apt to serve as a yardstick for assessing the seriousness of the situation; this naturally also holds true when the number of animals available for examination is small.

It further stands to reason that the frequency of rats showing marked macroscopic lesions will vary according to the type of plague prevailing in the area in question. Thus it was claimed that, in Brazil, rat plague was generally manifest in an unusually mild and atypical form. In regions where the rat population is becoming resistant to the infection, results of macroscopic examination are also apt to be less clear-cut than in areas with a fully susceptible rodent population.

Autopsy findings

While the description of the postmortem findings in plague-affected rats is based mainly on the classical studies published by the Plague Research Commission in India in 1907, further contributions to the knowledge of this subject have been made by other workers, recently, for instance, by Prado, Macchiavello, de Smidt, de Moura & Remião, and Hundley & Nasi. The results of these investigations may thus be summarized:
Rigor mortis. As pointed out by the Plague Research Commission, rigor mortis, often present in plague-affected rats and sometimes persisting even after putrefaction has set in, is somewhat characteristic, the limbs projecting stiffly from the body in a distinctive manner.

Subcutaneous tissues. One of the common features of rat plague is subcutaneous congestion, which may manifest itself by a reddish hue of the skin before the rats are dissected, being specially visible on the plantar surface of the hind feet. However, as stressed by the Commission, this sign may be absent in plague-affected rats and present in animals succumbing for other reasons. Among subsequent workers, Paisley and Gomila laid great stress upon pink discoloration not only of the feet, but often of the abdomen and thorax also, Gomila finding “pink feet” in 75% of his plague-positive rats.

Another point noted by the Commission and other observers was a peculiar purplish-red appearance of the thoracic and abdominal muscles, due to the presence of congested vessels. In conjunction with the reddish-pink colour of the subcutaneous tissues, this appearance was considered as strongly suggestive of plague infection.

The Plague Research Commission found subcutaneous haemorrhages to be quite frequent in plague-infected rats and emphasized, at the same time, the absence of this feature in animals not so infected. The haemorrhages were most often situated in the submaxillary region even if no bubo was present in the neck or elsewhere; next in frequency came the region of the flank, while in young or medium-sized rats the haemorrhages were apt to be widespread.

In the experience of the Plague Research Commission a general subcutaneous oedema, which forms a characteristic feature in experimentally infected guinea-pigs, was rare in naturally plague-affected rats.

Lymph-nodes. In describing the changes in the lymph-nodes of plague-affected rats, the Commission drew a sharp distinction between a process of general involvement and primary buboes. Enlargement and congestion of the lymph-nodes in general was met with in septicaemic plague and also, as the result of secondary invasion through the blood-stream, in rats showing a primary bubo. Infection could also spread from the original bubo through the lymphatics to neighbouring lymph-nodes, thus leading to the formation of “primary buboes of the second order”.

The morbid appearances in primary plague buboes depended upon the stage of the infection. At first the affected lymph-nodes showed only some enlargement and congestion, with haemorrhagic points on the cut section. Later, signs of necrosis became manifest, affecting either the medullary portion (or even merely part of it) or, in advanced cases, the whole substance of the nodes involved. Ultimately disintegration took place, with the formation of usually rather dry purulent material.
In well-developed infections the tissues round the primary buboes were as a rule affected, often showing infiltration as well as marked congestion and haemorrhages, and sometimes a localized subcutaneous, occasionally gelatinous, oedema.

While, as a rule, the prominent swelling caused by the relatively-large primary buboes was apt to attract attention, their existence was sometimes suggested merely by slight asymmetry and confined to signs of some necrosis on the cut section. The Plague Research Commission therefore recommended the incision of all lymph-nodes, even if they were not outwardly changed and did not exhibit the peculiar hard consistency often noted in plague buboes. Axillary buboes were particularly liable to be overlooked as they might be small, flattened, and lying parallel to the inner surface of the upper extremity under the lateral insertion of the pectoral muscle. It was therefore necessary to cut through this muscle to reveal the axillary cavity.

Analysing the results of 4,000 autopsies of naturally infected rats, the Plague Research Commission found involvement of the lymph-nodes in 84.72% (buboes in single situation in 73.05%), and absence of buboes in 15.25%. The distribution of the buboes in single situation was as follows:

<table>
<thead>
<tr>
<th>%</th>
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<tbody>
<tr>
<td>Neck</td>
</tr>
<tr>
<td>Axilla</td>
</tr>
<tr>
<td>Groin</td>
</tr>
<tr>
<td>Pelvis</td>
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</table>

The bubo distribution recorded by other workers was sometimes not in full accord with that shown above, but it should be kept in mind that, as a rule, they based their conclusions on much smaller samples than those available to the Plague Research Commission. In the fairly large amount of material examined by Pollitzer in China, cervical buboes were the most frequent.

Liver. Although the liver of rats yielding ample bacteriological evidence of infection with *P. pestis* may show at autopsy only slight and uncharacteristic changes, if indeed any, plague-infected rats often display lesions of the organ which may be considered pathognomic for this infection. As established by the Plague Research Commission, these characteristic lesions were twofold in nature.

On the one hand appearances were frequently present which macroscopically seemed due to fatty changes but, as revealed by the microscope, were actually caused by necrosis. In the early stages of this process, a characteristic mottled appearance was produced by the contrast between the yellowish aspect of the parts with "fatty" changes and the reddish colour of congested areas. In advanced cases the liver had a pink tinge and no signs of division into lobules were seen. The organ appeared as
though modelled in wax with the outer surface peculiarly dome-like and the edges sharply defined; it was no longer elastic but rather friable. Although these changes appeared to be pathognomic in fresh carcasses, a similar condition of the liver was sometimes found in putrid rats which had not succumbed to plague.

Another change found in 58% of the 4,000 rats, occasionally side by side with "fatty" appearances, consisted of the occurrence of necrotic foci scattered over the surface and throughout the substance of the liver ("granular" liver). Typically these nodules were of pin-point size and, if superficially situated, not bulging. However, in some cases—apparently of longer standing—the granules were coarser, those on the surface were raised above the liver tissue and, although good cultures were obtained, only a few bacilli could be seen in microscopic preparations from the organ.

Spleen. In well-marked cases of rat plague the spleen was enlarged and firm with a moulded appearance so that it was found lying over the stomach instead of being out of sight like a soft normal spleen. It must be noted, however, that a similar enlargement of the spleen may, not infrequently, be met with in rats not suffering from plague. More characteristic was the presence of smaller or even confluent larger nodules but, according to the Plague Research Commission, such nodules were found in only 4.5% of the plague rats, being thus considerably less common than in the liver.

Kidneys and suprarenals. The kidneys were often congested and not infrequently showed subcapsular haemorrhages. "Fatty" changes were frequent, but the presence of plague nodules was exceptional. The suprarenals were often enlarged and congested.

Stomach and intestines. In the experience of the Plague Research Commission, no characteristic changes were found in the stomach and intestines of plague-infected rats; subserous haemorrhages, occasionally seen on the stomach, were rarely present in the intestines.

Although most workers confirmed these observations, a few found marked changes in the gastro-intestinal tract. Paisley and Connal et al. noted in some of the plague-infected rats in Lagos, Nigeria, subserous haemorrhages and bloody effusion in the small intestines. Mesenteric buboes were present in such cases. Prado in Brazil found plague-infected rats with enlarged and haemorrhagic mesenteric lymph-nodes and also noted sometimes the presence of the same changes or of caseous pus in retroperitoneal lymph-nodes of the lumbar region. In rare instances he observed in the intestines elliptic white patches, corresponding in size to Peyer's patches, which were in a process of cicatrization or seemed to have become cicatrized. It was assumed that the rats showing these intestinal lesions had contracted infection by devouring plague carcasses.
**Pleurae and lungs.** Haemorrhages were fairly often noted in the pulmonary pleurae and lungs but apparently never in the parietal layer of the pleurae.

Pleural effusion was a fairly frequent sign in plague-infected rats; it was met with in over 70% of the animals found to be plague positive by the Plague Research Commission. Macalister & Brooks,69 who had the opportunity of comparing the findings in a small number of plague-affected rats with those in large numbers of plague-free animals, laid stress upon the clear appearance of the fluid, because in the case of trypanosomiasis a blood-stained exudate was found. However, as noted by the Plague Research Commission and confirmed by other workers, a slightly or moderately blood-stained effusion may be found in plague-infected rats as well. Moreover, though trypanosomiasis is fairly frequent among the rats in China, Pollitzer failed to observe pleural effusions in them unless they were infected with plague.

While it may be asserted that until recently the presence of an appreciable or abundant amount of clear pleural fluid proved the existence of plague in the rats concerned, it must be noted that nowadays a pleural effusion of the same character may be encountered in rats which have been poisoned with α-naphthylthiourea (ANTU).

Though a certain degree of congestion and/or oedema might be present in the lungs of plague-affected rats, more marked or characteristic changes were as a rule absent. Nodules were rare, being present in only 2.5% of the Plague Research Commission series, and primary pneumonic changes quite exceptional.

**Pericardium and heart.** Pericardial effusion was fairly frequent in plague-affected rats. Epicardial haemorrhages were occasionally present and the superficial vessels were often congested. The walls of the heart were relaxed, the right cavities usually engorged with blood and the left empty.

"**Chronic** plague in rats

Kolle & Martini 55 seem to have been the first observers to note the existence of a chronic form of plague in rats which had been experimentally infected months before. The animals in question showed caseation of the bronchial lymph-nodes, induration of the lungs, and encapsulated foci in the submaxillary lymph-nodes with positive bacteriological findings. Similar results were afterwards obtained by Hata (quoted by Pollitzer 98) in rats and guinea-pigs treated first with small doses of weakly virulent *P. pestis* and then with fully virulent material. Thus an experimental basis seemed to have been furnished for the belief of early workers, such as Simond 107 and Gotschlich,45 that chronic rat plague might be the means for perpetuating the infection during the off-seasons between epizootics.
A profound study of this problem was carried out by the Plague Research Commission in India (1906-12). This work, supplemented by the exhaustive histological studies of Ledingham, showed the presence of different categories of apparently "chronic" lesions in naturally plague-infected rats. As summarized by Petrie, a distinction could be made between "peripheral" lesions, consisting of encapsulated abscesses corresponding in size and relative frequency to the primary plague buboes, and "visceral" lesions, usually found in the spleen and consisting either of thick-walled abscesses, often attached by adhesions to the surrounding serous membranes, or of necrotic areas in the spleen substance. Plague bacilli were often absent from such lesions; if present, they were not abundant, though usually virulent. In other instances, in which bacteriological findings were invariably negative, adhesions of the spleen to the surrounding structures, scars on this organ or fibrous thickening of its capsule were found singly or in varying combinations.

The Commission was careful to point out that, except in cases with positive bacteriological findings, it was not possible to obtain direct proof that such lesions were caused by plague. In fact, similar appearances were found in Madras and Poona rats which had definitely not been exposed to plague infection. It could be shown, however, that:

1. Similar lesions were present in certain experimentally infected rats. Specially noteworthy was their presence in 11% of rats from different parts of India which had survived inoculation with small doses of plague bacilli and had been killed three weeks afterwards; the largest proportions of such lesions were found among the survivors of the most susceptible group.

2. The lesions found in rats exposed to natural infection were of an identical character, regardless of whether or not plague bacilli could be detected in them; in fact sometimes both foci, yielding positive and negative bacteriological findings, were present in one and the same animal.

3. Rats with such lesions increased in number during and after plague epizootics.

The Commission found no evidence whatsoever to show that lesions still containing plague bacilli had been the source from which an acute infection had been re-established in a particular rat. On the contrary, these lesions seemed to represent successive stages in the natural process of recovery from acute plague infection. For this reason the Commission proposed for them the designations "resolving" and "resolved" plague, in preference to the term "chronic" plague, in order to avoid the erroneous impression that rats with such lesions were instrumental in perpetuating the infection.

While these views have been accepted by most investigators, a few expressed divergent opinions.
Swellengrebel & Otten\textsuperscript{112} described a “mitigated” form of plague in a naturally infected rat and were able to produce identical lesions in partly immune rats as well as in guinea-pigs inoculated with material of low virulence or infected cutaneously with minimal doses of virulent plague bacilli. The changes observed in such animals consisted of multiple small axillary or inguinal buboes, haemorrhages in all organs, and sometimes exudates in the serous cavities. Plague bacilli could not be directly demonstrated and infection of susceptible animals was apt to produce at first a similar process. However, further animal passage finally led to typical plague. Swellengrebel & Otten ascribed some importance to this “mitigated” form of plague, believing that the process might pass, under natural conditions, to the acute form.

Bordas et al.\textsuperscript{16} and Williams & Kemmerer\textsuperscript{122} drew attention to rats, showing only trivial or no lesions and found especially at the end of the epizootics, from which plague cultures of supposedly low virulence could be isolated. Tanon (quoted by Petrie\textsuperscript{91}) expressed the idea that by continued passage from rat to rat such strains might regain virulence and lead to a severe epizootic.

Williams,\textsuperscript{121} besides reporting on two naturally infected rats which had suffered apparently from resolving plague yet showed bacteraemia, experimented with 2,278 \textit{R. norvegicus}, killing the animals surviving cutaneous or subcutaneous plague infection at different intervals. Among the rats showing signs suggestive of resolving plague, one killed 21 days, and four killed 30 days, after inoculation showed plague bacilli in the heart blood immediately after death. Williams suggested that these experiments completed the proof of a perpetuation of the infection through plague strains of low virulence, as suggested by Tanon, and formed the tentative hypothesis that plague produced among the rats a certain proportion of carriers in which bacteraemia might appear at times with consequent opportunities for infection of fleas and a spread of the disease to other rats.

Recently Savino et al.,\textsuperscript{102} finding that one rat out of almost 120,000 dissected showed features of resolving plague and yet yielded positive results in cultural and animal tests, expressed the opinion that rats suffering from this form of the disease might be the means of carrying over the infection.

Even apart from the fact that the claim made by some of these authors to have found in their rats plague strains of a low virulence has not been supported by sufficiently exact tests, the evidence recorded, and the theories put forward by them, cannot be considered as convincing.

Nobody will deny that, in a small proportion of rats suffering apparently from resolving plague, bacteraemia may still persist or, in other words, that no sharp dividing line can be drawn between instances of acute or subacute, and those of resolving, rat plague—a fact stressed by the Plague Research Commission. That bacteraemia could persist in such animals
for more prolonged periods or that it could reappear in the course of the resolving process has, however, never been proved. As Petrie maintained with much reason:

"such an event is unlikely to happen, because, during the process of recovery from an acute attack, rats presumably acquire a considerable immunity, and, moreover, there is no experimental evidence, either in plague or in any other similar infection, to support the idea that a chronic focus is able to kindle a fresh septicaemia."

A few workers have drawn attention to the occurrence in commensal rats of an "inapparent" form of plague in which macroscopic signs of the infection were absent and direct bacteriological examination yielded negative results, yet animal experiments proved positive. However, it was not claimed that such animals played a role in the perpetuation of the infection. On the contrary, Peryassu denied that his "carrier" rats were responsible for plague outbreaks and Devignat, who established the presence of the infection in apparently healthy rats through animal experiments with their pooled bone-marrows, noted that human plague was absent from the localities where such rodents had been found.

Commensal Mice

As a rule, naturally plague-infected commensal mice show no characteristic postmortem changes. General signs of infection, particularly swelling of the spleen, may be present but these are also met with in animals which do not succumb to plague. However, Pollitzer not infrequently noted, in the commensal mice he dissected, a brick-red discoloration of the liver, sometimes associated with a mottled appearance due to the presence of yellowish areolas. Almost invariably mice showing such liver changes were found to have succumbed to acute plague.

Wild Rodents

Although, as will be discussed later, numerous naturally plague-infected wild-rodent species have been found, few detailed descriptions of the morbid appearances met with in such animals have been recorded. The available information may be summarized as follows:

Siberian marmots (tarabagans)

As stated by Wu Lien-teh, 15 Siberian marmots (Marmota bobak) found naturally plague-infected up to 1924 could be classified thus:

<table>
<thead>
<tr>
<th>Type of Plague</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td>bubonic plague</td>
<td>11</td>
</tr>
<tr>
<td>? pneumonic plague</td>
<td>1</td>
</tr>
<tr>
<td>? septicaemic plague</td>
<td>1</td>
</tr>
<tr>
<td>? residual plague</td>
<td>2</td>
</tr>
</tbody>
</table>

Not all these animals were in a condition suitable for an exhaustive examination. As far as could be established, cervical buboes preponderated,
being found in 9 of the 11 animals showing features of bubonic plague, together with axillary buboes in two cases.

The spleen was usually more or less swollen and softer, sometimes showing small greyish nodules. The liver showed, as a rule, signs of congestion, and occasionally also yellowish-white nodules and/or haemorrhages. Secondary lung involvement was frequent, as confirmed by the histological studies of Wu Lien-teh and Lin Chia-Swee.127

Rodents of south-east Russia

Sisels (susliks). In naturally infected susliks (Citellus pygmaeus) all forms of plague—bubonic, pneumonic, and intestinal—have been diagnosed; according to Nikanoroff,85 transitions among these types have been frequently met with. Susliks displaying no marked macroscopic lesions, yet yielding virulent plague cultures, were also often seen.

Among the animals with well-marked features of plague, the bubonic type preponderated, Suvoroff (quoted by Nikanoroff 55), for instance, finding buboes in 44 (84.6%) out of 52 naturally infected susliks. The inguinal lymph-nodes were most frequently involved.

Signs of residual plague were often manifest. Thus Nikanoroff,84 investigating a district where plague had been active the year before, found lesions of this type in the lymph-nodes of the susliks, particularly in those in the groin.

Gerbils. Studying the morbid anatomy and histology of experimentally infected gerbils (Meriones meridianus), Lobanov & Fedorov 66 found evidence for the existence of two different types of plague with a distinct seasonal incidence. In spring and early summer (April to July) the character of the disease was fairly benign with a tendency towards the formation of localized abscesses, which became encapsulated and absorbed, and were finally replaced by scar tissue. In late summer and autumn (August to October), on the contrary, an acute type of generalized plague was prevalent.

South African rodents

According to Pirie,92 experimentally infected wild rodents involved in the plague outbreaks in South Africa as a rule showed no marked gross changes, the most constant signs being slight and general subcutaneous congestion, and more-marked engorgement of the vessels behind the sternum and front of the chest wall. Since the animals had been inoculated percutaneously on the inside of the left hind-leg, inguinal buboes were usually present, but were not conspicuous in animals which had survived less than five or six days: in those succumbing after three or four days, at most a slight, soft, sometimes haemorrhagic swelling of the inguinal lymph-nodes was apparent. The spleen was usually somewhat enlarged and presented a duller and more opaque appearance than in normal
animals. In the lungs small patches of incipient pneumonic plague were frequent.

It was not possible to say definitely whether similar alterations were present in naturally infected animals because only small numbers of these could be examined. The available evidence suggested that in nature also an acutely septicaemic type of plague prevailed. Chronic lesions were never met with in the multimammate mice and were extremely rare in the gerbils.

**Californian rodents**

As summarized by Wu Lien-teh, McCoy found, in a representative series of 246 naturally infected Californian ground-squirrels (*Citellus beecheyi*), acute plague in 60 (24.4%), all with buboes; subacute plague in 152 (61.8%), 73.7% with buboes; and residual buboes in 34 (13.8%). Inguinal buboes were most frequent, followed by involvement of the cervical and, lastly, of the axillary lymph-nodes. Primary lung involvement was exceptional, but secondary lung lesions, consisting of the presence of nodules or areas of grey hepatization, were common.

Again dealing with the pathology of plague in Californian wild rodents, Meyer stressed the frequency of lesions in the lymph-nodes as well as a striking tendency towards "latent" infections without the presence of gross features. Meyer also stated that secondary, or even primary, pulmonary localizations were much more common in these animals than in rats. Involvement of the lymph-nodes adjacent to the gastro-intestinal tract—apparently due to gastro-intestinal infection as the result of cannibalism—was, according to Meyer's observations, by no means infrequent.

The diversity of the morbid lesions met with in the plague-affected rodents of California was, in Meyer's opinion, connected with a varying susceptibility of the animals during and after hibernation. Subacute and resolving plague were the prevailing types during winter.

In contrast to the opinion of Kellogg, Meyer found no evidence that the plague strains isolated either from Californian wild rodents or, during the pneumonic epidemics at Oakland and Los Angeles, from human sources possessed any peculiar pneumotropic properties.

**Argentinian rodents**

As maintained by Alvarado & Barrera (see also Barrera), a subacute type of plague without marked gross lesions was prevalent among the various species of *Microcavia*, *Graomys*, and *Galea* involved in the plague outbreaks in Argentina. Instances of inapparent infection, where animal experiments alone yielded a positive result, were met with (Outes). An atypical form of plague was described by Barrera, in which hypertrophy, congestion, and haemorrhages of the testicles were present.
"Inapparent" Plague in Wild Rodents

A form of plague occurring in naturally infected wild rodents and characterized by the absence of gross findings, negative results of direct bacteriological examination, yet positive experimental results with the organs of the animals in question, has been described by workers in the Union of South Africa, south-east Russia, and California (Meyer et al.\textsuperscript{79}), and recently also by Outes\textsuperscript{88} in Argentina and by Baltazard & Mofidi\textsuperscript{5} in Persia. The Russian observers of this type of the disease (variously designated as "plague with invisible lesions", "latent" or "inapparent" plague) advanced the hypothesis that, if the rodents involved became subjected to adverse environmental influences, they might develop bacteremia and, being thus apt to infect their fleas, might then be instrumental in perpetuating the disease.

Thus far no proof for this assumption has been brought forward. In fact, as Baltazard & Mofidi\textsuperscript{5} seem to suggest, it is by no means certain that "inapparent" plague is a type sui generis, because the animals in question might have been either in an initial stage of the infection or in the process of recovery from it.

As will be discussed in chapter 6, definite evidence exists that, in hibernating wild rodents, the infection may remain localized throughout the winter sleep at the portal of entry and/or in the regional lymph-nodes, to become generalized after the animals have awakened in spring. In Pollitzer's opinion, it is so far not justifiable to identify this "latent" type of plague with the "inapparent" form of the infection claimed to exist in non-hibernating rodents.

HUMAN PLAGUE

A classification of human plague has been attempted in various ways, the point of difference being usually the position accorded to the "septicemic" type. As commonly defined, this is a form of the disease where, as in bubonic plague, the causative organisms enter through the skin (or rarely through superficial mucous membranes) but where, owing to an overwhelming infection or to a lowered resistance of the host, the regional lymph-nodes are, as it were, overrun so that an immediate invasion of the blood-stream takes place.

It must be noted, however, that an apparently primary septicaemia may be actually due to a secondary invasion of the blood-stream either because buboes developing in deep-seated lymph-nodes may escape clinical detection (the "hidden" buboes of Wright\textsuperscript{123}) or because, as the result of a rapidly progressing infection, the reactions taking place in super-
ficial regional lymph-nodes may remain so inconspicuous as to be quite overshadowed by the most serious general condition of the patients. It is of great interest to note in this connexion the contention of Girard that what is considered as primary septicaemic plague is really a form of the disease with inconspicuous buboes.

It must be further emphasized that, in contrast to the commonly held beliefs, not only an entry of the infection through the skin but also a direct invasion of the deep parts of the respiratory tract may produce a rapidly generalized type of the disease without conspicuous local reactions. Girard recently spoke in this connexion of a "clinically septicaemic" form of pneumonic plague.

For these reasons it seems preferable to follow the lead of Crowell who distinguished between two main types of human plague only—the primary bubonic and the primary pneumonic (or, more correctly, pulmonary) form. As will be discussed in chapter 8 (p. 439), recently the terms "zootic" and "demic" have been proposed to designate these two main forms of plague.

**Pathology of Bubonic Plague**

**Route of infection**

The skin forms the usual portal of entry in bubonic plague, infection being produced in the overwhelming majority of cases through the bite of infected rodent fleas, rarely through that of other blood-sucking insects or directly through contact with infected rodents, human victims or contaminated inanimate objects. Another rare group of cases are those where infection entered not through the skin but through superficial mucous membranes, such as tonsils and conjunctiva.

**Bacteraemia**

The statements of different observers as to the frequency of bacteraemia in bubonic plague vary considerably, but no doubt can exist that these discrepancies were due in the first place to the different amounts of blood used for examination. In particular, as shown by the Plague Research Commission, microscopical examination of blood smears alone was unreliable. As established by the Commission, severe bacteraemia might be present at a comparatively early stage of the disease, but as a rule its degree was in an inverse relation to the length of time between examination and death of the sufferers. While bacteraemia was thus usually progressive in character, "diminishing", "irregular", and "fluctuating" types were met with. Patients showing bacteraemia might recover.

Later observations confirmed that bacteraemia was by no means always restricted to fatal cases of bubonic plague. Thus Teissier et al. came to the conclusion that, even in mild cases of bubonic plague, an early bacillaemia was nearly always demonstrable and that its presence in the
later stages alone rendered the prognosis unfavourable. Ohoto obtained positive results in 72.2% of his material; bacteraemia disappeared in mild cases as early as the second day and not later than the tenth, while persisting in severe cases. Kirschner, using preliminary cultivation in bile, found bacteraemia in 212 out of 237 definite plague cases; Bonebakker, who also used bile media, in 85% of his bubonic-plague patients. Schoeb stressed the diagnostic value of blood cultures in plague. Gonzaga, coming to the same conclusion, stated that bacteraemia occurred early in ordinary bubonic plague when diagnostic advantage might be taken of its presence.

The results obtained by Jawetz & Meyer, when infecting guinea-pigs, mice or rats intracutaneously or subcutaneously with relatively small, non-toxic doses of \( P. \) pestis, serve as a corollary for the above-recorded observations. As stated by these two workers, their findings "tend to confirm Teissier's claim because an invasion of the blood stream always follows the early lymphatic spread in experimental animals, after the local resistance has been overcome. The initial bacteraemia may last for only a very short time, due to the effective filtering action of the liver and spleen. The filters are overcome and bacteria appear again in the circulating blood only after the massive multiplication of organisms. Active multiplication of \( P. \) pestis in the blood stream is probably always terminal."

**Action of endotoxin**

While, as discussed above, even in apparently uncomplicated cases of bubonic plague a passing bacteraemia seems the rule and this incessantly progresses in cases ending fatally, the important role played by the endotoxin liberated in the course of the disease must duly be taken into account.

As stated by Dieudonné & Otto, the marked prostration and the disturbances of the sensorium conspicuous in severe plague cases are no doubt due to an action of the endotoxin which, according to Girard & Sandor and Girard, exhibits a marked neurotropism.

The widespread haemorrhages and degenerative changes in the internal organs conspicuous in plague victims are likewise the result of a generalized absorption of the endotoxin, while the foci of necrosis associated with bacillary emboli in the organs are ascribed by Petri to local liberation of the endotoxin.

It is interesting to note in this connexion that the German Plague Commission found in three foetuses from plague-infected mothers haemorrhages and parenchymatous degeneration of the organs but no bacilli which, in contrast to the toxin, were apparently unable to pass through the placenta. However, some cases of true intra-uterine infection with \( P. \) pestis have been noted since (Wu Lien-teh et al.; Haffkine, quoted by Wu Lien-teh; Bikov).

Of special importance is the fact that the high concentration of toxin within, and in the vicinity of, the primary buboes leads to haemorrhagic
infiltration of the walls of the neighbouring veins (first noted by Childe\textsuperscript{19}) and consequently to the passage of the bacilli into the blood-stream.

\textit{Morbid anatomy}

\textit{External appearances.} The general appearances noted in plague victims naturally depend a great deal upon the time that has elapsed after death and the temperature prevailing in the interval. Generally it may be maintained that rigor mortis sets in quickly, becomes marked, and lasts for a long time. On the other hand, signs of decomposition in the dead bodies do not develop rapidly, except in cases of mixed infection.

It is striking that, although in the overwhelming majority of cases the skin forms the portal of entry for bubonic infection, as a rule no reaction is noticeable at that site or in the lymph-vessels leading to the primary buboes, which usually form the first manifestation of the process. Sometimes, however, a primary plague pustule or a carbuncle may be present at the site of infection, and inflamed lymph-vessels may be seen leading to the bubo.

Secondary skin manifestations, such as pustules and carbuncles due to an invasion of the skin through the blood-stream, are likewise rare in modern plague outbreaks though they seem to have been more conspicuous in some epidemics than in others. Skin haemorrhages, usually of a petechial character, situated either in the vicinity of the primary bubo or in other skin regions, may not infrequently be detected if properly looked for; Franca\textsuperscript{30} found them in Oporto, Portugal, 46 times in 110 autopsies and also noted carbuncles 11 times (7 of a primary nature), pustules 6 times, and once a pemphigus-like skin affection.

\textit{Lymph-nodes.} The classical studies of Albrecht & Ghon\textsuperscript{1, 2} have led to a distinction between:

\begin{itemize}
  \item \textit{(a)} primary buboes of the first order, developing in the lymph-nodes draining the site of infection and, as a rule, representing the local manifestation of the morbid process;
  \item \textit{(b)} primary buboes of the secondary order, i.e., lymph-nodes situated near to, as a rule proximally from, the originally invaded lymph-nodes or tissues and receiving infection through the lymph-stream;
  \item \textit{(c)} secondary buboes, infected by way of the blood-stream and consequently apt to be present in all types of plague where a septicaemia develops.
\end{itemize}

The primary buboes of the first order are characterized in typical cases by a marked reaction in the surrounding tissues as well as by a process of haemorrhagic inflammation and coagulation necrosis in the lymph-nodes themselves. Many gradations of these morbid processes are met with.
One or several lymph-nodes may be affected; in the latter case the individual nodes are often matted together, and with the surrounding tissues, so that considerable swellings are produced.

All degrees of inflammation may be present in buboes according to the intensity of the process and the length of illness, varying from simple lardaceous or medullary swelling to gelatinous infiltration and bloody infarction, and from softening and liquefaction to pus formation and complete necrosis (Dieudonné & Otto). The degree and extent of the reaction around the glands may also vary considerably, though usually marked oedema and infiltration are present which may involve, in addition to the surrounding connective and adipose tissue, other structures such as veins (see above), fasciae, and muscles.

The primary buboes of the secondary order may show similar changes to those described above but, as a rule, the reaction in the surrounding tissues is less marked in degree and extent.

The secondary buboes are usually smaller in size than the primary ones; with the possible exception of some oedema, the process is limited to the lymph-nodes themselves which may show congestion, medullary swelling, and discrete haemorrhages.

As will be gathered from the above descriptions, inconspicuous primary buboes may show appearances macroscopically indistinguishable from those exhibited by secondarily invaded lymph-nodes.

**Mouth and fauces.** Generally speaking, no characteristic changes of the oral and faucial mucosa are found in plague. However, some congestion and also signs of cyanosis may be present, while the papillae at the base of the tongue and the pharyngeal follicles may be swollen. The tonsils as a rule show corresponding changes but sometimes they are the seat of marked inflammation and ulceration with membrane formation.

Though processes of this kind met with in plague victims need not necessarily be of a specific nature, no doubt exists that *P. pestis* may produce such lesions. However, the tonsillar involvement is by no means always of a primary nature; often the infection, entering in the usual manner through the skin, leads first to the formation of cervical buboes from which the process secondarily spreads to the tonsils (de Sousa, Crowell). Martinez Vinueza distinguished accordingly between primary and secondary anginose plague.

**Respiratory system.** The lungs may be invaded in bubonic plague by different routes. In cases where the tonsils are affected, a pneumonic process may be produced by aspiration; Albrecht & Ghon, for instance, saw two such cases in a series of 44 autopsies.

It should be noted, however, that tonsillar plague by no means invariably leads to such secondary localization of the infection in the lungs. Thus de Sousa stated that faucial involvement may remain localized
and clear up, the sputum never containing numerous plague bacilli. Crowell 22 found that in 11 cases with pharyngeal lesions, lung involvement was present seven times.

A number of observers laid great stress upon a direct spread of plague infection from cervical or axillary buboes to the lungs (see Wu Lien-teh 124). Gaud & Jorge 31 maintained in this connexion that, among the five groups of axillary lymph-nodes, the thoracic or "subpectoral" one was most often involved in plague, and as these lymph-nodes were in communication with the intrathoracic lymphatics, a direct invasion of the lungs was likely.

Though due attention should certainly be paid to a direct invasion of the lungs from cervical or axillary buboes, its importance should not be exaggerated. Secondary lung involvement is by no means restricted to cases with axillary or cervical buboes. On the other hand, localization of the infection in the axillary or cervical lymph-nodes is by no means invariably followed by secondary pneumonia.

Thus Crowell, 22 who paid special attention to this point, found pneumatic changes in only six out of ten cases with cervical buboes, and concluded that there was no constant relation between the lesions in tonsils, cervical lymph-nodes, and lungs. Wu Lien-teh 124 also drew attention to the fact that cervical buboes sometimes predominated in mild outbreaks of plague. It is also noteworthy that axillary buboes were frequent in Transbaikalia, especially in patients infected through tarabagan fleas or through handling infected rodents, and yet the incidence of secondary lung involvement was not particularly high.

Far more important still than such considerations is the fact that the lung lesions usually found in bubonic plague, and carefully studied by numerous investigators, could as a rule be definitely ascribed to an invasion of the lungs through the blood-stream.

Statements as to the frequency of secondary lung involvement by this usual route vary much and it is difficult to collate them because they are based upon different methods of clinical or postmortem examination. It has been maintained with much reason that some involvement of the respiratory tract is usually, if not invariably, present in bubonic infections followed by bacteraemia, varying in degree from simple catarrh to grave forms of congestion and specific bronchopneumonia. Di Mattei, 75 a strenuous advocate of this idea, could demonstrate P. pestis in the sputum of patients in whom no definite signs of pulmonary localization could be detected, and even recommended sputum examination as a suitable means for the early bacteriological diagnosis of bubonic plague infection.

**Circulatory system.** Frequently, subserous haemorrhages are found on the inner layer of the pericardium and the epicardium. Some accumulation of liquid in the pericardial sac (usually clear and without admixture of blood) is quite common.
The heart, especially on the right side, is usually distended and filled with dark-red blood showing little tendency to coagulate. Haemorrhages under the endocardium and the intima of the valves are occasionally noted. The heart muscle constantly presents alterations corresponding to cloudy swelling or fatty degeneration.

The conspicuous changes taking place in the veins located in the vicinity of buboes have already been noted (page 206). Small haemorrhages are frequently found under the intima of the veins in general. Vint 115 stressed that the haemorrhages found in plague victims were jet-black in colour, thus contrasting with the bright-red petechiae usually met with in septic conditions due to other causes.

Spleen. The spleen, though usually enlarged, is in a considerable minority of cases not much more than normal in size. It is further noteworthy that in plague the organ is often distinctly firmer in consistency than in other infectious diseases. It would seem that both enlargement and softening are more marked in instances of rapidly progressing infection; this holds true of cases with inconspicuous buboes as well as of instances of respiratory infection where no manifest pneumonia develops.

The capsule of the organ is tense, and more or less opaque; subcapsular haemorrhages are frequent. The substance of the spleen shows some congestion. The follicles, if at all visible, are small, not rarely surrounded by a ring of intensely congested tissue, the trabeculae usually not prominent. Anaemic infarcts are not infrequent.

Liver. The liver is often enlarged and, as a rule, more or less congested. Small haemorrhages may be present below the capsule. Usually there is evidence of cloudy swelling or early fatty degeneration. Sometimes a nutmeg appearance has been noted, but it seems an open question whether this is due to venous congestion or to an acute degeneration of the liver cells round the hepatic veins (Wu Lien-teh & Woodhead 128).

Besides these generalized alterations, many observers described nodes or spots of a yellowish colour either on the surface or in the parenchyma of the liver, due apparently to a localized fatty degeneration or to necrosis.

Kidneys. The kidneys show more-or-less advanced degenerative changes, sometimes true nephritis. Haemorrhages are frequently noted on the inner layer of the capsule and in the pelvis, more rarely on the surface of the organ. Smaller necrotic foci have been observed but are infrequent.

Gastro-intestinal tract. In the overwhelming majority of autopsies no marked changes are found in the gastro-intestinal apparatus. Haemorrhages beneath the peritoneal coats are comparatively rare, but are frequently noted beneath the mucosa of the stomach and intestines. Not rarely, hyperaemia of part of the mucosa of the stomach or intestines, or even congestion of some portion of the intestines throughout all layers, is
present. A catarrhal condition, sometimes accompanied in the intestines by a pinkish excretion, is not infrequent.

Advanced changes, especially ulcerations, were described by a few observers only. In some of these cases at least it is doubtful whether such unusual lesions were actually specific in nature.

**Brain and meninges.** In addition to the congestion and oedema of the brain and meninges usually met with at autopsy, instances of plague meningitis have been described by several workers. Although the meningeal process was usually due to a secondary invasion through the blood-stream subsequent to bubonic infection, a few authors (Lafont et al.; Williams; Lewillon et al., and Landsborough & Tunnell) claimed to have observed a primary form of plague meningitis. One cannot help feeling, however, that in these instances ill-defined or hidden buboes might have escaped detection.

Landsborough & Tunnell described the cerebrospinal lesions found at autopsy of their case as follows:

Brain congested, convolutions and sulci flattened, tissue soft; thick, canary-yellow, fibrino-purulent exudate covering most of the external surface of the brain; ventricles, especially the lateral ones, dilated and containing plenty of turbid fluid; spinal cord showing marked congestion but less exudate except in the region of the cauda equina.

As mentioned before, a very unusual case of chronic relapsing latent plague meningitis was described by Meyer et al.

**Pathology of Primary Pneumonic Plague**

Since, as has been mentioned before (page 207), plague infection entering through the deep parts of the respiratory system does not invariably lead to the formation of pneumonic foci but may result in a rapidly generalized infection with only insignificant changes (particularly congestion and oedema of the lungs) in the respiratory tract, it would be preferable to speak of "pulmonary" rather than "primary pneumonic" plague—the more so because not infrequently it is doubtful whether authors dealing with the problems of "pneumonic" plague refer to the primary type or to secondary lung invasions taking place through the blood-stream. On the other hand, the term "primary pneumonic plague" has become so familiar that one must be hesitant to replace it, desirable though this would be.

**Pathogenesis**

While most investigators believe that primary pneumonic (pulmonary) plague is caused by an infection entering through the deeper portion of the respiratory tract, a few, especially Koulecha, considered the tonsils or other parts of the upper respiratory tract as the portal of entry of the infection which then reached the lungs through the blood-stream. As
discussed earlier (page 207), experimental observations did not support this "tonsillar" theory and the same holds true of the findings in man:

(1) In instances where an infection initially entering the tonsils led to pneumonia, cervical buboes, if not pre-existent, invariably developed in the course of the disease. In primary pneumonic plague the cervical lymph-nodes showed slight or even no changes. In the experience of most investigators, the lesions in the upper respiratory tract as well were, as a rule, slight and obviously secondary in nature.

(2) As shown by the work of Girard, who made parallel examinations of the sputum and blood cultures in a series of pneumonic cases, plague bacilli were present in the former before they could be demonstrated in the blood. Identical findings were recently recorded by Wagle & Bedarkar, while Wu Lien-teh and, more recently, Hennessey referred to instances of primary pneumonic plague where even post mortem no bacteraemia was demonstrable.

(3) Histological examinations also convinced most investigators that the lung lesions in primary pneumonic plague evolved initially and were followed, instead of being preceded, by an invasion of the blood-stream. Specially noteworthy is a record of Jettmar concerning a patient who committed suicide early in the disease. Here the blood-stream was still free from plague bacilli and it could be seen that these micro-organisms were just on the point of penetrating into the blood-vessels through the lymph spaces.

Nattan-Larrier & Richard came to the conclusion that, in man as in experimentally infected animals, a bacterial alveolitis was the characteristic feature of primary pneumonic plague, the infection propagating from the respiratory lumina towards the lymphatics.

Pathology

Pneumonic plague is distinguished from the bubonic type by a more rapid course and—except in patients treated with the now available potent remedies—by an almost invariably fatal termination.

In order to explain these differences it must be kept in mind that in bubonic plague the defensive mechanisms of the body are apt to prevent, or at least to retard or modify, a massive generalized infection. Considering the problem of secondary pneumonic plague in particular, Heckenroth maintained that this type of lung involvement was caused by plague bacilli attenuated in virulence by the antibodies produced in the primary bubo.

In primary pneumonic plague, on the other hand, the initially invaded area is devoid or nearly devoid of defensive means (Wu Lien-teh) so that the morbid process can spread without hindrance, leading to an enormous agglomeration of the bacilli in the lungs. Even apart from the fact that bacteraemia, though undoubtedly secondary in nature, develops fairly
soon, a most ominous role is played by an early and massive absorption of endotoxins, van Loon\textsuperscript{67} aptly comparing the condition in primary pneumonic plague to that in acute peritonitis. Wu Lien-teh\textsuperscript{124} was therefore right in maintaining that the surprising thing was not that so many patients succumbed to the former disease but that even a few should recover.

\textit{Morbid anatomy}

In dealing with the morbid anatomy of primary pneumonic plague, it is sufficient to consider the lesions in the respiratory tract only, because appearances in the other organs are identical with those seen in victims to bubonic infection.

\textit{Upper respiratory passages.} The changes found in the upper respiratory passages of victims to primary pneumonic plaque are usually not prominent, thus being similar to those generally noted in the bubonic type.

\textit{Trachea and bronchi.} In well-marked cases the mucosa of the trachea and bronchi is markedly congested. The mucous membrane of the trachea is covered by a blood-stained, sometimes frothy, exudate. The bronchi occasionally show a similar covering; more often they contain a bloody and frothy serous fluid. Submucous haemorrhages are occasionally seen.

More advanced lesions are rare, but small necrotic patches were sometimes noted. On the other hand, in less characteristic cases the trachea may be pale and may contain non-bloody mucus or mucopurulent secretion, sometimes of a brownish colour. In cases without manifest pneumonia, secretion may be totally absent, but some degree of congestion is invariably noted.

\textit{Pleurae.} Recent fibrinous adhesions between both layers of the pleurae are frequent, but are generally present over the inflamed parts of the lungs only. The presence of any quantity of serous exudate, whether blood-stained or not, is comparatively rare.

Fibrinous deposits are often present over the surface of pneumonic areas, though varying much in extent and appearance. Purulent exudate, on the other hand, seems to be practically absent except in cases complicating pre-existing tuberculosis.

Haemorrhages of different sizes, rarely present beneath the parietal layer of the pleura, are very often noted beneath the visceral pleura. Sometimes they are scattered all over this or localized at areas free from pneumonic changes. In well-marked cases they are often concentrated above superficial pneumonic patches and form, together with the fibrin deposits, a rather characteristic pattern of red and yellow dots and bands, first described by Albrecht & Ghon.\textsuperscript{1, 2} However, insignificant changes may be present even over superficially situated pneumonic foci while in the case of their more central localization macroscopic alterations of the pleurae may be altogether absent.
Lungs. As postulated by workers such as Vint and Wright, a distinction may be made between three types of lung manifestations met with in primary pneumonic or, one should rather say, pulmonary plague:

(a) A form in which acute congestion and usually also lung oedema are marked but where, though plague bacilli abound, no consolidation is present;

(b) A type characterized by the presence of lobular pneumonic foci;

(c) A form where lobar involvement seems to be present.

Wright also referred to a fourth type characterized by the presence of scattered and apparently necrotic nodules but this process, also referred to by Vint and by Hennessey, was obviously secondary in nature, being due to an embolic invasion of the lungs through the blood-stream.

Hennessey maintained that whereas formerly instances of “broncho-lobular” pneumonic plague had predominated in Uganda, since 1933 the apparently lobar form was preponderant. He believed that the prevalence of this type was due to a loss of virulence of the local plague strains which had become manifest at the same time. It must be emphasized, however, that most workers who had access to sufficiently large material, noted the simultaneous presence of cases with lobular and cases with so-called “lobar” pneumonic changes, during the pneumonic-plague epidemics they observed. More than that, though it is convenient to adopt for descriptive purposes the classification given above, actually no sharp line of demarcation can be drawn between the three types of morbid appearances, a transitory group with slight pneumonic lesions standing between the first and second while, as discussed below, the so-called lobar form represents merely an advanced stage due to the confluence of originally lobular pneumonic foci.

The lobular foci encountered in primary pneumonic plague may be situated either near the surface or within the lobes of the lungs. One or several such patches may be present in one lobe; sometimes they were found to be arranged along one bronchus like “the flowers of the hydrangea” (Strong).

When situated near the surface, the lobular foci bulge somewhat and the adjacent pleura shows frequently the peculiar changes described above. On cut section, the foci appear wedge-shaped or round and are often surrounded by a zone of engorgement and oedema. Their colour is usually either dark or greyish-red in the centre with a deep-red periphery; only in rare instances do they show a uniformly grey colour. As in pneumonic plague in general, the cut section does not show the definite granulation typical of croupous pneumonia; no fibrin can be scraped off, but only a uniform greyish-white fluid. The foci situated away from the surface of the lungs are, as a rule, roundish in shape. They display in well-marked cases the same characteristics as the superficial foci, but in instances with slight pneumonia they may be ill defined.
In the opinion of most observers, the so-called lobar consolidations found in primary pneumonic plague are due to a confluence of originally lobular foci. It is therefore not surprising that grey hepatization of a whole lobe, so frequently seen in croupous pneumonia, is never met with in pneumonic plague and that even extended areas of red hepatization are rarely present. As a rule the extensively affected lobes show on cut section a variegated aspect, areas of grey hepatization adjoining those with red hepatization and these in turn shading into parts of the lung tissue which are merely in a stage of engorgement (Strong\textsuperscript{110}). Both lobular foci and more extensive pneumonic areas may be present side by side in the same lung or even lobe.

**Bronchial lymph-nodes.** Although in some cases the bronchial lymph-nodes may exhibit little or no change, they are usually markedly enlarged, soft, and hyperaemic; on cut section they may show punctiform ecchymoses or a general deepening of colour as a result of diffuse haemorrhage. Koulecha\textsuperscript{60} was of the opinion that the affected lymph-nodes finally displayed the appearances of Albrecht & Ghon’s primary buboes of the secondary order.

The tissues surrounding the lymph-nodes at the bifurcation of the trachea are often oedematous and show haemorrhages, but advanced periadenitis is absent. The oedema with extensive haemorrhages, frequently noted by Strong\textsuperscript{110} during the 1910-1 Manchurian epidemic, in the anterior mediastinum round the thymus gland was not conspicuous in the victims of primary pneumonic plague dissected by Pollitzer during the 1920-1 outbreak.

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Chapter 5

METHODS OF LABORATORY DIAGNOSIS

EXAMINATION OF RODENTS AND FLEAS

Before discussing the laboratory methods available for the detection of plague in individual rodents, it should be pointed out that a properly functioning intelligence service is of outstanding value for this work, since the laboratory staff is thus kept informed of the occurrence and extent of unusual rodent mortality. In the case of the commensal rodents in particular, the finding of an appreciable number of animals which have succumbed spontaneously may ordinarily be taken as prima facie evidence for the existence of a plague epizootic, while marked scarcity of live rodents in a locality which previously showed heavy infestation is indicative of a past major epizootic. In view of what has been discussed in chapter 4, every possible effort must be made to examine the rodents found dead because they offer the strongest chance of obtaining frankly positive evidence of infection. This point is well illustrated by the recently published observations made during the period 1940-50 by Gross & Bonnet in the Hamakua district of Hawaii. These workers detected plague 358 times in 4,808 rodents found dead as against 23 times in 20,623 killed animals and 20 times in 304,807 trapped rodents.

Evaluation of Methods

The principal methods used to detect plague infection in rodents are as follows:

1. autopsy;
2. bacteriological tests (smears and cultures);
3. serological tests;
4. animal experiments with material from individual rodents or with cultures isolated from them.
(5) animal experiments with pooled organs;
(6) animal experiments with pooled fleas.

Autopsy

Bearing in mind that the pioneer work of the Plague Research Commissi­on was done at the time when acute plague was rampant among the rats of India, it is not surprising to find that the Commissioners, comparing in 1907 the various methods available for the diagnosis of rodent plague, emphasized the outstanding value of the macroscopic findings. In the opinion of the Plague Commission, it was justifiable to diagnose the disease in rats on the basis of the presence of even one of the pathognomonic gross signs, regardless of whether or not subsequent smear examinations, culture tests, or animal experiments yielded positive results.

It is no longer possible to evaluate the results of macroscopic examina­tions as highly as the Plague Research Commission did—not only because animals may be found which, for the reasons set forth in the preceding chapter, show no gross signs of the infection, but also because observations made outside India have proved that morbid appearances macroscopically indistinguishable from plague may be produced by infective agents other than Pasteurella pestis. It is noteworthy in this connexion that, in the experience of Macchiavello at Antofagasta, Chile, rats which afterwards proved to be plague-infected were less apt to show suggestive gross signs than a large number of macroscopically suspect but actually plague-negative animals.

Nevertheless, the presence of signs such as typical buboes, a characteristic mottled or waxlike appearance of the liver, and clear pleural fluid has been found to be strongly suggestive in most plague areas; as has been noted before, frequency of rats showing such pathognomonic signs proves the presence of an acute epizootic, while their rarity is suggestive of a quiescent stage of the infection. In order to make reliable macroscopic observations, it is necessary, however, to perform dissection before putrefaction has set in.

Bacteriological tests

While subsequent workers, following the lead of the Plague Research Commission, continued to lay stress upon the direct results of rat dissection, they usually considered it necessary to confirm the diagnosis with the aid of laboratory methods, especially smear examination and culture tests.

Although, as will be discussed later (page 237), great care has to be exerted in interpreting the results of smear examination, the abundance of characteristic bipolar-stained bacilli or involution forms in smears prepared from the organs of the dissected rodents goes a long way towards supporting a positive diagnosis in the course of outbreaks previously confirmed by fully reliable methods.

Culture methods are fully satisfactory whenever it is possible to examine fresh material, but the presence of putrefaction renders it difficult or even
impossible to obtain reliable results from the buboes or internal organs of plague-suspect animals by such tests. However, the possibility of proving the existence of plague in putrified rodents was greatly increased by the recommendation—first made in 1926 by Pons (quoted by Murdock \(^{81}\)), then by Fusco (quoted by Devignat \(^{23}\)) and Micheletti,\(^{78}\) and again independently by Uriarte et al.\(^{111}\)—that in such cases the bone-marrow of the tibia or femur, rather than the internal organs, should be used for bacteriological examinations.\(^a\)

While the great value of this method was confirmed by most field workers and also through experiments made with rat and guinea-pig carcasses by Russo,\(^{93}\) a few investigators came to less satisfactory conclusions. Macchialvello & Paracampos\(^{73}\) in particular, working in the hot climate of north-east Brazil, had many failures with this procedure and expressed an urgent warning against the exclusion of the possibility of plague on the grounds of negative results thus obtained. They admitted, however, that the impossibility of cultivating \(P.\) pestis from many of their rodents appeared to be largely due to the fact that the plague strains prevailing in north-east Brazil were often too attenuated to cause septicaemia. Bone-marrow cultures made from animals suffering from acute or subacute plague were invariably positive and less contaminated than those from internal organs. Moreover, since the viscera of dead rodents were apt to be destroyed by insect larvae, bone-marrow examination was often the only method practicable for laboratory investigation.

**Serological tests**

Since serological tests yield results far more rapidly than do culture tests, they are of outstanding value for the confirmation of the prima facie evidence obtained from dissection and smear examination. The methods suitable for such tests have been referred to in Chapter 3 and are dealt with in greater detail on page 243.

**Animal experiments**

**Individual tests.** Animal experiments performed with the organs of individual rodents or with the growths isolated from them yield fully reliable results if conducted in an adequate manner. They are, therefore, of the greatest value in confirming the diagnosis both in incipient plague epizootics and in sporadic instances of rodent infection. However, it is hardly possible to perform experiments with material from each individual animal during the routine examination of large numbers of rodents.

\(^a\) Petragnani,\(^{44}\) while endorsing the value of bone-marrow examination, considered it still more advantageous to cultivate the brain of putrefied rats in a medium consisting of eight parts of ordinary and two parts of liver bouillon.
Pooling-tests.

(a) Organs: A most important innovation, permitting of the large-scale use of animal experimentation for the diagnosis of rodent plague, was introduced by Chapin 19 in 1909. Finding that many of the rats dissected by him at Seattle, USA, were too putrified to show gross lesions, and wishing not to overlook instances of "inapparent" infection, he resorted to cutaneous inoculation of guinea-pigs with the organs of not one but a whole group of rats. Chapin himself, perhaps because he used "the organs of every rat of an entire catch . . . sometimes 30 or even more in number" to infect single guinea-pigs, failed to obtain positive results in the 400 rats tested in this manner. However, the method soon afterwards proved successful in the hands of other workers, such as Lloyd 11, 68 (also at Seattle) in 1914 and Creel 11 at New Orleans in 1915, and has been much used since.

Swellengrebel & Hoesen 104 seem to have been the first to replace Chapin's method by subcutaneous infection of guinea-pigs with suspensions obtained by collecting small pieces of the organs of the rats to be examined in a sterile mortar and grinding up this material with a little sterile saline. Handling in this manner 7,625 apparently normal rats, the two workers were able to detect plague in 54 pools.

The value of this method, which has remained one of the standard procedures for ascertaining the presence of plague in commensal rats as well as in wild rodents, has been well demonstrated by the following results reported at the 1934 Calcutta Conference of Medical Research Workers (quoted by Pollitzer 89):

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of animals used or group tests</th>
<th>Number of animals found smear-positive</th>
<th>Number of groups tested</th>
<th>Number of positive group tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rattus rattus</em></td>
<td>2,107</td>
<td>2</td>
<td>141</td>
<td>13</td>
</tr>
<tr>
<td><em>Mus musculus</em></td>
<td>1,393</td>
<td>-</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td><em>Bandicota malabarica</em></td>
<td>306</td>
<td>1</td>
<td>95</td>
<td>15</td>
</tr>
</tbody>
</table>

These results are all the more noteworthy because rodents showing suggestive signs of infection were not included in the group tests. Cultures made from the suspensions prepared for guinea-pig infection were often unsatisfactory.

An interesting method, taking advantage of bone-marrow examination as well as of pooling, was used by Devignat 25 in the course of monthly rodent surveys in the Belgian Congo. The procedure consisted in cutting off the legs of freshly killed rodents (mainly *Rattus natalensis*) just above the knee, aspirating the bone-marrow with the aid of a syringe provided with a suitably thin and short-bevelled needle, and suspending the material in a bottle containing 3 ml of sterile normal saline. Repeating this operation with each animal of the daily catch, it was thus possible to
obtain a pool containing material from up to 500 rodents. The suspension was immediately used for subcutaneous inoculation of a guinea-pig, which was then dispatched to the laboratory. In the case of animals the bones of which were too small to yield bone-marrow, the spleens were successively used for cutaneous inoculation of a guinea-pig.

Practising these methods, Devignat repeatedly succeeded in demonstrating the existence of plague in rodents which otherwise appeared to be free from the infection.

Van Riel & Mol,\textsuperscript{92} working in the Kivu region of the Belgian Congo, also obtained good results with bone-marrow pools. They used the subcutaneous method for infecting guinea-pigs in the field, but preferred the cutaneous route when making tests in the laboratory.

It deserves attention, however, that the experiences made by Heisch\textsuperscript{53} in the Rongai area of Kenya, where inapparent plague seemed to be prevalent among the wild rodents, did not confirm the value of the above-described method. Heisch referred in particular to one instance, where the pooled spleens, bone-marrow, and fleas collected from a batch of \textit{Arvicanthis} had been used separately for the inoculation of different guinea-pigs, and positive results had been obtained only with the spleens. He urged, therefore, that pools from the internal organs rather than those from the bone-marrow should be used to demonstrate the presence of inapparent rodent plague and recommended in particular the technique adopted by Meyer et al.,\textsuperscript{77} who inoculated their test guinea-pigs with material collected from not more than five plague-suspect ground-squirrels.

\textit{(b) Fleas :} Several observers like Kitasato, the Plague Research Commission, Petrie & Todd and Jettmar (quoted by Pollitzer\textsuperscript{89}) had shown that while, particularly at the end of an intense epizootic, it was often difficult or even impossible to find plague-affected rodents, it was usually comparatively easy to collect infected fleas for laboratory tests. However, since smear examination and culture methods are, as a rule, rather unsuitable for demonstrating the existence of the infection in these insects, it was often necessary to resort to animal experiments in order to get reliable results.

An early attempt to use pooling methods when examining fleas was made by Swellengrebel & Hoesen\textsuperscript{101} who, working with 15,279 fleas, obtained positive results with 18 pools. However, the method attracted no attention until it was once more applied in 1936 by Eskey\textsuperscript{32} to detect foci of wild-rodent plague in California. Since then, ample use has been made of this procedure in order to ascertain the presence of the infection in commensal rats as well as in wild rodents.

No doubt can exist that the large-scale use of one or preferably both of the above-mentioned pooling methods is alone fully reliable for deciding whether or not plague is present in a given rodent population.
**Comparison.** Assessing the value of pooling-tests in comparison with other methods of rodent and flea examination, it must be realized that, paradoxical though it may seem at first glance, the extreme sensitivity of the pooling-tests limits their usefulness. Being apt to prove positive in the case of pools containing merely a few, or possibly even single, virulent plague bacilli, they furnish a reliable answer to the question of whether plague is present in the rodents or fleas examined, but do not show to what extent the infection is present. Although this second point may be rather immaterial under certain circumstances—for instance when making large-scale wild-rodent surveys—it is of paramount importance when dealing with plague manifestations in individual localities, where the proper conduct of anti-epidemic work depends on full awareness of the trend of infection. Under these circumstances, to neglect the methods available for the examination of individual rodents in favour of pooling-tests would be erroneous.

As has been noted above, the method of using flea pools has the great advantage of being applicable even when the number of rodents has become much reduced by an extensive epizootic. Moreover, since fleas are not merely vectors, but also to a considerable extent reservoirs of plague, positive results with flea pools may be obtained during the off-seasons, when it may be difficult or even impossible to prove the continued existence of rodent infection. It must also be admitted that examination of flea pools is more expedient and less dangerous than that of tissue pools. It is therefore not surprising to find that some workers place sole reliance on the former method, considering the routine examination of tissue pools to be generally superfluous. Humphreys et al.\textsuperscript{57} recently supported this opinion because they found that parallel examination of 5,019 tissue pools and 4,641 flea pools yielded only three instances in which the former proved positive and the latter negative. However, pointing out that in two of these three instances only one flea per animal had been obtained, Humphreys and his co-workers admitted the value of tissue-pool tests in cases where the rodents concerned were free, or nearly free, from ectoparasites. It must be realized as well that positive results with flea pools may prove misleading in so far as the rodents which appear to be concerned may have picked up fleas from plague-susceptible species without suffering from the disease themselves. It would appear, therefore, that examination of tissue pools, since it serves to check the results obtained with flea pools, should be used parallel with flea-pool tests periodically at least, if not as a matter of routine.

**Techniques**

Dealing with the technique of rodent examination, it seems permissible to concentrate attention on the methods suitable for commensal rats,
which in most plague areas form the bulk of the material to be handled; the more so because the procedures to be adopted in the case of other rodent species do not differ in principle from those required for rat investigation.

Collection and delivery of rats

If rats found dead, or killed by the people, are available for examination, they should preferably be put into individual paper bags or be individually wrapped in paper for transport to the laboratory.

An alternative adopted in China was to place large tin buckets or wooden boxes with lids, or large, covered earthenware jars, at strategic points of the plague-affected settlements. The people were urged to place the dead rodents in these collecting vessels, which were inspected twice daily; the rodents found were taken to the laboratory in covered tin boxes. In plague-affected localities where the people were un-co-operative, it was possible to obtain dead rats by paying rewards to the scavengers for the delivery of carcasses found by them in refuse.

Rats caught in snap traps should be transported to the laboratory in individual paper or canvas bags. An adequate procedure recently recommended in this connexion by Gross & Bonnet was as follows: taking care not to touch the animals, the trappers freed them by picking up the trap by the base and releasing the striker, allowing the rodent to fall into a suitable receptacle. If it was proposed to examine the fleas, the retrieved rodent was placed in a paper bag and one-quarter of a teaspoonful of calcium cyanide was inserted; the bag was then shaken and tightly tied by the neck. Otherwise the trapped rodents were put into gallon-cans containing kerosene.

Cage traps in which rats have been caught alive should be enclosed in canvas bags for transport to the laboratory.

Killing rats trapped alive

Although, as discussed by Omar, a considerable number of procedures has been recommended for killing rats delivered live to the laboratory, the following deserve prime consideration:

Submersion. While the method of submerging the rodents in water or other fluids is the least attractive of the available procedures, it is cheap and effective, the more so as it facilitates collection of the fleas harboured by the rodents. The use of antiseptic fluids in place of pure water offers no essential advantages and must be avoided when it is planned to use the fleas for culture examination or pooling-tests.

Volatile fluids. A simple and effective method for killing rats in cage traps enclosed in canvas bags is to put each bag with the trap inside into a suitably sized box or tin, partly to open the bag and to push in a cotton pad
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soaked in a volatile fluid such as chloroform, ether, or gasolene. The box or tin is then kept closed for about 20 minutes, a time sufficient to kill the rat and at least to stun its fleas. Gasolene is the cheapest of the suitable volatile fluids and is also preferable in so far as, according to Eskey & Haas, chloroform or ether is apt to exert an adverse influence on the infectivity of the fleas.

*Lethal gases.* Trapped rats as well as their fleas may be conveniently killed with the aid of calcium cyanide. For this purpose the cage traps in their canvas bags, which should first be opened up, are put into a tin or wooden box provided with a well-fitting cover and an opening suitable for pumping in the dust after the receptacle has been closed.

The rats and their fleas are killed rapidly and, if a tin of sufficient size is provided, several traps may be dealt with at the same time.

Calcium cyanide is also suitable for dealing with dead rats delivered to the laboratory, particularly those not enclosed in bags. For this purpose a layer of the chemical is put into a large tin bucket provided with a well-fitting cover. The rat carcasses are placed on the calcium cyanide and should preferably be kept in the closed tin overnight. Before they are taken out in the morning, the tin should be left open for at least 15 minutes. In the case of an acute epizootic, calcium cyanide may be applied once more before ventilation of the tin.

To avoid accidents, these methods should be applied in the open air by workers trained in their use.

*Collection of fleas*

Before carrying out dissections, it is necessary to free the rodents from fleas and other ectoparasites. This can be easily done by thoroughly brushing and combing all parts of the fur of the carcasses. These operations are best performed either in a large, deep, white enamel pan or while holding or suspending the rats over a large, white enamel basin filled with water. Before starting the work, the rats may be first plunged into the water; some workers prefer to keep them submerged while they are brushed and combed.

The fleas and other ectoparasites collected from each rat should be put into a separate stoppered ampoule to await classification and indexing.

*Dissection and preparation of material*

It would be redundant to deal in detail with the technique of rat dissection, which is performed according to the methods generally adopted for the autopsy of small laboratory animals—although, as is described on page 245, with special precautions. However, attention must be drawn to the necessity of examining with special care the lymph-nodes of plague-suspect carcasses. The recommendation of the Plague Research Commission that
the pectoral muscles should be cut so as to make the axillary lymph-nodes visible has been referred to in Chapter 4, and the advice of the Commission to inspect the cut surface even of lymph-nodes which appear to be unaffected externally has also been noted. In addition to the superficial lymph-nodes, the deep cervical, mesenterial, retroperitoneal, and pelvic lymph-nodes should receive attention.

Smears or impression films should be made not only from abnormal lymph-nodes but also from the heart-blood, spleen, liver, and lungs, all of which should be placed on one slide in order to save labour and material. Cultures and material for animal experiments or pooling-tests should be taken from any bubo present as well as from one or more of the above-mentioned internal organs, or, in the case of putrefaction, from the bone-marrow.

Recording of results

As recommended in the antiplague instructions published by the Office International d'Hygiène Publique in 1937, daily records should be kept showing (a) the number of rats and other rodents examined, classified by species; (b) the number of males, females, and pregnant females in each species; (c) the number of rats found infected in each species.

These figures and their totals are used to calculate plague indices, i.e., the percentages of rodents found infected in each species and in the totals. If, as is advisable, graphs based on these values are prepared, it is preferable to use for this purpose the five-day averages instead of the daily figures (Shih & Pollitzer 96).

Flea examination

While animal experiments with pooled fleas are of outstanding value in plague work, possibilities for taking advantage of bacteriological methods for the examination of plague-suspect fleas are rather limited. It has been stated by Webster 118 that smears of individual crushed fleas may be stained and examined for the presence of plague-like bacilli, but only a proportion of the plague-infected fleas will be detected thus, and the morphology is not really conclusive. Studying the suitability of culture methods for detecting plague in fleas, Tiflov 106 worked with suspensions prepared by crushing 26-100 living wild-rodent fleas (Nosopsyllus consimilis or Crenophthalmus wagneri), each batch containing but one infected flea. Results obtained on the one hand by injecting part of the suspensions intramuscularly into guinea-pigs, and on the other by cultivation were as follows:

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of tests made</th>
<th>Number of positive results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea-pig infection</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>Cultivation on agar</td>
<td>39</td>
<td>33</td>
</tr>
<tr>
<td>Cultivation on blood-agar</td>
<td>39</td>
<td>37</td>
</tr>
</tbody>
</table>
However, results obtained by most other workers when using suspensions prepared by crushing fleas for cultivation were so disappointing that it was recommended that the proventriculus and stomach of plague-suspect fleas should be dissected out and the contents used for cultivation. Satisfactory results were obtained in this way both with individual fleas (Gore 47) and when pooling the stomach contents of 60-70 fleas (Bichkov & Borzenkov 19), but this procedure is far too elaborate to be suitable for routine work. Animal experiments therefore remain the method of choice for routine examination of plague-suspect fleas.

EXAMINATION IN HUMAN PLAGUE

Patients

Bubonic plague

Puncture. Although ample use has been made of bubo puncture to collect material for the bacteriological diagnosis of bubonic plague, various objections have been raised against this method.

Some workers pointed out that in the acute stage of the disease it was difficult, with the aid of this procedure, to obtain sufficient material for examination. It must be noted, however, that this difficulty may be easily overcome if, as recommended by Girard,39 the needle and syringe are well washed out after puncture with a little saline and the latter is then used for the tests. The alternative, sometimes suggested, of first injecting a few drops of sterile saline or broth into the substance of the bubo and then aspirating, is less recommendable—if for no other reason than that this procedure considerably adds to the discomfort of the patient.

Another objection was that even in the acute stage of the disease the method was apt to yield negative results if, instead of the affected lymph-nodes themselves, the often conspicuous oedema hiding them were punctured. It was also pointed out that since, in the course of recovery, the causative organisms in the affected lymph-nodes first became scanty and then altogether disappeared, punctures carried out late in the disease often failed to confirm the diagnosis of plague. This held true particularly of suppurated buboes.

The fear was moreover expressed that puncture of plague buboes might prove harmful by facilitating entry of the infection into the lymph- or bloodstream. It is difficult to agree with this opinion if it is considered that extirpation of plague buboes has been successfully practised to treat the disease; it must, however, be admitted that the operation is rather painful.

b As aptly pointed out by de Smidt,294 the punctate of plague buboes should contain numerous cells as well as plague bacilli. Absence of the former indicates that the buboes have not been reached.
Blood culture. Dissatisfied with the method of bubo puncture, several observers advised the use, instead, of early blood culture for the diagnosis of bubonic plague. Some, such as Bonebakker, recommended bile media for this purpose; South American workers, such as da Silva and Barreto & Castro, preferred direct intraperitoneal inoculation of experimental animals with the blood of the patients.

As has been pointed out in chapter 4, particularly rapid results may be obtained with the latter procedure if, according to the proposal of Gotschlich, material for microscopic and culture examinations is taken in vivo from the peritoneal cavity of the infected animals.

In addition to the above-mentioned methods, serodiagnostic procedures may be used (see page 243).

Evaluation. When trying to determine the comparative value of these methods, it must be kept in mind that, in order to take full advantage of the potent remedies now available for plague treatment by starting their administration at the first possible moment, an early diagnosis of the disease is indispensable. Considering that in bubonic plague development of the gland affection precedes that of septicemia, it would seem at first glance that examination of material obtained through bubo puncture should be of greater value for making a quick diagnosis than blood tests, and still more than serodiagnostic procedures, which yield a positive result only after the morbid process has developed. It must be realized, however, that this holds true only as far as smear examination of the bubo contents is concerned, because confirmation of the diagnosis through culture tests and animal experiments is no less time-consuming when such material is used than when the blood of the patient is examined.

The finding of numerous and characteristic bacilli in smears made from the buboes goes a long way towards supporting the diagnosis, but it is questionable how far such results are superior in value to the prima facie evidence procurable in typical cases through clinical examination alone. It is felt, therefore, that bubo puncture followed by smear examination and confirmatory tests should be used only in clinically doubtful cases, or in order to establish the diagnosis in hitherto plague-free localities. Ordinarily a decision whether or not to initiate treatment can be reached on clinical grounds, and blood examination or (particularly in mild cases or those which have received early treatment) serodiagnostic methods should be used to confirm the diagnosis.

Primary pneumonic plague

A clinical diagnosis of primary pneumonic plague is easy as soon as the characteristic sputum is expectorated, and this evidence is strongly supported by the examination of sputum smears which in typical cases show an abundance of characteristic bacilli. Culture tests and animal
experiments carried out with such sputum yield comparatively rapid results.

As will be discussed later, primary pneumonic plague is ushered in by a stage lasting about 20-24 hours, during which cough is absent or insignificant and no characteristic expectoration occurs. At this stage, plague bacilli are either altogether absent from the sputum or saliva, or are present in such small numbers among other bacteria as not to be recognizable with certainty. Clinically, such patients show signs of an acute general infection but no lung involvement. To arrive at a definite diagnosis at this stage of the disease is therefore difficult or impossible. If plague is present, further clinical observation as well as repetition of sputum examinations at frequent intervals will eventually lead to a diagnosis. It is clear, however, that during epidemics, and particularly when dealing with patients who have been in contact with pneumonic-plague victims, it is imperative not to wait for such confirmation but to start energetic treatment as soon as the presence of the disease is suspected.

Septicaemic plague

A prima facie diagnosis of plague without manifest buboes or manifest lung involvement is not difficult in the ultimate stage of the disease, because it is then possible to detect large, or at least appreciable, numbers of plague bacilli in blood smears. Earlier in the disease the organisms are few and far between; the clinical signs are only those of a serious general infection and are hence without pathognomonic significance. However, as pointed out by Bouffard & Girard, puncture of the liver may be used with advantage at this stage, and it is also noteworthy that Goldstein was successful when puncturing the lungs in cases of pulmonary plague where no manifest signs of pneumonia could be detected. Conseil & Durand recommended lung puncture also in cases of pneumonic plague where no sputum was available, especially in children who, as a rule, did not expectorate.

The laboratory diagnosis of septicaemic plague can be easily established through culture tests or animal experiments with blood specimens taken even early in the disease, but by the time the results of such examinations become available the patients will be beyond hope, if not already dead. Their only chance lies in drastic treatment started as soon as the presence of the infection is suspected.

Dead bodies

Whenever a complete autopsy is possible, no difficulty will be encountered in establishing a prima facie diagnosis in the case of typical bubonic plague, even if the presence of the disease had not been previously suspected, because the characteristic features of a haemorrhagic type of adenitis
are hardly ever found in morbid processes due to other causes (van den Berg & Vos).

Cases where the bubonic lesions are not marked may be far more easily overlooked unless the possibility of plague is kept in mind and adequate laboratory examinations are undertaken.

The same may be said to hold true of instances of primary respiratory infection where pneumonic changes are slight or even absent. Cases of typical primary pneumonic plague usually show such distinctive changes in lungs and pleurae that they should lead, if not to a prima facie diagnosis, at least to the suspicion that some peculiar infection, necessitating careful bacteriological examination, is present. Still, the fact should not be overlooked that certain other infective processes, especially influenza, may produce gross lung lesions not dissimilar to pneumonic plague, and that some instances are on record where micro-organisms closely resembling \textit{P. pestis} were found in individuals showing pneumonic features at autopsy (Pollitzer).

Not only for lack of facilities but mainly on account of popular prejudices, it is often impossible to perform complete autopsies in instances where death from plague is suspected. Various methods have been recommended to obtain, under such circumstances, material for laboratory examination.

\textit{Excision}

Some workers, such as Lefrou and, more recently, Macchiavello, advised excision of the buboes and/or pieces of suitable internal organs (e.g., the liver, the spleen, or—if pneumonic plague was suspected—the lungs) in place of complete autopsies, but it must be feared that even this simplified procedure would be much resented in countries where any mutilation of dead bodies is abhorred. Serious consideration should be given, however, to the possibility of using the viscerotome in place of dissection procedures, as has been successfully done by plague workers in Ecuador.

\textit{Puncture}

A procedure much used in place of dissection methods for obtaining material for laboratory examinations was puncture of various organs of the dead bodies. In the case of bubonic plague, the buboes were usually advocated for this purpose, but satisfactory results were also obtained both in this form of the disease and in septicaemic plague by puncturing the liver (Bouffard & Girard). Opinions as to what organs should be chosen in the case of pneumonic plague were somewhat divergent, but most workers advised puncturing several parts of both lungs as well as the liver.
or heart (Girard; 38, 40 Petrie; 87 Kamal 59). Such elaborate procedures were recommended because sometimes lung punctures failed when material was taken from non-consolidated parts of the organ (Wu Lien-teh 114).

Generally speaking, it must be realized that negative results with materials obtained by puncturing the organs of dead bodies do not exclude the possibility that the individuals in question might have succumbed to plague, particularly if smear examinations alone are used to support the diagnosis. On the other hand, owing to the not infrequent presence in the dead bodies of coliform or other bacilli resembling *P. pestis*, the exclusive use of smear examination may yield false positive results. It should be noted, in this connexion, that Gram-negative bacilli of a suggestive appearance are conspicuous in smears made with material from the gastro-intestinal tract, which may be punctured in place of the liver or spleen by inexperienced workers. However, cells are practically entirely absent in such preparations.

A useful procedure recommended by Vincke 112 and Girard 40, 41 for facilitating animal experiments with the materials obtained by puncturing dead bodies was to wash out the syringe, after puncture, with sterile normal saline and to use the fluid for the inoculation of guinea-pigs. Vincke repeatedly punctured the buboes and/or various organs, washing out the needle and syringe after each puncture with the aid of 3 ml of sterile saline which had been placed in a 10-ml flask, and using the resulting suspensions to inoculate guinea-pigs cutaneously with the aid of cotton plugs. He found that material taken in this way within 24 hours after the death of the victims remained suitable for animal inoculation for 5 days, provided that the temperature did not exceed 35°C. A similar method was used by Girard who took material from any bubo present as well as from the liver and the upper, middle, and lower parts of both lungs.

**Bone-marrow examination**

Several workers obtained satisfactory results when using the bone-marrow of plague-suspect corpses for laboratory tests. Thus Ramos Diaz 91 was able to make a retrospective diagnosis of a pneumonic-plague outbreak in Peru by examination of the bone-marrow from a rib of one of the victims who had been buried for two months.

Some of the workers in South America recommended obtaining bone-marrow for laboratory tests from dead bodies by "digitomy", i.e., amputation of a finger (preferably the most easily amputated forefinger) and opening the second or third phalanx. Lobo & Silvetti 69 found this material, because scant in plague bacilli, unsuitable for smear examination, but obtained good results by cultivation or animal experiments.

Alvarado, 1 dealing with the technique of "digitomy", recommended disinfecting the finger, before amputation, with alcohol. After the opera-
tion, which could be safely performed without rubber gloves, the operation wound was covered up with a cotton pad dipped in formalin and fixed with a bandage, or the hand was simply placed under the clothing of the dead body. For transport to the laboratory, the amputated finger was placed stump down in a bottle containing absorbent cotton. Extraction of the bone-marrow was somewhat difficult but could be facilitated by opening the bones lengthwise instead of crosswise. Alvarado suggested that in the plague regions compulsory "digitomy" should be performed in all cases where death after an acute illness of less than ten days' duration had occurred.

The method was also recommended by Barreto & Castro,\(^3\) who advised using the bone-marrow extracted immediately after amputation for the preparation of a roll culture in sulfite agar which was forwarded to the laboratory. The same procedure was used to dispatch material obtained through bubo puncture or section of the veins.

Useful as these methods of bone-marrow extraction are, they would, one fears, meet with much opposition in some of the plague areas. It might be possible, however, under such conditions to obtain bone-marrow for postmortem examination from the sternal bone, as is done in clinical work. It is of importance to note in this connexion that Modica\(^7\) obtained positive cultural results in 10 out of 12 instances with the bone-marrow obtained through sternal puncture from plague patients.

**Preservation of Material**

Not only in the case of wild-rodent infection but also in that of rat-caused plague, which shows an increasing tendency to evolve in remote rural localities, it has become a matter of great importance to ensure that the materials to be forwarded for examination reach the often distant laboratories in a fit condition.

As has been noted, some workers, instead of relying on methods of preservation, preferred to use the material for examination immediately after its collection for the preparation of cultures (see Barreto & Castro\(^9\)) or the inoculation of test animals (see Devignat\(^2\) and van Riel & Mol\(^2\)), but for various reasons it is often impossible to take advantage of these procedures. In such cases it is necessary to preserve the vitality and virulence of the materials to be sent to the laboratories either by physical means or by keeping them in fluids or other substances counteracting putrefaction.

Into the first-mentioned category fall various procedures for keeping the materials for examination at a low temperature pending transport.

A method adopted for this purpose in Argentina was to use, for the transport of rats, large metal drums provided with double walls and to put ice into the outer compartments of these containers, which had a capacity
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for 20-60 rats. Henriques recommended putting the tissues to be forwarded into test-tubes with paraffined stoppers, and enclosing the tubes for transmittal in thermos flasks filled with broken ice. A similar method is used in the USA (Link). Macchiavello & Paracampos, studying the viability and virulence of P. pestis in the tropics, noted that if guinea-pig or rat organs were kept in the icebox, survival of the plague bacilli was better in the spleen than in the liver, in which apparently a rapid autolysis, inimical to the micro-organisms, took place. For this reason some laboratories did not recommend forwarding liver specimens for examination.

It is obvious that preservation on ice of plague-suspect carcasses or organs pending transit will prove useful only as long as the distances to be covered are not too great or if rapid means of transport are available. This is not universally the case and, moreover, in many of the rural plague foci, ice is not available. The recent proposal of Amies to use common salt instead of ice deserves, therefore, due attention. His method consisted of putting the suspect carcasses into a dry jar with a cap which was screwed down tightly after 2-3 ounces (60-80 g) of salt had been added. The salt was then made to cover the body of the animal by rotating the jar. It was best to examine the bone-marrow of animals preserved in this manner. Positive results were obtained in this way even from a plague-infected animal kept for 67 days.

Broquet advocated the preservation of plague-suspect organs in a fluid containing 20% glycerol and 2% calcium carbonate, stating that P. pestis in organs kept in this manner remained virulent for periods up to 13 days. The usefulness of this method was confirmed by several workers. Uriarte & Morales Villazon recorded in this connexion that, in the spleens of experimentally infected animals which had been preserved in Broquet’s fluid either at room temperature or in the refrigerator, plague bacilli usually remained virulent for 12-19 days, sometimes even longer. Prado jr. found Broquet’s fluid useful for forwarding the lymph-nodes of plague-suspect rats to the laboratory. After arrival the tissues were placed on sterile filter-paper to remove the excess of glycerol and were then used for cutaneous or subcutaneous inoculation of test animals. Issaly & de Issaly recommended the following formula for preparing Broquet’s fluid: 20 ml of pure neutral glycerol (Baume 30°), 2 g of calcium carbonate, and distilled water to 100 ml. The fluid was sterilized for 10 minutes at 100°C and then kept in bottles, the corks of which had been sterilized by dry heat and afterwards impregnated with sterilized melted paraffin to prevent development of fungi. These authors found that the spleens removed from experimentally infected guinea-pigs even two days after death continued to harbour virulent plague bacilli up to 15 days when kept in the fluid. They recommended its use for forwarding the organs of plague-suspect animals, preferably spleen or bone-marrow, to the laboratory. To prevent acidification of the fluid which, like the formation of fungi, biased its efficiency, they advised buffering with
bisodium phosphate and citric acid (1.1046 g and 0.0230 g, respectively, per 100 ml of fluid).

As established by Devignat and Henriques, infection of experimental animals could be produced with plague fleas kept in Broquet's fluid for periods up to 6 days.

Satisfactory results were also obtained by using saline solutions, in place of Broquet's fluid, for the preservation of plague-suspect organs or fleas. Webster, who seems to have been the first to suggest this method, recommended that in the case of fresh carcasses a few drops of heart-blood be taken with a sterile pipette through a seared portion of the heart surface and added to one ml of sterile citrated saline in a small test-tube. Such samples were apt to remain suitable for laboratory examination for some days.

As established by Girard, suspensions in normal saline obtained from experimental animals 3-10 hours after death by puncture and washing-out of the syringe remained virulent for up to 6 days when kept at a temperature of 16°-26°C, but for 3 days only when kept at 37°C. Suspensions from the carcasses of experimental animals which had been kept after death for 48 hours at 21°C remained virulent for 24 hours only, but the organs of such animals were still infective after 3 days.

Eskey & Haas recommended 2% solutions of sodium chloride for the transport of fleas to the laboratory. This concentration, while inhibiting the growth of contaminating bacteria, was not harmful to the plague bacilli. Meyer even used 3% saline for forwarding fleas during summer, but took care to wash them in normal saline before grinding them up for animal tests.

The methods of preserving plague-suspect organs in paraffin with a melting-point of 42°-44°C (Henriques) or in mixtures of vaseline oil and vaseline (or lanolin or paraffin), as recommended by Berlin & Bacheva, have not been widely used. For various reasons Girard considered the latter method of little practical value.

Synopsis of Diagnostic and Differential-Diagnostic Methods

Laboratory methods for the diagnosis and differential diagnosis of plague have already been discussed in chapters 3 and 4. Nevertheless, it seems advisable to summarize them here, at the same time indicating their characteristic values.

Motility tests

As has been mentioned when dealing with the bacteriology of plague, the differential-diagnostic value of motility tests is not absolute since *P. pestis* is invariably immotile while *P. pseudotuberculosis*, though typically
motile, may occasionally fail to show movement. Generally speaking these tests are, however, of importance, the more so since with their aid it is possible to establish quickly the fact that otherwise suspect strains which show motility are not those of plague.

In place of hanging-drop examinations some plague workers recommended the use of Levinthal's method. A useful modification of the latter was described by Himmelfarb:

Small drops of broth cultures of *P. pestis* are placed without spreading on thin agar; the plates are then incubated for 24-48 hours at 20°C. A small coverglass is placed next to a suitable colony and a second coverglass, with one edge resting on the first, is put in a slanting position over the colony. A little saline is placed over the colony with a capillary pipette. Motility is then studied with the aid of a high-power dry objective or the immersion lens.

To facilitate a distinction between active Brownian movement and true motility, Himmelfarb recommended replacing the saline by a 1% solution of mercuric chloride which, while not interfering with Brownian movement, rendered actively moving microorganisms immotile.

**Smears**

**Fixation.** Many workers, both because the usual method of fixation by passing the preparations through the flame does not ensure killing of the plague bacilli and because specimens treated in this way show less clear bipolar staining, prefer fixation of plague smears with alcohol. For this purpose the air-dried smears are treated for at least one minute with 95% ethanol, an ethanol-ether mixture, or preferably absolute methanol. Unpurified or denatured methanol such as that used as fuel, may be substituted. If many specimens have to be handled, it is expedient to immerse them in staining jars filled with alcohol. After fixation, the alcohol is poured off and the specimens are dried. Some workers advise the washing-off of the alcohol with water before drying the smears in the air or with the aid of blotting-paper. If smears have to be sent to a distant laboratory for examination, it is, according to Webster, best to fix them in alcohol for 10 minutes and, after drying, to dispatch them unstained.

**Staining.** Although different workers recommend various and sometimes rather elaborate methods of staining plague smears, it is as a rule quite satisfactory to use, in addition to Gram's method, the simple stains ordinarily available in laboratories, such as dilute carbolfuchsins, Loeffler's methylene blue, or 1% carbolthionin blue. Should bipolar staining be indistinct, the alcohol-fixed specimens may be treated before staining, for half a minute with 0.5% acetic acid (Gaffky, quoted by Dieudonné & Otto). Petragnani found it advantageous to use, instead of Loeffler's methylene blue, a stain made up by adding to 10 ml of distilled water 2-3 drops of cold-saturated methylene blue and 12 drops of a 1% solution of lactic acid, staining at slight heat for half an hour.
Gram’s method of staining is particularly indicated when dealing with sputum, pus from buboes, or material from putrified carcasses or dead bodies, so as to facilitate the differentiation of the Gram-negative plague bacillus from Gram-positive micro-organisms, such as the pneumococcus and certain spore-bearers which may resemble *P. pestis* in simply-stained preparations.

Webster\textsuperscript{113} advocated using Jensen’s modification of Gram’s method for plague smears, while de Smidt\textsuperscript{101} was in favour of the following method:

Alcohol-fixed smears were stained for 15 seconds in a solution consisting of 1 g crystal violet, 10 ml absolute ethanol, and 300 ml distilled water; after washing, the smears were treated for a few seconds with Gram’s iodine until brown or iron-grey, and acetone was then directly applied until no more colour was given off. After rapid washing the smears were counterstained with 1\% neutral red solution.

*Evaluation.* Modern workers are unanimously of the opinion that, particularly when dealing with incipient outbreaks or with sporadic instances of the infection, smear examinations alone are insufficient to establish the diagnosis of plague.

As has been discussed before (page 222), instances of “inapparent” infection, where smears yield a negative result but where animal experiments prove positive, are frequently observed in rodents. Similarly, culture tests or animal experiments may sometimes establish the diagnosis of human plague in instances where smear examinations gave a negative result. The presence of morphologically atypical plague bacilli may also confound the issue when sole reliance is being placed on an inspection of smears.

On the other hand, examination of smears is often apt to suggest the presence of plague in instances where the infection is absent. Pasteurellae other than the plague bacillus, showing morphological features similar to, or even identical with, those of the latter organism, have often been found in rodents, and occasionally also in man. Moreover, in decomposed carcasses or dead bodies, bacilli belonging to other genera may show microscopic appearances similar to those of the pasteurellae and, as was claimed by some observers, e.g., de Smidt\textsuperscript{101} and Hennessey,\textsuperscript{54} the presence of such organisms may prove misleading when sputum smears of patients suspected to be suffering from pneumonic plague are examined.

The limitations of smear examination are well illustrated by the following results obtained by Girard\textsuperscript{45} through the combined use of this method and of animal experiments in the case of materials which had been collected from human plague victims with the aid of punctures:

<table>
<thead>
<tr>
<th>Smears</th>
<th>Total number of combined examinations</th>
<th>Animal experiments Positive</th>
<th>Animal experiments Negative</th>
<th>Positive inoculations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>781</td>
<td>690</td>
<td>91</td>
<td>88.3</td>
</tr>
<tr>
<td>Suspicious</td>
<td>962</td>
<td>409</td>
<td>553</td>
<td>42.5</td>
</tr>
<tr>
<td>Negative</td>
<td>2,642</td>
<td>124</td>
<td>2,518</td>
<td>4.7</td>
</tr>
</tbody>
</table>
In spite of these shortcomings a judicious use of smear examinations is indispensable in order to watch the trend of fully confirmed epizootics. Smear examinations are also of some importance for the recognition of human plague, particularly in clinically obscure cases of bubonic plague.

**Cultures**

The methods of cultivation which appear to be of prime importance for the identification of *P. pestis*, and for its differentiation from other bacterial species, may be grouped according to whether they are intended to take account of:

1. morphological and growth characters;
2. growth requirements;
3. selective-growth requirements.

**Characteristics of morphology and growth.** (a) *Involution forms*: It is of importance to note that, as first shown by Hankin & Leuman, cultivation of the plague bacillus on agar containing 3% sodium chloride leads to the production of marked involution forms. True enough, claims that this phenomenon is specific for the plague bacillus to the exclusion of *P. pseudotuberculosis* have not been substantiated. However, the morphological features noticeable when the latter is grown on 3% salt-agar are apt to be different from those manifest in the case of the plague bacillus.

Thus Topping et al., growing a pseudotuberculosis strain of human origin on 3% salt-agar, noted the presence of longer rods and of coccoid and some swollen forms but stated that "it was not the characteristic pleomorphism of plague". Likewise Haas, cultivating a pseudotuberculosis strain of rat origin on 3% salt-agar, obtained involution forms which were "usually long, slender, slightly curved rods, or medium-sized rods lying in bundles in a manner suggestive of the diphtheria bacillus; less frequently there were enlarged hollow staining organisms resembling 'balloon' forms of involuted *P. pestis*".

It moreover appears that, as first noted by Zlatogoroff (quoted by Dieudonné & Otto) and confirmed by other observers, involution of plague bacilli on 3% salt-agar becomes manifest in 24 hours—that is, more rapidly than in the case of other bacterial species.

(b) "*Stalactite*" growth: The peculiar crumbly and "stalactite" growth of *P. pestis* in broth can no longer be considered of differential-diagnostic importance, because such features, while not invariably present if this micro-organism is cultivated, may be produced by the rough form of the pseudotuberculosis bacillus. Broth cultivation of an otherwise identified plague strain is, however, of value in so far as absence of turbidity renders it likely to be a pure culture of the organism.

**Requirements for growth.** (a) *Plain agar*: In order to distinguish between plague and pseudotuberculosis bacilli, advantage may be taken of
the fact that the former generally grows sparsely on plain agar while the latter develops abundantly after 24 hours' incubation (Bezsonova et al. 8).

To carry out such tests, care must be taken to use inocula sufficient in size to permit growth of the plague bacillus. On the other hand, it must be kept in mind that if material for cultivation is taken directly from the animal or human body, admixture of blood or tissue-fluids may lead to an unusually abundant growth of *P. pestis* on plain agar.

*(b) Small inocula:* Among the numerous methods which have been utilized to induce growth of *P. pestis* from small inocula, the following simple procedure recommended in the *Report of the Haffkine Institute for the year 1931* 105 is fully satisfactory for diagnostic and differential-diagnostic purposes:

With the aid of a 1-mm loop, dilutions of the micro-organisms to be tested are made in tubes each containing 10 ml of normal saline. Blood-agar slants are then inoculated with 1-mm loopfuls of the dilutions, and plain agar slants with 5-mm loopfuls. In the case of the plague bacillus, discrete colonies appear on the blood-agar slopes and none at all on plain agar, while pseudotuberculosis bacilli develop equally well on both media.

*(c) "Exhausted" media:* Basing his recommendation on earlier work by Fabiani, 83 Petragiani 86 advocated, for the differentiation of plague bacilli from other bacterial species, the use of old culture media on which plague bacilli had been grown, and which had been sterilized after the requisite amount of water had been added. Plague bacilli failed to develop on such "exhausted" media, while other micro-organisms developed freely.

*(d) "Hungry" media:* Bezsonova 6 (see also Bezsonova et al. 7) found "hungry" agar, prepared without peptone, suitable for differential-diagnostic work; pseudotuberculosis bacilli developed fairly well on such media while plague bacilli grew poorly, if at all.

*(e) Bile media:* It should be added that bile media, although not suitable for differentiating the plague from the pseudotuberculosis bacillus, have been used with advantage for the enrichment of primary blood cultures. Kirschner 60 found pure bile, previously recommended by La Rosa, 82 sterilized either by filtration or by heating for 20 minutes at 110°C, satisfactory for preliminary cultivation of blood or pus. By this method good growth could be obtained with small inocula (10-40 plague bacilli per ml); in fact, even the addition of one drop of blood to 5 ml of bile was sufficient to show up a slight bacteraemia. Ohoto 88, 84 first advocated the use of Conradi and Kayser's bile medium for taking blood cultures in plague work, but afterwards used a medium consisting of 500 ml of fresh ox bile to which 5 g of peptone had been added. This was steam-sterilized for two hours, divided into 50 test-tubes, and again heated for one hour.

For routine work the simple procedure recommended by Sokhey & Wagle 108 is, however, fully satisfactory. This consisted of using 0.5 ml of
blood, obtained from a vein of the patient, to inoculate two agar slants (0.25 ml per slant) which were then incubated for two days at room temperature.

Requirements for selective growth. (a) Low temperatures: In order to isolate plague bacilli from contaminated materials, advantage has been taken of the ability of this micro-organism to grow at low temperatures. Petragnani recommended for this purpose incubation at 10°C but, as has been noted when dealing with the growth of *P. pestis* on gelatin, even freezing of media inoculated with contaminated material has been employed.

(b) Media counteracting contaminants: Besides the above-mentioned method, cultivation on media able to counteract the growth of contaminants was recommended for work with such materials. Drennan & Teague advocated an agar medium prepared from beef-heart to which 0.025% sodium sulfite and 0.00143% of crystal-violet had been added. Similarly Meyer and Batchelder recommended addition of 0.025% sodium sulfite and 0.0025% gentian violet to hormone beef-heart agar. Kister found Endo's medium useful for work with putrefied rats, since it restricted the growth of *Proteus vulgaris*. However, it was necessary to establish empirically the amounts of sodium sulfite and of fuchsin most favourable for this purpose. The use of the two media mentioned above is therefore more expedient.

Biochemical reactions

Carbohydrate substances. (a) Techniques: As discussed by Pollitzer, solid media have been used to study the action of *P. pestis* on carbohydrate substances, and Francis obtained good results with the semi-solid medium of Enlows; liquid media have, however, been utilized for such tests by most recent workers, and seem to be preferable. The technique adopted by Chen on the advice of K. F. Meyer and Pollitzer was as follows: 1% peptone water (prepared with a brand of peptone not containing carbohydrate-like fractions which might give false positive results) served as base, to which the fermentable substances were added in 1% proportions. Andrade's indicator was incorporated and the final pH of the medium was adjusted to 7.4. The cultures to be tested were first streaked out on blood-agar plates to check for contamination. If found pure, loopfuls of the growths were transferred into the appropriate media. The tubes were then incubated at 37°C. As recommended by de Smidt, the growths were aerated by shaking the tubes daily. Readings were taken daily for a period of 21 days. In order to make sure that this procedure kept the bacilli alive, subcultures on blood-agar plates were made once weekly from each tube under test.

Another possible source of error to be guarded against, in conditions prevailing in the tropics, is the spontaneous hydrolysis of saccharose;
constant control with Fehling's solution is therefore necessary (Devignat & Boivin 27).

In their recent studies, Devignat & Boivin 27 took advantage of the "micro-glucide dish" devised by the former author.26 To utilize this expedient and economical method, Devignat & Boivin first grew the plague strains to be tested for 48 hours at 30°C in 12 ml of peptone water containing Andrade's indicator. 0.5-ml quantities of these growths were then placed in the tubes of the dish, each of which contained 0.5 ml of one of the carbohydrates to be used, in a 4% solution made with twice-distilled water. An observation period of 8 days was found sufficient when applying this method.

In addition to the tests described above, a simple and expedient method devised by Uriarte & Morales Villazon 109 deserves mention. These workers used for their test 2% peptone water, slightly alkaline to litmus, with 8 g of glucose or laevulose and 4-6 ml of a 1% aqueous neutral red solution per litre. After sterilization at 110°C, this was poured into Durham tubes. Uriarte & Morales Villazon claimed that growth of plague bacilli in this medium produced a characteristic triad of signs: (a) slight acidity with a corresponding colour change to yellow; (b) absence of gas production; (c) formation of a flocculent precipitate which settled down so that the fluid remained clear. They claimed that none of the micro-organisms likely to be confused with the plague bacillus showed this combination of reactions.

Devignat & Boivin, 27 while confirming these results in general, found in three out of 36 instances slight turbidity instead of formation of a flocculent deposit.

Another simple method, recommended by Webster,113 involved the use of 1% peptone water containing glucose, mannite, lactose, and saccharose respectively besides an indicator. After incubation at 37°C for 48 hours, there should be production of acid but not of gas in the Durham tubes containing glucose and mannite, and no change in those containing lactose and saccharose.

(b) Evaluation: As has been discussed previously, the claim made by earlier observers that tests with glycerol-containing media were of value for a differentiation between plague and pseudotuberculosis bacilli can no longer be considered as valid. The importance of tests with rhamnose-containing media is still stressed but it must be noted that: (a) according to Russian observers glycerol-positive plague strains isolated in south-east Russia and the interior of Asia were apt to dissociate into rhamnose-acidifying and rhamnose-negative variants; (b) late rhamnose acidification produced by plague strains has been observed; and (c) Devignat & Boivin, 27 working with 40 strains isolated in the Belgian Congo, observed slight rhamnose acidification in one instance. It would be unwise, therefore, to deny the plague character of an otherwise suspect strain merely because it shows activity towards rhamnose media. At the same time, however,
exceptions of this kind do not invalidate the rule that, in contrast to pseudo-
tuberculosis bacilli, plague bacilli (as well as the pasteurellae in the strict
sense) produce no acidity in rhamnose media.

*Hydrogen sulfide and indole.* Tests for the presence of hydrogen sulfide
and indole are of limited differential-diagnostic importance, because both
plague and pseudotuberculosis bacilli fail to produce these substances in
the course of cultivation. A simple test devised by Webster 113 is as follows :
A tube containing 1% peptone water should be inoculated with the bacilli
to be tested and a few drops of 5% lead acetate solution put on the inner
end of the plug, which should be made of white cotton-wool. If hydrogen
sulfide is absent, the plug should show no blackening after incubation at
37°C for 24 hours. To the inner end of the plug of the same culture (covering
the plug if necessary with a new layer of white cotton-wool) a drop of
1% potassium persulfate and then a drop of Ehrlich’s rosindole reagent $c$
should be added. If indole is absent, there should be no pink colour on
the plug after incubation for 24 hours at 37°C.

*Milk.* Tests with litmus milk (see also chapter 2) cannot be considered
as fully reliable for a differentiation of the plague from the pseudotuber-
culosis bacillus, because some strains of the latter species are poor alkali-
producers. Results obtained through cultivation of the plague bacillus in
litmus milk are also somewhat inconsistent, Devignat & Boivin, 27 for
instance, finding that the medium remained unchanged in 33 out of 38
strains tested, while slight acidity was produced 4 times and slight alkalinity
once.

*Nitrates.* As discussed in chapter 3, procedures demonstrating respec-
tively a reduction of nitrates to nitrites, and production of nitrous acid,
are not universally valuable for a differentiation of plague and pseudo-
tuberculosis bacilli; even in areas where positive reactions were usually
given by *P. pestis*, occasional negative results were noted. Webster’s 113
simple reaction test is as follows : A 5-mm loopful of the same culture
recommended for his hydrogen sulfide test is placed on a white opal glass
plate and mixed with a 2-mm loopful of Ilosvay’s reagent $d$. If nitrites are
present, a pink colour should appear within a minute.

Attention has been drawn to the potential value of Fauconnier’s 36
proposal to differentiate between plague and pseudotuberculosis bacilli
with the aid of tests showing the urease activity of these organisms. How-
ever, the favourable results reported by this author require verification.

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$c$ Ehrlich’s rosindole reagent is prepared by taking 1 g of *p*-dimethylamo-benzaldehyde, 95 ml of
absolute ethanol, and 20 ml of concentrated hydrochloric acid, and mixing the whole with an equal quantity
of rectified spirit.

$d$ Ilosvay’s reagent is prepared by mixing equal parts of (a) 1 g of sulfanilic acid, 14.7 ml of glacial acetic
acid, 285 ml of distilled water, and (b) 0.2 g of naphthylamine, 14.7 ml of glacial acetic acid, and 325 ml of
distilled water.
Serodiagnostic tests

As has been stated previously when dealing with the problem of serodiagnosis, advantage should be taken of agglutination to test unknown strains with plague immune sera of established potency, and to examine, on the other hand, the sera of suspect patients with the aid of known cultures.

While satisfactory procedures, including slide-tests convenient for the rapid diagnosis of human plague, have been worked out, the problem of to what extent agglutination may be relied upon for the identification of unknown strains, and consequently for a differentiation of plague and pseudotuberculosis bacilli, is not yet solved. The methods proposed by Bhatnagar and by Seal appear to be promising but their differential-diagnostic value should be confirmed by large-scale investigation.

In addition to the methods of agglutination, haemagglutination, and complement fixation dealt with in chapter 3, the following serological tests seem to possess practical value for the diagnosis, particularly the retrospective diagnosis, of human plague: (a) the flocculation test, introduced by Girard (see chapter 3), using the serum of patients or convalescents and toxic filtrates or extracts of P. pestis; and (b) the allergic reaction recommended by da Silva, jr & de Albuquerque (see also da Silva, jr.). These authors used an antigen prepared by removing the bubo of a subcutaneously plague-infected guinea-pig immediately after death, boiling this material for two hours in normal saline, grinding it in a sterile mortar at the proportion of 1 g per 20 ml of saline, filtering through sterile gauze, and finally adding 0.5% phenol. If found sterile, this material was used in quantities of 0.1 ml for intradermal injection. A control injection with an equal amount of milk was made 4 cm from the site of the antigen injection. Readings were taken after 24, 36, and 48 hours. In positive cases a papule surrounded by an inflammatory zone developed, the reaction becoming maximal after 36 hours.

Some workers, such as Cambosu, Menezes, and Tumansky, recommended the use of serological methods for the rapid diagnosis of rodent plague. A further precipitin test was recently recommended by Larson et al. These workers established, by preliminary investigations, that boiling of the materials to be tested, as formerly employed in carrying out thermoprecipitin tests (Piras and others, quoted by Pollitzer), led to a decrease in the antigen content. Diethyl ether was therefore used to sterilize the culture or tissue suspensions to be tested with plague immune sera. Precipitin tests performed with antigens obtained in this way from tissues of animals which had died of plague showed the presence of soluble antigens in sufficient quantities to be of diagnostic value in instances where the material had been stored at 37°C for periods of at least 14 weeks.

In places where adequate laboratory facilities are available, complement-fixation tests, carried out according to the method of Chen et al. (see chapter 3, p. 169), may prove valuable for the diagnosis of rodent plague.
Bacteriophage tests

As discussed when dealing with the bacteriophage problem, Gunnison et al. recently found that tests carried out with plague phage at 20°C offered a means of differentiating between plague and pseudotuberculosis bacilli.

To facilitate the application of such tests, Cavanaugh & Quan soaked sterilized strips of filter paper in broth cultures of *P. pestis* which had been seeded with a potent plague phage and then subjected the strips to lyophilization or desiccation in vacuo. If such strips were applied to freshly made plague subcultures on blood-agar plates and these were incubated at 20°C, a 1-mm wide zone of lysis usually became visible in 18-24 hours round the bacteriophage-coated strips while no such clearance was produced round noncoated strips of filter paper used as controls. Tests made under identical conditions with growths of *P. pseudotuberculosis* gave negative results. As far as established up to now, the bacteriophage-coated strips preserved by lyophilization remained capable of producing lysis for periods of three months, if sealed under nitrogen and stored at room temperature. Desiccated strips did not display such keeping qualities.

Should the value of this procedure be confirmed by extensive trials, it would form an easy means of differentiating plague and pseudotuberculosis bacilli, the more so as it might be possible to supply field workers or persons operating in areas without facilities for bacteriophage work with test-strips prepared in central institutions.

Animal experiments

In evaluating the methods available for the laboratory diagnosis of rodent plague, stress has been laid on the importance of animal experiments performed with pooled organs or pooled fleas. At the same time, however, it was pointed out that, invaluable though these methods are for giving a true overall picture of the situation, they fail to yield information on the extent and degree to which plague is present. Other methods for filling this gap must therefore be used side by side with pooling-experiments. Merely to carry out animal experiments with material from individual infected rodents would not be sufficient to establish to what degree they had been infected, and would, moreover, be quite out of question in the course of considerable epizootics. However, such experiments are called for when dealing with initial or sporadic manifestations of rodent plague.

The position in regard to human plague is similar. Here also it is essential to verify the diagnosis in initial or sporadic cases by all available methods, including animal experiments; to continue these with each patient during a considerable epidemic would, however, be impossible.

The choice of experimental animals to be used for plague diagnosis depends upon the nature of the work to be performed. Generally speaking,
guinea-pigs should be chosen for pooling-tests and these animals are also most suitable for confirming the diagnosis in initial or sporadic plague manifestations. As recommended by Lloyd, three guinea-pigs, infected respectively through the unbroken skin, subcutaneously, and intraperitoneally, should be used when dealing with initial or sporadic manifestations of the infection. While the most rapid results may be expected from the intraperitoneally infected animal, the likelihood of obtaining a pure culture is greatest in the case of the percutaneously infected one. In tropical climates particularly, it is advisable to kill moribund animals (i.e., those which lie down on their sides) with the aid of chloroform, because this procedure, besides saving time, increases the chances of isolating pure growths.

Intraperitoneal infection of white mice is a method of unsurpassed value for establishing the existence of a bacteraemia and, at the same time, confirming the diagnosis in instances of human plague. Moreover, it will often be possible to use these animals, which can be easily bred and kept at small expense, on a larger scale than guinea-pigs. A drawback is that white mice are far more susceptible than guinea-pigs to pneumococcus infections (Girard).

White rats are of outstanding value for differentiation between plague and pseudotuberculosis bacilli. It is true that a few strains of the latter species have been found pathogenic for white rats, but such aberrant results may also be obtained when using most of the other methods available. When performing differentiation tests, the cumulative evidence obtained in various ways, rather than the results of any individual method, should be taken into account (Schütze).

Precautions Advisable

The dangers confronting plague laboratory workers are threefold:

1. the possibility of contracting bubonic infection through the bite of blood-sucking insects, especially rodent fleas;
2. the possibility of contracting direct bubonic infection when performing postmortems or otherwise handling contaminated material;
3. the possibility of contracting pneumonic infection through splashing or spraying material laden with plague bacilli.

Bubonic infection through fleas

When it is necessary to enter plague-infected houses, or when coming into contact with persons or dead bodies, or with rodents possibly harbouring infected fleas, it is safest to wear a special costume, consisting principally of a gown similar to that used by clowns which, made of one piece, covers
the whole body except the head and hands. This is donned through the opening in the neck and is then tied firmly round neck and wrists. The costume is completed by rubber or high leather boots, a linen cap, and, as far as necessary, by rubber, canvas, or leather gloves. A mask must be added if there is danger of pneumonic infection.

Since it is rather trying to wear the above-mentioned gown under tropical conditions, long linen stockings covering the feet and legs and tied up above the knees may be substituted, or high boots (made from rubber or leather), into which the trousers are tucked, alone, may be used.

It has been recently recommended that protection against fleas should be ensured by impregnation of the underwear with DDT, or impregnation of the clothes with other modern insecticides (Elishewitz; 30 Linduska et al.; 66 Smith & Burnett 102). A combination of such applications with the use of long linen stockings and/or high boots would seem particularly effective.

Safe methods for handling rodents delivered to the laboratory for examination and for freeing them from fleas have been described earlier (see pages 225, 226). Experimental animals kept in the laboratory should be housed in rat-proof rooms, or at least in rat-proof cages. To protect infected animals against stray fleas, the containers accommodating them may be covered with gauze, surrounded by tanglefoot paper, or suspended.

Direct bubonic or pneumonic infection

If one works with the cleanliness and carefulness indispensable for laboratory work in general, no special precautions need be observed when examining plague material by bacteriological methods. To minimize the danger of accidents, glassware of the best quality should be used exclusively for plague laboratory work. Slides should be immersed immediately after examination in a jar containing an antiseptic fluid (preferably industrial spirit) and should be boiled before cleaning.

When performing human autopsies, a proper costume should be worn, including a rubber apron and high rubber-boots. Solid rubber-gloves are indispensable. In his autopsy work, done before sulfonamides and antibiotics had become available for treatment, the present writer used two pairs of rubber gloves, the cuffs of the gown being tied down over an inner pair of the usual medium-weight pattern, and hands and forearms then being covered by a long pair of solid postmortem-gloves.

To avoid danger from occasional splashing, masks and preferably also goggles should be worn.

Less stringent precautions are necessary when dissecting plague-infected animals, the more so because plague workers should learn to perform autopsies with the aid of suitably long instruments, without touching the carcasses. However, even if this is done, rubber gloves should be used as a precaution against accidental touching of the animals or splashing of infectious material.
The carcasses of the dissected animals should be burned immediately after autopsy, together with the contents of the containers where the animals have been confined. Cremation is also preferable in the case of dissected human plague victims. If this is impossible, the dead bodies should be wrapped in shrouds soaked in strong antiseptic solutions before being put into leak-proof coffins or, preferably, quicklime should be used to cover them.

Experimental infection of test animals should be carried out with the precautions recommended above for dissection. When using injection methods, care must be taken to avoid the danger of spraying about infectious material; it is best to wear masks whenever infecting test animals with the aid of syringes.

All possible care must be taken when manufacturing vaccines or sera with virulent plague strains, and when carrying out experimental research work, because, as shown by several deplorable incidents, it is in the course of such activities that pneumonic infections are most likely to occur.

A strict supervision of the assistant and lower staff employed in plague laboratories is essential to make sure that they are not remiss in adopting all necessary precautions.

If in the course of plague laboratory work any mishap occurs, adequate methods of disinfection of the clothes and persons of the workers concerned, as well as of the whole room, should be started at once. Any wounds on fingers or hands, whether pre-existent or contracted during the accident, should be attended to first, preferably by thorough soaking in alcohol.

If the risk of infection of workers involved in laboratory accidents seems appreciable, prophylactic administration of sulfonamides should be resorted to.

It is, on the other hand, necessary to make sure that laboratory workers, in order to protect themselves against imaginary dangers, do not use sulfonamides indiscriminately. Pollitzer saw an instance of serious kidney affection (haematuria and oliguria) in a technician detached for field work who, with the idea of protecting himself against plague, took 1-2 g of sulfadiazine daily for several weeks. Fortunately, no permanent harm resulted.

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Chapter 6

HOSTS OF THE INFECTION

RODENTS AND LAGOMORPHA

Reviewing in 1928 the then rather limited knowledge available concerning the occurrence and importance of plague in rodents other than the common rats and mice, Jorge felt justified in drawing a clear-cut distinction between the pandemic type of plague introduced into human settlements and houses all over the world by the "domestic" rats and mice, and "peste selvaticque", which is dangerous for man only when he invades the remote endemic foci populated by wild rodents.

Although Jorge's concept was accepted, some discussion arose regarding the appropriateness of the term "peste selvaticque" or, as Stallybrass and Wu Lien-teh translated it, "selvatic plague". It was pointed out by Meyer that, on etymological grounds, the name "sylvatic plague" would be preferable, and this term was widely used until Pozzo and Hoekenga doubted, and Girard denied, its adequacy on the grounds that the word "sylvatic" implied that the rodents concerned lived in forests, whereas that was rarely the case. Girard therefore advocated the reversion to the expression "wild-rodent plague" which was used before the publication of Jorge's study—a proposal it has seemed advisable to accept for this monograph.

Much more important than the difficulty of adopting an adequate nomenclature is that of distinguishing between rat and wild-rodent plague—a distinction which is no longer as clear-cut as Jorge was entitled to assume. Since 1928, the list of rodents other than the commensal rats and mice known to suffer from natural plague has grown incessantly so that, instead of little more than a score, almost 200 species or subspecies are now known to be implicated. Besides varying in their etiological importance, they

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a It is important to note, however, that—as has been pointed out by an erudite reviewer of the above passage—the Latin name sylvaticus, when applied to animals, actually means wild rather than forestal and the names derived from it in Spanish, Italian and Portuguese convey the same meaning so that Jorge's nomenclature is fully justified on etymological grounds, though as it is likely to mislead English and French readers, it may be preferable to use the name "wild-rodent plague".
show marked differences in their ecology; many live near to man rather than in the remote haunts of sylvatic plague, and thus become apt to take part in the perpetuation and spread of the infection in settlements and even houses. It follows that it is no longer possible to deal merely with wild-rodent and rat plague as was done in the past; attention must also be paid to those species other than the commensal rats and mice which, on account of their peri-domestic or semi-domestic habits, take an intermediate position and are, in part, responsible for a transition of the infection from the wild to the commensal rodents, or vice versa.

Wild Rodents

When trying to trace the gradual evolution of present-day knowledge on the existence and importance of wild-rodent plague, it is not surprising to find the earliest evidence in Central Asia where the infection probably originated and where it has certainly existed since time immemorial.

As first stated by laymen such as Tsherkasoff—in his *Memories of a hunter in Siberia* (1856-63)—in the case of Transbaikalia and Outer Mongolia, and Prjevalski for the Ordos country of Inner Mongolia, and later confirmed by medical men, e.g., Rudenko, Skchivan, and Barykin, the inhabitants of these areas had been aware for generations of the periodical occurrence of a fatal, infectious disease in the tarabagans (Siberian marmots) which was apt to spread to man, and had taken surprisingly adequate measures to protect themselves against this danger. The mysterious illness of the marmots was not only the subject of numerous legends, but was also referred to in the old Tibetan sacred books. In the opinion of the local practitioners, small worms invisible to the naked eye caused this and other infectious diseases, a claim curiously similar to that arrived at in 1658 by Athanasius Kircher with regard to human plague.

The infection prevailing among the tarabagans, and among the human beings who had come in contact with them, seems to have been first identified with plague in 1895 by Bjeliavski & Rjeshetnikoff, probably because their attention had been attracted to this disease by its spectacular appearance at Canton and Hong Kong in 1894. However, the evidence brought forward by these two observers was not supported by laboratory examinations and it was not until 1905 that bacteriological proof of the existence of human plague in Transbaikalia was obtained. Two years later, a tarabagan dissected by Barykin was also found positive, but it was only in 1921 that the existence of widespread epizootics among these animals was confirmed (Sukneff and others, quoted by Wu Lien-teh).

These records leave little room for doubt that the foci of wild-rodent plague in Central Asia belong to the category of "natural" foci defined

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As discussed later, species belonging to the orders of Lagomorpha and Rodentia are involved in what for the sake of brevity, is commonly called "wild-rodent plague".
by Pavlovski as arising and continuing to exist independently of factors connected with the presence of man.

Whether any other foci of wild-rodent plague fall into the same category, or became so established long ago, is a difficult question to answer. The foci in south-east Russia and in Kurdistan deserve consideration in this respect, but too little is known of their early history to permit of any definite conclusion. It should be noted in connexion that, though wild rodents were occasionally suspected of playing a part in the spread of plague in south-east Russia during the first decade of the present century, or, perhaps, even since the Vetlianka outbreak in 1878-9, it was not until 1912 and 1913 that the existence of natural plague in the sels (susliks) was bacteriologically confirmed by Deminski and by Berdnikov, respectively. Proof of the existence of the infection among the gerbils (Meriones) of Iranian Kurdistan has been obtained quite recently by Baltazard et al.

As mentioned in chapter 1, claims have been made that the origin of wild-rodent plague in the western parts of the USA was due not to a recent importation of the infection by the sea-route, but to an early immigration from Central Asia of certain of the species involved; however, as was pointed out, the evidence supporting the latter assumption is not convincing. There is no doubt that the foci of wild-rodent plague existing in South Africa, and in parts of South America, became established through a spread of the infection from the commensal to the free-living species during the present century.

It is of historical interest to add that, as far as can be established, the first authentic record of natural plague in wild rodents was made by Simond who, in 1898, reported positive findings in Indian palm-squirrels (Funambulus palmarum) at Karachi. As recorded by Bruce Low in the following year, several porcupines, together with some monkeys, died of plague during an outbreak at Mysore. However, since these animals had been kept in a zoological garden and no other records on porcupines are available, one cannot justifiably include them in the lists of rodents, other than the commensal rats and mice, in which the existence of natural plague has been confirmed or suspected.

Species involved

In order to show the occurrence of natural plague in rodent species or subspecies other than the commensal rats and mice, and in Lagomorpha, lists enumerating (a) the animals in respect of which positive proof of the infection has been obtained, and (b) the suspected animals, are contained in Annex 1, tables I and II (pp. 623 and 633).

Particular attention has been paid to make Annex 1 not only as accurate as possible, by closely following the standard nomenclature of Ellerman and of Ellerman & Morrison-Scott, respectively, but also as complete as possible, though it is realized that entire success has not been obtained in
the latter respect. Indeed, it seems doubtful whether it will ever be possible
to compile a really satisfactory list of plague-affected rodents and Lago-
morpha other than the commensal rats and mice because it is most probable
that, in addition to the known foci, unrecognized foci of sylvatic plague
exist, and because, even in the areas where the presence of this type of the
infection has been ascertained, by no means all the species or subspecies
involved have been detected. In chapter 1 attention was drawn to the
statement of Davis that over 100 rodents or other small animals are at
risk of infection in South Africa, and it is also noteworthy that Pozzo 157
believed that 70 rodent species were involved in Argentina.

| TABLE XIV. FAMILIES AND SUBFAMILIES OF RODENTIA AND LAGOMORPHA
| IN WHICH THE PRESENCE OF NATURAL PLAGUE HAS BEEN CONFIRMED |
| Family       | Subfamily | Number of species or subspecies found infected | Area                                                   |
| Bathyergidae | —         | 1                                              | Angola                                                 |
| Caviidae     | Caviinae  | 9                                              | Argentina, Brazil, Ecuador, Peru                      |
| Chinchillidae | —         | 1                                              | Argentina                                              |
| Dipocidae    | Dipodinae | 5                                              | Iranian Kurdistan, south-east Russia, Transbaikalia    |
| Echimyidae   | Echimyinae| 1                                              | Brazil                                                 |
| Geomyidae    | —         | 2                                              | USA (western States)                                  |
| Heteromyidae | Dipodomyinae | 3                                          | USA (western States)                                  |
|              | Heteromyinae | 1                                          | Venezuela                                              |
| Muridae      | Cricetinae| 48                                             | Argentina, Bolivia, Brazil, Ecuador, Louisiana (USA), Peru, south-east Russia, South Africa, USA (western States), Venezuela |
|              | Dendromyinae | 4                                           | Belgian Congo, South Africa                           |
|              | Gerbillinae| 12                                             | Belgian Congo, India, Iranian Kurdistan, south-east Russia, Russian Turkestan, South Africa, Transcaisia |
|              | Microtinae | 12                                             | Iranian Kurdistan, south-east Russia, Transbaikalia, USA (western States) |
|              | Murinae    | 30                                             | Belgian Congo, Burma, Ceylon, East Africa, Egypt, Gold Coast, India, Kenya, Senegal, South Africa |
|              | Otomyinae  | 6                                              | Belgian Congo, East Africa, Kenya, South Africa        |
| Pedetidae    | —         | 1                                              | South Africa                                          |
| Sciuridae    | —         | 49                                             | Canada, Ceylon, Ecuador, India, Manchuria, Mongolia, Peru, south-east Russia, Russian Turkestan, Senegal, South Africa, USA (western States), Transbaikalia |
| Leporidae    | —         | 14                                             | Argentina, Bolivia, Brazil, England, Ecuador, Peru, South Africa, Transcaisia, USA (western States) |

Total: 199
Natural plague has been confirmed in the families and subfamilies of the orders of Rodentia and Lagomorpha listed in table XIV.

In order to deal adequately with wild-rodent plague in the strict sense, table XV has been prepared to show: (a) the reservoir hosts in the definitely established foci where this type of the infection is known to be, or to have been, active; (b) other families or subfamilies, species or subspecies of which have also been found infected, and which are therefore apt to play a subsidiary role and, if living near man, are able to act as intermediaries between the strictly wild and the commensal rodents.

The data of table XV well bear out the statement of Meyer \(^{130}\) that

\[\text{"as a rule, a principal species belonging to the family \([\text{of}]\) Sciuridae or \([\text{to the subfamily of}]\) Gerbillinae, living in subterranean colonies, in families or singly, presides over the exchanges of the plague bacillus..."}\]

A study of the relative distribution of these two main groups of reservoir hosts in the wild-rodent foci in or near Asia clearly shows that three zones may be distinguished:

**TABLE XV. LIST OF DEFINITELY ESTABLISHED WILD-RODENT PLAGUE FOCI SHOWING RESERVOIR HOSTS AND OTHER FAMILIES OR SUBFAMILIES FOUND NATURALLY INFECTED**

<table>
<thead>
<tr>
<th>Plague focus</th>
<th>Main reservoir</th>
<th>Also found infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Cavilinae (Cavia, etc.)</td>
<td>Chinchillidae, Leporidae</td>
</tr>
<tr>
<td>Iranian Kurdistan</td>
<td>Gerbillinae (Meriones)</td>
<td>Dipodinae, Microtinae</td>
</tr>
<tr>
<td>Manchuria, Mongolia, and Transbaikalia</td>
<td>Sciuridae (Marmota)</td>
<td>Dipodinae, Microtinae</td>
</tr>
<tr>
<td>Peru</td>
<td>Cricetinae (Groomys)</td>
<td>Cavilinae</td>
</tr>
<tr>
<td>Peru/Ecuador border region</td>
<td>Cricetinae (Akodon)</td>
<td>Leporidae</td>
</tr>
<tr>
<td>Russian Turkestan</td>
<td>Sciuridae (Sciurus)</td>
<td>Cricetinae</td>
</tr>
<tr>
<td>South Africa</td>
<td>Sciuridae (Marmota)</td>
<td>Gerbillinae</td>
</tr>
<tr>
<td>South-east Russia northern foci</td>
<td>Sciuridae (Cilellus)</td>
<td>Dipodinae, Microtinae</td>
</tr>
<tr>
<td>southern foci</td>
<td>Gerbillinae (Meriones)</td>
<td>Leporidae</td>
</tr>
<tr>
<td>Transcaisia</td>
<td>Gerbillinae (Meriones)</td>
<td></td>
</tr>
<tr>
<td>USA: western States Arizona and New Mexico</td>
<td>Sciuridae (Cynomys)</td>
<td>Dipodinae, Gaomyida</td>
</tr>
<tr>
<td>coastal regions and northern part of Intermountain plateau</td>
<td>Sciuridae (Cilellus)</td>
<td></td>
</tr>
<tr>
<td>southern deserts</td>
<td>Cricetinae (Neotoma)</td>
<td>Leporidae, Sciuridae</td>
</tr>
<tr>
<td>Washington</td>
<td>Microtinae (Lagurus)</td>
<td>Cricetinae, Dipodomyinae, Sciuridae</td>
</tr>
<tr>
<td>Venezuela</td>
<td>Heteromyinae, Heteromys, Cricetinae, Sigmomys</td>
<td>—</td>
</tr>
</tbody>
</table>

* See Link \(^{109}\)
(1) Central Asia, where large Sciuridae (marmots) form the plague reservoir (Manchuria, Mongolia, Russian Turkestan, and Transbaikalia, and perhaps other adjacent areas mentioned in Annex 1, table II (p. 633));

(2) South-east Russia, northern part, where small Sciuridae (sisels) are of prime importance;

(3) South-east Russia, southern part, Iranian Kurdistan, and Transcaspia where Gerbillinae (Meriones) are the reservoir hosts.

This separation into a north-eastern area, where Sciuridae are the fons et origo mali, and a south-eastern area, where a corresponding role is played by the Meriones, is a matter of great interest.

Sciuridae (ground-squirrels and prairie-dogs) also form reservoirs in some of the western States of the USA and in the focus situated at the border between Peru and Ecuador, while Gerbillinae are of paramount importance in South Africa.

The Cricetinae form reservoirs in three wild-rodent foci—namely, Argentina, the Huancabamba area of the Peruvian Andes (Macchiavello 119, 120), and the southern deserts of some western States of the USA—while the Caviinae, Heteromyinae, and Murinae each act correspondingly in only one area. The comparatively inconspicuous part played in wild-rodent plague by the Murinae, to which subfamily the commensal rats and mice also belong, seems striking when the ominous role played by the latter species in the pandemic type of plague is given consideration. However, as will be discussed later, the observations made in respect of the multimam-mate rat, Rattus natalensis, prove that this rule is by no means without exceptions.

**Characteristics of principally involved species**

A short description of the species mainly involved in wild-rodent plague, and of those of their habits which are of importance in the perpetuation and spread of the infection, may be given thus:

1. The marmots (tarabagans) of Central Asia and the adjacent plague areas are big animals attaining a length of about half a metre and a weight of 4 to 7.5 kg. They dig deep burrows in firm ground and live in settlements of varying size, each family inhabiting a separate burrow. Following hibernation, which lasts from about October to April, they mate in spring. After a pregnancy lasting 6 weeks, the female gives birth to 2 to 7 young.215 As far as can be ascertained, the young continue to stay in the maternal burrow even after they have become independent, while the mother seeks new quarters for herself.

2. The small sisels or susliks (Citellus pygmaeus) of south-east Russia look like miniature tarabagans and have a body-weight of 60 to 200 g. They use two kinds of burrows: shallow ones during the summer and deeper ones in the winter. Their hibernation lasts longer than that of the
tarabagan, the adults beginning to sleep in July, the young animals in August or September. The mating period begins immediately after the end of the hibernation period in early spring. After a pregnancy lasting probably not longer than three weeks, litters of 8 to 10 young are born.

The young animals leave their mothers about a month after birth, each settling down in an individual burrow, preferably a previously used one. The sisels are therefore most active:

(a) in early spring, when the animals, which otherwise lead an individualistic life, meet to mate, and when the females prepare the burrows for their litters;

(b) in early or late June, according to the weather, when the young disperse, sometimes travelling as far as 2 to 5 km before settling down;

(c) a month later, when the winter burrows are prepared and occupied (Wu Lien-teh; 215 Kalabuchov & Raevsky 91).

3. The *Meriones meridianus* of south-east Russia are non-hibernating animals which leave their burrows mainly at night. They are ratlike in appearance, but their hind-legs are longer and stronger. Their burrows are shallow and have several hidden entrances in addition to the principal one. The latter is kept closed with sand by the female, the sand being accumulated by several strokes of the hind-legs. The female discontinues blocking up the entrance when her young mature, in order to let them go out on the surface. The mating season of these fertile animals extends from May to October; pregnancy lasts from 25 to 28 days. Though up to three litters may be born to one female per year, 85% have only one litter annually. The young leave the maternal burrows after 25 to 30 days (Rall 162).

As stated by Baltazard et al., 4 the *Meriones* of the Iranian Kurdistan keep away from man and dig their burrows in non-cultivated areas far from settlements. They are of sedentary and peaceful habits, frequently visiting the burrows of their neighbours, particularly during the mating season. Though accumulating food reserves for the winter, they leave their burrows even during the coldest weather.

4. The *Tatera brantsi*, which, together with the Namaqua gerbil, *Desmodillus auricularis*, form the main plague reservoir in South Africa, are active, non-hibernating animals of strictly nocturnal habits. Their size is approximately that of rats. Their burrows have many entrances and are arranged in colonies, the distribution and size of which vary according to the nature of the soil and the available food-supply. There are two main breeding seasons—one after midwinter towards spring, and one after midsummer towards autumn. Three to five young are born per litter; they leave the parent burrows when maturing and may range over a wide area before settling down to breed at the age of three months (Wu Lien-teh; 215 Davis 39).
According to Powell (quoted by Schulz 175), the Namaqua gerbils are less active and less sociable animals than the Tatera species. They live in families of 2 to 8 members, inhabiting warrens which have many entrances. They reach sexual maturity at the age of three months, and have four litters annually of 4 to 6 young per litter.

As stated in chapter 1, the coucha rat, Rattus natalensis, a fertile, mouselike animal of nocturnal habits, plays an ominous role as a link in the transition of plague from the gerbils to man. Being rather inactive, these rats prefer occupying burrows deserted by gerbils or other rodents to providing their own shelters. Since they prefer to frequent, rather than shelter permanently in, human settlements and houses, and may even live away from human habitations, these animals seem to deserve a place among the wild rodents (Davis; 37 Fourie 53, 54).

5. The ground-squirrels of the western States of the USA fall, according to Eskey & Hass,48 into three size-groups:

(i) large animals comparable in size to the grey tree-squirrels, e.g., Citellus beecheyi, C. columbianus, and C. variegatus grammurus;
(ii) medium-sized animals, somewhat more than half the size of the larger species, including C. armatus, C. beldingi, C. richardsoni, and C. washingtoni;
(iii) small ground-squirrels including, among others, C. spilosoma, C. townsendi, and C. tridecemlineatus.

The prairie-dogs (Cynomys) are comparable in size to the large ground-squirrels and may be divided, according to Eskey & Haas,48 into two groups—the white-tails, comprising Cynomys leucurus, C. gunnisoni, and C. parvidens, inhabiting the Rocky Mountains, and the black-tails, C. ludovicianus, which range over the Great Plains. The former live in a manner similar to that of the ground-squirrels and often on common ground with them; the latter live in peculiar circumscribed colonies or "towns", marked by prominent mounds and the absence of all low vegetation in the immediate vicinity.

Eskey & Haas state that the wood- or pack-rats (Neotoma) also fall into two groups: bushy-tailed species, such as Neotoma cinerea, living in the more forested country of the colder zones and found infected in California; and round-tailed species, found in the southern deserts, among which, according to these authors, N. desertorum plays the most important role. It should be noted that these rats, like most rodent species found in the southern deserts, are of strictly nocturnal habits.

The Peromyscus, suspected by Mohr 138 to be of etiological importance, are white-footed meadow-mice, apparently apt to frequent the outbuildings of farms (Meyer & Holdenried 134). The Lagurus curtatus, recently incriminated as an important plague reservoir in Washington State by Link,105 is a vole inhabiting sage-brush areas.
Dealing generally with the ecology of the rodent species found involved in the western States of the USA, Eskey & Haas 48 emphasized that

“human habitation and agriculture produce conditions attractive to many wild rodents. In irrigated valleys, around orchards, or in camps and resorts the various species often congregate, easily adapting themselves to an association with humans and enjoying the artificially enhanced food supply.”

While such species are apt to shelter in haystacks, empty spaces beneath buildings, lofts, and other man-made structures, the rodents which stay away from human settlements generally live in tunnels, the location and architecture of which varies with the species. Most of these rodents congregate in colonies, though in a degree differing according to the species. However, the observations of Brown 21 in Alberta, Canada, prove that there are exceptions to this rule. Brown found that C. richardsoni did not live together but were widely scattered and moved extensively over the prairie, whereas C. columbianus, common in the mountains and foothills, lived in colonies and moved within a restricted range.

Generally speaking, the wild rodents in the western States of the USA appear to produce a single litter each year, the number of offspring varying from four to a dozen. The young remain in the nests for the first few weeks of their life, after which they build tunnels of their own and forage for themselves (Eskey & Haas 48).

It is of great importance that many of the species undergo hibernation or aestivation, or a combination of both. It appears, however, that such periods of inactivity do not occur as regularly and universally as in the case of the tarabagans and of the susliks in south-east Russia. Meyer 130 noted in this connexion that the young ground-squirrels did not hibernate or aestivate, while Evans & Holdenried, 49 working at the Calaveras dam in California, found that

“there was evidence of both aestivation and hibernation for varying periods but not all of the squirrels were inactive at the same time”.

Meyer 128 laid some stress on the cannibalistic tendencies of the rodents found plague-affected in the western States of the USA, and pointed out that “involvements of the lymph-nodes adjacent to the upper and lower gastrointestinal tube as a sequel of cannibalism are by no means infrequent”. Though these findings deserve attention, one cannot conceive that this mode of infection could have played a really important role.

6. *Heteromys anomalus*, incriminated together with *Sigmodon hirsutus* as a primary plague-reservoir in Venezuela, is stated to frequent cultivated fields, granaries, and even houses in search of food, whereas the latter rodent lives in forests, rarely visiting fields and never entering houses (Isaac Riaz 86).

7. The “cuis” (Caviinae), which play a principal role in the wild-rodent plague foci in Argentina, generally live underground but may make
tunnels between plants in locations where the vegetation is sufficiently abundant for this purpose (Barrera 7). These animals do not penetrate into occupied buildings and, in general, seem to have little contact with man (Macchiavello 119). However, exceptions to this rule seem to exist; Barrera, for instance, noted that, in the north of the country, the Indians used the “cuis” as food. The breeding season of these species is usually in spring.

*Graomys griseoflavus* (which probably also serves as a reservoir host in Argentina), although originally an arboreal rodent, may nest in various locations, even under the roofs of houses. As stated by Barrera 9 when dealing with the manifestations of wild-rodent plague in Mendoza Province, these rodents were hunted for their furs, as well as for culinary purposes.

8. Discussing the plague focus in Huancabamba, Peru, Macchiavello 119 stated that the *Akodon* (Cricetinae) which, together with an *Oryzomys* species, formed the primary reservoir of the infection, were attracted to the farms at harvest times. In the Peru-Ecuador border regions, humans contracted the infection in the fields where the secondarily involved Cricetinae lived. *Sciurus stramineus nebouxi*, which formed the primary reservoir, was an arboreal rodent.

**Movement-range**

The movements of free-living animals like the wild rodents fall into two categories:

1. a “normal dispersal” (Meyer 129) caused by physiological needs such as the daily requirements for food, the search of the maturing young for shelter, and the periodical movements of groups to the vicinity of fields or even settlements at harvest-times;

2. migrations due to abnormal causes, e.g., food scarcity, or catastrophes such as floods, which bereave the animals of their shelters as well as of their food-supplies.

It is impossible to make a clear-cut distinction between these two categories as far as the range of the movements is concerned. As already noted, the young, when in search of homes, and even the adults (especially the males) of some species, may roam quite far. Still more important, certain species which play a subsidiary role in wild-rodent plague may normally cover wide distances. This is true in particular for the Lagoathracis; the South African hares, for instance, travel far in the course of a single night.

That large-scale true migrations of wild rodents do take place, is well confirmed by the observations made of the lemmings in Scandinavia (Elton 47). Evidence is also available that migrations undertaken on a minor scale have helped the spread of wild-rodent plague. For instance,
Barrera stated that the *Graomys*, driven by hunger to human habitations, were responsible for a plague outbreak in northern Argentina. Similarly, multimammate mice were considered to have played an important role in the 1944-5 plague epidemic in Ngamiland, Bechuanaland Protectorate. At the height of the gerbil epizootic, the multimammate-mouse population became unusually large; a flood forced them to leave their burrows in the swamps and they invaded the villages, bringing with them the infection contracted from the gerbils.

It would appear, however, that such instances of a spread of wild-rodent plague through true migrations are exceptional.

**Susceptibility and resistance**

As stated earlier, laboratory tests have proved that different wild-rodent species may vary markedly in their susceptibility to infection with *Pasteurella pestis*. Several observers have claimed that the differences found to exist in this respect are not merely of academic interest, but are of great actual importance. Tikhomirova denied that the highly susceptible sisels (susliks) could be the preliminary plague-reservoirs in south-east Russia, and incriminated the rather resistant *Meriones meridianus* as the fons et origo mali. Similarly, Krumbiegel stated that, compared with the suslik, *Citellus pygmaeus, C. fulvus* was of no great importance in the perpetuation of plague in south-east Russia because it succumbed to a fulminant type of the infection which could therefore not persist long in this species or spread far. On the other hand, Davis noted in South Africa that epizootics in the Namaqua gerbil, *Desmodillus auricularis* (a species found by Pirie to be rather resistant to plague), took a protracted course, which no doubt favoured a perpetuation of the infection.

In a recently published paper, Baltazard et al. not only stated that the *Meriones* subspecies—which were somewhat resistant to infection with *P. pestis*—were an important reservoir of the disease in the Iranian Kurdistan, but also maintained that, in general, slightly susceptible rather than highly sensitive species were instrumental in the perpetuation of wild-rodent plague.

The occurrence and importance of seasonal variations in the susceptibility to the infection within any one species have been claimed by observers in south-east Russia as well as in the western States of the USA.

In this connexion mention may be made of the investigations made in south-east Russia by Nikanoroff to study the influence of the seasons on experimental plague in the sisels (susliks). Starting his experiments in the middle of June, he successively tested four batches of susliks, each consisting of 30 animals, at fortnightly intervals by subcutaneous injection with uniformly virulent cultures. While most of the animals in the first batch quickly succumbed to acute plague, the disease displayed an increasingly slow evolution in the three subsequent batches. Thirty days after
infection there were no survivors in the first group, as compared with 3 in the second, 5 in the third, and 18 in the fourth.

In 1934, Tinker & Kalabuchov found that young ground-squirrels born in that year were most susceptible to plague, adult females less so, and adult males least. The two workers assumed that these differences were related to deteriorations in the physiological condition of the animals, found to evolve in the females during gestation, and in the young animals during the period of dispersal.

Carrying out laboratory tests with Meriones meridianus, Lobanov & Fedorov noted that the gerbils infected during a period from April to July showed a localized and apparently resolving type of plague, characterized by the presence of minute abscesses which became encapsulated and were eventually replaced by scar tissue; cultivation and animal experiments gave negative results in such instances. The majority of the animals infected during a period from July to October showed an acute and generalized form of the disease.

The presence of a most acute form of plague among the young ground-squirrels in California was claimed by Harrison and was confirmed by Meyer, who stated that laboratory tests had proved that the young animals were highly susceptible to the infection. He also maintained, in contrast to Tinker & Kalabuchov, that the adult males, and not the adult females, came second in order of experimental susceptibility.

Although the evidence quoted above is noteworthy, it is the present writer's feeling that the higher susceptibility of young wild rodents might sometimes have been more apparent than real. Their chances of infection are particularly great because they may roam far during the period of their dispersal, and also because they may settle down in burrows containing infected fleas, the former inhabitants having succumbed to the disease. The outcome of the laboratory tests might have been the result not of an increased susceptibility of the young animals, but of a decreased susceptibility of the adults due to aestivation or approaching hibernation. This may have been particularly true in the case of the Californian squirrels, the young of which, in contrast to the adults, neither aestivate nor hibernate.

Role of hibernation

The importance of hibernation in the epizootiology of wild-rodent plague has been proved by several observers.

1. Siberian marmots (tarabagans). The idea that plague infection might remain quiescent in the bodies of hibernating tarabagans and that it might kill the animals after they awaken in the spring, seems to have been first put forward by Le Dantec. Experimental support for this hypothesis was obtained by Dujardin-Beaumetz & Mosny who found in two alpine
marmots (*Marmota marmota*) which had been infected while hibernating and which died after 61 and 115 days, respectively, foci of chronic pneumonia teeming with plague bacilli.

These results were fully confirmed by further experiments recorded by Wu Lien-teh,\(^\text{212}\) \(^\text{214}\) Wu Lien-teh & Pollitzer,\(^\text{216}\) and Gaiski\(^\text{58}\) which definitely proved that:

\(a\) Siberian marmots infected with *P. pestis* while hibernating may continue to sleep and not succumb to a generalized infection until after awakening in spring; in the instances recorded by Wu Lien-teh,\(^\text{214}\) the longest periods of survival were 88 and 130 days, respectively;

\(b\) animals infected percutaneously or subcutaneously during hibernation and killed at various intervals during further sleep may show signs of resolving plague or evidence of a peculiar form of “latent” plague in which virulent bacilli persist at the site of inoculation and/or the regional lymph-nodes.

No doubt can exist that the persistence of such a localized infection in the hibernating animals was responsible for the appearance of generalized plague after they awakened in spring.

Gaiski\(^\text{58}\) assumed that the peculiar evolution of plague in the hibernating tarabagans “was in causal connexion with the process of bacteriophagy”. However, though he had been able to demonstrate the presence of plague phages in some of his experimental animals, this assumption cannot be considered as generally valid. The extensive work carried out by Wu Lien-teh and Pollitzer with both hibernating and non-hibernating tarabagans produced no evidence of the presence of plague phages.

A most interesting point established by Gaiski\(^\text{58}\) was that three tarabagans, which had been infected while hibernating, rapidly succumbed to an acute type of the disease when re-infected with *P. pestis* soon after they awakened. In all three animals, localized signs of the past infection were noted at autopsy. As Gaiski stated, these observations explained “why in spring animals which had been infected during hibernation and had carried plague bacilli to the moment of awakening, may succumb ”.

It is curious to note in this connexion that, according to the observations of Rudneff,\(^\text{173}\) hibernating sisels (*C. pygmaeus*) showed, during hibernation, a leucopenia with a specially marked diminution of the neutrophiles.

2. **Susliks.** Some observations on the evolution of plague in hibernating susliks seem to have been made by early investigators in south-east Russia, for an editorial appearing in the *Lancet*\(^\text{96}\) in November 1913 stated that “it has been shown by experiment that infection of this animal [suslik] may be greatly prolonged, especially during the hibernating season”.

Churilina\(^\text{28}\) also reported that hibernating susliks, when plague-infected, were apt to survive up to five months while the controls died in two to seven days.

Further evidence was obtained by Gaiski\(^\text{56}\) who studied, during the course of a year, the seasonal changes in the susceptibility of the susliks
to plague. He used for this purpose 242 animals, grouped in 27 batches, infecting each succeeding group with a plague strain isolated from the preceding one. Seasonal differences were noted in two directions:

(i) the mean length of illness varied, showing a minimum (3 days) in June and a maximum (25 days) in winter;

(ii) in June and July, 100% of the animals had bacteraemia, as compared with 60% in winter, and 40% in March. The other animals either had a localized form of plague, with bacilli confined to the site of infection, or harboured the causative organisms in their organs, but not in their blood. The incidence of the localized type of the disease was highest in winter (30%).

Of the 30 susliks infected during hibernation:
21 awoke and succumbed after 2 to 22 days (the average was 8 days);
3 were killed after infection (2 after 15 days, and 1 after 35 days);
6 succumbed after 45 to 138 days.

Of the three animals killed, two (one killed after 15 days, the other after 35 days) showed latent plague with positive findings at the site of the infection only; the third animal showed bacteraemia.

Three animals died after 96, 120, and 138 days, respectively, at the end of hibernation. Two of these showed abscesses at the site of infection while, in the third animal, plague bacilli were present in the internal organs as well as at the site of inoculation.

Some field observations made in south-east Russia seem to lend support to the experimental evidence furnished by Gaiski. Thus, Tumansky noted the existence of two periods when plague assumed epizootic proportions among the susliks—a major one, commencing at the time of the dispersal of the young and lasting until the onset of hibernation, and a minor one in early spring at the time of mating, soon after the end of the winter sleep.

It is of interest to note in this connexion that, according to the inhabitants of Mongolia and Transbaikalia, spring outbreaks of plague did occur among the tarabagans. However, these statements ought to be received with caution because instances of a high mortality in spring due to other causes have been observed in these animals.

3. North American ground-squirrels. Two series of experiments undertaken by Prince & Wayson gave the following results:

(a) two hibernating ground-squirrels (C. richardsoni) were inoculated with plague. One succumbed to an acute form of the infection after two weeks. The second—and two further animals which had been infested with plague-infected fleas—did not contract the disease.

(b) out of four ground-squirrels (C. townsendi) which survived four months in hibernation after intracutaneous infection with P. pestis, one sickened seven days after awakening and died on the eighth day, showing an acute inflammatory reaction at the site of inoculation and bacteraemia. The plague nature of the process was
fully confirmed. The three other animals remained well and were killed 15 days after awakening. They appeared normal at autopsy.

A point of great interest is the relation between the latent type of plague characterized above and the "inapparent" form of the infection referred to in chapter 4. As stated, it is so far not justifiable to identify these two types. Up to the present, it has merely been claimed that inapparent plague may lead to a generalized infection while, in the case of latent plague, positive proof of such an evolution has been obtained.

Some evidence is available which shows that, as has been fully proved in the case of the commensal rats, in wild rodents also, the prolonged existence of plague may lead to a progressive increase in the number of animals which are resistant to the infection.

Noting that in plague-stricken localities of California the ground-squirrels were rather resistant to laboratory infection with *P. pestis* while those captured in plague-free districts were uniformly susceptible, McCoy expressed the view that the preponderance of insusceptible animals in the foci "may mean a gradual extinction of the disease or it may indicate that this partially resistant race of rodents will, if not vigorously attacked, perpetuate the disease for many years."

The early findings of McCoy were fully confirmed by Meyer by the examination of approximately 450 healthy ground-squirrels collected partly from a known plague-focus and partly from an area where the disease had not been demonstrated. Meyer did not feel sure that the insusceptibility to infection with *P. pestis* found in the rodent populations of plague foci was solely the result of a process of "natural selection" by which the receptive strains of the animals were gradually wiped out while the resistant strains survived. He suggested that an immunity induced in individual animals by previous plague attacks might also be of some importance. It also seemed significant that, according to the observations of Bychkoff, guinea-pigs which had not become manifestly ill when bitten by plague-infected fleas had proved resistant to subsequent challenge-infection with *P. pestis*.

Evaluating the actual importance of the insusceptibility becoming increasingly manifest in the rodent population of plague foci, Meyer aptly summed up the situation as follows:

"There is every reason to believe that the interplay between infection and immunity may be influenced by an infinite variety of ecological factors which accelerate or retard it. For example, even a coalescing epidemic spread of the plague infection in a rodent population may fail to reach every colony. Thus, at the apparent termination of the epizootics, both susceptible and resistant rats or squirrels may survive and interbreed, furnishing sufficient hosts to maintain the infection in a smoldering non-readily recognizable state."
Effect of density of wild-rodent populations

It is obvious that:

(a) if plague spreads to or reappears in a locality populated by wild rodents, the situation is far more likely to become serious if the rodent population-level is high than if the rodents are scarce;

(b) in the case of wild rodents living in colonies, an intercolonial spread of the infection will be facilitated if these colonies are close together and impeded if the colonies are widely separated;

(c) severe epizootics in a highly populated area may reduce the number of wild rodents to a level comparable with that of sparsely populated districts and thus curb the further progress of the infection.

It is important to note in this connexion that the density of the rodent population in any given group of burrows and colonies is not uniformly high throughout the year, but reaches a maximum during the period between the birth and dispersal of the young. Thus, according to Kalabuchov & Raevski, the population level in the sisle colonies of south-east Russia was 3.2 to 3.6 times higher during this period than during the rest of the year. These authors maintained that, if plague was pre-existent or was introduced at that time, the temporary overcrowding of the burrows considerably aggravated the situation and was at least partly responsible for the high mortality (85%) of the suslics during their first year of life.

As has been established by investigation of the epizootics or has been suggested by studies on the occurrence of human outbreaks, the incidence of plague in the wild-rodent foci often showed periodic fluctuations, the peaks of which generally coincided with a maximal abundance of the rodents concerned. The length of these cycles was usually 3 to 5 years, but was sometimes more extended (Wu Lien-teh; Fourie; Meyer; Davis; Macchiavello).

These observations attracted much attention since extensive studies on the population fluctuations of wild mammals, particularly of the lemmings, had proved that periodical epizootics were of paramount importance in regulating the numbers of these animals. As summarized by Elton: "the method by which most rodents regulate their numbers is as follows: increase in numbers over several years up to a point at which an epidemic of some sort occurs, which kills off a large proportion of the population. Increase then takes place again, and is followed by another epidemic, and so on indefinitely."

The question naturally arose as to whether plague was one of the epidemic or, one should rather say, epizootic diseases apt to act as a population regulator. While this was considered likely by some workers, e.g., Wu Lien-teh, Barrera, Meyer, and Evans & Holdenried, Davis definitely spoke of "the establishment of so potent a regulatory factor as plague" in the South African wild-rodent foci.
Seasonal incidence of plague

That wild-rodent plague often shows a seasonal incidence, being active during certain months only, and more or less quiescent during the rest of the year, has been proved by many observations, some of which have shown that in addition to the rodents themselves, their fleas may play an important role in this respect.

As far as the part taken by the rodents is concerned, stress has already been laid upon seasonal changes in the susceptibility to infection with *P. pestis* found to exist in some species. Mention has also been made of two extrinsic factors which are apt temporarily to exacerbate plague incidence—namely,

1. the overcrowding of the burrows in late spring and early summer due to the presence of the young as well as the adult animals;
2. the dispersal of the young which, for the reasons previously enumerated, greatly enhances their chances of infection.

It is clear that, in hibernating species, plague can remain manifest during the warm season only. However, as discussed earlier, the infection is apt to persist in the hibernating animals in a latent form and also, as will be discussed in a future chapter, in the rodent fleas.

Since, in the western States of the USA, only the adult squirrels hibernate and/or aestivate (the young animals and the non-hibernating species involved in plague outbreaks remain active throughout the year), the seasonal incidence of plague in these areas was less clear-cut than in the regions where the tarabagans or susliks were the reservoir hosts. Indeed, Meyer stated that plague was sometimes found to be active among ground-squirrels in December and January. Generally speaking, however, summer epizootics prevailed and "the lesions observed in squirrels shot in winter are those of subacute and resolving plague".

In the wild-rodent foci of Argentina, epizootics prevailed in winter but, as pointed out by Barrera, this seasonal incidence was mainly due to a low flea-index prevailing in summer. However, a decrease in the rodent population, caused by epizootics during the preceding winters, was also apt to reduce the incidence of the disease during the warm seasons.

In regard to South Africa, it was stated by Davis that "man is at risk of infection during the summer 'plague season', whereas rodent epizootics, whether in wild or domestic species, do not show any marked seasonal incidence".

Trend of epizootics

Reporting on the 1935 anti-plague campaign in the Bechuanaland Protectorate, Gerber stated that it usually took 8 to 12 months for an epizootic to burn itself out. About 90% of the wild rodents (mainly gerbils)
were killed, and about three years elapsed before the remainder increased to the previous density. Epizootics then reappeared.

An excellent description of the "epizootic cycle" in *Tatera brantsi* foci was given by Davis \(^9\) thus:

"The development of epizootic conditions is preceded by sporadic outbreaks of epizootic plague in a few smouldering foci. The gradual diffusion of *P. pestis* from these initial foci involves more and more colonies, until the majority are in various stages of decline. It takes from 4 to 6 months for an isolated colony to die out, but as cross-infection from one colony to another is erratic, it may take 12 or 15 months for a major epizootic to run its course. Major epizootics recur on the average every 5 or 6 years. Man is at risk of infection before, during and after this major epizootic, which, in effect, means for at least 3 in every 6 years."

A more detailed description of the evolution, course, and decline of the epizootics in the South African gerbils has recently been given by Schulz \(^175\) who stated that, after a major epizootic, the gerbil population was restored to normal density in 3 to 5 years.

Investigating ground-squirrel epizootics in Kern County, California, Evans et al.\(^50\) found that these exacerbations of the plague situation "coincided fairly well with the period of dispersal of the young squirrels". The epizootics ran a separate course in each of the localities involved, the active phase apparently lasting for 2 to 3 weeks only.

**Persistence of infection**

It may be gathered from the preceding discussion that the persistence of wild-rodent plague depends upon the establishment of a kind of equilibrium between the various factors favouring or counteracting the establishment of the causative organism in the host herds.

No doubt can exist that, in some of the species involved, a reduced susceptibility of the animals to infection with *P. pestis* is instrumental in creating such an equilibrium. This mechanism is at work in the case of species such as the Namaqua gerbils of South Africa, the *Meriones meridianus* of south-east Russia, and also, as recently claimed by Baltazard et al.,\(^4\) in case of the *Meriones* forming the plague reservoir in Iranian Kurdistan.

While fully admitting the great importance of this mechanism for the perpetuation of plague in some of the wild-rodent species involved, the present writer cannot agree with the thesis of Baltazard and his colleagues that the presence of such a reduced susceptibility of the host herds is a sine qua non for the continuation of the infection. For, as shown below, a persistence of the infection, even in fully susceptible species, is rendered possible in various ways.

A most important stabilizing influence is exerted in some of the fully susceptible wild-rodent herds by the hibernation period which, while cutting short the decimation of the species through acute or subacute plague,
at the same time permits a carry-over of the infection in the form of latent plague.

Although this regulating mechanism is absent in the case of non-hibernating species, several other factors exert a similar influence. As has been noted, even in the non-hibernating species plague often shows a seasonal incidence, i.e., periods during which epizootics are apt to occur alternate with seasons during which the infection, assuming an enzootic character, becomes far less fatal.

The decrease in the rodent populations during severe epizootics and the erratic progress of the latter, which often spares individual colonies within the affected areas, form important means of preventing a wholesale extinction of the hosts.

On the other hand, during the off-seasons, when the continued existence of the invaders is threatened, there will usually be a sufficient number of rodents which, because suffering from acute or subacute plague with bacteraemia, are capable of serving as links in the perpetuation of the infection. Moreover, even if this mechanism should fail, fleas which continue to harbour *P. pestis* are apt to carry over the infection.

Wild-rodent species other than the primary-reservoir hosts may also take part in the perpetuation of the infection. Their involvement is bound to become more serious at the same rate as the intensity of plague increases among the primarily affected species. Consequently, if the numbers of the latter are reduced to such a degree as to lessen the perpetuation of the disease, the subsidiary species are apt to be sufficiently involved to carry over the infection until the primary hosts become numerous once more.

As is suggested by an interesting observation of Meyer, 132 species which formerly played a subsidiary role in a focus of wild-rodent plague may, under exceptional circumstances, become the primary reservoirs of the infection. He recorded in this connexion that, in a focus originally maintained by *Cieillus beecheyi*,

"under the influence of a control program which was directed solely against the squirrels, *Pasteurella pestis* had transferred its activities to the mice (mainly *Microtus californicus*) and thus had protected its persistence and perpetuation..."

Discussing the perpetuation of wild-rodent plague in south-east Russia, Nikanoroff 146 and Gaiski 37 laid stress upon the existence of a "transitional" zone between the northern steppes, where sises (susliks) were the primary hosts, and the southern, sandy stretches where, in their opinion, mice were principally involved. They maintained that, in this border region, summer epizootics among the susliks were regularly followed by winter outbreaks among the mice. Thus, a vicious circle favouring the perpetuation of the infection seemed to exist.

Tikhomirova, 199 while confirming that the epizootics in *Meriones meridianus*, which she considered as the reservoir host in the southern
regions, took place in autumn and early winter, did not share this belief in the existence of a transitional zone, but maintained that a spread of plague northwards from the *Meriones* foci led to secondary infection of the susliks.

In a note entitled "Persistence of sylvatic plague", Meyer & Eddie recorded an observation in San Mateo County, California, according to which

"two of the locations in the County where infected [ground-squirrel] fleas were found in 1936 were recognized as the same colony or series of burrows proven to harbor diseased squirrels in the summer of 1916".

They added that "these and similar observations indicate that sylvatic plague persists probably indefinitely in an area once invaded...

Though this is undoubtedly the case, it does not seem very likely that the same burrows or colonies remain continually infected throughout prolonged periods.

**Spread of infection**

Though, as previously discussed, a spread of wild-rodent plague at distance may be effected through the agency of migrations or of animals like hares which normally travel far, these and similar means of disseminating the infection per saltum are of comparatively little importance, or become operative only under exceptional circumstances as holds true of the migrations.

Ordinarily, the spread of wild-rodent plague is by contiguity, due probably to "an accumulation of small movements among rodents" (Meyer 180).

Discussing this problem, Davis drew an apt distinction between an intracolonial and an intercolonial spread of the infection, stating that

"the movement of individuals brings each warren a contact by relay movement during a night's activity. In consequence, the spread of plague throughout the warrens of a colony is not hindered, but the spread from one colony to another, mainly by adult males and by the maturing young at rather rare intervals, is sporadic ".

That wild rodents may be carried to settlements by rail is suggested by an observation in the port of Quequen, Argentina, where *Galea leucoblephara* were found in the grain supplies imported from the hinterland. It was assumed that the transportation of grain by rail was responsible for the rat epizootic present in the port at the time.

As stated in chapter 1, the epizootic prevailing among the commensal rats of Tacoma, Washington, in 1942-3 was suspected to have been due to an importation of plague from wild-rodent foci in the hinterland, but it is noteworthy that Hundley & Nasi spoke in this connexion of the findings of rats and mice in the grain-cars. That, however, possibilities of a transport-
ation of plague-infected wild rodents do exist in the western parts of the USA, is shown by recent observations of Ecke & Johnson in Colorado. As pointed out by these workers, ranchers in the southern part of the State, prompted by the desire to control the prairie-dogs on their property, were prone to pay attention to epizootics among these rodents in New Mexico and to fetch diseased animals from there in order to release them on their land. Ecke & Johnson obtained definite knowledge of three instances of this kind, in one of which the rancher in question drove 250 miles from his home to get diseased prairie-dogs. According to them two workers

"reliable sources state that control by the above method was very good, as may be expected. It is evident, however, that the ranchers are unaware of the dangers to which they are exposing themselves by practicing this form of prairie dog control. If this practice is as common as is believed, it probably is the explanation for some of the 'abnormal jumps' of plague epizootics over great distances.

Ecke & Johnson also referred to a rancher's boy, "reported to be catching prairie dogs from an epizootic zone and transporting them to a nearby town to be sold as souvenirs to tourists", but considered this practice as very uncommon.

In South Africa, great stress was laid on preventing the transportation of infected rodents by rail or even trucks (Thornton), but one must wonder whether the transportation of wild rodents was frequent. According to Fourie, coucha rats (R. natalensis) were seldom carried in farm produce. That field rodents may be transported in this manner is proved by an observation in the Cumbum Valley of India where four such animals were detected among rice-bags on bullock-carts, but this was apparently an exceptional occurrence.

In a study on "The focality of rodent plague in the light of ecologo-geographical ideas", Rall distinguished between "mechanical" barriers against the spread of infection, such as rivers and mountain ranges, and "biological" barriers, created through the presence of "sterile zones" where susceptible rodent-hosts, if present at all, occurred either in small numbers or were patchily distributed.

The importance of such biological barriers is illustrated by the observations made by Fedorov et al. of a sandy stretch on the left bank of the Ural River. Plague did not become established in this locality because the latter was sparsely populated by susliks and mice and also, as Fedorov and his co-workers believed, because the seasons during which the various rodent species became comparatively numerous did not coincide.

However, observations in South Africa, particularly, have shown that zones which were ecologically unsuitable for the establishment of plague, or belts which had been artificially freed from rodents, do not absolutely bar the spread of the infection because they may be overrun or outflanked by waves of severe epizootics.
Referring to the likelihood that wild-rodent plague will not spread much further in the Union of South Africa, Davis made the important statement that

"the geographical distribution of *X. eridus* and *X. piriei* is a useful biological indicator of the conditions in which *P. pestis* can be perpetuated, but it does not explain them".

One might venture to suggest that these two fleas are efficient components of rodent-vector combinations or "teams", which, according to the concept of Mohr, are of fundamental importance in the genesis and perpetuation of plague manifestations.

**Interrelation between wild-rodent and rat plague**

Since the interrelations existing between wild-rodent and rat plague differ most markedly in the various areas concerned, it is necessary to consider each area separately instead of dealing with the subject in a comprehensive manner.

**India.** Though India, where no convincing evidence for the independent existence of wild-rodent plague has so far been found, does not seem to fall within the compass of the present discussion, some observations made in that country in regard to the bandicoots deserve mention because they throw an interesting sidelight on the relations between wild and commensal rodents.

Since plague workers have used various Latin names to designate the bandicoots, it is necessary to note first the classification of these animals recently adopted by Ellerman:

<table>
<thead>
<tr>
<th>Species</th>
<th>Subspecies</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bandicota bengalensis</em></td>
<td><em>B. bengalensis bengalensis</em></td>
</tr>
<tr>
<td>Lesser bandicoot-rat or &quot;Indian mole-rat&quot;</td>
<td><em>B. bengalensis kok</em></td>
</tr>
<tr>
<td><em>Bandicota indica</em></td>
<td><em>B. bengalensis gracilis</em></td>
</tr>
<tr>
<td>Large bandicoot-rat</td>
<td><em>B. bengalensis varius</em></td>
</tr>
<tr>
<td></td>
<td><em>B. bengalensis wardi</em></td>
</tr>
<tr>
<td></td>
<td><em>B. indica indica</em></td>
</tr>
<tr>
<td></td>
<td><em>B. indica nemorivaga</em></td>
</tr>
<tr>
<td></td>
<td><em>B. indica savilei</em></td>
</tr>
<tr>
<td></td>
<td><em>B. indica siamensis</em></td>
</tr>
<tr>
<td></td>
<td><em>B. indica jabouillei</em></td>
</tr>
</tbody>
</table>

According to this classification, *Bandicota malabarica* Shaw, previously classed as a separate species by Ellerman, is now being considered as falling into the subspecies *B. bandicota indica*. Similarly, *B. bengalensis kok* (often called "Gunomys kok" in plague publications) and *B. bengalensis gracilis* no longer appear as species, but as subspecies in the new list.

c Ellerman states that "many specimens of this form have been examined, and the conclusion reached is that there is only one (individually variable) subspecies in the area just listed" (i.e., India and Ceylon).
The first available record indicating that the bandicoots might be implicated in plague outbreaks is a statement made in 1906 by Hossack 79 according to which "Nesokia bengalensis" (the small bandicoot-rat) seemed to be intimately concerned in the spread of the infection in Calcutta.d

As reported by the Plague Research Commission in 1907 and 1910,134,155 in Belgaum and other places of Bombay Presidency (now Bombay State), the large bandicoots (B. indica), which had formerly visited the houses, had completely disappeared with the advent of plague. Since no reliable evidence of migration could be obtained, it seemed highly probable that these animals, which had been found susceptible to experimental infection with P. pestis, had been wiped out by epizootics.

It should be noted in this connexion that, according to ecological studies recently carried out by Sharif & Narasimham 179,180 in the Barsi, Belgaum, and Dharwar Districts of Bombay State, Bandicota malabarica (scilicet indica), a rodent of domestic habits, "was very common in localities situated on soft ground".180 Gunomys kok (fig. 26 (p. 281)) was found in the open fields but was rarely discovered near human habitations. In Sharif & Narasimham’s examinations no instance of natural plague was found in either of these two species.

Observations made in respect of the bandicoot rats in Bombay City are of great interest. Referring to these animals in the 1937 report of the Haffkine Institute, Sokhey & Chitre 189 stated that :

"during 1907 when the plague epidemic was still active in Bombay, the Indian Plague Commission noted that Nesokia bengalensis (Gunomys varius) ["] was not a common rodent in Bombay City and that it formed only 1 per cent. of the rat population of the City and that the rest of the rat population was made up almost entirely of Mus rattus (Rattus rattus) and Mus decumanus (Rattus norvegicus); Mus rattus forming 66.2 per cent. of the rat population. During 1937, June to December, we have classified 164, 787 rats collected in the City of Bombay ... and find that Gunomys varius now (1937) forms over 30 per cent. of the rat population while Rattus rattus forms only about 25.7 per cent. Some of the difference between the observations carried out in 1907 and 1937 may well be due to personal factor in classification and possibly methods of trapping. The Indian Plague Commission also found, working in 1907, that both Rattus rattus and Rattus norvegicus were equally and highly susceptible to experimental infection. They found that 45 per cent. of the rats tested succumbed to infection. While, in 1937, [we] find that both Rattus rattus and Rattus norvegicus are highly resistant to plague (10 per cent. susceptible), and have in course of time been replaced to a considerable extent by Gunomys varius which is highly susceptible (about 70 per cent. susceptible). This is an interesting observation and may have some bearing on the mechanism of pandemics, how they come to an end and how they start again after a lapse of time".

As far as the figures of table XVI are comparable, they show that, from 1941 onwards, the incidence of Gunomys kok continuously increased to

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d According to recent information, lesser bandicoots (" Gunomys varius ") were frequent in Calcutta showing an incidence of about 26% (Rao 149) and 68.8% (Lal & Seal 71). B. indica were rarely found but were also more conspicuous in the material of Lal & Seal (4.5%) than in that of Rao (under 1%). Of the 19 rodents examined and found plague-infected by Lal & Seal in 1949, 5 were " Gunomys ".

e As stated in the report of the Haffkine Institute for 1940-1, a reclassification of the Bombay rodents established that " Gunomys varius " was identical with Gunomys kok; " Bandicota indica " with Bandicota malabarica; " Rattus rattus " with Rattus rattus rufescens; " Mus musculus " with Mus dubius and the insectivore " Crocidura caerulea " with Suncus caeruleus.
reach a maximum of 52% in 1947, and then gradually declined. It will also be noted that, in 1948, 42 of these rodents were found infected as against 2 *Rattus rattus rufescens*. However, in 1949, only a few specimens of each species proved positive and no instances of rodent plague were found in 1950 and 1951.

<table>
<thead>
<tr>
<th>Year</th>
<th><em>R. rattus rufescens</em></th>
<th><em>R. norvegicus</em></th>
<th><em>Gunomys kok</em></th>
<th><em>Bandicota malabarica</em></th>
<th>Incidence of rodent plague</th>
</tr>
</thead>
<tbody>
<tr>
<td>1938</td>
<td>24.7</td>
<td>25.4</td>
<td>27.3</td>
<td>1.2</td>
<td>—</td>
</tr>
<tr>
<td>1939</td>
<td>23.8</td>
<td>24.4</td>
<td>35.5</td>
<td>1.2</td>
<td>—</td>
</tr>
<tr>
<td>1940</td>
<td>24.9</td>
<td>23.9</td>
<td>34.3</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>1941</td>
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<td>23.0</td>
<td>37.1</td>
<td>0.8</td>
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<tr>
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<td>22.5</td>
<td>45.7</td>
<td>0.8</td>
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</tr>
<tr>
<td>1943</td>
<td>20.3</td>
<td>22.7</td>
<td>46.3</td>
<td>1.1</td>
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<tr>
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<td>21.9</td>
<td>22.1</td>
<td>47.0</td>
<td>1.1</td>
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<tr>
<td>1945</td>
<td>21.0</td>
<td>21.6</td>
<td>49.2</td>
<td>1.8</td>
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<tr>
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<td>49.0</td>
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<tr>
<td>1947</td>
<td>18.9</td>
<td>23.0</td>
<td>52.0</td>
<td>0.6</td>
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<td>18.3</td>
<td>47.4</td>
<td>0.8</td>
<td>2 <em>R. r. rufescens</em> and 42 <em>Gunomys kok</em> found infected</td>
</tr>
<tr>
<td>1949</td>
<td>23.3</td>
<td>20.0</td>
<td>42.2</td>
<td>0.4</td>
<td>4 <em>R. r. rufescens</em> and 2 <em>Gunomys kok</em> found infected</td>
</tr>
<tr>
<td>1950</td>
<td>23.9</td>
<td>17.6</td>
<td>39.3</td>
<td>0.7</td>
<td>—</td>
</tr>
<tr>
<td>1951</td>
<td>21.1</td>
<td>16.5</td>
<td>36.7</td>
<td>0.8</td>
<td>—</td>
</tr>
</tbody>
</table>

* The figures for the period 1938-48 are taken from the reports of the Haffkine Institute, Bombay, while those for 1949-51 were kindly supplied by Dr. P. M. Wagle, Director of the Institute. The rodents were reported to have been trapped alive only.

Some further observations on bandicoots were made in South India during the course of plague inquiries instituted by the Indian Research Fund Association (now the Indian Council of Medical Research).

Dealing with the results of this work in the Cumbum Valley, Madras Presidency (now Madras State), George & Webster noted that bandicoots were occasionally found dead from plague in the villages and actually proved the presence of the infection in two *Gunomys kok* found near settlements. Another report on this work even stated that

"it is usual in several villages of the Cumbum Valley for the epizootics to commence in bandicoots. The infection then passes to house rats and mice".

According to Wu Lien-teh, a statement to the same effect was made at the 1934 Conference of Medical Research Workers in Calcutta.
George & Timothy, reporting on a preliminary study of plague at a hill-station in the Nilghiris, also viewed the mole-rats (*Gunomys kok*) and *Bandicota malabarica* with suspicion. They stated that, in that locality, the mole-rats lived around human habitations and sought shelter in the houses during rainy seasons or when food was scarce outside. *Bandicota malabarica*, on account of its semi-domestic habits, seemed apt to act as "the intermediary in the extension of plague from house rats to wild rodents". However, though conditions seemed suitable for the establishment of a sylvatic-plague focus, the two workers failed to find infected wild-rodents.

Later work at Coonoor in the Nilghiris led to the suspicion that, besides *R. rattus* and some mouse species, bandicoots (*Bandicota malabarica*) and mole-rats (*Gunomys kok*) were also "concerned with the spread of plague". It was stated that the latter animals, which were found in large numbers in the potato-fields and compounds of dwelling-houses, migrated into the houses when the potato season was over. Their fleas were mostly *Stivalius*, but occasionally other fleas, including *Xenopsylla cheopis* and *X. brasiliensis*, were also found on them.

While no convincing evidence has so far been brought forward that the bandicoots of India play a role in the establishment and maintenance of sylvatic-plague foci, the findings made in Bombay City are of importance since they prove that plague-susceptible rodents (other than the commensal rats and mice) which are able to accommodate themselves to a life near man may become dangerous not only because they are apt to act as intermediaries in the spread of the infection, but also because they may take the place of less-susceptible rat-strains in the houses.

*Mongolia, north Manchuria, and Transbaikalia*. As noted in chapter I, plague, evidently spreading from a wild-rodent focus in Inner Mongolia, became entrenched among the commensal rats of south Manchuria. A similar evolution was presumably responsible for the frequent appearance of bubonic epidemics in Shan-si and Shen-si. On the contrary, in north Manchuria, Outer Mongolia, and Transbaikalia, such a transition of the infection from the wild to the commensal species seems never to have been observed. However, Wu Lien-teh assumed that the absence of rat plague in Outer Mongolia and Transbaikalia was due to a scarcity of these animals. Conditions for their involvement would have been more suitable in north Manchuria, but the occasional appearance of the disease there was due to an importation through human agency resulting in a spread of pneumonic plague directly from man to man without involvement of the rats.

*South-east Russia*. In south-east Russia also, the commensal rats did not seem to have become involved in the wild-rodent epizootics. However, in the southern part of this area, an important role was played by commensal mice, *Mus musculus musculus* and *Mus musculus wagneri*. 
The latter, living mainly in the open during summer but retiring, at the approach of cold weather, to haystacks and human dwellings (Tikhomirova & Zagorskaya) played an important role in bringing the infection to man, particularly as they were in the habit of visiting the burrows of wild rodents (Obolenski).

Central Africa. The interesting relationship existing in Central Africa between *R. coucha ugandae* auctt. (*R. natalensis*) and *R. rattus* seems to merit attention.

As stated in chapter 1, *R. rattus* formed the sole reservoir of the infection in Kenya and Tanganyika, as well as in those parts of Uganda where these rodents, having arrived at a comparatively recent date, were able to replace the native *R. coucha ugandae*. It was stated that *R. rattus* not only sheltered in the thatch of roofs, in the walls, and under the floors of the houses, but also sometimes lived, during the dry seasons, in sweet-potato fields near the settlements. The common field-rat, *Arvicanthis abyssinicus*, was sometimes seen to seek shelter in houses from which *R. rattus* was absent, and was then apt to harbour *X. cheopis* in addition to its usual flea, *X. brasiliensis*.

*R. coucha ugandae*—the rodent by far the most frequently found in human habitations—was the plague reservoir in the focus at Lake Albert, Belgian Congo, but, in the Lake Edward focus, seems to have shared this causative role with *R. rattus alexandrinus*. *Arvicanthis abyssinicus* was also regularly trapped indoors but, although more conspicuous in the huts of the Lake Edward focus, appears, in that area, to have remained free from plague so far.

South Africa. Both *R. natalensis* and *R. rattus* act as intermediaries between the primary gerbil-reservoir and man. The former—a rather inactive animal which prefers settling down in a deserted gerbil-burrow to digging a new one, and, at the same time, has a strong tendency to enter human habitations or even to seek shelter in them—is particularly apt to act as an intermediary.

The problem of the comparative importance of these two species is curious. In his 1948 study on sylvatic plague in South Africa, Davis stated that

"the semi-domestic multimammate mouse (*Mastomys coucha*), [scilicet *R. natalensis*] is the intermediary between the primary gerbil reservoir and man. The house-rat (*Rattus rattus*) brings infection into even closer contact with man than the multimammate mouse. The risk of plague to man is greatest in areas where one or both of these species commonly frequent farm buildings, especially when they are abundant and in close contact with wild-rodent colonies during an epizootic ".

Referring to this subject again in 1950, Davis, while once more emphasizing the importance of *R. natalensis* as a link between the wild-rodent reservoir and the commensal rodents and man, stated that
"in South Africa *R. rattus* stands at the end of the chain of infection from the sylvatic reservoir and is now largely responsible for human infections, especially in the hyper­enzootic areas ".

At the same time Davis stressed that, in South Africa, *R. rattus*

"does not however act as a permanent reservoir of plague in rural areas nor in urban areas where plague has from time to time shown itself... It appears therefore that the climatic conditions are against the permanent circulation of *P. pestis* in *R. rattus* populations in spite of the fact that they are heavily parasitised with well known vectors... ".

Referring to the plague manifestations in the Cape midlands during the period 1925-31, Davis pointed out that both *R. rattus* and *R. natalensis* were absent from the central and western karroo, *Mus musculus* being the only commensal rodent present. Consequently, conditions for a spread of the infection to man were not as suitable as in the Orange Free State. Human infection was mostly spread by means of a direct contact with wild rodents or their fleas.

*USA: western States.* In their fundamental study on plague in the western areas of the USA, Eskey & Haas stated that the commensal rat species, which were not indigenous to the western hemisphere, had been introduced into the Pacific regions more than 100 years ago. As a result, the rats became "well established from the coast to the western ramparts of the Sierra Nevada-Cascades, enjoying both rural and urban distribution" but, they added, "most of the vast region lying between the Sierra Nevada-Cascades and the Rocky Mountains—the great intermountain plateau—is entirely rat-free ".

On the eastern side of the Rocky Mountains, commensal rats were numerous in all States except Montana. In New Mexico, the valley of the Rio Grande was well populated by these rodents, as was the country east of this valley.

It would seem that, thus far, no instance of rat plague has been recorded in the interior of the western States of the USA. That this is not solely due to an absence of these animals, is suggested by a recent observation of Link. Investigating an epizootic among the cotton-tail rabbits of Lea County, New Mexico, he found commensal rats to be present but free from plague; none of the 32 specimens trapped had fleas.

In the coastal areas of the western States of the USA, a spread of plague not only from the commensal rats to wild rodents, but also from the latter back to the "domestic" species, seems to have been observed. As noted in chapter 1, it is usually assumed that an importation of plague into California by the sea-route led first to rat plague, the infection then spreading to the wild rodents. However, attention was drawn to the belief of some workers that pre-existing sylvatic plague had secondarily involved the San Francisco rats. It was also stated that a transition of the infection from the wild to the commensal species had probably been responsible for the 1924 Los Angeles epidemic and for the rat epizootics present
in 1942-3 at Tacoma, Wash. During the former outbreak, in which mainly *R. norvegicus* were involved, a few infected ground-squirrels were found but it was assumed that these animals might have become secondarily infected from the rats.²¹⁵

Attention was also drawn in chapter 1 to the fact that, on several occasions, wild-rodent fleas had been detected on rats and that, in one of these instances, Meyer & Holdenried,¹³⁴ working in California, succeeded in establishing the presence of plague in *R. rattus rattus* and *R. norvegicus* some of which were infested with ground-squirrel fleas. These authors stressed the potential danger of a spread of the infection from wild rodents living away from human habitations to species like *Peromyscus* and *Microtus* or to the commensal rodents, all of which had, to some extent, close contact with man. It should be noted, however, that, so far, instances of a transition of plague to the commensal rodents have been fairly infrequent, and that much attention is being paid to rodent and flea control in and around human settlements.

**Venezuela.** According to the descriptions given by Isaac Riaz,⁸⁶ conditions suitable for an exchange of the infection between the commensal and wild rodents existed in the Aragua State of Venezuela. Among the rats—which appear to have been immediately responsible for the infection of man—*R. rattus rattus* in particular was often of peri-domestic rather than of domestic habits, while *Heteromys anomalus*, one of the two wild rodents involved, frequented the fields and granaries and even entered the houses in search of corn. Guinea-pigs kept in houses (acmes domesticos) seem to have been involved in the 1943 outbreak.

**Argentina.** As stated in chapter 1, the relations between wild-rodent and rat plague were not uniform in the various plague-affected areas of Argentina. Since contact between the two groups of rodents was brought about only by the attraction of the wild rodents to the food-supplies in settlements and houses, secondary involvement of the rats was bound to take place mainly in well-cultivated areas. In sparsely populated and little-cultivated regions, e.g., Mendoza Province, there was hardly any chance for a transition of plague from the wild rodents to the rats.¹⁰,¹²

Barrera¹¹ pointed out with much reason that the greatest potential danger of the transition of plague to the commensal rats was the possibility of an establishment of the infection in these animals which would lead to successive epizootics. He claimed that such a stage had not been reached, the major rat-epizootics observed so far in Argentina having evolved in the coastal areas where no wild-rodent plague existed.

Quite naturally, human plague showed a sporadic incidence in the non-cultivated areas where man could contract the infection only when entering the haunts of the wild rodents. A secondary involvement of the
rivers through contact with wild rodents invading the settlements led, on
the contrary, to the infection of groups of people.11,109

Peru. In chapter 1 attention was drawn to a curious observation
made by Ramos Diaz164 during a plague outbreak at Lambayeque. The
rats (R. rattus) in this mountainous locality lived in the fields, but visited
the houses at night and thus came in contact with guinea-pigs kept by
the people. No doubt the infection of the latter animals, which was the
immediate cause of the epidemic, had been derived from the free-living rats.

In the wild-rodent plague focus in the Peruvian-Ecuador border region
commensal rats were altogether absent (Macchiavello118,119). The human
victims contracted plague when harvesting corn in fields infested with
Cricetidae which thus served as a link for passing the infection originally
present in tree-squirrels (Sciurus stramineus nebouxi) to man.

Discussing the recently discovered plague-focus in Huancabamba,
Peru, Macchiavello119 stated that there, also, commensal rats and their
fleas were absent. An invasion of the houses by the Cricetidae (the primary
reservoirs of the infection) after the harvests was responsible for violent
outbreaks of human plague.

Ecuador. As mentioned when dealing with the plague situation in
Ecuador in chapter 1, in that country also, the guinea-pigs (Cavia aperea)
kept in the houses, though often the immediate cause of epidemics,
were merely instrumental in passing the infection perpetuated among
free-living commensal rats to man. While Sáenz Vera174 stated that the
rats came in direct contact with the guinea-pigs and even cohabited with
them, Macchiavello117 came to the interesting conclusion that infection
of the latter was effected by an invasion of the houses by the rat-fleas,
rather than by the rats themselves.

In 1936, when dealing with the problem now under review, Wu Lien-
teh215 maintained that "generally speaking the danger of a spread of
plague from wild to domestic rodents is more apparent than real ".

Bold as this statement seems, no cogent reasons exist to refute it, parti-
cularly if it is kept in mind that increasing emphasis is now being laid on
reducing and even abolishing this danger by adequately controlling the
commensal rodents.

Commensal Rodents

Biology and ecology

Before entering into a discussion of the subject now under review,
an explanation is due as to why the term "commensal rodents" has been
used in these studies in preference to the usual designation of "domestic
rodents". The reasons for adopting the former name—which has also been
used by some other recent writers, e.g., Schwartz176—are: (a) to indicate
that the common rats and mice, though often forced in their struggle for
existence to shelter in human habitations, have really nothing in common with truly domestic animals, and (b) to indicate that it is by no means unusual for these rodents to lead a more independent existence instead of living in constant contact with man. Since, even when away from man, they usually depend on food-supplies destined for human consumption, the designation “commensal” seems to remain appropriate.

While, as discussed earlier (see p. 253), several families or subfamilies and numerous species are involved in wild-rodent plague, apart from some local exceptions, the commensal rodents implicated in plague outbreaks belong to three species of Murinae—Rattus norvegicus, Rattus rattus, and Mus musculus. As aptly maintained by Hinton,73

“possibly these species are the most highly organized members of their family; but unquestionably they are the most successful of mammals. They are clearly of Asiatic origin; but uninvited, and unfortunately for us, they have linked their fortunes with those of humanity. Human enterprise, in all its phases, and human negligence have disturbed the balance of Nature in favour of these species, have afforded them an unnatural degree of protection from their many enemies, a large and unmerited share of the world’s food-stuffs, together with perfect travelling facilities. Small wonder then that these creatures have invaded and colonized all lands...; that they have developed into serious pests, taking a heavy toll from human prosperity, and forming a most deadly menace to public health”.

**Description**

According to Hinton,73 these three species of commensal rodents may be described thus:

*R. norvegicus* is a large species of heavy and rather clumsy build; muzzle blunt, ears small, densely clad with fine and short hairs, thick and opaque; tail stout, never as long as the combined length of the head and body (fig. 25).

*R. rattus* is a smaller and slenderer animal of elegant build; muzzle sharp, ears large, almost naked and translucent; tail slender, at least as long as, and often considerably longer than, the combined length of the head and body (fig. 24).

*Mus musculus* is a small animal, looking like a miniature *R. rattus*; ears moderately large, clothed almost everywhere with short, fine hairs; tail about as long as the combined length of the head and body, frequently longer, rarely shorter.

Other easily ascertainable characteristics of the three species are enumerated in table XVII, the measurements quoted referring to adult specimens.67, 208, 215

As shown by table XVII, the coloration of the two rat species shows marked variations.

Black varieties of *R. norvegicus* are to be found, thus stultifying the name of “brown rat” which is often given to this species.

The differences in coloration found in the case of *R. rattus* have been used to distinguish between three subspecies—namely, *R. rattus rattus*, which is black or slate-coloured on both back and belly; *R. rattus alexandrinus*, which has a tawny back and a greyish-white belly; and *R. rattus*
FIG. 24. RATTUS RATTUS RATTUS

Size = 35.6-46 cm (including tail)

FIG. 25. RATTUS RATTUS NORVEGICUS

Size = 33-47 cm (including tail)

FIG. 26. BANDICOTA BENGALENSIS KOK (GUNOMYS KOK AUCTT.)

Size = 39.8-47 cm (including tail)
frugivorus, which also has a tawny back but has a white or lemon-coloured belly. However, many intergradations exist between these three subspecies and it is the opinion of some experienced observers, e.g., Jorge and Webster, that, for the practical purposes of plague work at least, these colour distinctions should be disregarded. It is interesting to see that this opinion has been endorsed by Ellerman who now considers R. r. alexandrinus and R. r. frugivorus not as true subspecies, but merely as forms or colour phases of a typical rattus race.

**Distribution**

General agreement seems to exist that the three rodent species now under review are of Asiatic descent, the original home of R. norvegicus and of M. musculus having been Inner Asia, and that of R. rattus, Burma and India. It is in agreement with this concept that R. norvegicus is the predominant species in north China, where it presumably arrived long ago by a land-route, while R. rattus, which is restricted to the coastal districts, appears to be a comparative newcomer. In central and south China on the other hand, both rat species usually coexist in varying proportions.

In contrast to what has been found in north China, R. rattus alone is found in the inland districts of India, R. norvegicus being present in seaports only (Webster; Taylor; George & Webster; George & Timothy). Referring to R. rattus, Ellerman stated that “in India wild (whitish-bellied) and commensal (dark-bellied) races occur together extensively. There is doubtless much interbreeding between the two.”
In India, as in China, commensal mice (*M. musculus*), belonging to various and, in part, not well-identified subspecies or races, are widely distributed.

Regarding the time of arrival of the three species of commensal rodents in Europe, it seems to be universally agreed that *M. musculus* came from Asia “with a people not older than the Neolithic” (Hinton). The question as to when the rats penetrated into Europe is, on the other hand, still debated.

Dealing with this problem in 1936, Wu Lien-teh felt entitled to refer to the “generally accepted opinion . . . that the ships of the returning crusaders were responsible for the importation of these pests (*Rattus rattus*) in the 12th century”—an opinion endorsed by several subsequent writers, e.g., Holsendorf, Morgan et al., Shrewsbury, and Tricot-Royer. However, as has been mentioned in chapter 1, in a recently published note, MacArthur quoted evidence to show that the rats have been known in Europe since ancient times.

While the possibility that *R. rattus* have been present in Europe longer than has usually been assumed should be given attention, it is difficult to believe that *R. norvegicus* became widespread there at an early date. Voicing the opinion of most authorities, Hinton stated in this respect that “the people of western Europe had no knowledge of the species [*R. norvegicus*] until 1716, when it was introduced to Copenhagen as the result of a visit by the Russian fleet. In the year 1727, a 'mouse year' in the Caspian region, vast hordes of these rats, according to Pallas, moved westwards after an earthquake (but probably in search of food); they swam across the Volga and swarmed into the houses of Astrakan. Thence they spread across Russia into western Europe.”

As a result of this mass immigration, *R. norvegicus* became the predominant rat-species in Europe. As maintained by Liston, this replacement of *R. rattus* by the Norway rat was due not so much to an actual struggle between the two species, but to the fact that the former rat could adapt itself less well to changes in the habits of man which led to the expulsion of the rats from human dwellings.

The validity of Liston’s contention was proved by further observations which showed that *R. rattus* was gradually apt to regain more and more of its lost territory. In this connexion, Jorge pointed not only to the importance of an incessant importation of this species by vessels, but also to the environmental changes produced by a further progress of civilization which put the Norway rats at a disadvantage. Thus, in Great Britain, it was found that structural improvements such as impermeable floors, ferro-concrete construction, and separate sewerage-systems were inimical for *R. norvegicus*, while *R. rattus* profited not only by the handicaps of its competitor, but also by the availability of fixtures like telephone-wires which facilitated its progress from building to building.
It has to be noted, however, that the conditions arising during the second World War were once more apt to favour the Norway rats. Morgan stated in this connexion that

"as the result of enemy action sewers have been opened up and other barriers against the brown rat have been destroyed. It is anticipated therefore that the black rat \( R. \textit{rattus} \) will have a tough fight to maintain his hold against an invader whose tastes are more catholic and who will live and breed under conditions that are not acceptable to his brother."

With regard to Africa, reference was made in chapter I to the statement of Jorge that \( R. \textit{norvegicus} \) was preponderant in the Mediterranean part of the continent, while \( R. \textit{rattus} \) reigned supreme in "Greater Africa". Attention was also drawn to interesting observations in Central Africa according to which \( R. \textit{rattus} \), though now forming the principal plague-reservoir in Uganda, seemed not to have arrived there before the beginning of the present century. Considering the rather conflicting views as to how this introduction took place, Hopkins reached the tentative conclusion

"that \( R. \textit{rattus} \) was brought into the inland areas of Kenya and into Uganda by the railway, which was begun at Mombasa in 1896 and reached Kisumu at the end of 1901."

As shown by a recent invasion of \( R. \textit{rattus} \) into the Kasenyi region of the Belgian Congo, the infiltration of this species into Central Africa is still continuing.

The distribution of the two rat species in South Africa seems similar to that noted in India, i.e., \( R. \textit{norvegicus} \) are found in the ports and their immediate vicinity, while \( R. \textit{rattus} \) are widely distributed in the interior (Davis).\(^{40}\) \( \textit{Mus musculus} \) apparently shows an even wider distribution; as noted earlier, it was the only commensal rodent in parts of the Cape midlands.

Dealing with the rat problem in the USA, Holsendorf stated that

"the 'black rat', \textit{Rattus rattus rattus}, otherwise known as the English rat or ship rat, was introduced to Europe in the twelfth century and was transferred to America about 4 centuries later. This antedated the arrival of the brown [Norway] rat by at least 200 years."

As noted earlier, Eskey & Haas were of the opinion that the importation of the two species into the coastal areas of the west took place at a time "well in excess of 100 years."

Most interesting data on the present distribution of the commensal rats in the USA is given in a handbook entitled \textit{Rat-borne disease: prevention and control}, published by the Communicable Disease Center of the US Public Health Service.

Contrary to formerly-held beliefs, it is stated in the handbook that, like the common mice, \( R. \textit{norvegicus} \) have become established in nearly
all parts of the USA. It was noted in particular that, though more than 99% of the rats on ships arriving in the USA from abroad were *R. rattus*, this species predominated in only some of the South Atlantic and Gulf Coast ports; in many seaports of these areas Norway rats were preponderant.

It appears on the other hand that, recently, *R. rattus*, probably because of its inability to compete with the Norway rats in the southern seaports, "has moved inland more and more each year and now predominates in a number of inland cities... How far it will spread is uncertain, but it is not likely to become widely distributed in the Northern States as the temperature may limit its spread in that direction."²⁰⁸

In the USA, as elsewhere, changes in local conditions were found apt to bring about shifts in local dominance from one rat species to another.

In Canada, commensal rats (mainly *R. norvegicus*) have been known to exist "since the earliest times" on the west coast of British Colombia.²⁸ A few places in the interior of this province also became infested, e.g., Nelson, where *R. rattus* were found. The prairie regions seem to have remained free until about 1900 when rats appeared at the international boundary of North Dakota and Manitoba. A gradual invasion of Manitoba and Saskatchewan followed so that rats are now established in all the larger municipalities of these two provinces. So far, however, rats do not seem to have colonized in Alberta.²⁸ Brown²³ stated in this connexion that they were repeatedly imported into this province by the railways, but were always found and destroyed.

The distribution and comparative importance of the two species of commensal rats in the South American plague-areas has been dealt with in chapter 1. Generally speaking, *R. norvegicus* appears to be the predominant species in the various Latin American countries (Moll & O’Leary³⁹).

General habits of rats and mice

From Hinton’s description⁷³ of the general habits of the commensal rats it may be gathered that:

(a) both *R. norvegicus* and *R. rattus* are normally nocturnal in habits, spending the day in their nests;

(b) while establishing their shelters as near as possible to their food-supplies, they may go further away in search of food. When regularly undertaking such foraging trips, they usually establish temporary hiding-places en route which, if convenient, may eventually be turned into the permanent dwellings;

(c) The rats invariably follow definite runs when leaving their shelters, proving so conservative in this respect that they may be caught by unbaited traps set in their path.
The general habits of the commensal mice are similar to those of the commensal rats. As a rule, however, they move about only in the immediate vicinity of their shelters, going, as maintained by Harrison, not further than 10 feet (approximately 3 m) from them. Another important point is that, in contrast to the commensal rats, particularly the Norway rats, *M. musculus* do not display shyness to alterations in their environment ("new-object reaction" quoted by Barnett).

**Gnawing.** Like all rodents, the commensal rats do not gnaw only to get access to food-supplies, but they must also do so in order to keep their rapidly growing incisors short enough for use.

Describing the gnawing abilities of the rats, the handbook on rat-borne disease states that these animals "will gnaw through any material with a gnawing edge and with a degree of hardness less than the hardness of the enamel of their teeth" including many synthetic building-materials, unhardened concrete, and even lead-pipes.

**Burrowing.** While *R. rattus* are more proficient in gnawing than the Norway rats, the latter show outstanding prowess in burrowing. However, in India *R. rattus* were seen to dig in soft materials like earthen floors and walls, and in the USA were also found able to burrow underground shelters (Milmore; Perolio).

It is important to note that although burrows for shelter and nesting rarely exceed 18 inches (approximately 45 cm) in depth, rats will dig much deeper and further to gain access to food-supplies.

**Gaining entrance.** Rats have been found able to gain entrance through openings admitting a cylinder with a diameter of half an inch (1.27 cm).

**Reaching and jumping.** As stated in the above-mentioned handbook, "rats have been observed reaching successfully from one point of vantage to another almost as far as their own length along smooth vertical walls. For this reason the figure of 18 inches [approximately 45 cm], the maximum reach of a large rat plus a small allowance for safety, represents the distance which must be completely clear of possible holding points."

It was also noted that "a rat can be expected to do a standing high jump of nearly 2 feet [approximately 60 cm]. With a running start, and with a trick of bouncing against the vertical surface two-thirds of the way up to gain momentum, the rat can jump 3 feet [approximately 90 cm] and occasionally a little more than 3 feet. Jumping out and down from a standstill, a rat can cover a horizontal distance of about 8 feet [approximately 2.5 m] while dropping less than 15 feet [approximately 4.5 m], and it can do even better with a running start."

Rats have been observed to drop 50 feet (approximately 15 m) without being killed. A roof rat swinging under a rafter is shown in fig. 27.

**Climbing.** While *R. rattus* is undoubtedly more able and prone to climb than the Norway rat, the latter will climb almost as well as the
former when necessary. In fact, as recently stressed by Jany,\textsuperscript{86a} \textit{R. norvegicus} by adopting tree-climbing habits may become dangerous, not only by depredating fruit-trees, but also by entering the upper storeys of houses from trees planted too close to the buildings.

**FIG. 27. ROOF RAT SWINGING UNDER A RAFTER**

Occasionally, Norway rats will also walk along telephone-wires almost as well as \textit{R. rattus} do habitually.\textsuperscript{208}

Rats are able to climb any vertical surface on which they can get a toenail-hold, but do not do so regularly in order not to rub their foot-pads raw.\textsuperscript{208} However, they can easily climb vertical wires which are not rat-guarded, and vertical pipes if the diameter of the latter does not exceed 3 inches (approximately 7.5 cm); they can also climb on the outside of any vertical pipe which is within 3 inches of a wall or other continuous support. Likewise, they can climb on the inside of vertical pipes, which are not rat-guarded, with a diameter ranging from 1\textfrac{1}{2} to 4 inches (approximately 4 to 10 cm).\textsuperscript{142}
Within the limits imposed by their size, the commensal mice climb well. It may be added that these agile animals are also proficient in jumping and burrowing.

Swimming. As shown by many observations, the Norway rats are excellent swimmers and divers. They are able to "swim through the water-seals in toilets and floor-drains without fear or hesitation" and habitually infest sewers and drains, the more so because—being by no means dainty in feeding habits—they find there food and drinking-water. Likewise, they may establish their shelters in the banks of surface-waters in order to prey on fish-food or even young fish (Cottam).

*R. rattus* and *M. musculus* do not enter water voluntarily. However, resembling the proverbial sailor who, though spending his life at sea, has never learnt to swim, *R. rattus* is the species of commensal rat usually found on ships.

**Feeding habits and food requirements**

As has been generally observed, and has been confirmed by baiting tests with Norway rats, owing to their nocturnal habits the commensal rats and mice usually feed, even in a quiet environment, at night. However, if their food-supply is short, they may snatch some nourishment at any time of the day, and may then devour the food available at the place of discovery instead of carrying it back to their shelters for consumption or storage, as they usually do.

Recent observations by Calhoun seem to suggest that rats differ in social standing within their colonies, each colony having a domineering bully which partakes first of the best food available and lays in no stores, in contrast to the weaker rats which try to do so whenever they have a chance.

As previously noted, *R. rattus* and also the commensal mice, being dainty feeders, are rather dependent on food-supplies destined for human consumption, while the Norway rats may subsist on any nourishment available to them on garbage heaps, in sewers, and the like.

No doubt can exist that, if given a choice, commensal rodents will show marked preferences for certain kinds of food, but they can adapt themselves to the staple foods available, in particular, often living mainly on cereals. It is interesting to note in this connexion that, Davis, on the basis of studies at Baltimore, Md., maintained that

"Norway rats must have a substantial amount of grain (usually available in the form of bread) in their diet. Rats will starve in the midst of plenty of raw or cooked vegetables".

While little credence can be given to many of the statements made in regard to the amount of food a commensal rodent can consume in a given period of time, some reliable figures have recently become available.
Thus, Chitty & Shorten estimated that, on an average, a Norway rat consumed 30 g of wheat per day (0.35 g to 0.50 g per gulp). An estimate made in India was that R. rattus consumed about 3,908 g of rice per year or 10.7 g per day—a figure which tallies well with the 11 g of wheat found by McDougall to represent the daily food-consumption of R. conatus.

**Hosts of infection**

R. rattus appears to be far less in need of drinking-water supplies than the Norway rat. Still, according to the above-mentioned observations in India, R. rattus consumed 143.5 ounces (approximately 4 kg) of water per year, or 0.4 ounce (approximately 11 g) per day. It was found that this species could subsist on dry rice without water up to 24 days, and on water alone for 10 days, while controls given neither food nor water subsisted up to 6 days.

**Water requirements**

Though R. norvegicus has been found able to subsist under laboratory conditions for quite considerable periods without water, in general, water is indispensable to these rats as they are used to drinking freely. If necessary, they may climb the roofs of houses to get to water-tanks.

**Harbourage**

As emphasized in the handbook on rat-borne disease, the possibilities for the harbourage of commensal rodents are almost endless in variety. The animals may prepare shelters by burrowing in embankments, near or under buildings, or may settle in the latter where, as stated by Holsendorf, three types of harbourage are available:

1. **Structural**—offered by permanently protected spaces between double walls, under double floors, above false ceilings, behind enclosed stairways, raised platforms, or boxed-in pipes, etc.

2. **Incidental**—offered by furniture and equipment.

3. **Temporary**—offered by mass storage of material or merchandise, rubbish heaps, old furniture, odds and ends piled in cellars, attics, and closets, and similar accumulations which, if left undisturbed for periods of several weeks, can and will be used by rats for homes and breeding places.

Commensal rodents are apt to harbour not only in or near premises inhabited by man, but also in those parts of houses or in outhouses where domestic animals are kept, in buildings where foodstuffs are processed or stored, and in establishments such as slaughterhouses.

Morgan et al. found colonies of R. norvegicus and M. musculus flourishing in cold-storage plants where these animals had adapted themselves to an existence at 17°F (−8.3°C) by growing slightly thicker furs and layers of fat underneath. The ability of M. musculus to live and breed in cold-storage plants was confirmed by Laurie.
As indicated by the common names often given to them, the "sewer rat", *R. norvegicus*, as a rule prefers to live underground, whereas the "roof rat", *R. rattus*, harbours in or under the roofs of buildings, or in the upper storeys.

The Norway rat is well suited for an underground existence, not only because it is much more hardy and far less dainty in habits and food requirements than *R. rattus*, but also because, though not keen to climb as a rule, it is outstandingly proficient in burrowing shelters. One must also suppose that, in hot climates, this rat prefers the comparative coolness of its burrow to an existence above ground.

The reasons why *R. rattus*, an excellent climber, prefers to shelter in the upper parts of buildings, appear to be threefold: it is less able to withstand hardships, particularly a cold environment, than the Norway rat; it is much cleaner in habits and feeding than *R. norvegicus*; by harbouring high up, it is able to keep out of the way of any Norway rats living in the same premises underground, or on the ground floors.

The importance of the last-mentioned factor is proved by observations which have shown that, in the absence of Norway rats, *R. rattus* may live on the ground floors or even underground. For instance, according to Girard, in Madagascar, where Norway rats are absent, *R. rattus* inhabits the sewers in their place.

If the two rat species coexist in a locality, their relative distribution may be irregular. Thus, Milmore noted that, under such circumstances, sometimes buildings or whole city-blocks were infested by *R. rattus* and *M. musculus* only, whereas adjacent houses or blocks also harboured *R. norvegicus*.

**Breeding habits**

The commensal rats and mice are extremely fertile. They reach sexual maturity long before they have completed their growth. The classical investigations undertaken in this respect by the Plague Research Commission in Bombay (quoted by Hinton) yielded the following results:

<table>
<thead>
<tr>
<th></th>
<th>Minimum weight of sexually mature animals</th>
<th>Average weight of adult animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g)</td>
<td>(ounces)</td>
</tr>
<tr>
<td><em>R. norvegicus</em></td>
<td>100</td>
<td>about 3.5</td>
</tr>
<tr>
<td><em>R. rattus</em></td>
<td>70</td>
<td>about 2.5</td>
</tr>
</tbody>
</table>

As confirmed by the recent investigations of Davis & Hall, male rats remain fertile throughout the year. According to Hinton, the females have a sexual season extending for any one female for 9 months of the year, during which time they come "on heat" at intervals of about 10 days. These periods last for a few hours only and if no copulation takes place during that time, a female cannot be impregnated until her next heat. During
the sexual season some evidence of the regular occurrence of heat is shown even by a pregnant female; parturition during the season is immediately followed by heat so that a female may be impregnated a few hours after the birth of a litter.

   The period of gestation is usually about 21 days, but may be prolonged for a further 10 days in a female nursing her young.

   According to figures supplied by the Plague Research Commission (quoted by Wu Lien-teh 215), the average and maximum numbers of young rats per litter were:

   \[
   \begin{array}{ll}
   & R. \textit{norvegicus} & R. \textit{ratus} \\
   \text{average} & 8.1 \text{f} & 5.2 \\
   \text{maximum} & 14.0 \text{g} & 9.0 \\
   \end{array}
   \]

   The menopause usually appears at the age of 15 to 18 months, but particularly good environmental conditions tend to delay its appearance. 73

   Dealing with the breeding habits of \textit{M. musculus}, Hinton 73 stated that these animals

   "attain sexual maturity when three months old. The sexual season of the females is a very long one. The period of gestation is normally from nineteen to twenty-one days; it may in certain circumstances be shortened to twelve or thirteen days. 'Heat', not lasting longer than twelve hours, rapidly succeeds parturition. Many litters are born throughout the year, but fewer in the cold months. The number of young per litter is between five and six, but it may be as many as nine or as few as two."

   Scrutinizing the evidence available in regard to annual pregnancy- and lactation-rates, both from cities in the USA and other places, e.g., Bombay and Dakar, Davis 33 arrived at the following figures for \textit{R. norvegicus}:

   \[
   \begin{array}{ll}
   \text{pregnancies per year} & 4.3 \\
   \text{lactation periods per year} & 3.8 \\
   \end{array}
   \]

   However, since there was a great loss of sucklings, Davis reached the conclusion that "an adult female weans rats at the rate of about 10 rats per year."

   It is important to note that this figure is rather below the estimates given by earlier plague-workers in regard to the number of young rats produced per year.

   Though rat-breeding continues throughout the year, particularly under optimal environmental-conditions, it has been observed that, as a rule, there are one or two seasons during which the number of pregnant and nursing females is maximal. Studying this problem, Buxton 24 concluded that, in temperate and subtemperate climates, the main breeding-periods coincided with the warm season. That great heat exerts an unfavourable

\footnote{8.7, according to Davis,33}

\footnote{8. According to Holsendorf,14 litters of more than 20 have been observed.}
influence on the breeding-rate seems to be suggested by the observation of Raynald\(^1\) that, at Shanghai, which has an excessively hot summer, the breeding season of the rats coincided with the end of winter and the beginning of spring.

Buxton\(^2\) was of the opinion that the degree to which the rats breed is mainly determined by the state of their nutrition. Their breeding-rate might, therefore, be influenced by an abundant or poor harvest which, in turn, is governed by climatic conditions.

It is quite likely, therefore, that in the case of the rats, as in that of the wild rodents, a mechanism regulating their numbers according to Elton’s concept\(^3\) might be at work. There can be no doubt, however, that in the case of the rats, many extrinsic influences would be bound to interfere with the operation of this “natural” regulatory mechanism.

*Density of rat populations*

One cannot doubt that a relation exists between the density of the rat population and that of the human population. Thus, it was recently pointed out by Gracie\(^4\) that in Great Britain the greatest concentrations of rats in sewers were present beneath the most densely-populated precincts.

At the same time, however, one should give no credence to the time-worn statement that the number of rats in a given locality invariably equals that of the human population—an estimate which, as pointed out by Davis & Fales,\(^5\) was originally derived from statements regarding the rat incidence on English farms. Davis & Fales announced as the result of their observations in Baltimore, Md., that, in 1947, the estimated total number of rats in Baltimore was 200,000, or not more than 1 rat per 5 human inhabitants. They added, however, that this figure was much lower than that of 400,000 rats estimated to have been present in Baltimore in 1944 and ascribed this reduction in the rodent population on the one hand to rat-control measures and general sanitary improvements, and to an influence of the cold, wet spring of 1947 on the other.

Obviously, therefore, it is impossible to set standards for the density of rat populations in different places, or at different times in any one place. On the contrary, it is necessary to ascertain in each instance the number of rats present through a preliminary survey, and then to watch the trend of the rodent population with the aid of the methods which will be described in a future chapter.

As far as one is entitled to draw conclusions from the scanty information available, it would seem likely that, in other well-sanitized cities, as in Baltimore, less than one rat per head of human population would be found. There is reason to assume that in less well-sanitized cities, and in smaller settlements in general, the number of rats equals or even exceeds the number of people. This is presumably the rule on farms (Tice\(^6\)).
Population dynamics

In a profound study on the characteristics of global rat populations, Davis drew a distinction between two types of changes in rat populations:

(1) an increase up to the capacity of the area in question to support the rats, and

(2) fluctuations due to changes in this capacity.

As pointed out by Davis in the latter connexion, the rat population in a place at any given moment is the result of an interaction of two factors—reproduction and mortality. The effect of a possible third factor—namely, the increase or decrease of the rodent population through movements—is actually of no importance.

With regard to the force of mortality, Davis maintained that, as indicated by data from a farm, only about 5 out of 100 rats lived for 12 months, the average life of a rat in a population being about 6 months.

Dealing in greater detail with the quantitative relations of the various regulatory factors, Davis stated the following:

(a) Environment. A shortage of either food-supplies or nesting-sites (harbourage) limits the number of rats in a given locality or at a given time. This limitation is “density-independent”, i.e., it is operative regardless of the size of the rat population in question.

(b) Predation. Davis used the term “predation” to indicate all causes of rat mortality, including that caused by cats, viruses, traps, or poisons. He emphasized that, in contrast to the above-mentioned environmental factors, predation was “density-dependent”, the proportional effect exerted by predators decreasing pari passu with a decrease in the rodent population according to the law of diminishing returns.

(c) Competition. As is obvious, the effect of competition among the rodents for food, living space, and mates is also “density-dependent”, increasing hand in hand with population increases.

Comparing the practical value of predation with that of sanitation, which increases competition between the rodents for food and living space, Davis came to the important conclusion that, while it is generally feasible to increase competition, it is rarely possible to increase predation to a satisfactorily high degree.

Movements and migrations

As in the case of wild rodents, when dealing with the movements and migrations of the commensal rodents, it is necessary to distinguish between:

(a) short-range daily (or, one should rather say, nightly) excursions, necessitated by the search for food and water;
(b) seasonal or, as Macchiavello\textsuperscript{119} called them, “reversible” migrations undertaken periodically to fields under cultivation, and return to the settlements after the harvests have been gathered;

(c) true, progressive migrations covering considerable distances.

Recent observations have confirmed that the urban rats and mice spend most of their life within a very limited home-range. Davis,\textsuperscript{33} summarizing the evidence available in this respect, maintained that movements of rats even from one street-block to another were rare, and that, if a few rats left their home-range, the chances of their survival were slight.

Commensal rodents living in rural environments may also have a strictly limited home-range\textsuperscript{34, 209} but, as noted above, they are able to make seasonal migrations.

Some observers have noted that commensal rats living in rural environments are also apt to undertake progressive migrations covering wide distances. Recently, Macchiavello\textsuperscript{119} stated that these animals may move en masse over hundreds of kilometres, possibly in search of vitamin E in grain-germs.

Nevertheless, it would seem that, in most plague-areas, such mass migrations are exceptional. It is true, as discussed earlier, that, even now, commensal rats invade areas previously unoccupied by them, but it is probable that these movements are not mass migrations in the true sense, but rather gradual infiltrations.

Passive transportation

As discussed in chapter 1, a passive transport of rats by ships and railways was of most fateful importance for the spread of plague. Further, there can be no doubt that, in addition to rail-transport, in some plague-areas, motor-vehicle transport—particularly trucks—led to a spread of the infection by the carriage not only of infected fleas, but also of infected rats.

It is less certain whether this holds generally true of primitive means of transport. Summarizing the evidence available in this respect, the annual report for 1937 of the Eastern Bureau of the League of Nations Health Organisation, Singapore, pointed out that observations made in the Cumbum Valley of south India did not support the view that rodents or even fleas were frequently carried in consignments of rice transported on bullock-carts. It was noted, however, that, in the opinion of some observers, even “pack horses are common means in Java of conveying infected rats and their fleas”\textsuperscript{102}

It is curious to note that, according to a report made in 1942,\textsuperscript{181} a live mouse was found in the galley of an aeroplane arriving at Miami, Florida, from Puerto Rico. It was stated in this connexion that

“considering the increase in the size of transport planes, the carrying of foodstuffs that are attractive for rats, and the ingenuity of these animals in boarding vessels, seeking
food supplies, establishing nesting places and avoiding man's devices for destroying them, the possibility of rats boarding airplanes is certainly not remote).

**Damage caused**

Calling the rat "Public Enemy No. 1 among the animal pests", Holsendorf aptly quoted a statement by Creel & Akin who considered this rodent not only as the least useful, but also as one of the most dangerous, of nature's parasitic animals living at man's expense.

A recently published indictment of the commensal rats stated that, apart from their ominous role as the reservoirs of plague and other diseases, they caused tremendous harm by consuming or spoiling grain, merchandise, fruits, vegetables, nuts, and eggs; by killing chicks and other young birds, as well as young pigs and sheep; by damaging sugar and other plantations; and by starting fires by gnawing the insulations on electrical conductors, and in other ways.

Though many authors have tried to assess the monetary equivalent of the depredations caused by the commensal rodents, one must fully agree with Kalmbach's statement that hardly any reliable data is available in this respect. This holds particularly true in the case of foodstuffs because, as has been pointed out with much reason, rats will feed on any edible substance from valueless garbage to expensive packaged-products, which they may damage to an extent far in excess of the value of the amount of food they actually consume.

That the damage done by rats to foodstuffs may far exceed the loss caused by actual consumption, has been clearly demonstrated by the recent experiments of Barnett. Forty rats were divided into 4 groups of 10 and each group was put into a separate room. One ton of wheat, divided into 9 sacks, was placed in each of the 4 rooms, and left for 12, 20, 23, and 28 weeks, respectively. The rats fouled 70.4% of the wheat while causing a weight-loss of only 4.4%. The total monetary loss amounted to 18.23% of the value of the wheat and sacks; most of this was due to the damage done to the sacks.

With regard to the diseases caused by the commensal rodents, it has been recently stated by Mohr that these animals are responsible, or probably responsible, for the following infections or infestations.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Reservoir</th>
<th>Mode of transmission to man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plague and murine typhus</td>
<td>Rats and to a much lesser extent</td>
<td>Through rat fleas</td>
</tr>
<tr>
<td></td>
<td><em>Mus musculus</em></td>
<td></td>
</tr>
<tr>
<td>Salmonellosis</td>
<td><em>M. musculus</em> and rats</td>
<td>Contamination of foodstuffs by droppings and possibly also through rat fleas</td>
</tr>
<tr>
<td>Disease</td>
<td>Reservoir</td>
<td>Mode of transmission to man</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>----------------------------------</td>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>Leptospiral jaundice</td>
<td>Norway rats in particular</td>
<td>Contamination of food and water by urine of infected animals</td>
</tr>
<tr>
<td>Rat-bite fever</td>
<td>Rats, and probably also M. musculus</td>
<td>Bite by an infected rodent</td>
</tr>
<tr>
<td>Rickettsialpox</td>
<td>M. musculus</td>
<td>Through mouse-mite (Allophanus-sus sanguineus)</td>
</tr>
<tr>
<td>Lymphocytic choriomeningitis</td>
<td>Probably M. musculus</td>
<td>Possibly through direct contact, or through contaminated food</td>
</tr>
<tr>
<td>Amoebiasis and tapeworm infections</td>
<td>Rats</td>
<td>Food contaminated by droppings</td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>Dogs and possibly Norway rats</td>
<td>As stated by Mohr, both rats and human beings might acquire infection from a common source, but rats might take a share in infecting man</td>
</tr>
</tbody>
</table>

### Plague in Commensal Rodents

As shown by the studies of Abel,¹ Tiraboschi,² and others, the now generally accepted concept that, as a rule, plague epizootics in the commensal rats precede and cause human manifestations of the disease had been arrived at by some early observers. Thus, a sacred, poetical work, the Bhagavata Purana, written centuries ago in Sanskrit, warned householders in Hindustan to leave their homes as soon as rats fell from the roofs and died.¹⁰⁷ To judge from a statement recorded by Forbes ⁵² (quoted by Simpson ¹⁸⁶), this advice was still followed by the population of Marwar during the Pali plague of 1836-8.

A great rat mortality, preceding and accompanying the human outbreaks, was noted by a British official during the Kumaon outbreak of 1834-5. The same phenomenon was observed and commented on by medical men during several of the later epidemics in that area, and during earlier outbreaks in Garhwal (Simpson ¹⁸⁶).

The Chinese in Yunnan were also well aware of the fact that rat mortality presaged outbreaks of human plague.²¹⁵ Thus, during the epidemic at Chaochow in 1792, Shih Tao-nan (quoted by Wu Lien-teh ²¹⁵), in a poem entitled “Death of rats” which he wrote a few days before he died of the disease, deplored that:

> "Few days following the death of the rats,<br>Men pass away like falling walls!"

In China also, some medical men appear to have suspected that the rats were of etiological importance in plague. Lowry,¹¹¹ noted that, during the 1882 outbreak at Pakhdoi, “in nearly every house where the disease broke out, the rats had been coming out of their holes and dying on the
floors” and dissected some of these animals, but found nothing definite microscopically.

Niles recorded that, during the 1894 outbreak in Canton, one of the Chinese officials urged the population to kill rats, and to collect dead ones, offering to pay, out of his own pocket, 10 cash for every carcass brought to him.

Rennie, who examined a considerable number of the rats thus collected, noted that 90% of them showed enlargement of lymph-nodes which, however, was present “in a much less marked degree than in the human subject”, and he raised the question “is the disease in man and animals identical?”

In marked contrast to the advanced views held in the East, no convincing evidence seems to be available to show that the role played by the rats in the causation of plague outbreaks was realized at an early date in Europe. Though it was commonly believed that animals as well as humans were affected by the plague poison, which was supposed to be fermenting in the ground, no particular attention, or even none at all, was paid in this connexion to the rats (MacArthur). It is also significant that the rats and mice often depicted in illustrated texts and in pictures after A.D. 1250 invariably seem to be healthy (Neustätter).

One of the main reasons why the rats attracted no attention was probably that their dying seemed of no importance in comparison with the havoc caused by simultaneous “pestilences” among domestic animals which, though actually due to other infectious agents, were then thought to be identical with plague.

Far more distressing than this early ignorance is the fact that, even when evidence proving the existence and importance of rat plague became available about sixty years ago, it was not accepted forthwith.

It should be noted in this connexion that Yersin (quoted by Lagrange) obtained positive findings in rats almost immediately after the discovery of the plague bacillus in June 1894, for he noted in his diary under the date of 23 June 1894: “I search and find the organism in the corpses of dead rats, and there are many throughout the city.”

Yersin amplified this statement in the first elaborate report on his work at Hong Kong, reaching the conclusion that “plague is therefore a contagious and inoculable disease. It is probable that the rats are the principal vector.”

Convincing as Yersin’s evidence was, it did not impress the other workers at Hong Kong. Lowson’s official report on the 1894 epidemic, for instance, contained the following statement: “The question of the infection of rats previous to the epidemic being noted in human beings has been made too much of.”

\[\text{\textsuperscript{h}}\] “la peste est donc une maladie contagieuse et inoculable. Il est probable que les rats en constituent le principal véhicule.”
Since, as Millot Severn \(^{178}\) (one of Lowson’s successors) put it, the early plague investigators in Hong Kong were “obsessed with the idea that bubonic plague was primarily a gastro-intestinal infection”, it was not until 1901 that rat destruction was started there (Brown \(^{20}\)), even though this method had been expressly recommended by Yersin \(^{218}\) as early as 1897.

Similarly, as recently stated by Link \(^{106}\) in an article entitled “Plague on the high seas”, the importance of the rats in ship-borne plague was not recognized until 1906, even though, between the years 1900 and 1904, infected animals had been found on vessels on nine different occasions.

Nevertheless, Yersin’s findings were confirmed by some early plague-workers, among whom Ogata \(^{149}\) in Formosa (1897), Simond \(^{185}\) and Hankin \(^{68}\) in India (1898), Ashburton Thompson \(^{196}\) in Sydney (1900), and Blackmore in Port Elizabeth (1902) were particularly praised by Liston \(^{107}\) who, himself, played a worthy part in plague research. In 1905, the British Secretary of State for India, following proposals made by the Lister Institute a year earlier, established a Joint Advisory Committee, appointed jointly by him, the Royal Society, and the Lister Institute. The Committee appointed a Plague Research Commission and, from 1905 onwards, Liston and other workers (notably, Lamb, Petrie, and Rowland) took part in its activities. The findings of this Commission formed an outstanding monument to scientific teamwork and were instrumental in defining the fundamental problems of plague in rats as well as in rat fleas.\(^{215}\)

As summarized by Lamb,\(^{98}\) at the time the Plague Research Commission commenced its work, even those who were agreed upon the causal role of the rats differed in their views as to how the infection was spread among these animals. Some observers were still influenced by time-honoured beliefs ascribing importance to an infection through the air, through direct contact, or through contaminated inanimate objects (fomites). Others, laying stress upon the cannibalistic habits attributed to rats, considered that plague might be transmitted through the rats feeding on one another. Through a series of well-planned experiments the Commission was able to refute these beliefs and, at the same time, to establish that plague was principally an insect-borne—and, particularly, a flea-borne—infecction.

**Relative importance of roles of rats and mice**

**Rats.** In the past, as well as recently, it has been argued by some writers that *R. norvegicus* is less susceptible to infection with *P. pestis* than *R. rattus*, and therefore cannot independently act as a plague reservoir.

However, no convincing evidence has ever been brought forward to support such claims. Reliable laboratory-investigations have proved that both species are equally susceptible to the infection and, as pointed out earlier in this monograph, observations made in the various plague-areas have
shown that each of the species is capable of taking an independent part in the causation and perpetuation of the disease.

At the same time it must be realized that, if coexistent in a plague-affected locality, the two rat species may play different roles. This point was well illustrated by the investigations made by the Plague Research Commission in Bombay. 98

The Commission's observations showed that, as far as could be judged from statistical evidence, R. norvegicus, though considerably less common in India, appeared to be twice as liable to natural plague-infection as R. rattus. There seemed no doubt, however, that this higher incidence of the disease in the Norway rats was the result not of a higher susceptibility to the infection, but of their far heavier flea-infestation. Evidently it was mainly due to the same reason that R. norvegicus was of prime importance for the carry-over of the infection during the inter-epizootic periods.

It was further found that, at the onset of the plague season, the infection became rampant among this species first. Then, after an interval of about ten days, epizootics appeared among R. rattus and these, in turn, were mainly responsible for the appearance of human plague after a further interval of 10 to 14 days. However, it was certain that this evolution was due merely to the fact that R. rattus lived in houses and therefore in close contact with man, whereas R. norvegicus, though infesting the ground floors of houses, were more numerous in other locations, such as gullies, compounds, stables, warehouses, and shops.

The conclusion reached by the Plague Research Commission that differences in the habitat of the rats and in their flea-infestation, rather than differences in their susceptibility to the infection, determined the role played by these animals in plague, has been endorsed by other workers, recently by Roberts 169, 171 in Kenya. Though R. rattus alone were involved in the Kenya outbreaks, two types of plague manifestations could be distinguished—namely, an urban one characterized by the occurrence of epidemics, and a rural one of an endemic type. In the opinion of Roberts, it was of importance in this connexion that, in the urban localities, the rats lived underground so that both they and their fleas were apt to come into close contact with man, whereas in the rural areas there was little, if any, possibility for such a close contact because the rats there lived in the thatch of roofs. In fact, as maintained by Cormack 29 and Roberts, 171 the roof-rats did not enter the interior of human dwellings at all; during the day they hid in the thatch, and emerged at night on the outside of the huts, and then proceeded to the cereal stores to feed.

Mice. As pointed out by Jorge, 89 M. musculus usually lives in close contact with man and would be rather dangerous were its role in plague similar to that of the rat. Actually, however, though often found infected during the course of rat-epizootics, the mouse is usually a victim of the
disease rather than an agent in its perpetuation. The main reasons for this fortunate circumstance are:

(a) as established by the Plague Research Commission and confirmed by other workers, these animals were often less susceptible to plague infection than the rats collected in the same localities;

(b) the flea-index of the mice was often considerably lower than that of the rats and, still more important, the specific flea of the mice, *Leptopsylla segnis*, is not an efficient plague-vector and hardly ever attacks man.

However, as shown by several observations, *M. musculus*, if infested by other than its usual fleas, may be of some, or even of considerable, importance in the perpetuation and spread of plague. Mention has already been made of the role ascribed to these animals in south-east Russia, and also of the observation by Herivaux & Toumanoff in Indochina of an epizootic among *M. musculus* infested with *X. cheopis*. Some importance was also ascribed to this rodent species in Brazil. Girard, while stating that the mice usually did not appear to be involved in the plague outbreaks of Madagascar, referred to one instance where, in a rural environment, a considerable epizootic had been found present among these animals as well as among the rats.

*Spread of rat epizootics*

It is obvious that, when paying attention to the spread of plague not from one individual rat to another, but from one group of these animals to other groups, a distinction has to be made between what Gill adequately called the "intramural" spread of the disease from one house, block, or part of a settlement to adjacent houses, blocks, or precincts, and spread at distance or, as observers in Java have called it, "metastatic" spread.

It is easy to understand how plague, conveyed either by the rats themselves or by their fleas, may creep from one house or group of houses to others, the more so since, as observed by the Plague Research Commission, these rodents may desert infected buildings, particularly when the human inhabitants have died or have left.

Such a spread of the infection may be extremely slow; one instance was recorded where a rat epizootic in India took six weeks to travel 300 feet (approximately 91 m). A further fact of great importance is that the intramural spread of plague often takes place in an irregular manner, leaving the rat population of some houses, blocks, and even whole precincts unaffected.

The question as to whether active movements of the rats are responsible for the long-distance spread of plague, and if so, to what extent, has been the subject of considerable debate. In the opinion of the Plague Research Commission, this factor was of no importance, the spread of the infection
per saltum being effected by the passive transportation of rats or their fleas by human and goods traffic. This opinion has since been shared by most subsequent workers.

**Factors limiting the spread of rat plague**

It may be maintained that the spread of plague among the commensal rodents is limited mainly by:

(a) factors governing the role of the insect vectors, particularly the fleas;

(b) a diminution in the number of rats by severe epizootics which reduces the chances of infection among the scattered survivors;

(c) the irregular spread of the infection which may by-pass groups of animals living in the vicinity of plague foci;

(d) a state of non-susceptibility to the infection developing in individual animals or in rodent-herds which have been exposed to plague.

With regard to the last-mentioned factor—the only one which can be evaluated at present—it should be noted that some writers stressed the importance of an immunity acquired by the commensal rodents in the course of plague epizootics. They claimed that the animals became immune by surviving an attack of the disease, and sometimes even maintained that rats may acquire an active immunity by exposure to subinfective doses of *P. pestis* without passing through a stage of manifest illness. More important still, these writers postulated that the immunity acquired by the commensal rodents through exposure to plague was passed on to their offspring.

While no doubt can exist that commensal rodents, particularly those which have recovered from an attack of plague, may become immune against the infection, it is probable that such an active immunity is maintained for short periods only; Gill\(^{62}\) was of the opinion that the immunity did not last more than a few months.

The rather unlikely assumption that the acquired immunity of commensal rodents is passed on to their offspring has been definitely disproved by investigations made by Sokhey & Chitre.\(^{155,159}\) Experimenting with the offspring of white mice which had survived plague infection owing to having been previously immunized with Haffkine vaccine, these workers demonstrated that the young mice, when inoculated with standard infective doses of *P. pestis* at an age of 3 to 4 months, showed no evidence of immunity.

For these reasons, it would appear that the immunity acquired by some commensal rodents in the course of plague epizootics usually plays no important, and certainly no permanent, role in the limitation of the
epizootics. However, it is of the utmost importance in this respect that, as first shown by the Plague Research Commission\textsuperscript{156} and confirmed by other observers, particularly Sokhey & Chitre,\textsuperscript{188,189} the presence of plague in a herd of commensal rodents leads to a gradual extinction of the susceptible strains of the animals, whereas resistant strains survive and produce offspring which are also plague-resistant.

In this connexion the Plague Research Commission\textsuperscript{156} established that:

(a) a considerable number of the Bombay rats were resistant to laboratory infection with \textit{P. pestis} produced either through flea-bites or through subcutaneous injection of infective doses standardized according to the rather unsatisfactory methods then available;

(b) the survivors of experimental epizootics were also highly resistant when challenged with such test doses;

(c) rats caught in plague-free localities in India showed a high susceptibility to the infection, amounting to 97\% to 100\% in the case of Madras City, whereas rats from plague-affected localities were more or less resistant, the degree of their resistance to the test doses generally being proportional to the extent to which the places in question had suffered from the disease.

Sokhey & Chitre\textsuperscript{188,189} repeated these investigations with accurately standardized test-doses. They compared the aggregate human-plague death-rate (calculated per thousand of the population) of numerous Indian cities and towns during the period 1899-1929 with the percentage death-rate from plague in batches of \textit{R. rattus} obtained from the same localities. As a rule, about 50 adult animals were taken from each locality. The results showed that, on the whole, the percentage rates of infection were inversely proportional to the human-plague death rates, i.e., the resistance of the rats to test infection was higher the more the locality in question had suffered from plague.

Confirming some earlier observations, e.g., those of Spencer\textsuperscript{191} at Alabama, Sokhey & Chitre also established the important fact that, even in localities which had never suffered from plague, a considerable minority of the rat populations could be resistant to the infection. It is therefore obvious that, normally, both plague-susceptible and plague-resistant rat-strains exist side by side, the occurrence of plague upsetting the balance between the two groups by killing the susceptible animals.

The question of whether this also holds true of the rat populations in rural endemic-areas—only urban settlements were investigated by the above-mentioned workers—seems, thus far, to have received insufficient attention. Rao\textsuperscript{166} stated that he had found evidence of resistance to the infection in a group of 22 rats caught in a village of Hyderabad State. However, George & Webster\textsuperscript{60} established the reverse in the Cumbum
Valley and, similarly, Roberts reported that he had found no evidence of plague resistance among the rats of a rural endemic-area in Kenya. It would be desirable to confirm these findings through further and more extensive investigations. As far as evidence is available, it would appear that the rat populations in rural endemic-areas may remain susceptible to infection with *P. pestis*.

Considerable time may elapse before the preponderance of resistant rats becomes sufficiently marked to exert an influence on the plague situation in the localities concerned. For, as summarized by Wu Lien-teh, this process of selection "may show an indefinite variety in different localities and certain influences may tend to retard it. Liston for instance pointed out with reason that an epizootic may leave susceptible rat colonies untouched so that at its termination both resistant and susceptible rats may survive and interbreed. A similar situation may be created if susceptible rodents are imported into a locality where the local rats have become more or less resistant. In fact the Plague Research Commission found that town rats in Bombay showed a higher immunity to plague infection by feeding and by flea transmission than ship-rats from the harbour, which latter had presumably not been exposed to infection.”

No doubt can exist, however, that when reaching a sufficiently high degree, the resistance of the commensal rodents to the infection is apt to exert a profound influence on the plague situation in the areas concerned. Liston maintained in this connexion that "the evolution of an immune race of rats following on a long series of epidemics may explain the gaps in the continuity of epidemics which are known to have occurred in the history of plague in different countries.

and that this may also

afford an explanation for the cessation of epidemics of plague in countries in which changes in the social life of the inhabitants fail to supply us with an adequate solution”

A point of great interest and importance is how long the resistance, becoming manifest in the rat populations of infected localities, will persist in the absence of plague. The Plague Research Commission (quoted by Wu Lien-teh) noted, in this respect, that the rats of Vaniyambadi which had suffered severely from plague in 1901-3 were highly susceptible when tested eight years later. Further information on this point was furnished by Sokhey & Menezes (see table XVIII).

Table XVIII shows that apparently the herd susceptibility to plague became nil in the second year during which rat infection was absent from Bombay, but began to rise again in the following year. However, as shown by table XVI (see p. 274), rat plague continued to be practically absent from Bombay.
TABLE XVIII. SUSCEPTIBILITY OF RATS TO PLAGUE IN BOMBAY DURING THE YEARS 1931-9

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number examined at Bombay</th>
<th>Number found plague-infected</th>
<th>Number infected</th>
<th>Percentage mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1931</td>
<td>220,316</td>
<td>748</td>
<td>118</td>
<td>9.3</td>
</tr>
<tr>
<td>1932</td>
<td>272,018</td>
<td>600</td>
<td>106</td>
<td>8.5</td>
</tr>
<tr>
<td>1933</td>
<td>256,900</td>
<td>393</td>
<td>139</td>
<td>7.9</td>
</tr>
<tr>
<td>1934</td>
<td>237,854</td>
<td>34</td>
<td>33</td>
<td>6.5</td>
</tr>
<tr>
<td>1935</td>
<td>226,289</td>
<td>0</td>
<td>40</td>
<td>5.0</td>
</tr>
<tr>
<td>1936</td>
<td>182,727</td>
<td>0</td>
<td>20</td>
<td>0.0</td>
</tr>
<tr>
<td>1937</td>
<td>186,987</td>
<td>0</td>
<td>90</td>
<td>7.8</td>
</tr>
<tr>
<td>1938</td>
<td>187,276</td>
<td>0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1939</td>
<td>207,230</td>
<td>0</td>
<td>50</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Seasonal incidence of rat plague

While, as will be discussed later, the seasonal incidence of plague is largely governed by the influence which climatic conditions exert on the flea vectors of the infection, some observers have laid stress also on a role played in this respect by the breeding periods of the rats.

This point seems to have been brought up first by Gotschlich who, finding that the season during which plague became epidemic at Alexandria coincided with the main breeding-period of the rats, postulated that a causal connexion existed between these two events, the young rats furthering a recrudescence of the epizootics.

While Martin expressed disbelief, other observers, e.g., Gill, were of the opinion that, in India also, a seasonal increase of the rat populations caused by peaks in breeding gave impetus to the epizootics. In Lamb’s summary of the work of the Plague Research Commission, it was stated in this connexion that

“both in Bombay and in the Punjab ... breeding of rats goes on all the year round, but that it is especially vigorous during the season between the end of one epizootic and the beginning of the next. During this interval there would, therefore, be added to the rat population a large number of young susceptible individuals, a factor which would evidently influence the rise of the epizootic.”

Considering the knowledge now available on the population dynamics of rats, and bearing in mind the observation of Roberts that, in Kenya, the breeding-rate of R. rattus was much higher in the endemic than in the unaffected areas, one might postulate that a vicious circle exists in this
respect, i.e., a decrease of the rat population through an epizootic leads to more frequent births during the off-season which in their turn, help to promote the subsequent epizootic.

OTHER HIGHER ANIMALS

Mammals

As will be seen in Annex 1, table IV (see p. 636), a number of animals belonging to various orders of the class Mammalia, other than rodents and Lagomorpha, have been found naturally plague-infected, or have been suspected of suffering from plague.

Camels

The occurrence of plague in camels was suspected by some of the earlier workers in south-east Russia and strength was lent to their assumptions through the investigations of Nikanoroff, who succeeded in infecting these animals through administration of \( P. pestis \) by the subcutaneous route, per os, and by inhalation. He pointed out that contamination of the forage of the camels through the faeces of plague-infected rodents might lead to their natural infection, and that such infection might prove dangerous for man because the Kirghese were wont to kill diseased domestic animals and to consume their meat. In the opinion of Nikanoroff, a number of human outbreaks had been recorded which appeared to be due to this mode of infection.

It has to be noted that these claims have not been accepted universally. Petrie, for instance, stated that Nikanoroff's postulations "conflict so sharply with experience gained elsewhere, under both natural and experimental conditions, that it seems best to await further information".

Dogs

Fujinami claimed to have found a dog suffering from pneumonic plague during the 1910-11 Manchurian epidemic. The validity of this observation was upheld by Strong & Teague because they found that dogs were susceptible to experimental infection with \( P. pestis \) by inhalation.

In the course of their work in Morocco, Blanc & Baltazard had the opportunity of examining a dog which had died in a plague-affected household. The spleen of this animal was somewhat enlarged. Smears made from this organ, as well as from the liver, lungs, and nasal mucus, showed

\[\text{Further reference to the subject of rodent plague will be made when discussing the problems of epidemiology.}\]
numerous suspicious bipolar-stained bacilli. The diagnosis of plague was supported by the positive results obtained through inoculating white rats with the pooled fleas (Ctenocephalides canis) of the dog. Fleas (Ct. felis) from a cat which had died in the same house soon after the dog, likewise proved positive for plague.

It would therefore seem that, on rare occasions, dogs may contract plague under natural conditions. Certainly, however, instances of this kind are of little importance when contrasted with the far more dangerous role dogs are apt to play by picking up plague-infected rodent-fleas and conveying them to their masters.

_Cats_

While experience gained in all major plague-areas has confirmed that cats may suffer from plague under natural conditions, it has been much debated whether they can contract infection by feeding, and whether their infection occurs frequently enough to be of importance for the spread of the disease.

Araujo ³ and Henriques, ⁷¹ who recently devoted attention to the former problem, confirmed the conclusion previously reached by Dujardin-Beaumetz (quoted by Henriques ⁷¹) that, under natural conditions, cats could contract infection by feeding only if their buccal or intestinal mucosa was traumatized by bone fragments.

In the opinion of several observers, e.g., Henriques, ⁷¹ and Silva & Valença, ¹⁸⁴ cats were not, in general, very susceptible to experimental infection with _P. pestis_. Uriarte & Morales Villazon ²⁰⁷ found that such infection produced a chronic disease characterized by emaciation and abscess formation, but that the causative organisms could not be recovered at autopsy. As shown by Henriques, ⁷¹ the death of experimentally infected cats could be due to the action of the endotoxin of _P. pestis_, and not to a bacteraemia.

According to Moll & O'Leary, ¹³⁹ plague-workers in Argentina often noted instances where the presence of plague in cats seemed to be responsible for human cases. Pozzo, ¹⁵⁷, ¹⁵⁸ while admitting that in some parts of the country a high mortality among these animals was observed during plague outbreaks, was not certain whether this was invariably due to infection with _P. pestis_.

In Brazil, the frequency of a rapidly spreading and acutely fatal disease among cats was noted in the plague foci, and sometimes also in the plague-free areas (Silva). ¹⁸² Bezerra Coutinho & Macchiavello who studied this problem closely, ascribed this fatal cat-disease, which they called "adenomyelo-enterosis", to infection with a filterable virus. ¹⁶, ¹¹⁵, ¹¹⁶, ¹²¹, ¹²², ¹²³, ¹²⁴ This conclusion was questioned by Silva ¹⁸³ who claimed that the cat mortality was due, in part, to plague.
Be this as it may, generally speaking the incidence of plague among cats was not high enough to play a conspicuous role in the spread of the infection to man. However, the cats, like the dogs, are apt to be dangerous by bringing infected rodent-fleas into the houses.

Shrews

The shrews, which live side by side with the rats and regularly visit houses at night, play a not inconsiderable role in the spread of plague. As shown in Annex 1, table IV (see p.636), they have been found liable to contract natural plague in several plague-areas. More important still, being infested with rat ectoparasites, they are apt to convey infected fleas to human habitations.

Sharif & Narasimham also stressed that Suncus murinus, besides wandering from house to house, could undertake excursions from village to village.

Plague-Insusceptible Beasts and Birds of Prey

Ample evidence exists to show that carnivora and birds of prey may play a role in the spread of plague, even though they are resistant to the infection, because they are apt to pick up, and later to disperse, infected rodent-fleas. Some workers have also stressed the fact that birds of prey regurgitate the indigestible portions of their meals some time after they have fed and have postulated that these "casts", if consisting of the remnants of plague-infected carcasses, may be a means of conveying the infection to rodents eating such morsels. Egorov, who seems to have been the first to propound this view, found that virulent plague bacilli were present in "casts" regurgitated two days after an infectious meal by an eagle (Aquila hipalensis) which had been fed in the laboratory with the carcasses of plague-infected guinea-pigs.

Experiments performed by Araujo did not lend support to Egorov's contention because it was not possible to demonstrate the presence of P. pestis in the contents of the gizzards and intestines of buzzards (Urubus) which had been fed with plague-infected materials. However, Jellison repeatedly succeeded in isolating plague bacilli from the casts of owls and falcons which had been infected in this manner. In the course of field investigations he was able also to demonstrate several times the presence of P. pestis in the remnants of ground-squirrels which had been partly eaten by birds of prey, sometimes after the latter had transported the rodent carcasses for some distance. Believing that the ground-squirrels were often carnivorous, Jellison postulated that they could contract plague by feeding on remnants of infected carcasses.

Even if one is ready to subscribe to such views, it is likely that infections caused by the consumption of casts or remnants of plague carcasses are of
less importance than those due to the transport of infected fleas by birds of prey.

Sergeev, a worker in south-east Russia, noted in the latter connexion that many species of birds were apt to seek temporary refuge in the burrows of wild rodents, and that some nested there permanently. On the other hand, one species (Oenanthe oenanthe), which had been proved to harbour wild-rodent fleas known to be plague-vectors, nested in houses, and therefore seemed capable of conveying the infection from the fields to human settlements.

Brown, like Jellison, drew special attention to a North American species, the burrowing-owl (Speotyto cunicularia), which lived in close contact with ground-squirrels, often sharing their burrows. The dangerous role of this owl in the spread of plague was confirmed by Wheeler & Douglas through inoculation tests with pools of sticktight fleas (Echidnophaga gallinacea), common ectoparasites of the ground-squirrels, which had been collected from one such bird.

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Chapter 7

INSECT VECTORS

FLEAS

Classification

While it is generally agreed that the fleas belong to the class Insecta of the phylum Arthropoda, different names have been used to designate the order into which these insects fall, British and American workers preferring the term “Siphonaptera”, continental European authors that of “Aphaniptera”.

The known genera of fleas are placed in two superfamilies—namely, Pulicoidea and Ceratophylloidea. The family of Pulicidae, which belongs to the former superfamily, is of greatest importance as far as plague is concerned, because it comprises notorious vectors like certain Xenopsyllinae. Descriptions of the outstanding characteristics of these and other species of interest to plague workers will be found in Annex 2 (see page 648).

Development

There are four stages in the life-cycle of a flea, the larvae hatched from the eggs passing through a pupal stage before becoming adults. The early stages of this life-cycle may be described as follows.

The eggs, minute, glistening, and white in colour, are deposited either on the hosts, or in their nests or haunts. Sometimes, eggs laid on the hosts stick to the pelt or plumage of the latter, but, even then, they usually fall off sooner or later.

The number of eggs laid by the female flea apparently differs in different species. As stated by Patton & Evans, a female Xenopsylla cheopis lays 300-400 eggs during her lifetime—2 to 6, or even more, at a time. Bacot observed a female Pulex irritans depositing 448 eggs during a period of 196 days, while, according to Patton the female cat-flea (Cieno-
cephalides felis) lays over 800 eggs throughout her life-span. Oviposition occurs only when the female has regular blood-meals.

As described by Jordan,\textsuperscript{131} "the larvae that hatch from the eggs are rather like small legless caterpillars; they are pallid or slightly brown and bear sparse long hairs on all segments of the body. When disturbed they throw themselves about with a twisting motion, are very lively and dislike light . . . they sometimes occur on the hinder part of mice, in the pelts of dirty dogs and cats, on nesting birds and occasionally on human beings of unclean habits ", but usually live on floors, or in the nests or lairs of the hosts where they are able to feed on organic matter contained in the dust.

Dealing with the next stage in the development of the fleas, Jordan\textsuperscript{131} stated that

"when ready to pupate the larvae make semi-transparent cocoons by sticking bits of earth, sand or organic refuse together amongst the debris, or they may fill small cavities in hollow trees, etc. with a loose network of silken threads so as to support the cocoons of numerous individuals ".

The larvae remain quiescent within the cocoons for a time and then change to pupae, from which the adults eventually emerge; the adults may remain fully formed within the pupal skin for some time before emerging. Since the walls of the cocoon are opaque, it is usually impossible to tell whether it contains a larva, a pupa, or an adult flea, and the period passed within the cocoon is therefore termed, for convenience, the "cocoon stage ".

While, under optimal conditions, the life-cycle of a flea may be completed within three weeks, unfavourable climatic influences or conditions of starvation may considerably prolong the various stages of development. Laboratory observations on the maximal duration in days of this development, and also on the length of adult life, in four common flea-species were summarized by Bacot\textsuperscript{6} as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Egg stage</th>
<th>Active larval stage</th>
<th>Cocoon stage</th>
<th>Length of active adult life fed</th>
<th>Length of active adult life unfed</th>
<th>Total days (fed adults)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nosopsyllus fasciatus</td>
<td>10</td>
<td>114</td>
<td>450</td>
<td>106</td>
<td>95</td>
<td>680</td>
</tr>
<tr>
<td>Xenopsylla cheopis</td>
<td>10</td>
<td>84</td>
<td>182</td>
<td>100</td>
<td>38</td>
<td>376</td>
</tr>
<tr>
<td>Pulex irritans</td>
<td>12</td>
<td>202</td>
<td>239</td>
<td>513</td>
<td>125</td>
<td>966</td>
</tr>
<tr>
<td>Ctenocephalides canis</td>
<td>8</td>
<td>142</td>
<td>354</td>
<td>234</td>
<td>58</td>
<td>738</td>
</tr>
</tbody>
</table>

**Length of adult life**

It has often been maintained that unfed fleas live longer than those which have partaken of single blood-meals, because a flea which has fed once must continue to do so in order to survive. However, as noted below, the experiences of some observers did not bear-out this contention.

General agreement exists that female fleas survive longer than the males of the corresponding species. In the opinion of some observers, the lower ratio of surface-area to mass in the females renders them less sensitive to untoward climatic conditions, particularly low humidity.
Since fleas may find more favourable conditions for survival on their specific hosts, and still more in inhabited burrows or other sheltered locations, than under artificial conditions, it is difficult to deduce, from laboratory observations, the normal length of life of these insects. Commenting on the findings tabulated earlier, Bacot maintained that 

"... on this basis, and allowing for the longest recorded unfed imaginal lives, it will be seen that there is no difficulty in accounting for active adults being found, in favourable situations, where there have been no hosts for considerable periods. We may safely estimate for C. [N.] fasciatus 22 months, P. irritans 19 months, X. cheopis 10 months, Ct. canis 18 months ...".

Observations made in the case of the species infesting the sisels (susliks) of south-east Russia have shown that wild-rodent fleas may also survive for prolonged periods. Golov & Ioff maintained in this connexion that suslik fleas could stand low temperatures—down to \(-25^\circ C\) \((-13^\circ F)\)—and prolonged starvation up to 10 months. Fleas kept in test-tubes at temperatures corresponding to those of the rodent burrows in winter, remained active and able to feed upon hibernating susliks. Large-scale investigations carried out later by Tiflov & Ioff showed that:

1. Fleas of the south-east Russian wild-rodents, kept starving in a moist atmosphere, could survive for prolonged periods—at room temperature up to 292 days, in the cellar up to 369 days. The fleas inhabiting the nests instead of dwelling upon the hosts themselves appeared especially well adapted to prolonged starvation.

2. Fleas fed once soon after leaving their cocoons, generally survived a little longer than those of the preceding series.

3. Those fed periodically survived up to a maximum of 1,725 days, i.e., 4 years, 8 months, and 25 days, in the case of a female Neopsylla setosa.

4. Under unfavourable conditions of humidity the female fleas proved harder than the males.

Further studies on the longevity of adult fleas were made by Burroughs with over 10,000 specimens of different fleas, which were kept in the laboratory at various temperatures and humidities within ranges encountered under natural conditions. The maximal periods of survival of six species infesting wild rodents in the USA were found to compare with the length of life of X. cheopis and N. fasciatus thus:

<table>
<thead>
<tr>
<th>Species</th>
<th>Maximum length of life in days by sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfed</td>
</tr>
<tr>
<td>Diamanus montanus</td>
<td>45</td>
</tr>
<tr>
<td>Oropsylla idahoensis</td>
<td>37</td>
</tr>
<tr>
<td>Malaraeus telchinum</td>
<td>15</td>
</tr>
<tr>
<td>Opisodasys nesiotus</td>
<td>15</td>
</tr>
<tr>
<td>Orchopaeas sexdentatus</td>
<td>14</td>
</tr>
<tr>
<td>Megabothris abantis</td>
<td>10</td>
</tr>
<tr>
<td>Xenopsylla cheopis</td>
<td>40</td>
</tr>
<tr>
<td>Nosopsyllus fasciatus</td>
<td>13</td>
</tr>
</tbody>
</table>
Burroughs concluded from his observations that:

(a) generally speaking, a single blood-meal proved beneficial for the fleas, those fed once before starvation surviving a little longer than those never fed;

(b) the longest-lived fleas were those kept at high humidity (relative humidity of 90% or more) and fed daily;

(c) with one exception (Opisodasys nesiotus, which could not be kept satisfactorily), the maximum periods of longevity were among the females.

Influence of climatic conditions

Many observations have shown that a close relation exists between the prevailing climatic conditions and the incidence of the various flea-species. If the climatic conditions are favourable for the development and survival of a given species, its incidence will be high. Conversely, deviations from such favourable weather conditions are bound to lead to a decreased occurrence of the fleas in question. It is clear, therefore, that periodic changes of the climatic conditions are apt to be followed by seasonal fluctuations in the incidence of the fleas and that, since their climatic requirements vary considerably, different species of fleas may be predominant at different seasons of the year. Consequently, it is often not so much the total frequency of the fleas as the relative frequency of the different flea-species which is at variance in a given locality, or on a given host, during the different seasons.

The problem of how climatic factors influence the flea incidence has been the subject of many investigations. Some observers, e.g., Nicoll, Bacot & Martin, and Goyle, came to the conclusion that a high saturation-deficiency ("drying power of the atmosphere") exerted an unfavourable influence on the adult fleas. Bacot and Martin stated in this connexion that:

"1. The survival of fleas (X. cheopis) apart from their host is approximately in inverse proportion to the saturation deficiency of the air, provided the temperature and air movement are constant. In other words, it is proportional to the rate at which they lose water.

2. Under similar conditions but with constant saturation deficiency, their length of life is reduced to between one-half and two-thirds by a 10°C. [18°F] rise in temperature. Compared with the effect of saturation deficiency, that of temperature upon the longevity of fleas is, within the range of climatic conditions over the greater part of India, a smaller one.

3. A variation in saturation deficiency from 5 mm. to 35 mm. [0.2-1.4 inches] such as occurs in the plains of Northern India at different seasons would, accordingly, shorten the average duration of life of wandering rat fleas in the proportion of 15 to 1. As a rise in mean temperature occurs simultaneously with the increase in saturation deficiency and may amount to a difference of 20°C. [36°F] between January and June this would reduce the length of life of wandering fleas to about one-third. The effect of saturation deficiency and increased temperature will be additive and would go a long way to explain some of the climatological features of the epidemics."
The validity of the above statements was denied by later observers, first, apparently, by Leeson, who found that there was "no direct proportion between survival of unfed fleas and saturation deficiency of the atmosphere at any temperature". He again came to the same conclusion when working with fleas which had been fed before being subjected to starvation.

Mellanby found that, while adult fleas were comparatively resistant to dryness of the atmosphere, the larvae possessed no such resistance. His results were corroborated by Buxton, who pointed out that, owing to the presence of regulating mechanisms, the loss of water by adult fleas was insignificant, even at high temperatures and low humidities, and could be made good at the next meal. The flea larva, on the contrary, was "not capable of much resistance to desiccation, losing water with its excrement, and probably also in respiration ...". As established by Sharif, an adequate moisture-content of the food, which formed the chief source of water-supply for the flea larvae, was essential for their development. However, an excessively high humidity, because it rendered the food unsuitable, was as inimical for the development of the larvae as too low a humidity.

It is of great importance to realize that, even if the extrinsic climatic conditions become unfavourable, the "micro-climate" prevailing in the rodent burrows may continue to be suitable for the development of the fleas. Ingram noted in this connexion that the temperature in the rodent burrows was higher during the winter, and lower during the summer, than the outside temperature, whereas the humidity in the burrows was constantly higher than that outside. A series of observations made by George & Webster showed the following:

<table>
<thead>
<tr>
<th></th>
<th>Maximum</th>
<th>Minimum</th>
<th>Daily range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outside temperature</td>
<td>86.5°F</td>
<td>63°F</td>
<td>12.5°F to 21°F</td>
</tr>
<tr>
<td></td>
<td>(30.3°C)</td>
<td>(17.2°C)</td>
<td>(7°C to 13.8°C)</td>
</tr>
<tr>
<td>Temperature in burrows</td>
<td>79°F</td>
<td>72°F</td>
<td>1°F to 7°F</td>
</tr>
<tr>
<td></td>
<td>(26.1°C)</td>
<td>(22.2°C)</td>
<td>(0.55°C to 3.8°C)</td>
</tr>
</tbody>
</table>

Thus, as summarized by these workers, the temperature in the burrows was affected only to a small extent by the daily variations of the outside temperature.

Comparisons of the state of humidity showed that the saturation deficiency of the air in the burrows varied from 0.11 inch to 0.14 inch (2.8 mm to 3.6 mm), whereas that of the outside air varied from 0.65 inch to 0.19 inch (16.5 mm to 4.8 mm).

The conclusions drawn from further observations recorded in India in 1936 were that the temperature of rat burrows almost corresponded to the minimum temperature of the outside air and exhibited only a limited range of diurnal variation, and that the saturation deficiency was decidedly lower than that of the outside air.
Macchiavello \textsuperscript{156} laid great stress upon the fact that, in some areas of Brazil where the weather was exceedingly hot, \textit{X. cheopis} found suitable conditions for survival in the rat burrows where the temperature was 5°-8°C (9°-14°F) lower than outside.

Interesting observations were also recorded by Sharif \& Narisimham \textsuperscript{231} in connexion with differences in flea infestation detected by them in the case of three wild-rodent species. Ascribing these differences in the flea-index of the animals to the different arrangement and depth of their burrows, the two workers stated that:

"\textit{M. [Millardia] meltada} usually lives in a straight burrow which hardly goes more than 9 inches [23 cm] deep into the soil and is open at both ends. As the burrow is not deep, the direct heat of the Sun raises the temperature of the nest and keeps it hot and dry. This makes it impossible for any flea to breed in the nest of this rodent. The burrows of \textit{T. [Tatera] indica} go as deep as 40 inches [1 m] and they have a tortuous course. The heat of the Sun cannot penetrate this depth and ventilation is very poor on account of the tortuous course of the burrow. The temperature in the nest can only be lower and humidity higher than those of the atmosphere. The temperature and humidity of such deep burrows are least affected by atmospheric changes. They remain almost constant with slight seasonal variations at such a depth. The nest of \textit{T. indica} is thus an ideal place for breeding of fleas, and consequently it is always full of fleas, and as the result of that this species always harbours the largest number of fleas. The burrow of \textit{G. [Gonomys] kok} is intermediate between the two burrows. The nest is found at a depth of 12 to 18 inches [30-45 cm] but the course is such as to encourage ventilation. It is not so favourable for breeding of fleas as that of \textit{T. indica}. Consequently, very few fleas are found in it... On the whole, there was [a] slight diminution in the flea infestation of \textit{T. indica} during the hot months; but it was observed that the flea infestation was directly proportional to the depth of a burrow during these months."

Estrade \textsuperscript{56} pointed out the important fact that \textit{X. cheopis} could thrive in accumulations of dust containing cereal debris, where the temperature was apt to be constant, little ventilation took place, and the saturation deficiency varied little. As a result, young fleas could develop by the hundred in dark corners of the huts in Madagascar.\textsuperscript{74-76, 81, 217} In the opinion of Girard,\textsuperscript{76} such "free-living" fleas were apt to obtain their first meal from man rather than from rats and, if feeding on a patient with plague bacteraemia, might become a means of conveying the infection to the rats.

Observations by Herivaux \& Toumanoff\textsuperscript{98} had shown that, in Saigon, Indochina, \textit{X. cheopis} was mainly found in houses, the floors of which were paved with tiles made from baked clay, or in huts with a soil of sandy clay, whereas \textit{X. astia} was prevalent in huts with a sandy soil. Attempting to explain the reason for these differences in the distribution of the two flea-species, Toumanoff \& Herivaux \textsuperscript{259} suggested that the evaporation of water accumulated in the sandy clay or from the baked-clay tiles led to a lowering of the temperature and thus created favourable conditions for \textit{X. cheopis} which preferred, in comparison with \textit{X. astia}, a lower temperature.
Observations made in India\textsuperscript{118} showed that the maximum temperature in cracks of the walls of houses and in thick thatch-roofs was $50-10^\circ\text{F}$ ($3^\circ-6^\circ\text{C}$) below that of the outside air. Possibly, therefore, such locations are also suitable for the development of fleas.

It should be noted in this connexion that the humidity requirements for the development of different flea-species may vary considerably. Thus, de Meillon\textsuperscript{188} found that \textit{X. brasiliensis}, because it was able to complete its life-cycle at a relative humidity of 51 \%, could breed in debris accumulated on the floor of huts, sheds, or garages, even though the humidity there was much lower than that prevailing in rodent burrows.

\textit{Ecology}

Although dependent upon their hosts for nourishment, the adult fleas of many species do not stay on them constantly, but spend a considerable part of their lives in the nests of the rodent burrows, or even lead a rather independent existence as free-living fleas.

It is important to note that, depending on the species concerned and on the prevailing climatic conditions, the proportion of time the fleas spend on the hosts and away from them, respectively, is subject to much variation.

As far as the common rat-fleas are concerned, it holds generally true that \textit{N. fasciatus}, which feeds at long intervals, spends most of its time off the body of its hosts, whereas \textit{X. cheopis}, which feeds frequently, has a far greater tendency to stay in the fur of the rats\textsuperscript{98}.

Making comparative observations on \textit{X. cheopis}, \textit{X. astia}, and \textit{X. brasiliensis}, Webster \& Chitre\textsuperscript{275} stated that

"... a very large proportion of all the fleas ... was found on the host. It is not impossible that the wandering may be largely nocturnal, but the figures suggest that the three species show no marked difference in the proportions on hosts and abroad respectively ".

The probability that the fleas which shun daylight move around mainly after dark deserves attention. Some observers, e.g., Sanguy,\textsuperscript{221} maintained that the fleas convey plague to man mainly at night.

Though the rat-\textit{Xenopsylla} no doubt have a tendency to infest their hosts more permanently than \textit{N. fasciatus}, it is important to keep in mind that, as has been noted earlier, they have been found able to lead, under suitable environmental conditions, a rather independent existence as free-living fleas. This observation has been made in India as well as elsewhere,\textsuperscript{137}

Reporting on their observations in the Lake Albert plague-focus of the Belgian Congo, Vincke \& Devignat\textsuperscript{265} stated that, in contrast to \textit{X. cheopis} and \textit{X. brasiliensis}, which were apt to lead an independent existence on cereal debris accumulated in dark and unswept corners of the straw huts, \textit{Leptopsylla segnis} was found exclusively on the hosts. These observations on \textit{L. segnis}, which is a blind flea, stand in an interesting contrast to those of Tiflov \& Potapov\textsuperscript{259} in south-east Russia; these workers maintained that flea species with well-developed eyes, such as \textit{Citellophilus tesquorum} and
Frontopsylla semura, had a tendency to stay on their wild-rodent hosts, while fleas with rudimentary eyes (N. setosa and Ctenophthalmus pollex) inhabited the nests. However, no stringent conclusions should be drawn because, in Madagascar, L. segnis was found in the burrows as well as on the rodents. 217

Little doubt can exist that the proportion of fleas found on their hosts, and away from them, is influenced by the prevailing climatic conditions. Indeed, it seems likely that, in this respect, the basic differences found between the Xenopsylla, living in warm countries like India, and N. fasciatus, which is the prevalent rat-flea in countries with a cooler climate, are not wholly due to intrinsic causes, but are partly the result of climatic influences.

That climatic conditions are apt to exert an influence upon the frequency of fleas on their hosts has been proved by observations like that of Hirst, 102 who found that rainy weather led to temporarily lowered flea-counts.

Impressed by the role which fleas leaving the carcasses of plague rats played in the spread of the infection, early workers were led to believe that this exodus took place rather rapidly. In this connexion, Webster & Chitre 275 quoted a statement by Mason 164 to the effect that the fleas began to leave a carcass 15 seconds after a rat had died, and that all had departed after two hours and a quarter. However, Webster & Chitre pointed out that, in their experience, even when live rats were close at hand, active fleas could be regularly recovered from the carcasses after the latter had begun to putrefy. Similar observations were recorded by other workers, for instance, by Martinez, 163 who stated that he had found more than 30 fleas on one dead rat. Roberts, 216 while agreeing that "statements relating to all fleas leaving their late hosts immediately or very soon after death are not accurate", emphasized that certain species, particularly the rat-Xenopsylla, left their hosts at greater speeds than others, e.g., the field-rodent fleas, Dinopsyllus ellobius lupusus and Ctenophthalmus cabirus.

Cole & Koepke, 35 analysing the results of a rat-flea survey at Savannah, Ga., computed that the rats delivered dead to the laboratory had lost 70.4% of their X. cheopis, but only 56.7% of their L. segnis. These authors added, however, that this difference was not statistically significant.

In the course of his work in China, Pollitzer (unpublished observations) gained the distinct impression that the number of fleas on rats delivered dead to the laboratory was larger when the weather was temporarily inclement. It is noteworthy in this connexion that, according to Robic, 217 a lower temperature exerts an adverse influence on the activity of the fleas.

As proved by the observations of Symes & Hopkins, 249 fleas of the species Echidnophaga gallinacea usually remain attached to the dead rodents.

Nutritional requirements

Larvae. Marked differences exist in the nutritional requirements of the larvae of different flea-species. Some, the N. fasciatus larvae for instance,
depend upon the presence of blood-substances in their food which, under natural conditions, are supplied to them in the faeces of the adult fleas. For other flea larvae, e.g., those of the *Xenopsylla*, such substances are not indispensable, although favourable for their development.

It will be perceived that, in contrast to fleas like the *Xenopsylla*, the propensity of adult *N. fasciatus* to dwell in the nests, rather than on the bodies, of their hosts is a factor of great importance in the survival of this species.

Profound studies on the nutritional requirements of the larvae of *N. fasciatus* and the Indian rat-fleas (*X. cheopis*, *X. astia*, and *X. brasiliensis*) by Shariff\(^{227, 228}\) showed that:

1. Though blood was essential for the larvae of *N. fasciatus*, it was not fully sufficient for larval development, the larvae requiring additional food-substances which, in nature, were supplied to them "by the organic refuse present in the bed of the host of the adult fleas".\(^{227}\)

2. Pure blood also proved inadequate for the larval development of the *Xenopsylla*, because it was deficient in accessory growth-factors, particularly vitamins of the B-group.

3. In nature, the larvae of these fleas apparently derived the accessory food-substances from micro-organisms, possibly fungi, admixed to their food. It was obviously due to the presence of such micro-organisms that the *Xenopsylla* larvae could subsist on wheat-flour.

Confirming the statements of earlier observers, e.g., Hirst,\(^{98}\) Sharif reached the conclusion that the distribution of the various rat-flea species was governed by the character of the food-supply available to their larvae as well as by climatic factors. Particularly important was that, in contrast to *X. astia* which needed, comparatively, the most nutritive diet, the larvae of *X. cheopis* and *X. brasiliensis* had simple nutritional requirements. Consequently, they were apt "to survive transport in grain, even without rats, to places far from their original home".\(^{228}\)

Nevertheless, since the temperature tolerance of *X. brasiliensis* was low, its incidence in India was confined to cooler regions. *X. cheopis*, being more adaptable in this respect, was, on the contrary, widely distributed.

*Adults.* Though adult fleas of both sexes are normally dependent for subsistence on the blood which they suck directly from their hosts, it is curious to note that, according to Jordan,\(^{131}\) "a starved flea will imbibe blood oozing from a pin-prick, will suck at a drop of water, or even insert its mouth-parts into the skin of a caterpillar and suck its body fluids".

The frequency with which adult fleas obtain meals from their hosts seems to depend not only on the species concerned, but also on the sex of the fleas and on climatic conditions.
With regard to the differences existing in this respect between different flea-species, it has already been noted that, among the common rat-fleas, *N. fasciatus* feeds at considerably longer intervals than the *Xenopsylla*.

That there may be a difference, in respect of the frequency of feeding, between the sexes of the same flea-species has been shown in the case of *X. cheopis*, *X. astia*, and *X. brasiliensis*, the males of which have been found to attack their hosts more frequently than the females.\(^97,^{273}\)

Discussing the influence exerted by climatic factors on the frequency with which the fleas bite, Hirst\(^98\) considered it likely that a high saturation-deficiency, by rendering the fleas more thirsty, would induce them to feed more often. That a high temperature may act in an analogous manner has been shown by the investigations of Cole\(^34\) who maintained, however, that temperature variations exerted an influence only on *X. cheopis* males, which fed more frequently in hot weather and less frequently if the temperature was lower.

*Host selectivity*

Though most rodent fleas specifically infest more than one species of animal, as a rule they restrict themselves to hosts belonging to the same generic group.\(^51\) Though they may be reluctant to do so, they are nevertheless capable of attacking hosts belonging to other generic groups, if driven by hunger. Therefore, as aptly pointed out by Wayson,\(^272\) the host preferences of the rodent fleas merely retard, but do not prevent, their transition from one genus of hosts to another.

Of particularly ominous importance is the fact that, with the exception of *L. segnis*, the fleas commonly infesting the commensal rodents quite readily attack man if thoroughly starved. The rat-*Xenopsylla*, in particular, have been found able to subsist for considerable periods on human-blood alone, as shown by the following data supplied by Webster & Chitre.\(^274\)

<table>
<thead>
<tr>
<th>Rat-fleas fed on human-blood only</th>
<th>Species</th>
<th>Length of life offered (days)</th>
<th>Feeds accepted</th>
<th>Percentage accepted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>X. cheopis</em>, female</td>
<td>162</td>
<td>136</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td><em>X. cheopis</em>, male</td>
<td>63</td>
<td>53</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td><em>X. astia</em>, female*</td>
<td>53</td>
<td>47</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td><em>X. brasiliensis</em>, female</td>
<td>127</td>
<td>57</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td><em>X. brasiliensis</em>, male</td>
<td>68</td>
<td>60</td>
<td>29</td>
</tr>
</tbody>
</table>

*No male *astia* was kept alive on human-blood for more than a few days, although many fed readily.

Webster\(^278\) compared the percentages of food acceptance by the three species of Indian rat-fleas when exposed daily on rats, guinea-pigs, and a human subject, respectively, and recorded the following results:
Species | Percentages of acceptance when fed on rats | guinea-pigs | man
---|---|---|---
X. cheopis, female | 51 | 53 | 32
X. cheopis, male | 67 | 75 | 46
X. astia, female | 64 | 55 | 38
X. astia, male | 84 | 91 | 52
X. brasi/ensis, female | 47 | — | 42
X. brasi/ensis, male | 72 | — | 44

It will be noted that, even when fed on rats, the fleas did not accept daily feeds. No marked differences existed between the three flea-species, particularly as far as the tests made on the human subject were concerned. Webster’s conclusion was therefore that “the three Indian rodent Xenopsylla readily feed on man in the absence of a more suitable host, even at temperatures of over 80°F. [27°C]”.

Though, as shown in the lists of wild-rodent fleas given in Annex I, table V (see page 638), a considerable number of the species have been found capable of biting man, it has been often maintained, particularly by workers in the western States of the USA,\(^5\)\(^1\)\(^,\)\(^5\)\(^2\)\(^,\)\(^1\)\(^7\)\(^3\)\(^,\)\(^1\)\(^7\)\(^4\) that these fleas attack man far more reluctantly than the rat fleas, and are therefore by no means invariably responsible for the transmission of plague from the wild rodents to man. Apparently, however, differences exist in this respect between different flea-species. Thus, Meyer & Holdenried,\(^1\)\(^7\)\(^8\) referring to ecological studies instituted to trace the origin of human plague-infection in rural areas of California, stated that “fleas of the Muridae—mice (Peromyscus), meadow mice (Microtus), and pack rats (Neotoma)—in the vicinity of human habitations, were the most likely transmitters of Pasteurella pestis”.

Natural enemies

Observations by several authors have shown that fleas are preyed upon by certain other insect species. In this respect, an important role is played by the ants, which are able to attack not only flea eggs and larvae,\(^7\)\(^2\) but also adult fleas.\(^2\)\(^7\)\(^3\) The latter are also destroyed by spiders,\(^2\)\(^7\)\(^3\) while other insect species attack the eggs, larvae, or pupae.

Fedina\(^6\)\(^0\) and Flegontova\(^6\)\(^2\) found that certain beetles, particularly those of the Staphilinidae family, preyed upon the larvae and adults of fleas in the burrows of Citellus pygmaeus. Flegontova expressed the opinion that the activity of the beetles reduced the likelihood of a survival of the fleas in the wild-rodent burrows and, consequently, the chances for a carry-over of plague by the fleas.

Commensal-Rodent Fleas

Classification\(^a\)

The fleas found on the commensal rodents of the various plague areas may be classed as follows.

\(^a\) The more important taxonomic characters of fleas as well as a key to the identification of some common fleas are contained in Annex 2 (page 648).
(1) Those specific to the commensal rodents which show a wide distribution or are found in several plague-areas. *X. cheopis* falls into the first category, *X. brasilienensis* and *N. fasciatus* into the second. *L. segnis*, though considered by most authorities to be specific to the commensal mice, should also be included in the second group because it is regularly found on the commensal rats in many areas; usually the numbers found are small, but occasionally the percentage is higher.

(2) Those specific to the commensal rats which show a more limited, or even a quite restricted, geographical distribution, such as *X. astia* and some other fleas which are mentioned later.

(3) Fleas of wild rodents which may infect the commensal species either accidentally or, even, with some regularity.

(4) Flea species which, because they are frequent in the environment of the commensal rodents, are often found in limited numbers on the latter, although they are not specific for them. *Echidnophaga gallinacea* and *P. irritans*, two species with an almost cosmopolitan distribution and a remarkable faculty for adapting themselves to a large range of hosts, stand foremost in this group, but some other species, particularly *Ct. felis*, are also included.

Factors governing incidence

It has been claimed by some authors that *X. cheopis* is a parasite of *Rattus rattus* rather than of *Rattus norvegicus*, but no doubt can exist that the observations which seemed to prove this contention are not generally valid. As a rule, in localities where *R. norvegicus* and subspecies of *rattus* coexist, *X. cheopis* infests both indiscriminately and, often, even predominates, to some extent, on *R. norvegicus*. Occasionally, an unusually high *cheopis*-infestation of *R. norvegicus* has been observed. Eskey\(^52\) collected at San Francisco, Calif., 1,600 *X. cheopis* from 10 Norway rats trapped within a period of 10 days in the basement of one building. *X. cheopis* has also been found to be the main rat-parasite in localities where Norway rats greatly preponderated—for instance, in Manchuria.\(^110,191\) Likewise, Hecht\(^91\) found a 98% incidence of *R. norvegicus* and a 95% *cheopis* incidence at Caracas, Venezuela.

It seems safe, therefore, to state that *X. cheopis* shows no specific preference for the *rattus* subspecies, but parasitizes the Norway rats at least to the same degree. As a rule, this flea is considerably less common on commensal mice and shrews.

According to observations made in India, other rodent species with opportunities for contact with the commensal rats showed, as a rule, slight infestation with *X. cheopis* but were apt to be more highly infested with *X. astia* than the two rat-species.\(^71,282,275\) As stated by Hirst,\(^104\) the latter flea was also widespread on insectivores.
Incidence in relation to climatic conditions

The statement made earlier that different flea-species react differently to the influence of climatic conditions is well supported by many observations made in respect of the rat fleas.

As far as the rat-Xenopsylla are concerned, it may be stated that X. astia possesses a poor power of adaptation to temperate climatic conditions, being particularly sensitive to low temperatures.\textsuperscript{96, 104, 273}

According to observations in India,\textsuperscript{123, 273} X. brasiliensis was, on the contrary, adversely affected by hot weather, whereas the coolest season was congenial for it. It is in accord with these findings that X. brasiliensis is prevalent in the temperate section of Brazil.\textsuperscript{14, 15} However, Roberts\textsuperscript{214} pointed out that, in Kenya, this flea was able to thrive in the hot, humid coastal areas as well as on the cold, damp mountain-slopes, and also in very dry areas with a high temperature. Nevertheless, X. brasiliensis showed a limited geographical distribution in Kenya, a fact partly explained, in Roberts' opinion, by its failure to colonize in the underground burrows of R. rattus.

As shown by its wide geographical distribution, X. cheopis is able to adapt itself to a considerable range of climatic conditions. It is, as has been previously noted, the common rat-flea in Manchuria and is also the prevalent species in the tropical section of Brazil.\textsuperscript{14, 15}

Comparing the distribution of X. cheopis with that of X. astia, Hirst\textsuperscript{104} drew attention to the observations of King & Pandit,\textsuperscript{137} who came to the conclusion that the prolonged duration, rather than the intensity, of the hot weather accounted for the scarcity of X. cheopis in the south-east of Madras Presidency (now Madras State). Hirst maintained, however, that

"... the intense hot and dry weather of the Ganges plains exerts no more than a temporary check on the great cool weather abundance of this flea in most parts of that vast plague-stricken region. Undoubtedly, climate has an important influence on the distribution of these two fleas, but the salient point seems to be, the sensitiveness of astia to cold, not that of cheopis to heat ".

Nevertheless, there can be no doubt that, generally speaking, a moderately warm and moist climate is optimal for X. cheopis. Thus, Estrade\textsuperscript{56} established that, on the Madagascar plateau, the optimum conditions for this flea were a temperature of 150-200°C (59°-68°F) and a relative humidity of 85% to 95%. Mohr,\textsuperscript{182} dealing with the situation in the USA, found that the cheopis populations were restricted to warm, humid zones, becoming increasingly sparse in the cold, arid areas. The incidence of X. cheopis was generally highest in localities where the January mean-temperature was 40°F (4.5°C) or more.

It is in accordance with these climatic predilections that, if established in a tropical climate, X. cheopis prefers cooler locations as a rule.\textsuperscript{131} Park,\textsuperscript{195}
discussing the situation in India and Ceylon, aptly pointed out in this connexion that this flea "is so hardy that it can thrive in the warm coastal strips, but in such cases prefers the commercial areas, where certain artificial conditions associated with commerce possibly compensate for more favourable climatic conditions naturally found on the hills".

A point of great interest is that at the highest elevations of Ceylon (5,000-8,000 feet (1,524-2,438 m)), *X. cheopis* is replaced by two indigenous rat-fleas, *Ceratophyllus tamilanus* and *Stivalius phoberus*. Similarly, it was found by Macchiavello that, in the highlands of the Andes above 3,000 m (about 9,850 feet), *Nosopsyllus londinensis* took the place of *X. cheopis*.

In view of their palaeo-arctic origin, it is not surprising to find that *N. fasciatus* and *L. segnis* are both apt to be more abundant during the cold seasons than in summer. Hirst found that the latter species, together with *X. cheopis*, replaced *X. astia* in the highlands of Ceylon at 4,000-5,000 feet (1,219-1,524 m).

As shown by the findings of Mohr in the USA, *N. fasciatus* was adversely affected by an arid or an extremely cold climate.

*Monopsyllus anisus*, which largely replaces *N. fasciatus* in temperate, eastern Asia, was, according to observations made in Shanghai, prevalent in spring.

**Incidence in relation to environmental conditions**

In presenting the problem now under review, it has seemed advisable to deal separately with the influences exerted on the incidence of commensal-rodent fleas by climatic factors and by environmental conditions, respectively. It must be realized, however, that no sharp line of demarcation can actually be drawn between these two factors, environmental conditions often exerting an indirect, rather than a direct, influence in this respect because they provide a microclimate favourable for the fleas.

Workers in Ceylon, India, and Java are generally agreed that *X. cheopis* has a marked predilection for comparatively dry situations, as found, for instance, in granaries and warehouses. Expressing this view, Hirst added that observers in Java, e.g., Otten, "have stressed the point that it *X. cheopis* is not adapted to multiply in nesting places out-of-doors or anywhere liable to marked excess of ground moisture. In such situations the natural enemies of the larva (moulds, mites and so forth) gain an ascendency".

King & Pandit found that insanitary surroundings seemed to favour *X. cheopis* and suggested that this factor might be of importance in the spread of this species by compensating for the adverse influence of climatic conditions generally unsuitable for it.

Studying the ecology of plague in two districts of Bombay State, Sharif & Narasimham found that, whereas *X. cheopis* predominated in com-
paratively dry and well-ventilated houses, *X. brasiliensis* was prevalent in cool, damp, ill-ventilated houses. However, no general deductions should be drawn from the latter observation because, as mentioned earlier in this chapter, in South Africa and also in Kenya, *X. brasiliensis* was found capable of thriving in excessively dry environments. Generally speaking, it would seem that local peculiarities of the environment are apt to cause marked differences in the incidence and distribution of the various rat-flea species.

It is probable that changes in environmental conditions are apt to lead to changes in the incidence of rat fleas. This is suggested by an observation of Roberts, who found that *X. brasiliensis*, which was the predominant species in the initial stage of a plague outbreak in Kenya, steadily decreased in numbers during the outbreak, whereas *X. cheopis* increased and finally assumed a dominant position. Since it had been previously established that the latter flea mainly infested the rats living underground, Roberts was of the opinion that structural and other changes carried out in the houses during the outbreak created favourable conditions for an increase of *X. cheopis* because more rats were forced to live underground. *X. brasiliensis*, which mainly infested the rats living in the roofs, was, at the same time, adversely affected by these environmental changes.

Eskey, reporting in 1938 on a rat-flea survey in San Francisco, Calif., noted an almost total absence of *P. irritans*, whereas 30 years earlier this species had averaged nearly one per rat trapped. It is quite likely that this decrease of *P. irritans*, which is an accidental parasite of the rats, was related to a gradual improvement in environmental sanitation.

It is of the greatest importance to state, in conclusion, that the incidence and species distribution of the commensal-rodent fleas in any given locality are by no means invariably static, but—even apart from seasonal fluctuations—are apt to be subject to marked changes caused, on the one hand, by an importation or infiltration of fleas and, on the other hand, by alterations in the local situation which influence the breeding and living conditions of the fleas either favourably or unfavourably.

It is clear that increased facilities for flea importation, combined with favourable local conditions, are apt to lead to the colonization of species hitherto not found or established in the locality in question.

For instance, such a situation favourable for *X. cheopis* seems to have developed in the port of Mattanchery in south-west India. As stated by Ali, a rat-flea survey made there in 1930 had shown a low *brasiliensis* and *astia* incidence combined with an apparently total absence of *X. cheopis*. A second survey in 1937 established the presence of this species as well as an increased rat-flea incidence in general. Ali ascribed these changes to a considerable increase in maritime traffic which had taken place during the period between the two surveys and which had obviously not been paralleled by provision of adequate storage-facilities in the port.
Original homes and geographical distribution

Xenopsylla astia. General agreement exists that X. astia is an oriental flea. Most observers consider India as its original home, but Hirst\(^{104}\) maintained it to be also indigenous in the lowlands of Ceylon, stating that "it is very nearly the sole rat-flea of the low-wet zone outside Colombo".

Apart from Ceylon and India, X. astia is also established in adjacent countries such as Arabia, Burma, Indochina, Iran, Iraq, and Java. In Malaya, it was rarely found at Singapore by Gilmour.\(^{72}\) Though introduced, X. astia apparently became well established in a number of ports on the East African coast.

According to a rat-flea survey carried out in Bombay harbour in 1934-5, X. astia was rarely found in ships, even though it was frequent in the dock area of that port.\(^{238}\) However, this flea was abundant on barges surveyed in the river-port of Rangoon\(^{115}\) and it was found in small numbers on grain-ships in Colombo harbour, particularly on those coming from Rangoon.\(^{104}\) Moreover, there can be no doubt that the appearance of X. astia on the east coast of Africa was due to its introduction by the rats of ships, probably Arabian dhows.

Xenopsylla brasiliensis. The original home of X. brasiliensis, the only member of its group which is found outside the Ethiopian region, was, in all probability, tropical Africa (Hopkins, personal communication). X. brasiliensis is not only found in areas like the Belgian Congo, Kenya, and Uganda, which lie within, or near, the zone of its original habitat, it also occurs in the south of Africa.

Since this flea frequently infests ship rats, it is not surprising to find that it became established in a number of areas overseas. It was found in the Canary Islands\(^{184}\) and, according to Jordan,\(^{131}\) also in England. In Brazil, it vies in importance with X. cheopis, being predominant in the temperate zone of that country, and according to Hecht\(^{91}\) it was also found in Caracas, Venezuela. In the East, it is a most important rat-parasite in the central and southern uplands of peninsular India,\(^{87}\) but is apparently rare in Ceylon.\(^{104}\)

Xenopsylla cheopis. The question as to where the original home of X. cheopis was situated has been the subject of considerable debate. Hopkins, whom the present writer consulted in regard to this difficult problem, stated that X. cheopis is now so widely distributed, partly as a result of natural spread and partly through the unintentional assistance of man, that it seems impossible to decide whether it originated in the Nile Valley, as was suggested by Jordan & Rothschild\(^{133}\) or in "Iran and Iraq and adjacent countries", as suggested by Jordan.\(^{130}\) There is, of course, no direct evidence, because no flea specimens collected before the early nineteenth century are known to be in existence, but the balance of the indirect evidence possibly inclines a little in favour of a Nilotic origin for the species.
The incidence of *X. cheopis* in the recently or currently plague-affected areas has been dealt with in chapter 1. Generally speaking, though capable of adapting itself to a wide range of climatic conditions, it is most prevalent in warm countries. As emphasized by Jordan, it occurs "especially often, outside its original range, in ports and centres for the shipment of cereals". Widespread though *X. cheopis* is, it has, even in recent times, invaded new territories and apparently still continues to do so.

A point deserving attention in this connexion is the date when *X. cheopis* made its appearance in India. Elaborating a suggestion made by Hirst, King & Pandit suggested that

"... the explanation of the recent history of plague in India from 1896 onwards and why this is different from previously recorded epidemics which rapidly died out, is that whereas previous epidemics occurred in the absence of *cheopis*, the 1896 infection occurred when *cheopis* was fairly widespread throughout India as a whole.

"We suggest that the dissemination of *cheopis* occurred after the extension of human intercourse and trade (particularly the cotton trade) with Egypt following the opening of the Suez Canal in 1869. Probably the process of *cheopis* importation began even before this. Thus, Choksy suggests that the actual infection was imported into Gujarat from Egypt in 1815 with cotton and caused the epidemic of that year. Whether this was so or not, it is apparent that the Bombay Presidency was importing Egyptian cotton at that time."

King & Pandit concluded, therefore, that

"... whereas all previous epidemics seem to have behaved like typical 'asia epidemics' in being localized and in not carrying over from one year to another, the epidemic since 1896, as we in India know to our cost, has behaved very differently."

There can be little doubt that the ingenious hypothesis of King & Pandit deserves great attention as far as peninsular India is concerned. Whether it also holds true of northernmost India, particularly the plague areas in the foothills of the Himalayas, where the infection appears to have been more firmly entrenched, seems an open question. It would be most desirable to make a detailed review of the early history of the disease there, and in India in general, in the light of King & Pandit's hypothesis.

It has been claimed by some observers, e.g., Hirst, Roubaud, and Girard, that in past centuries *X. cheopis* was frequent in Europe, and was thus apt to play the main role in the spread of the then frequent plague-outbreaks there. It was suggested that the subsequent disappearance of *cheopis* from Europe might have resulted from the replacement of *R. rattus* by *R. norvegicus*, which is supposed to be a less suitable host for this flea.

Fascinating as this hypothesis is, it is difficult to accept it. Mention has already been made of the observations proving that *X. cheopis* has no specific predilection for *R. rattus*, being, for instance, abundant in Manchuria where Norway rats greatly predominate.

It might be argued that since *X. cheopis* had been able to establish itself in Manchuria, it could have done the same in Europe where the winters are considerably less cold. However, it is striking to note that
the colonies of *X. cheopis* recently detected in Europe were almost invariably located in the basements of steam-heated buildings where, even in winter, the temperature was suitable for the development and survival of this warmth-loving flea. It seems rather doubtful whether similarly suitable temperatures were present during winter in the houses of mediaeval Europe. It is of importance to note in this connexion that the frequency of *X. cheopis* in Manchuria is probably due to the peculiar heating-arrangements in many of the houses. These are provided with wide, elevated platforms made from brick which, because they serve for sleeping as well as living purposes, are kept warm with the aid of flues day and night. Identical conditions probably account for the frequency of *X. cheopis* in Korea (Barnett & Tashioka 11).

*Nosopsyllus fasciatus*. As summarized by Jordan, 131 *N. fasciatus*, which is of European origin, "has almost become cosmopolitan, but does not occur in all countries and, outside its normal range, is usually found in ports and adjacent districts". According to this author, it is rare in India where several similar, but distinct, species of *Nosopsyllus* have been found. *N. fasciatus* is also rather rare in China where it is replaced by indigenous species, particularly *M. anisus*. As has been noted earlier, *M. anisus* is also present in other countries of temperate, eastern Asia.

*Leptopsylla segnis*. *L. segnis* (often called *L. musculi*) is, like *N. fasciatus*, a palaeo-arctic flea which has become almost cosmopolitan.

With rare exceptions, *L. segnis*, when present on mice in a given locality, also infests the commensal rats, sometimes to a considerable degree. For instance, surveys made of the fleas found on commensal rodents in Brazil and Malta, respectively, gave the following results:

<table>
<thead>
<tr>
<th>Locality</th>
<th>rats</th>
<th>mice</th>
<th><em>X. cheopis</em></th>
<th><em>X. brasiliensis</em></th>
<th><em>N. fasciatus</em></th>
<th><em>L. segnis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>São Paulo, Brazil, 1934 4</td>
<td>90.38</td>
<td>9.61</td>
<td>21.48</td>
<td>38.60</td>
<td>rare</td>
<td>38.30</td>
</tr>
<tr>
<td>Malta, 1936 27</td>
<td>94.10</td>
<td>5.80</td>
<td>37.50</td>
<td>—</td>
<td>13.75</td>
<td>48.75</td>
</tr>
</tbody>
</table>

Still more remarkable is the fact that Suárez 243 discovered, during a flea survey at Riobamba, Ecuador, that *L. segnis* was the only flea-species found on the commensal rats.

According to Mohr, 182 *L. segnis* was common on the commensal rats in the USA, but rare on *M. musculus*.

*Other species found on commensal rodents*. Apart from the wild-rodent fleas which are described later (see page 337), the following flea-species found on commensal rodents in the various plague-areas deserve attention because they have been proved, or have been suspected, to be vectors of *P. pestis*.

1. *Stivalius* species, infesting rats and other rodents, have been found capable of transmitting plague in Java and south India. The species involved
in Java, formerly called Pygiopsylla ahalae, has since been identified as Stivalius cognatus. Several Stivalius subspecies seem to be concerned in south India.

2. Synosternus pallidus, a flea occurring "from Senegambia and Morocco to India" and infesting a variety of hosts including rodents, hedgehogs, and carnivora (Jordan 131), was found to be frequent in French West Africa, mainly on the floors of the houses, but also to some extent on the commensal rodents. It is known to bite man.1, 136, 141

3. Synopsyllus fonquerniei, a species occurring on the high plateau of Madagascar. Originally identified on a small lemur and formerly apparently rare, this flea has become, since 1931, increasingly frequent on rats as well as on hedgehogs and insectivores (tanrecs).75, 78, 144, 217, 219 According to Girard,78 it is unknown whether it attacks man.

4. Paractenopsyllus kerguisteli, a flea closely allied to L. segnis, found on the Madagascar plateau on commensal rodents and dogs, but probably also infesting many other species of mammals living in forests.219, 267

5. Xenopsylla vexabilis hawaiensis, frequent on the commensal rodents in Hawaii, where it probably plays a role as a plague vector in the fields.50, 88

6. Pulex irritans, an Old-World flea now of almost cosmopolitan distribution. Though often called (or, one should rather say, misnamed) the "human flea", it has actually a remarkably wide host-range. In addition to infesting man and, usually in small numbers, the commensal rats, it often attacks pigs and goats as well as foxes and badgers, from which, according to Jordan,131 cave-dwelling man probably derived the parasite.

P. irritans is the predominant flea of the domestic dog in China, particularly in north China where Ct. canis is almost totally absent. It has also been found on dogs in other parts of the world, e.g., in Brazil,263 England,134 South Africa,168 and the western States of the USA.126 In Manchuria, P. irritans was also common on the domestic cat.191

This flea was also found on a wide range of wild animals including rodents, hares, and foxes in Argentina;13, 36, 205 foxes and wild cats in Alberta, Canada;126 Cavia aperea and rabbits in Ecuador;49, 159, 163 domestic guinea-pigs and Sigmodon peruanus in Peru;132 and rodents and carnivores in the western States of the USA.109, 111, 126, 179

7. Ctenocephalides species. Ct. felis felis and Ct. canis, of palaeo-arctic origin, have now become almost cosmopolitan, occurring not only on their specific hosts—the cat and dog, respectively—but also on other species of mammals, often in small numbers on the commensal rats, and occasionally on other rodents. Both species of fleas are prone to attack man and not infrequently become serious pests in houses.

Ct. felis orientis, considered by Jordan 131 as the specific flea of the dog in the Orient, was also found on jackals and on rodents, in India,291 and in
Ceylon \textsuperscript{104} in small numbers on the commensal rats. In the Cumbum Valley of south India this flea was abundant on cattle, particularly buffalo calves.\textsuperscript{71}

\textit{Ct. felis strongylus} replaces \textit{P. irritans} in the Belgian Congo \textsuperscript{44, 45, 270} and in Uganda as the flea of man. According to Hopkins,\textsuperscript{108} it is abundant on dogs and cats in East Africa, and also on goats and sheep. It occurs commonly on many other mammals of medium or large size, and also not infrequently in small numbers on the commensal rats.

8. \textit{Echidnophaga gallinacea}, an Old-World flea, has now become widely distributed, being not only common on poultry, but also infesting many other animals, including commensal and wild rodents. According to some observers, e.g., Mohr,\textsuperscript{182} it is more frequent on \textit{R. norvegicus} than on \textit{R. rattus}. It is commonly called the “stick-tight” flea, because the females, in particular, often attach themselves firmly to the head or neck of the hosts. \textit{E. gallinacea} attacks man.

\textit{Local distribution}

It is of great importance to realize that the commensal-rodent fleas present in any given locality often do not show a uniform distribution, but display marked predilections for particular locations. In this connexion, Toumanoff & Herivaux \textsuperscript{259} aptly spoke of “microsites” favourable for the fleas in question, but it must be kept in mind that the suitability of such sites is largely governed by the suitability of the microclimate prevailing in them.

The differences in the local distribution of the various species of fleas infesting the commensal rodents may be conveniently discussed in relation to the occurrence of the latter, i.e., inside or outside buildings, and in special types of premises or quarters; underground, or at higher levels; in urban or rural localities.

General agreement exists that, in contrast to species such as the palaeoarctic \textit{N. fasciatus}, and also \textit{X. vexabilis hawaiensis} and \textit{S. fonquerniei}, \textit{X. cheopis} mainly infests the commensal rodents living inside buildings. This holds true to a lesser extent of \textit{X. astia} and \textit{X. brasiliensis} because these species infest rodents other than the commensal rats and mice far more often than \textit{X. cheopis}, and are sometimes more prevalent on the wild-rodent species than on the common rats and mice. Davis \textsuperscript{43} noted in this connexion that, in South Africa, \textit{X. brasiliensis} was “essentially a flea of certain veld and bush rodents that thrives in domestic premises as well”.

As proved by observations in various regions, e.g., Ceylon, south India, Uganda, and the southern areas of the USA, as a rule, the incidence of \textit{X. cheopis} is higher in the commercial, than in the residential, quarters of the settlements, especially in premises where foodstuffs—particularly cereals—are handled or stored, and in cotton warehouses and mills.\textsuperscript{104, 137}
As previously mentioned, *X. cheopis* is benefited by the comparatively dry situations it is apt to find in granaries and warehouses, but there are two additional factors of at least equal importance in bringing about a high incidence of this flea in locations where cereals are stored or handled, namely, that such establishments are attractive to rats, and that cereal debris forms a most suitable pabulum for the *cheopis* larvae.

The suitability of cotton for the shelterage of *X. cheopis* is illustrated by observations in the Cumbum Valley which showed that the incidence of this flea in rat-nests containing cotton was higher than in ordinary nests. Compared to *X. cheopis*, the indigenous *X. astia* showed in general a more universal distribution in the settlements of Ceylon and south India. It was sometimes restricted to the residential quarters, but was occasionally found to be predominant in places where rice was stored or handled.

In places into which *X. cheopis* had been recently imported, its incidence was naturally restricted to the locations where cereals and cotton, which form suitable vehicles for the transport of this flea, had been stored. Though subsequently, an eccentric spread of *cheopis* was apt to take place, this led to an invasion of the commercial quarters rather than of the residential precincts. However, as pointed out by King & Pandit, a reversal of this distribution could be observed in localities where the sanitary conditions were worse in the houses of the residential quarters than in the bazaar areas. Sometimes *X. cheopis* became prevalent in one part of a town whereas *X. astia* remained the predominant species in the other precincts. In the opinion of King & Pandit, this pointed to a struggle for existence between the two species.

*E. gallinacea* was often found to be comparatively most abundant on rats in the residential or suburban zones, or in rural areas, particularly on animals caught near, or in, chickenyards and poultry houses; *Ct. canis* and *Ct. felis* were most abundant in houses where dogs or cats were present.

Dealing with the incidence of *X. cheopis* and *X. brasiliensis* in the settlements of Kenya, Cormack, Roberts, and Symes & Roberts stressed that the former flea was found on rats living underground, the latter on rats in roofs, particularly thatched roofs. Hopkins, considering these statements in the light of his observations in Uganda, came to the conclusion "that Roberts is correct in considering *cheopis* to be unsuited for life on roof-dwelling rats but that *brasiliensis* can live either on these or on burrowing species".

Studies on the incidence of fleas in rat-nests, carried out in the Salem District of Madras State, also confirmed that *X. cheopis* was more numerous in underground burrows than in nests found in ceilings or roofs. However, the workers in the Cumbum Valley found that this flea did not show a predilection for any particular level.

According to Hirst, *X. astia* showed an affinity for rats living either in roofs or in burrows in the walls of domestic premises. In the Cumbum
Valley, on the contrary, this flea was found to predominate in rat-nests recovered from underground holes.\textsuperscript{118,120}

While, in view of the discrepant findings of some workers, it is not possible to lay down hard and fast rules, the bulk of the available evidence shows that \textit{X. cheopis} has an affinity for rats living underground rather than for those sheltering on higher levels of the houses. This also seems to hold true of \textit{N. fasciatus}.\textsuperscript{216}

Referring to the distribution of the commensal-rodent fleas, observers in Kenya\textsuperscript{37,216,250} unanimously stated that \textit{X. brasiliensis} was almost the only species present in rural areas, whereas \textit{X. cheopis} was the predominant species in the mixed flea-populations of urban localities.

The preponderance of \textit{X. brasiliensis} in the rural areas appeared to be related to the affinity of this flea for rats living in thatched roofs, for Roberts\textsuperscript{216} recorded that, in Pumwani, Kenya—an area inhabited by various tribes—where all the houses had iron roofs, \textit{X. cheopis} preponderated at a ratio of 3.3 to 0.1. Cormack\textsuperscript{37} seemed inclined to ascribe the preponderance of \textit{X. cheopis} in towns to the fact that many of the rats there lived underground and were then exclusively \textit{cheopis}-infested.

According to Hopkins,\textsuperscript{109} \textit{X. brasiliensis} was, in general, almost the only flea found on the hut-rats in the rural plague-areas of Uganda, while \textit{X. cheopis} was rare or even altogether absent from those parts. The latter flea was common in some townships situated within the plague-areas, living in permanent buildings, especially shops, warehouses, and similar places. In the northern, plague-free zone of Uganda, \textit{cheopis} was abundant on the house-rats as well as on the field-rats.

\textit{X. brasiliensis} was also predominant in the rural areas of South Africa.\textsuperscript{42,43} In the plague-enzootic areas of the Orange Free State, in particular, the rats on farms were mainly parasitized by this flea, whereas in the towns and villages they were also infested by “representatives of the common cosmopolitan species, that accompanied \textit{R. rattus} on its introduction, namely \textit{X. cheopis}, \textit{N. fasciatus}, \textit{N. londinensis} and \textit{L. segnis}”.\textsuperscript{43}

Less extensive evidence available from India\textsuperscript{137,275} also indicates that \textit{X. brasiliensis} is predominant in rural areas rather than in urban localities. Sharif & Narasimham\textsuperscript{232} stressed that this flea was found in large numbers on the commensal rats of localities situated in the hilly, wooded tracts of Bombay State. In Dharwar town, the distribution of \textit{X. brasiliensis} was irregular; although absent from one part of that community, it formed over 50\% of the rat-flea population in another precinct. In sections where both species occurred, certain houses were infested exclusively with either \textit{X. cheopis} or \textit{X. brasiliensis}, the former fleas showing a predilection for comparatively dry and well-ventilated premises, the latter being able to exist in cool, damp, ill-ventilated quarters.

In the experience of Sharif & Narasimham,\textsuperscript{231,232} \textit{X. astia} was absent from the centre of the towns and comparatively most numerous in houses
on the outskirts which bordered on fields. It was the predominant species on the rats of farm-houses, and also in premises infested by bandicoots.

Wild-Rodent Fleas

Annex 1, table V (see page 638) shows that, in contrast to the commensal rodents which are infested by a few, but, as a rule, widely distributed, flea-species, the groups of wild rodents found in the various plague-areas are usually parasitized by diverse flea-species which show, almost invariably, a local, or at most a limited, distribution. It is, however, important to note that, as far as the individual wild-rodent species are concerned, marked differences exist in this respect, some of the animals having only one or two specific fleas, while others have several. In this connexion, Eskey & Haas \(^{54}\) recorded the interesting observation that the rodents parasitized by only one or two flea-species usually showed a higher degree of infestation than those harbouring several kinds of fleas. It is impossible to decide to what extent this is due to the presence of an antagonism between the various flea-species — as is supposed to exist in the case of \(X.\ cheopis\) and \(X.\ astia\) — but it seems more likely that the difference is due to the advantages derived by the fleas from close adaptation to one host or a few closely-related hosts.

Eskey & Haas further noted that the degree of flea infestation depended also on the size of the rodents concerned. These findings, which seem to have been confirmed by the field observations of Humphreys et al.,\(^{112}\) are in accord with those made in case of the commensal rats.

As far as the incomplete information available goes, it would appear that, generally speaking, in the case of the wild-rodent fleas, no sharp line of demarcation can be drawn between host-dwelling and nest-dwelling species, as even those fleas which are known to have a tendency to stay on their hosts also occur in considerable numbers in the burrows of the latter. This situation is well illustrated by the case of the tarabagan flea, \(Oropsylla\ silantievi\). Observations made in Manchuria and Transbaikalia\(^{281}\) suggested that this flea had a marked tendency to stick to the carcasses of its hosts and to the pelts removed from them. Yet, Golov & Ioff,\(^{54}\) who had opportunities for more thorough observations in south-east Russia, found that, even though \(O.\ silantievi\) had a propensity for staying on its hosts, the majority of these fleas nevertheless dwelt in the burrows.

However, in the case of some flea species, more clear-cut differences seem to exist in this respect. Thus, Kusenkov\(^{141}\) found that, in the North Caucasus area, \(Citellophilus\ tesquorum\) was found principally on the susliks, while \(N.\ setosa\) was found mainly in the suslik-burrows. The seasonal incidence of these two predominant suslik-fleas was also different. \(N.\ setosa\) was most frequent in spring, diminished in numbers in summer, and became frequent once more in autumn. The host-dwelling \(C.\ tesquorum\), on the contrary, became apparently more numerous pari passu with the increase
of the suslik population due to breeding, showed its maximal incidence in July, and then began to decrease.

As shown by the observations of Nikanoroff & Gaiski and of Borzenkov et al., wild-rodent fleas are not merely capable of subsisting during the hibernation period of their hosts, but may even remain numerous throughout the winter. However, as has been established by most observers, they do not survive or stay long in uninhabited burrows, even under favourable extrinsic conditions.

Eskey & Haas stated in this connexion that

"... the assumption has been advanced at times that fleas may continue to survive in abandoned nests and burrows for many months and, if plague infected, such fleas are believed to be capable of transmitting the infection over long periods. Field observations, however, did not support this view. Practically no nests yielded fleas that did not show evidence of recent occupation. Even nests which were excavated within a month after ground squirrels had abandoned them were devoid of fleas."

The opinion that wild-rodent fleas do not survive long in burrows deserted by their hosts was shared by Kolpakova & Lippert and by Fedina, who found that, under such circumstances, the fleas sooner or later emigrated.

Tiflov & Potapov, who also noted that the suslik fleas left empty burrows, stated that \textit{N. setosa}, while capable of surviving well on the ground outside the burrows for 24 hours, rarely survived there for 48 hours or more. No doubt can exist, however, that wild-rodent fleas may be present in large numbers on the ground near the openings of inhabited burrows. Eskey & Haas stated in this connexion that, by simply drawing a white cloth over the ground, they were able to collect in such a location over 200 ground-squirrel fleas in a few hours.

It is of interest to note that, owing no doubt to the abundance of free-living fleas not only in the nests of the burrows, but also in their runways and round their openings, wild rodents which were released after they had been captured, marked, and de-fleaed, were found to be heavily re-infested when caught again. Holdenried and his co-workers were thus able to take 1,835 fleas in 37 collections from a single squirrel.

Probably, wild-rodent fleas, although capable of staying away from their hosts in or near the burrows, are not well adapted to a free existence in more remote locations. This is suggested by the observation of Vincke & Devignat that, in contrast to the rat-Xenopsylla and \textit{Ctenocephalides}, two wild-rodent species—namely, \textit{Dinopsyllus} and \textit{Chiaastopsylla}—were incapable of subsisting in debris on the surface of the ground.

**Interchange of Fleas between Commensal and Wild Rodents**

Observations in some plague areas have shown that, in locations where commensal and wild rodents could meet, an interchange of their fleas was
apt to take place. As pointed out with much reason by Hopkins, the relative frequency of such a flea exchange indicates the degree to which contact between the two types of rodents takes place.

In the western areas of the USA, the occurrence of ground-squirrel fleas on rats was recorded by some early observers, e.g., Doane, Fox, and McCoy & Mitzmain. Identical findings were made in the course of a recent extensive survey by Prince.

However, Eskey & Haas, who examined a considerable number of ground-squirrels and rats obtained from locations where these rodents intermingled, found an interchange of their fleas exceedingly rare. In their experience "many more fleas normal to native rats and mice than fleas of ground squirrels were removed from domestic rats".

Findings made during a survey undertaken on a limited scale in a rural area of California by Meyer & Holdenried did not confirm the rarity of ground-squirrel fleas on commensal rats because about a quarter of the species collected from these animals consisted of such fleas, mainly D. montanus. On the contrary, 53 ground-squirrels taken from dumps and ranches heavily infested with rats yielded no rat fleas. Meyer & Holdenried assumed, therefore, that "the transfer of rat fleas to squirrels is less frequent than the reverse".

As established in the course of a recent study by Holdenried, in the southern and southwestern areas of the USA, Polygenis gwyni, an efficient plague-vector, regularly infested Norway rats as well as the cotton-rat, Sigmodon hispidus.

Less detailed evidence indicates that an interchange of wild-rodent and commensal-rat fleas also occurred in other plague-areas.

Thus, to judge from a record by Hecht, Rhopalopsyllus subspecies were predominant on the field-inhabiting rats, as well as on the wild rodents, examined during a survey in Aragua State, Venezuela. Jordan examined collections of fleas from Peru, and noted the occasional presence of X. cheopis and L. segnis, respectively, on some wild-rodent species; Ramos Díaz made a similar observation of X. cheopis on guinea-pigs in the Ecuadorian Sierra.

As previously noted, X. brasiliensis was found on both wild and commensal rodents in South Africa and Uganda. In Uganda and in the Belgian Congo, this flea, as well as X. cheopis, was found on the semi-domestic Arvicanthis abyssinicus. According to Heisch, "X. cheopis and D. lypusus were the predominant wild rodent fleas" in the Rongai area of Kenya, where X. brasiliensis appeared to be rare.

X. astia was sometimes found to be abundant on semi-domestic rodents in India. X. cheopis and X. brasiliensis were far more rarely recovered from such animals or their nests.

It will be gathered, therefore, that an interchange of fleas between commensal and wild rodents is by no means uncommon. Fortunately,
as has been discussed in chapter 6, instances where such fleas served as vectors of P. pestis were far less frequent.

**Role of the Rat Flea in Plague**

It is often overlooked that the credit for the discovery of the role played by the rat fleas in the transmission of plague belongs to Ogata and to Simond, who was obviously unaware of Ogata’s publication when starting his own investigations in 1897.

Working in Formosa, Ogata reached, on epidemiological grounds, the assumption that plague was an insect-borne infection and suspected the rat fleas in particular, stating that “one should pay attention to insects like fleas, for as the rat becomes cold after death, they leave their host and may transmit the plague virus direct to man”.

Obtaining positive results in mice which had been inoculated with a suspension of triturated fleas collected from plague rats, he was able to prove that these insects had actually ingested P. pestis.

As dramatically described in a paper published by Simond in 1936, his attention was attracted in 1897 to a possible role played by the fleas in the transmission of plague through observations on the presence of plague pustules (“phlyctènes précoces”) in about 5% of the patients. These pustules, often found on the legs of the sufferers and invariably on sound, unexcoriated parts of the skin, seemed to represent the portal of entry of a flea-borne infection, the more so as their appearance sometimes suggested that they were due to flea-bites.

On the other hand, Simond established that, under natural conditions, infection by feeding could not play an important role in the spread of plague from rat to rat because, as shown by laboratory tests, enormous numbers of P. pestis had to be ingested to produce an infection per os and, moreover, the pathological findings in animals infected by this route were altogether different from those found in naturally infected, or cutaneously inoculated, rats.

He obtained direct proof of the role of fleas in the transmission of plague by:

1. demonstrating the presence of P. pestis in smears prepared from fleas which had been collected from plague rats and the absence of these bacilli in specimens from healthy rats;
2. obtaining positive results with the technique used by Ogata;
3. successfully infecting healthy rats which had been confined in a wire cage and suspended above a plague-infected rat in a glass jar, and failing to get positive results when conducting such experiments with rats which had been freed from ectoparasites.
Finding that fleas which had ingested plague bacilli could continue to harbour them for prolonged periods, Simond correctly assumed that these insects were apt to play a role in the perpetuation, as well as in the transmission, of the infection. In fact, the only one of his postulations which was not confirmed by further research was that the conveyance of plague by the fleas was invariably due to an entry of their infected faeces into the bite-wounds.

Though the findings of Ogata and Simond found the early support of some investigators, such as Tidswell and Gauthier & Raybaud, they were, in general, ignored or even ridiculed. Lowe, who in 1942 published an enthusiastic appreciation of Simond's work, noted in this connexion that an editorial published in the *Indian Medical Gazette* in 1902 had spoken of the "worthlessness" of Simond's findings, the validity of which had been "pretty completely demolished" by the work of some other observers.

However, further evidence of the importance of fleas in the transmission of plague was furnished by other workers, e.g., Verjbitzki and Liston, and from 1905 onwards a systematic investigation of this problem formed one of the principal objects and, at the same time, one of the monumental achievements of the Plague Research Commission. As aptly stated by Wu: "... these classical researches ... still constitute the foundation of our knowledge of the transmission of plague by the rat-flea. Later workers have added considerably to our conception of the mechanism of infection, the respective roles of *X. cheopis* and *X. astia*, the problem of 'preservers' and of the 'infective' and 'infected' flea, the significance of the zoological and climatic factors, and so on, but their observations, epoch-making and conclusive though these are, serve to round out the picture rather than to alter the perspective in any important particular."

At the 1909 Bombay Medical Congress, Lamb read a paper on "The etiology and epidemiology of plague" which summarized a more elaborate report on the early work of the Plague Research Commission published by him in 1908, and quoted the following evidence in support of the role of the rat fleas in the spread of plague.

1. Attempts to convey plague from rat to rat by direct contact, aerial transmission, or soil infection, or by means of contaminated food, had shown that these modes of infection either played no role at all or did not play a role under natural conditions.
2. An enormous number of successful transmission-experiments had been carried out with rat fleas, all other means of infection having been rigorously excluded.
3. Experiments carried out, mainly with guinea-pigs but also with rats and monkeys, in specially constructed warehouses had proved that:
   (a) close and continuous contact with plague-infected animals (including contact with faeces and urine of the infected animals and
the eating of food contaminated with such faeces and urine) did not give rise to epizootics among the healthy animals as long as fleas were excluded;

(b) when fleas were present, epizootics were apt to start which varied in severity and rate of progress according to the season of the year and the number of fleas present;

(c) the seasons in which such experimental epizootics were readily produced and spread rapidly, coincided with the seasons during which plague was epidemic;

(d) epizootics could occur without direct contact between the originally infected and the healthy animals, the infection being effective in proportion to the access the fleas had to the healthy animals.

4. The site of the primary buboes in animals found plague-affected in nature and those infected in the laboratory by means of fleas was identical.

5. Work carried out in houses where plague had occurred showed that:

(a) guinea-pigs allowed to run about in such houses contracted the infection in 21% of the houses, regardless of whether or not these had been disinfected with a “strong solution of acid perchloride of mercury”;

(b) animals which had been placed in cages to which fleas had access in plague-infected houses often contracted the infection, while those exposed in flea-proof cages remained healthy;

(c) more than 30% of the fleas trapped in plague-infected houses contained abundant living and virulent plague-bacilli in their gastrointestinal tracts;

(d) fleas derived from the carcasses of plague rats found in the houses, or recovered from test animals kept in them, were capable of infecting healthy animals in the laboratory;

(e) houses which were definitely proved to be plague-infected contained, on an average, nearly three times as many rat fleas as houses which were only presumably plague-infected, and twelve times as many as houses which were free from suspicion.

6. Observations on the pathogenesis of human plague and on the time relationship between epizootics and epidemics were consistent with the assumption that the rat fleas, which were found to bite man readily when hungry, were responsible not only for the transmission of plague from rat to rat, but also for the conveyance of the infection from the rats to man.

These results are all the more striking when it is considered that the members of the Commission, though believing that they worked with
X. cheopis only, probably also experimented to some extent with X. astia — a then unidentified, less-efficient plague-vector.

Though both Simond and the Plague Research Commission had obtained some evidence that natural plague occurred in wild as well as in commensal rodents, the findings in the wild rodents were not sufficiently impressive to lead to investigations on the role played by their fleas. However, this gap was soon filled by McCoy, who showed that plague could be transmitted from ground-squirrels (Citellus beecheyi) to guinea-pigs, as well as between ground-squirrels, through one of their fleas, now called Diamanus montanus.

Infectibility of fleas with plague

The only way in which fleas can take up plague bacilli is by obtaining a blood-meal from an animal or from a patient suffering from plague septicaemia. Fleas which ingest P. pestis in this manner do not contract infection in the true meaning of this term, but merely become capable of harbouring this organism in their gastro-intestinal tracts. Nevertheless, it is customary to consider such pestiferous fleas as being "infected" with plague. As discussed later (see page 350), a great multiplication of the ingested plague bacilli may take place in a flea's stomach and/or proventriculus, and may result in a mechanical obstruction or "blockage" which sooner or later may prove fatal to the insect.

Observations by numerous workers have shown that in order to "infect" fleas with plague under laboratory conditions, they must be exposed on experimental animals in which bacteraemia has reached a high degree. This necessity is well illustrated by the following data supplied by Eskey & Haas:

<table>
<thead>
<tr>
<th>Degree of septicaemia in the hosts (guinea-pigs)</th>
<th>Number of fleas fed</th>
<th>Percentage of fleas plague-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 or more organisms per field in smear</td>
<td>439</td>
<td>32</td>
</tr>
<tr>
<td>2-10 organisms per field in smear</td>
<td>457</td>
<td>29</td>
</tr>
<tr>
<td>1 or less organisms per field in smear</td>
<td>546</td>
<td>25</td>
</tr>
<tr>
<td>Smears negative, culture strongly positive</td>
<td>469</td>
<td>17</td>
</tr>
<tr>
<td>Smears negative, culture slowly positive</td>
<td>344</td>
<td>10</td>
</tr>
</tbody>
</table>

It will be noted that, even under optimal conditions, only about one-third of the fleas used in these experiments became infected. It was found, however, that marked differences existed in this respect between the different flea-species. In a series of fleas previously examined by Eskey, it had been established that the infection in X. cheopis was 66.0% as compared with about 25% in the case of N. fasciatus, D. montanus, and Hoplopyllus anomalus, and 10% in the case of L. segnis.

In another paper published at the same time, Eskey maintained, with much reason, that the different infection-rates observed in the various flea-species might depend on differences in feeding habits, those fleas which
fed at long intervals being less likely to obtain a meal during the time their hosts had numerous plague bacilli in their blood. Possibly, therefore, the high incidence of infection in *X. cheopis* was due to its voracious feeding-habits.

Findings similar to those of Eskey were recorded by Eskey & Haas who, feeding 2,031 fleas of different species 3,521 times on plague-infected guinea-pigs, produced infection in 894 fleas. They noted that practically all the 28 rat and wild-rodent flea-species tested could be infected to a greater or lesser extent regardless of differences in sex or size. Rabbit fleas (*Hoploplectus glacialis affinis*) and cat fleas (*Ct. felis felis*) were found much less liable to contract infection than the rodent fleas.

It is difficult to decide to what extent fleas may become plague-infected under natural conditions. As summarized by Petrie, the Plague Research Commission established in this connexion that the average stomach content of a flea was about 0.5 mm³ and that, therefore, a flea imbibing rat-blood containing 10,000 or more plague bacilli per ml would take at least 5 bacilli into its stomach. It was estimated that 66% of the plague rats in Bombay had more than 10,000 bacilli per ml of their blood either before or immediately after their death.

The Commission also established that approximately 50% of fleas collected from the carcasses of these rats harboured *P. pestis*.

Eskey & Haas considered it as entirely problematical whether fleas were more readily plague infected under natural than under experimental conditions. Since, in the laboratory, the fleas were fed to capacity, their chances of ingesting *P. pestis* seemed as great as when they would have fed on their infected natural hosts. It seemed permissible, therefore, to assume that

"... the average plague infection of fleas in nature rarely exceeds one-third of those feeding on their plague-infected normal hosts, as this was the proportion of flea infection from exposure to guinea-pigs having over ten organisms per field in smears of their blood, indicating about as great a bacteremia as would likely occur in any animal".

**Disappearance of the bacilli**

As summarized by Petrie, the Plague Research Commission found that some of the fleas which had ingested *P. pestis* were able to rid themselves of the bacilli, and that the clearing mechanism was probably a phagocytic one. Experiments showed that a rise of temperature of about 8°C (14°F) between the limits 24°C and 32°C (75°F and 90°F) doubled the rate at which the bacilli disappeared from the gastro-intestinal tracts of the fleas. This seemed explained through the observation of Ledingham that, for a rise of 10°C (18°F), the rate of phagocytosis increased about 2.2 times.

That fleas were able to get free from plague bacilli was also observed by other workers—more recently by Douglas & Wheeler, Eskey & Haas, Burroughs, and Holdenried.
Experimenting with *D. montanus* and *X. cheopis*, Douglas & Wheeler noted that 62% of the *D. montanus* were free from plague bacilli 24 hours after one infective meal, while only 2% of *X. cheopis* became free after an interval of more than 48 hours.

Eskey & Haas tabulated their observations as follows:

<table>
<thead>
<tr>
<th>Flea species</th>
<th>Number originally infected</th>
<th>Percentage that became free</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Xenopsylla cheopis</em></td>
<td>140</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Thrassis pandorae</em></td>
<td>58</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Nosopsyllus fasciatus</em></td>
<td>51</td>
<td>8.0</td>
</tr>
<tr>
<td><em>Anomopsyllus nudatus</em></td>
<td>9</td>
<td>11.0</td>
</tr>
<tr>
<td><em>Opisocrosis labis</em></td>
<td>178</td>
<td>12.0</td>
</tr>
<tr>
<td><em>Orchopeas sexdentatus</em></td>
<td>81</td>
<td>13.0</td>
</tr>
<tr>
<td><em>Thrassis acamantis</em></td>
<td>8</td>
<td>13.0</td>
</tr>
<tr>
<td><em>Malarasius telchinum</em></td>
<td>74</td>
<td>16.0</td>
</tr>
<tr>
<td><em>Megalothrillus divisus</em></td>
<td>6</td>
<td>17.0</td>
</tr>
<tr>
<td><em>Thrassis petiolatus</em></td>
<td>6</td>
<td>17.0</td>
</tr>
<tr>
<td><em>Thrassis arizonensis</em></td>
<td>58</td>
<td>19.0</td>
</tr>
</tbody>
</table>

Burroughs, who carried out transmission studies with nine flea-species, established that the percentages of fleas which had become free from plague bacilli at the time of their death were as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Xenopsylla cheopis</em></td>
<td>28.0</td>
</tr>
<tr>
<td><em>Orchopeas sexdentatus</em></td>
<td>30.0</td>
</tr>
<tr>
<td><em>Opisodasys nesiotus</em></td>
<td>46.0</td>
</tr>
<tr>
<td><em>Nosopsyllus fasciatus</em></td>
<td>58.0</td>
</tr>
<tr>
<td><em>Megalothrillus abantis</em></td>
<td>60.0</td>
</tr>
<tr>
<td><em>Pulex irritans</em></td>
<td>69.0</td>
</tr>
<tr>
<td><em>Diamanus montanus</em></td>
<td>74.3</td>
</tr>
<tr>
<td><em>Malarasius telchinum</em></td>
<td>83.0</td>
</tr>
<tr>
<td><em>Oropsylla idahoensis</em></td>
<td>93.0</td>
</tr>
</tbody>
</table>

Burroughs noted that the *X. cheopis* which were found free from plague at death had survived much longer than those in which *P. pestis* had become established.

Holdenried worked with *D. montanus* and *X. cheopis* which, after an infective meal, were kept in jars at a temperature of 68°-73°F (20°-23°C) and 60%-90% relative humidity, and were given opportunities to feed on normal mice. In the *D. montanus* group, 25% were found to be free from *P. pestis* as early as three days after an infective meal; the last flea of this species which retained viable plague-bacilli died on the 35th day. In the *cheopis* group, the presence of infected fleas was demonstrated up to 52 days, and only 5 out of a total of 76 fleas became free from the bacilli.

Examining fleas which had been killed at intervals of 22-31 days after infection in the course of transmission experiments, Holdenried found that
80% *Polygenis gwyni* and 68% *X. cheopis* contained plague bacilli, as compared with 13% *D. montanus*.

Holdenried came to the conclusion that the disappearance of *P. pestis* from infected fleas probably did not depend on phagocytosis which ought to have exerted an identical action in the case of all flea species. He also disbelieved that the mechanical flushing-out of the alimentary tract with blood ingested from normal rodents after an infective meal offered an explanation, as had been adduced. He stressed in this connexion that *P. gwyni* was observed to defaecate profusely after the first few feedings, yet, as noted previously, 80% of these fleas continued to harbour plague bacilli until their death. In his opinion, therefore, the mechanism by which fleas became plague-free was still unknown.

As shown by these observations, no doubt can exist that, at least under experimental conditions, fleas which have ingested plague bacilli can become free from them. It is of importance to note in this connexion that the percentage of such a clearance was markedly lower in the case of highly efficient vectors, such as *X. cheopis* and *P. gwyni*, than in the case of less-efficient vectors.

**Virulence of the bacilli harboured by fleas**

It was suggested by some workers, e.g., Bacot & Martin, Otten, Meyer, Blanc and Macchiavello, that plague bacilli harboured by fleas may undergo a loss in virulence. As assumed by Otten, such weakly virulent bacilli, instead of producing infection, might be immunizing agents.

It would be unwise to disregard the possibility that plague bacilli might become less virulent in the fleas—the more so, because bacteriophage, the presence of which in *X. cheopis* has been demonstrated by Girard, might exert an influence in this respect. However, it must be emphasized that, in the experience of most observers, prolonged survival in the fleas did not lead to an abatement of the virulence of *P. pestis*. As has been shown by some workers, particularly Blanc and Blanc & Baltazard, the virulence of these organisms is apt to remain unimpaired for prolonged periods even in dried-up carcasses of plague fleas.

**Mechanism of Plague Transmission by Fleas**

As previously noted, Simond, when investigating the role of fleas in plague, was led to believe that the entry of infected flea-faeces into bite-wounds, rather than the flea-bites themselves, was responsible for the transmission of the infection. The Plague Research Commission also suspected a role of the flea-faeces, and suggested that a transmission of plague by fleas might be effected as well by:

(a) the rodents eating infected fleas;
INSECT VECTORS

(b) "the proboscis of the fleas mechanically conveying the bacilli from the infected to the healthy animal";

(c) a regurgitation of the stomach contents through the oesophagus and the pharynx, the bacilli being then injected with the saliva or on the pricker or being rubbed into the wounds made by the pricker".

These, as well as some other modes of plague transmission contemplated by later investigators, may be described as follows.

Faeces

The Plague Research Commission paid serious attention to the possibility that the faeces of infected fleas might play a role in the transmission of plague because it had been proved, by animal experiments, that these dejecta contained virulent plague bacilli, and also, in the case of some species, the fleas were apt to defaecate while feeding.

As has been pointed out by later observers, infected flea-faeces may prove dangerous not only at the time when the fleas feed, but also long afterwards, because virulent \( P. \text{pestis} \) have been found able to survive for prolonged periods, even in faeces which have become quite dry. \(^{16, 20, 51, 54, 84} \)

Likewise, it was claimed that the plague bacilli voided in the flea faeces might gain entry not only through the bite-wounds, but also through abrasions which were pre-existent or were produced when the flea-bitten animals scratched themselves.

However, other workers maintained that, for various reasons, the flea faeces did not play an important role in the transmission of plague. Thus, Swellengrebel \(^{246} \) noted that, in his experience, \( X. \text{cheopis}, \text{Pygiopsylla ahalae} \) (\( Stivalius \text{cognatus} \)) and \( N. \text{fasciatus} \) did not defaecate while feeding. The dejecta of \( X. \text{cheopis} \) and \( S. \text{cognatus} \) were so sticky that they adhered to the fur of the rodents instead of soiling their skins. Swellengrebel was able to obtain positive results invariably when infecting guinea-pigs through flea-bites under precautions which excluded contamination of the animal’s skins through the faeces of the fleas. He felt convinced, therefore, that the infection was introduced by the proboscides of the fleas, and not by their faeces.

Swellengrebel’s experiments were successfully repeated by a number of subsequent observers. The viscous consistency of the flea faeces was noted also by Burroughs, \(^{25} \) who pointed out, moreover, that the posture of the fleas during the act of biting led to deposition of the faecal droplets on the hair, rather than on the skin, of the animals.

Several workers adduced proof that the excretion of \( P. \text{pestis} \) by infected fleas was apt to be of irregular instead of continuous occurrence. Note-worthy recent observations made in this respect may be summarized as follows:

Eskey \(^{52} \) stated that plague bacilli were irregularly voided in the case of some flea species. Only one-third of the stool specimens obtained from
N. fasciatus proved positive, while those from D. montanus were invariably positive. This also held true of X. cheopis, but no prolonged observations of individual fleas were possible in the case of this species.

Eskey & Haas noted that only 55% of the faecal specimens obtained from N. fasciatus and Thrassis francisi gave positive results, while those from other plague-infected fleas yielded 80%—90% of such results.

Douglas & Wheeler found that excretion of P. pestis in the faeces of D. montanus and X. cheopis was quite irregular; of the daily samples obtained from these fleas, 56% and 25%, respectively, contained viable plague bacilli. The number of organisms voided in the faeces during 24 hours varied from less than 10 to a maximum of 400, with a daily average of 200.

In a later publication, Wheeler & Douglas added that, in the case of Hoplopsyllus anomalus, the percentage of plague-positive stool-samples was 63.0 as compared with 36.0 (about one-third) in the case of Ct. felis felis.

According to Tiflov, only 50.3% of faeces samples collected from Nosopsyllus consimilis during periods varying from 12 to 108 days proved positive, even though all the fleas were found infected upon dissection. Tiflov quoted earlier observations by Golov & Ioff which gave similar, though less conspicuous, results, e.g., 33.4% negative findings in the case of faecal specimens from Citellophilus tesquorum. In his opinion, the absence of plague bacilli from the faeces by no means indicated their absence from the gastro-intestinal tracts of the fleas in question.

Burroughs recorded that "dead fleas were frequently proved to be infected upon their inoculation in mice, even though organisms could not be cultured from the faeces during life."

Infection per os

It has been maintained by some observers that plague infection may result not only from the chewing and ingestion of infected fleas (as originally assumed by the Plague Research Commission), but also by the ingestion
of their faeces, or by an entry of the causative organisms contained in either fleas or faeces through the mucosa of the mouth or fauces.

The natural occurrence of infections due to the swallowing of plague fleas or their faeces is altogether improbable. As stated earlier, a gastrointestinal type of infection is practically absent in naturally infected rats, even though they might not only swallow infected fleas or their faeces, but also devour the carcasses of their infected mates.

The third of the above-mentioned possibilities deserves some attention on account of observations made of a rare, tonsillar type of plague, the appearance of which was ascribed to the habit of the people concerned of killing the vermin infesting them by biting. However, it is unlikely that rodents contract natural plague by contamination of the mucosa of their mouths or fauces through plague fleas or their dejecta. True, the preponderance of cervical buboes in plague rats might seem suggestive, at first glance, of a frequent entry of the infection through the mucosa of the mouth or fauces. However, the total absence of any marked local reaction in these organs, the occurrence of primary buboes not only on the neck, but also in other parts of the body, and the morbid appearances of plague rats in general are strongly suggestive of an entry of the infection through the skin and not through the mucosae. Even Blanc & Baltazard, who succeeded in infecting one white mouse through oral installation of a suspension prepared from infected \textit{P. irritans} faeces, concluded that a contamination of the mouth mucosa with the aid of such material, though possible, was neither easy to accomplish nor frequent.

\textit{Mechanical transmission}

The Plague Research Commission noted that, in transmission experiments carried out with numerous fleas which had fed first on plague-infected rodents and then on healthy animals, the largest number of infections occurred when using fleas which had been infected within 36 hours before being transferred to normal hosts. These observations seemed to speak in favour of the assumption that a mechanical transmission of the infection was effected through the proboscides of the fleas which had been recently contaminated with \textit{P. pestis}. However, the fact that the fleas used in such mass experiments sometimes continued to be infective while feeding on healthy rats, seemed to speak against an important role of a mechanical conveyance of the infection. Not much attention was paid, therefore, to this mode of plague transmission, the less so because, as aptly pointed out by Burroughs, there soon developed "a tendency to concentrate study on the individual flea rather than to work with fleas en masse as was previously done".

The chance that manifest plague could be produced mechanically by the bite of one flea with contaminated mouthparts is altogether remote because "the amount of materia morbi transmissible by the single puncture
of a flea’s proboscis is infinitesimal” (Hirst 97 after Walker 269). On the contrary, since it has been shown by Bychkov 30 that, under experimental conditions at least, guinea-pigs can become immune through the bites of plague-infected fleas, it is conceivable that the introduction of sub-infective doses of *P. pestis* on the proboscis of a flea which had recently fed on a plague-affected rodent might induce a state of immunity in a healthy animal later bitten by it.

However, as confirmed by the recent work of Blanc & Baltazard, 20 and Burroughs, 25 no doubt can exist that a transmission of plague may be effected through mass attacks of fleas with contaminated proboscides. Blanc & Baltazard concluded from experiments carried out with numerous fleas that “in the case of *P. irritans* as well as in that of *X. cheopis* one has the right to affirm that less than 24 hours after having left its dead host, that is, practically at its first bite of a new host, the flea is able to transmit plague to the latter”.

Exposing laboratory rats to the bites of 60-100 *X. cheopis* which had been plague-infected 24 hours previously, Burroughs 25 found that the animals died of acute plague 3-5 days after the introduction of the fleas, i.e., after a time interval which “disallows transmission by blocked insects as the causal factor in the transference of the organisms”.

Analogous experiments carried out with wild-rodent fleas were frequently negative, Burroughs stating in this connexion that “perhaps one reason some fleas transmit more efficiently *en masse* than others is because they feed more frequently, and so bite a new host animal while viable plague organisms remain on the mouthparts”.

Burroughs concluded with much reason that a mechanical transmission of plague through the recently contaminated proboscides of fleas was bound to be of considerable importance at the time of epizootics.

*Transmission through “blocked” fleas*

As has been noted earlier, the Plague Research Commission considered the possibility that a regurgitation of the stomach-contents of plague fleas might play a role in the transmission of the infection. No evidence to support this hypothesis seemed to be available, even though Liston 150 had found in 1903 through histological examinations that, after an infective meal, fleas showed numerous plague bacilli in their proventriculi. However, neither he nor anybody else thought at the time that these findings were related to a transmission of plague through the regurgitated gastrointestinal contents of infected fleas, and it was not until 1914 that the existence and paramount importance of this mode of infection was proved through the epochal work of Bacot & Martin. 8

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8 "Aussi bien pour pulex que pour xenopsylla, on est en droit d'affirmer que moins de vingt-quatre heures après avoir quitté son hôte mort, c'est-à-dire pratiquement à sa première piqûre sur un nouvel hôte, la puce est apte à lui transmettre la peste."
Bacot & Martin worked with *X. cheopis* and *N. fasciatus* which had been fed on plague-infected mice and which had been proved to harbour *P. pestis* by the examination of their faeces. Observing these fleas while they were feeding individually on normal rats, Bacot & Martin noted that some of them merely filled their oesophagi despite their persistent attempts to suck up blood into their stomachs. Further observation showed that the reason why these fleas continued their efforts to feed was that they were in a starving condition, their stomachs and/or proventriculi having become blocked through a great multiplication of the plague bacilli originally ingested.

This condition was apt to prove disastrous not only for the fleas themselves, but also for the hosts from whom they tried to obtain meals because, owing to the elastic recoil of the oesophageal and pharyngeal walls, the blood overfilling the oesophagus was apt to be driven back into the bite-wounds, carrying along plague bacilli detached from the bacterial plug which was sometimes seen to protrude into the oesophagus. As shown later by Bacot, fleas which become partially unblocked through the formation of a cleft in the centre of the proventricular plug are particularly dangerous. In such cases, blood may penetrate from the oesophagus into the stomach proper, but will run out again because the proventriculus, owing to the bacterial masses stuffing its lateral recesses, remains unable to fulfil its normal valve-like function, thereby preventing the regurgitation of the stomach contents into the oesophagus.

As has been demonstrated by Hirst, completely blocked fleas become particularly dangerous when they have fed on a host in which plague septicaemia is present because, when they attempt to feed again, such fleas will inject concentrated suspensions of *P. pestis* into their new hosts. It is obvious that the chances of such a transmission of the infection are bound to be best during active epizootics when the number of rodents in the ultimate stage of plague is apt to be large.

Studies on the process of blockage, particularly the valuable observations of Eskey, have shown that the formation of obstructing masses due to the multiplication of the originally ingested plague bacilli may commence to take place either in the proventriculus or in the stomach proper. This is not the result of chance, but of differences, probably of an anatomical nature, existing between the various flea species. In this connexion, Eskey and Eskey & Haas noted that, in the case of *X. cheopis* the proventriculus was much more frequently the primary site of blockage than in the case of other flea species.

According to Eskey, a blockage "originating in the stomach of a flea does not cause obstruction of that organ until it has invaded the tubular structure leading to the proventriculus, and usually not until it has extended into the proventriculus or oesophagus". It is clear, therefore, that, because the proventriculus of *X. cheopis* is apt to become primarily obstructed,
the process of blockage is bound to evolve more rapidly in this flea than in other flea species, the proventriculi of which are secondarily involved. In other words, the extrinsic incubation-period, i.e., the length of time elapsing between the ingestion of plague bacilli and the establishment of blockage, is bound to be considerably shorter in the case of *X. cheopis* than in the case of those fleas where blockage, because it starts in the stomach proper, evolves more slowly. According to Eskey \(^{53}\) and Eskey & Haas, \(^{54}\) *X. cheopis* had an extrinsic incubation-period lasting from 9-26 days, while *N. fasciatus* and wild-rodent fleas became blocked after intervals lasting from three weeks to over four months.

As established by these workers, the variations in the length of the extrinsic incubation-period in any one flea-species were due mainly to climatic influences. Eskey \(^{52}\) recorded in this respect that *X. cheopis* which had been kept at temperatures over 70°F (21°C) became blocked earlier than those kept at a mean temperature of 60°F (16°C), apparently because the increased temperature hastened the multiplication of *P. pestis*.

Eskey & Haas \(^{54}\) found that three lots of *X. cheopis* which had been kept in the incubator at 72°-80°F (22°-27°C) were capable of transmitting plague at an average of 15 days after they had been infected; this interval was six days less than the average extrinsic incubation-period in fleas of the same species kept at 66°F (19°C) in an unheated room. Transmission by five *cheopis* kept at 80°F (27°C) occurred after they had been infected for an average of 11 days.

In the opinion of the two workers, these findings indicated that “increased temperatures within the range of those of the experiments, should increase the rapidity with which fleas spread plague and that rodent epizootics should be more severe in warm climates than in cold, where *X. cheopis* are prevalent”.

Until blockage has become established at the end of the extrinsic incubation-period, the fleas remain capable of feeding in a more or less normal manner without producing infection in their hosts.\(^{52}\) However, it is important to note that, as established by Burroughs, *X. cheopis* became blocked more rapidly and in greater numbers when kept starving for several days than when fed daily. As Burroughs pointed out, this disproves the contention of Blanc & Baltazard \(^{18, 20}\) that the infectivity of fleas depends upon the multiplication of *P. pestis* in the fresh blood taken up at repeated feedings.

General agreement exists that, though blocked fleas often remain permanently obstructed, their gastro-intestinal passages may become temporarily, or even permanently, free. Eskey & Haas \(^{54}\) maintained in this connexion that temporarily blocked fleas never proved infective to the hosts on which they fed.

The survival period of blocked fleas varies according to the species concerned and also according to the prevailing climatic conditions. As a
rule, blocked *X. cheopis* succumb within a few days. This was confirmed by the observations of Eskey & Haas \(^{34}\) on the lifetime of infected fleas which showed that

"... infected *X. cheopis* did not live as long as other fleas and that increased temperatures lessened their length of life. Those kept at 80°F. (27°C) survived at an average only 10 days. Examination of *X. cheopis* after death revealed that nearly all of them died of starvation due to the bacterial process having caused obstruction of the stomach."

It is of the greatest importance to realize that by no means all the fleas which harbour plague bacilli become blocked. This is well illustrated by the recent observations of Burroughs,\(^{25}\) who recorded the following percentages of blocked fleas in the species studied by him:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number studied</th>
<th>Percentage blocked</th>
<th>Species</th>
<th>Number studied</th>
<th>Percentage blocked</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>X. cheopis</em></td>
<td>53</td>
<td>58.0</td>
<td><em>D. montanus</em></td>
<td>66</td>
<td>3.0</td>
</tr>
<tr>
<td><em>O. sexdentatus</em></td>
<td>53</td>
<td>28.0</td>
<td><em>P. irritans</em></td>
<td>57</td>
<td>1.75</td>
</tr>
<tr>
<td><em>N. fasciatus</em></td>
<td>47</td>
<td>23.0</td>
<td><em>E. gallinacea</em></td>
<td>48</td>
<td>0.23*</td>
</tr>
<tr>
<td><em>O. nesiotes</em></td>
<td>46</td>
<td>22.0</td>
<td><em>M. telchinum</em></td>
<td>115</td>
<td>0.0</td>
</tr>
<tr>
<td><em>M. abantis</em></td>
<td>75</td>
<td>12.0</td>
<td><em>O. idahoensis</em></td>
<td>61</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*"As these fleas could not be examined microscopically after each meal as were the others this data cannot be presented with certainty".*

As far as it is permissible to draw conclusions from these limited experiments and analogous observations of other workers, one might claim not only that certain flea-species are more apt to become blocked than others, but also that some species do not become blocked at all. However, while no doubt exists as to the validity of the former claim, one should be chary of accepting the latter. Since the blocking period varies considerably between the different flea-species, it is quite conceivable that, had more prolonged observation been possible, one might also have found evidence of blockage in the species which seemed incapable of becoming obstructed. For this reason, and also because one should not go too far in drawing conclusions from laboratory studies alone, one must fully agree with Eskey \(^{31}\) that

"... failure to obtain experimental transmission with certain species does not prove that they are incapable of acting as transmitting agents. In fact, the results of laboratory investigations tend to indicate that any flea, regardless of species, that feeds on septicemic blood may become plague infected, and later blockage of the esophagus may occur which would make the flea a potential vector."

At first glance, it is a puzzling but a well-established fact that, even though they have been completely blocked, fleas by no means invariably transmit plague to the hosts on which they try to feed. On the contrary, as summarized by Burroughs,\(^{25}\) it had been shown by previous workers, as well as in the course of his own observations, that "many blocked fleas never did transmit, and that others did not transmit the infection at each attempt to feed."
Eskey & Haas recorded the following interesting observations in this connexion:

"The persistent efforts of blocked fleas to secure blood do not necessarily signify that such bites will result in the transmission of plague. In fact during these experiments a great many fleas failed to transmit the infection although they fed from one to several times in a manner characteristic of complete obstruction to the stomach, and only a portion of the bites of fleas that transmitted plague to one or more guinea pigs were infectious after it was apparent that blockage had developed. In some instances bites of less than 1 minute's duration were infectious to guinea pigs while others lasting as long as 30 minutes failed to infect the hosts. However, plague transmission is more likely to follow prolonged efforts of fleas to feed than bites of short duration. At least, this applied to the bites made by fleas after they had infected one guinea pig, for 64 percent of their subsequent blocked feeding were infectious when the parasites made multiple attempts to obtain blood while only 28 percent transmissions occurred when the insects ceased to feed after one bite. During multiple bites the average attachment of the fleas was 13 minutes, as compared to 4 minutes during single bites."

Eskey & Haas were inclined to ascribe the frequent failure of the bites of blocked fleas to produce infection either to a lack of regurgitation or to the absence of P. pestis in the regurgitated blood, but they also considered the possibility that, in some of the fleas, the virulence of the organisms might have become so attenuated as to render them non-pathogenic.

Since, as will be gathered from the above statements, blocked fleas are, generally speaking, more likely to produce infection the more persistently they attempt to feed, it is obvious that voracious feeders like X. cheopis will be particularly apt to act as plague vectors. Observations made by Eskey have proved this contention. Blocked X. cheopis repeatedly tried to feed on the guinea-pigs on which they had been exposed and, in many instances, produced 2-5 foci of infection on the abdomens of the animals, whereas blocked fleas of other species rarely produced such multiple foci. Two N. fasciatus, which had each infected a guinea-pig, refused to bite the animals on which they were later exposed.

**Comparative importance of the various modes of transmission**

Though, as has been discussed, it has been claimed that a spread of plague through fleas may be effected in various ways, it seems safe to state that only two of these modes of transmission are of real importance—namely, a mechanical spread through mass attacks of recently infected fleas, and the "biological" transmission of the infection through individual blocked fleas.

Comparing the importance of these two modes of transmission, Burroughs postulated that

"... though, biologically, the individual flea is all-important in preserving plague in an enzootic state in areas where occasional sporadic cases of the disease occur in a sparse or highly resistant animal population, mechanical transmission by numbers of infected fleas is undoubtedly the more important means of disseminating the causative organism in a dense, susceptible population in epizootic times."
As stated earlier, one must agree that a mechanical transmission of plague by fleas is probable only during epizootics when, owing to the presence of numerous severely affected rodents, the "infection quantum" is great. However, one cannot agree with the concept of Burroughs that blocked fleas are of lesser importance during epizootics because, in contrast to ideas expressed by Eskey, Eskey & Haas, and Meyer, it fails to take into account the determinative influence exerted by variations in the length of the extrinsic incubation-period. Little doubt can exist that, under the climatic conditions prevailing during the plague seasons, the extrinsic incubation-period is short. Consequently, numerous fleas become blocked and therefore infective within short periods, quickly infect numerous rodents, and thus create ample opportunities for a rapid, further spread of the infection. A vicious circle is thus set up, to which momentum is added through the effect of mass attacks of recently contaminated fleas, and also through increased opportunities for a transmission of the infection by blocked fleas which have filled their oesophagi with the blood of rodents in the septicaemic stage of plague.

Conclusions

As is clearly shown by these observations, a marked discrepancy exists between the number of fleas which harbour \textit{P. pestis} and the percentage of those which are capable of transmitting the infection. Considering the comparative infrequency of such actual vectors of the infection, one must fully agree with the dictum of Webster & Chitre that "in the majority of infected fleas the plague bacillus has entered a blind alley" and, adopting the terminology used by George & Webster, one must realize that, though many fleas may be infected, only a minority become infective.

Factors Influencing Role of Fleas in Transmission of Plague

\textit{Climatic influences}

As has already been noted, the Plague Research Commission established, early in the course of its work, that the trend of experimental epizootics in rats and guinea-pigs corresponded to that of the outbreaks of rat and human plague in Bombay City. The experimental epizootics ran a rapid course with a high mortality during the plague seasons, while the infection spread far more slowly during the off-seasons, and many of the animals at risk remained unaffected.

That this different trend of the epizootics during the plague seasons and the off-seasons, respectively, was due to an influence exerted by the prevailing climatic conditions on the vectors of the infection, was shown through a series of well-planned experiments, the results of which have been summarized by Petrie.
A much higher percentage of successful flea-transmission experiments with Bombay
wild rats, and also with guinea-pigs, was obtained during the plague season than during
the off-season. Experiments of this kind were made in July-August, 1906, that is, during
the off-season; (1) at room temperature, with an average of 27 to 28°C. (80 to 83°F.),
and (2) in a specially constructed cool room kept at 21°C. (70°F.). Bombay rats, ship-rats
and guinea-pigs were used, and the proportion of successful transmissions at 21°C. was
nearly twice that at 27.5°C.

Similar experiments were made in January-February, 1907, that is, at the beginning of
the plague season, (1) at room temperature, namely, 24°C. (75°F.), and (2) in a specially
constructed hot chamber kept at from 29.5 to 32°C. (85 to 90°F.); guinea-pigs were used
and the ratio of successes was 84/32, or nearly 3 to 1 in favour of the lower temperature.”

In the opinion of the Commission, the failure of the infection to spread
rapidly and widely at temperatures of about 80°F (27°C), or more, was due
principally to a more rapid disappearance of P. pestis from the stomachs
of the fleas owing to an increased phagocytic activity of the white blood-
corpuscles of the host ingested together with the bacilli. That fleas kept
at higher temperatures were apt to become free from plague bacilli far
more quickly than those kept at room temperature was shown by animal
experiments with the faeces of infected fleas. The dejecta of fleas kept at
room temperature—24°C (75°F.)—were found to be infective for 21 days,
while fleas kept at 32°C (90°F) excreted virulent plague bacilli for three
days only.

Evaluating these experiments, Hirst 98 pointed out that they were open
to criticism because they were probably conducted not only with X. cheopis
(as the Commission assumed), but also to some extent with X. astia, a
less-efficient vector, and X. brasiliensis.

He further maintained that
“... the growth and virulence of plague bacilli at various temperatures in the stomachs
of fleas is probably dependent on a balance of several factors, the rate of multiplication
of the bacilli themselves, a factor influenced by temperature; the activity of the digestive
ferments of the flea itself and the amounts secreted by its gastric glands; autolytic digestion
of the plague growth; and the action of the ingested leucocytes”.

Believing that the digestive action of the tissues of the flea was apt to
alter the resistance of the ingested bacilli to phagocytosis, Hirst postulated
that the growth of P. pestis in the proventriculus was probably less affected
by the opsonic factor than the corresponding growth in the stomach
proper.

While the Plague Research Commission 203 had come to the conclusion
“that a plague epidemic is checked when the mean daily temperature
passes above 80°F [27°C] and especially when it reaches to 85°F or 90°F
[29°C or 32°C]”, some later observers who studied the influence exerted
by climatic conditions on flea-borne plague, postulated that dryness of the
atmosphere was also of great importance in this respect.

Bacot & Martin 8 maintained in this connexion that adverse climatic
conditions particularly affected the blocked fleas which, because they were
unable to imbibe fresh blood, were in danger of drying up when the tem-
perature was high and the degree of saturation of the atmosphere was low. The two workers felt certain, therefore, that the lifetime of blocked fleas was bound to become short as soon as the weather became hot and dry, and were “led to wonder whether this fact may not, to some extent, explain why in India epidemic plague is confined to cooler and moister seasons, and particularly why in Northern and Central India the epidemics are abruptly terminated on the onset of hot dry weather”.

The important role played in the epidemiology of plague by the dryness of the atmosphere was fully confirmed by the exhaustive studies of Brooks who found that:

1. Plague did not establish itself when the temperature rose above 80°F (27°C) accompanied by a saturation deficiency of over 0.30 inch (7.6 mm).

2. Plague epidemics were rapidly brought to an end in the presence of a high saturation-deficiency, even when the mean temperature throughout, and after termination of, an epidemic had been below 80°F (27°C).

3. Plague epidemics could commence and increase in intensity when the mean temperature was well above 80°F (27°C), provided that the saturation deficiency was below 0.30 inch (7.6 mm).

In the opinion of Brooks, “the adverse influence of high temperature and saturation deficiency may be explained by their effect on the duration of life of the rat flea, Xenopsylla cheopis, when separated from its host”.

Goyle, studying the influence of climatic conditions on X. cheopis and X. astia, came to the conclusion that, at a temperature of 68°F (20°C), X. cheopis was incapable of transmitting plague at a saturation deficiency of 0.6 inch (15.2 mm), while a saturation deficiency of 0.3 inch (7.6 mm) at the same temperature was sufficient to check the conveyance of the infection by X. astia.

Webster & Chitre, who published soon afterwards the results of large-scale transmission experiments carried out with these two flea-species and also with X. brasiliensis, obtained evidence to support the view that...

"... the chief factor in the spread of plague which is under the influence of climatic changes in Bombay is connected with the blocking phenomenon. The epidemic season seems to be controlled by the fact that within a rather limited range of temperature [68.8°-76.8°F (20.5°-24.9°C)] and humidity [saturation deficiencies ranging from 0.237 inch to 0.374 inch] a much larger proportion of infected fleas became capable of transmitting the infection”.

These observations tally with those of Eskey and Eskey & Haas on variations of the extrinsic incubation-period at different temperatures.

As is to be expected, findings analogous to those discussed above were made when, instead of variations of the saturation deficiency of the atmosphere, those of the relative air-humidity were observed. Thus, Campbell...
noted that a plague outbreak at Mwanza, Tanganyika, started, and ran its course, at a relative humidity of 80%, and came to an end two weeks after the relative humidity of the air had become less. Robie ascribed the rapid cessation of plague outbreaks in Madagascar at about 30°C (86°F) to variations in the relative humidity which were apt to occur at that level of temperature and which impaired the resisting power of *X. cheopis*.

While, as will be gathered from the evidence quoted, general agreement exists that climatic conditions exert a profound influence on the role of fleas in the transmission of plague, vastly different views are held by the different observers as to how this influence is exerted. It is claimed by some workers that changes in temperature and humidity are of importance because such changes exert an influence on plague-infected fleas, while other observers maintain that these climatic factors influence the blocked fleas in particular.

It is undeniable that variations of the temperature and humidity may regulate, to some extent, the number of fleas which continue to harbour *P. pestis* in their gastro-intestinal tracts. However, even under the most favourable climatic conditions, only a minority of these infected fleas become infective, and variations in their numbers, unless they become very marked, are probably not of great epidemiological importance. However, it is of paramount importance that favourable temperatures, because they shorten the extrinsic incubation-period, lead to a rapid increase of the number of blocked fleas, and that favourable humidities are apt to increase the length of survival of these infective fleas. One must therefore agree with the statement of Park that "summing up the... findings on plague transmission by fleas, it appears that it is not the total number of fleas or the specific flea counts that matter, not even the number of infected fleas, but the number of infective fleas that is of prime importance, and this depends perhaps entirely on climatic conditions".

However, it is of the greatest importance to remain aware of the fact that because the microclimate in the rodent burrows is apt to remain uniform throughout the year, it may continue to be favourable for a transmission of plague through the fleas, even during seasons when the generally prevailing climatic conditions are unfavourable for a spread of the infection.

**Vector capacity**

The existence of differences in vector capacity between the various flea-species was established early in the work of the Plague Research Commission, and this problem has engrossed the attention of numerous workers ever since. The methods used for a study of this question, although considerably varying in details, may be classed in two categories:

(1) Mass transmission-tests, carried out by the exposure of groups of fleas, previously fed on a plague-infected animal, on healthy laboratory animals, the fleas usually being put on the healthy animals a few days
after the original host had died so as to exclude the possibility of a mechanical conveyance of the infection.

(2) Individual feeding-experiments, i.e., those carried out with single, or a few, plague-infected fleas.

With regard to the value of these procedures, it should first be stated that both methods possess a common drawback—namely, that, for various reasons, particularly in order to obtain comparable results when testing different flea-species, it is often necessary to work not with their specific hosts, but with highly and uniformly susceptible laboratory-animals. As aptly pointed out by Burroughs, this drawback is of little importance in the case of some flea species, e.g., *X. cheopis*, for which favourable conditions may be created in the laboratory. It is, however, apt to vitiate results in the case of flea species which normally live in a cold climate, or when dealing with strains of species such as *P. irritans* which have become adapted to mammalian hosts other than rodents.

A great advantage of mass transmission-experiments is that they may be conducted under conditions resembling, to quite some extent, those in nature. In fact, by adding at suitable intervals new healthy animals to those initially introduced into the experimental cages, warehouses, or pits, veritable epizootics may be established and kept going. However, while valuable for epidemiological studies, mass transmission-experiments cannot be used to determine the vector capacity of fleas in an exact manner. Nevertheless, it is advantageous to use them before individual feeding-tests are carried out for this purpose, so as to arrive at a preliminary estimation of the vector capacity of the species to be examined.

Individual feeding-tests, as usually performed, possess the drawback of artificiality because, whereas the fleas in nature have as much access to their hosts as they feel impelled to seek, during these tests they are permitted to bite, or to try to bite, merely during limited periods and, usually, only once daily. In order to obtain comparable results when various flea-species are examined, this procedure must, inevitably, be followed. However, it must be kept in mind that the method does not necessarily give a complete picture of the capability of the individual species to transmit plague under natural conditions.

The following recently recommended methods to determine the vector capacity of fleas deserve mention.

Wheeler & Douglas first carried out tests en masse with each flea-species to be examined in order to determine whether it was able to transmit *P. pestis*. For this purpose, 100 fleas of the species in question were fed on a guinea-pig or a white mouse in the ultimate stage of plague. Two days after the death of the infected host, the surviving fleas were placed on a healthy guinea-pig, or were allowed to feed on healthy mice.

For individual feeding-tests, Wheeler & Douglas used, whenever possible, laboratory-reared fleas which were fed on a moribund plague-infected
mouse whose degree of septicaemia had been controlled. Some hours after the death of the mouse, the fleas were recovered, placed in a test-tube, lightly anaesthetized with ether, and examined under a dissecting microscope. Fleas which had not obtained a complete blood-meal were discarded. The others were placed individually in 75 mm by 15 mm glass shell vials, the mouths of which were closed by a square of fine-mesh, silk bolting-cloth held in place with a rubber band, and were kept at a temperature of 20°-23°C and an average relative humidity of 50%.

By inverting the vial and placing the open end securely against the shaved abdomen of an immobilized mouse, each flea was given a daily opportunity of feeding upon a healthy mouse. After feeding, each flea was transferred to a fresh, sterile vial and the faeces contained in the used vial were cultivated. At death, every flea was fixed and sectioned serially for histological studies.

The mice on which the infected fleas had fed were placed in individual glass-cages and observed for a period of 30 days. Each animal dying within this period was dissected, and smears and cultures were made to ascertain the presence of *P. pestis*.

The information obtained by the examination of the fleas and the mice was used to establish: (1) the infection potential, based upon the percentage of the fleas of a given species in which *P. pestis* had become established; (2) the vector potential, based upon the percentage of infected fleas capable of transmitting plague; and (3) the transmission potential, i.e., the mean number of transmissions effected by the infective fleas. The product of these three factors represented the number of transmissions effected by a given number of the fleas, or the vector efficiency.

Wheeler & Douglas thus illustrated the manner in which these potentials were established:

"Individual feeding trials have demonstrated *D. montanus* to have an infection potential of .85, a vector potential of .52, and a transmission potential of 2.58 with a vector efficiency of 1.14. That is, of 48 fleas used 41 or 85% became infected; of these 41 infected fleas 21 or 52% became infective and transmitted the infection to 50 mice or an average of 2.58 transmissions per infective flea. Dividing the percentages by 100 the potentialities of a single flea may be obtained. In other words a group of *D. montanus* given an infectious blood meal will yield an average of 1.14 transmissions per flea."

The results of the tests made with *D. montanus* and *X. cheopis* were as follows:

<table>
<thead>
<tr>
<th></th>
<th><em>D. montanus</em></th>
<th><em>X. cheopis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number used</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>Number infected</td>
<td>41</td>
<td>49</td>
</tr>
<tr>
<td>Number infective</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Number of transmissions</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Infection potential</td>
<td>0.85</td>
<td>0.98</td>
</tr>
<tr>
<td>Vector potential</td>
<td>0.52</td>
<td>0.29</td>
</tr>
<tr>
<td>Transmission potential</td>
<td>2.58</td>
<td>1.44</td>
</tr>
<tr>
<td>Vector efficiency</td>
<td>1.14</td>
<td>0.39</td>
</tr>
</tbody>
</table>
Wheeler & Douglas noted that, according to these results, *X. cheopis* had a higher infection-potential than *D. montanus*, but that only half as many *cheopis* became infective and transmitted only half as many times. They concluded, therefore, that

"...apparently then a high infection potential alone is no indication of high vector efficiency; likewise the vector potential alone without a consideration of the transmission potential will not give a true picture of vector efficiency. From the above data an accurate evaluation of vector efficiency must include a knowledge of the infection potential, the vector potential and the transmission potential."

In a second paper, Wheeler & Douglas recorded the results of further experiments made with *D. montanus* and *X. cheopis*, as well as with *N. fasciatus*, *Ct. felis felis*, and *Hoplopsyllus anomalus*. All five species were found capable of transmitting *P. pestis* in mass experiments. Results of individual transmission-trials in four of these species showed the following vector efficiencies:

<table>
<thead>
<tr>
<th>Species</th>
<th>Vector efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ct. felis felis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>H. anomalus</em></td>
<td>0</td>
</tr>
<tr>
<td><em>X. cheopis</em></td>
<td>0.43</td>
</tr>
<tr>
<td><em>D. montanus</em></td>
<td>0.84</td>
</tr>
</tbody>
</table>

Using techniques which were similar to, though more refined than, those of Wheeler & Douglas, Burroughs compared the vector capacities of the ten flea species studied by him by ascertaining the transmission-rate of each species, i.e., the quotient obtained on dividing the number of transmissions effected by the fleas in question, when given one daily opportunity to feed, by the total number of fleas. His results were as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number studied</th>
<th>Number of transmissions</th>
<th>Ratio of transmissions to fleas used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>X. cheopis</em></td>
<td>53</td>
<td>35</td>
<td>0.660</td>
</tr>
<tr>
<td><em>E. gallinacea</em></td>
<td>48</td>
<td>12</td>
<td>0.250</td>
</tr>
<tr>
<td><em>N. fasciatus</em></td>
<td>47</td>
<td>10</td>
<td>0.213</td>
</tr>
<tr>
<td><em>O. s. sexdentatus</em></td>
<td>53</td>
<td>9</td>
<td>0.170</td>
</tr>
<tr>
<td><em>O. nesiotus</em></td>
<td>46</td>
<td>3</td>
<td>0.065</td>
</tr>
<tr>
<td><em>M. abantis</em></td>
<td>75</td>
<td>4</td>
<td>0.053</td>
</tr>
<tr>
<td><em>M. telchinum</em></td>
<td>115</td>
<td>5</td>
<td>0.043</td>
</tr>
<tr>
<td><em>D. montanus</em></td>
<td>66</td>
<td>1</td>
<td>0.015</td>
</tr>
<tr>
<td><em>P. irritans</em></td>
<td>57</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td><em>O. idahoensis</em></td>
<td>61</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

As stated by Burroughs,

"...the transmission data obtained from individual flea feeding studies was analysed statistically to estimate the expected number of transmissions per flea of each species. These values are obtained as intervals which have a 90% probability of containing the true value. The true vector efficiency of *Xenopsylla cheopis* was found to be $0.660 \pm 0.234$ (expected transmissions per flea), that of *Nosopsyllus fasciatus* to be $0.213 \pm 0.157$, and that of *Orchopeas sexdentatus sexdentatus* to be $0.170 \pm 0.138$. *Opisodasys nesiotus*, *Megabothris abantis*, *Malaraeus telchinum* and *Diamanus montanus* transmitted very inefficiently".

INSECT VECTORS
Devignat made mass transmission-experiments in order to determine the vector capacity of the important flea-species found in the Belgian Congo. He found that the constants of vector capacity thus obtained equalled either 1 or 0. However, differences in the vector capacities of the fleas found able to transmit plague could be determined by comparing the surface areas of the curves illustrating the mortality of the flea-bitten mice with the surface area of a graph showing the mortality of mice infected with standard doses of \( P. \text{pestis} \) which was equal to 7,600 mm\(^2\). He tabulated his results as follows:

<table>
<thead>
<tr>
<th>Flea species</th>
<th>Surface area of graph showing mortality of flea-bitten mice (mm(^2))</th>
<th>Ratio to surface area * of graph showing results of virulence tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenopsylla cheopis</td>
<td>7,300</td>
<td>0.96</td>
</tr>
<tr>
<td>Xenopsylla brasiliensis</td>
<td>5,400</td>
<td>0.71</td>
</tr>
<tr>
<td>Dinopsyllus lypusus</td>
<td>6,900</td>
<td>0.90</td>
</tr>
</tbody>
</table>

\* 7,600 mm\(^2\)

Investigating the problems of flea-borne plague, some workers were led to believe that the capabilities of male and female fleas, respectively, of transmitting the infection were unequal. Hirst recorded in this connexion that all the fleas which he had found to be capable of conveying plague were females. On the contrary, Goyle postulated that male \( X. \text{cheopis} \) and \( X. \text{astia} \) carried the infection more readily than the females of these species.

The results obtained in this connexion by Webster & Chitre were discrepant. They concluded from some of their early experiments that female \( X. \text{cheopis} \) and \( X. \text{astia} \) were more capable transmitters than the males. Reporting on further investigations, they stated that

"... the question of the sex of the flea in connection with its transmitting power is not easy to answer. From the mixed flea experiments it would have seemed reasonable to conclude that, of the six groups, male \( \text{cheopis} \) and male \( \text{brasiliensis} \) are much the most regular transmitters. With the pit fleas, however, the cage experiments show female \( \text{cheopis} \) and female \( \text{astia} \) to be much more effective than their respective males. Male \( \text{astia} \) fleas have been found of low value as transmitters in all the experiments. The finding of an individual blocked male \( \text{astia} \) capable of infecting with a single bite was particularly fortunate ".

Eskey stated that, though he performed experiments with many males of different species, only two of the 45 fleas which he found capable of transmitting the infection were males.

The results of the experiments made by Eskey & Haas were similar to those of Eskey, but not as clear-cut. Moreover, as shown by the following figures, the number of female fleas observed was markedly in excess of the number of male fleas:

<table>
<thead>
<tr>
<th>Females</th>
<th>Males</th>
<th>Total fleas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number plague-infected</td>
<td>607</td>
<td>243</td>
</tr>
<tr>
<td>Number that were vectors</td>
<td>70</td>
<td>11</td>
</tr>
<tr>
<td>Percentage infected that were vectors</td>
<td>11</td>
<td>4</td>
</tr>
</tbody>
</table>
Wheeler & Douglas recorded the following observations:

<table>
<thead>
<tr>
<th></th>
<th>X. cheopis</th>
<th>D. montanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>males</td>
<td>females</td>
</tr>
<tr>
<td>Number used</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Number infected</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>Number infective</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Number of transmissions</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Infection potential</td>
<td>1.00</td>
<td>0.67</td>
</tr>
<tr>
<td>Vector potential</td>
<td>0.50</td>
<td>0.17</td>
</tr>
<tr>
<td>Transmission potential</td>
<td>1.00</td>
<td>1.50</td>
</tr>
<tr>
<td>Vector efficiency</td>
<td>0.50</td>
<td>0.17</td>
</tr>
</tbody>
</table>

It will be noted that, as far as these figures go, no marked differences in vector efficiency appeared to exist between the sexes in the case of *X. cheopis*. Male *D. montanus* were found to be less-efficient vectors than the females, but only 18 males were studied as compared with 62 females.

Holdenried, testing 39 male and 49 female *Polygenis gwyni*, found that 11 (28.2%) males were transmitters, as compared with 13 (26.5%) females.

In view of the fact that the various observers quoted above often used unequal numbers of male and female fleas, respectively, and considering that even their total samples were not large, one ought to accept the statement of Webster & Chitre that possibly "a larger series of experiments under any one set of conditions would abolish the apparent difference in the value of males and females as transmitters".

A question of great interest and possible importance is whether the vector capacities of different strains of the same flea species may be at variance. It is striking to note in this connexion that, as previously mentioned, *D. montanus*, while proving, in the experience of Wheeler & Douglas, an even more efficient vector than *X. cheopis*, figured last among the transmitters given in Burroughs' list. Results similar to those of Burroughs were recorded by Eskey & Haas, who noted that, out of 19 *D. montanus* tested, only 3 transmitted plague, and by Holdenried, who found that, out of 446 fleas of this species, only 9 conveyed the infection.

Discussing this problem, Burroughs was inclined to believe that the discrepancy between his results and those obtained by Wheeler & Douglas was due to biological differences between the strains used in the two experiments. There was no doubt that the fleas used by Wheeler & Douglas came from a locality different from that where Burroughs obtained his material.

The contention of Burroughs was supported by the observations of Holdenried who found that three of his *D. montanus* strains, obtained from different plague-foci, as well as a fourth strain from an apparently plague-free region, yielded no vectors at all, whereas strains from two other supposedly plague-free localities yielded vectors at a rate of 4% and 5%, respectively.
Considering the limited scope of these observations, one must agree with Holdenried that the evidence concerning the existence of racial differences in the vector capacity of fleas belonging to the same species, although suggestive, is not fully convincing.

Vector incidence

Before appreciating the influence exerted on the transmission of plague by variations in the incidence of the vector fleas, it is necessary to consider the methods recommended for flea surveys.

The early workers, though realizing the ominous role of the fleas which had left the carcasses of plague-infected rodents, paid no attention to these loose parasites when conducting routine surveys, but relied, for this purpose, on flea collections from rodents which had been trapped alive. They used this material to determine for each rodent-species, separately, (a) the total flea-index, i.e., the average number of fleas of all species per rodent, and (b) the specific flea-indices, i.e., the average number of fleas of each species found per rodent.

Though ample use was, and is, made of these procedures, it was pointed out by some observers, particularly by Hirst, and Cole & Koepke, that many variables coming into play were apt to detract from the value of flea-index determinations. Cole & Koepke stressed, in this connexion, that:

1. Indices computed from total fleas are unreliable because they are apt to include non-vectors, as well as species which are unequally effective as vectors.

2. The time fleas of different species spend on their hosts is apt to vary considerably.

3. No reliable measurements of the absolute number of the fleas can be made without considering the number of rodents because "a decreasing host population will concentrate the ectoparasites and raise the mean number per rat while an expanding host population will reduce the average counts".

4. The host species ought to be uniform in compared samples because, as in the case of R. norvegicus and R. rattus, for instance, different rodent-species show differences in flea infestation.

5. The age, as well as the size, of the individuals affects the counts, young and old rodents being liable to heavy infestation. The same may hold true of unhealthy animals.

6. Trapping techniques must be consistent in order to obtain comparable data.

7. Widely different indices may be obtained from rodents trapped in different sections of a town, or in different locations within one particular neighbourhood, or even on different levels of one building. X. cheopis,
for instance, particularly infests rats in grain stores, and is markedly more abundant on rodents trapped inside buildings than on those caught outdoors.

(8) Fleas do not uniformly infest all rodents of a locality, but show a patchy distribution so that, sometimes, a large part of a flea population is concentrated on a few animals.

Although it is desirable to do so, it is rather difficult to make exact determinations of the number of rodents in the course of routine flea-surveys, as recommended by Cole & Koepke. However, advantage may be taken of a simple method recommended by Hirst. The number of rodents caught per 100 traps set is ascertained and this factor is multiplied by the flea-index figures. As stated by Hirst, in this way “an index representing the gross ecto-parasitic flea population is obtained”.

In order to eliminate the errors arising out of an abnormally heavy infestation of a few rodents, Rumreich & Wynn recommended the adoption of a new index “derived by mathematically fitting an appropriate curve to the frequency distribution of parasite counts in any host population, and thus determining their normal upper limit”. However, as pointed out by a reviewer in the Tropical Diseases Bulletin, an index computed in this way “loses its great value of being able to be compared for different places”.

To improve the value of index determinations, Cole & Koepke recommended that the indices should be computed for flea-infested rodents only, and that male X. cheopis should be disregarded in such computations because, as shown by Cole, the females of this species are less variable than the males in their association with the hosts under varying temperature conditions.

In the opinion of some workers, more reliable results could be obtained in flea surveys by computing, instead of the indices, the percentage incidence of the various species or the infestation-rates, i.e., the percentages of rodents infested with the various flea-species.

Hirst maintained with much reason that computations of the percentage incidence of fleas could be legitimately used only when the general flea-index was more or less uniform because,

“... suppose the average number of all species of fleas per rat in one locality is two and in another twenty, and suppose there are 10 per cent. cheopis in a parallel collection from each, then the cheopis index in the first case is 0.2 and in the second 2.0. If the mean temperature is below 80°F. [27°C] the second figure is compatible with a severe epidemic, whereas a serious outbreak would be unlikely in the first instance”.

It follows that, in order to obtain fully reliable results, it is best to determine both the indices and the percentage incidences of the various flea-species, as has been recommended by King & Pandit.

Discussing observations made during a survey at San Francisco, Calif., Eskey found that “the cheopis index fluctuated so erratically from month..."
to month that it was of little value for determining the extent of seasonal infestation”. He therefore recommended that reliance should be placed upon the percentages of specific flea-infestation. Rumreich & Wynn,221 although establishing that there was a marked parallelism between these values and the specific indices, recommended the use of both methods in order to assess the incidence of the various flea-species.

Within recent years it has been urged by several workers that, in addition to surveys carried out with the aid of such methods, the incidence of free-living (loose) and burrow- or nest-dwelling fleas ought to be assessed.

Various methods have been recommended for the collection of loose fleas, including the exposure of guinea-pigs on the floors of houses, or of guinea-pigs or “de-fleaed” rats in cages; the use of flea traps; and the use of adhesive paper to which the fleas could become attached.201, 226

A simple and efficient trap, recommended by Lefrou & Wassilieff,148 consisted of a dish filled with oil and provided with a floating wick which was lighted at night to attract the fleas. However, it was found sufficient to fill the dishes with water, preferably adding a little alcohol or chloroform. Nowadays, a DDT suspension or solution might be substituted with advantage.

It was originally recommended that the trap should be used in houses with a soft floor so that the rim of the dish could be made level with the ground. However, as shown by M. A. Farid (unpublished observations in Lebanon), numerous fleas could be caught by placing 2.5-cm-high lighted traps on the ground. The provision of a 7.5-cm-wide sloping rim, the outer margin of which was made to rest on the ground to enable the fleas to crawl up instead of jumping, hardly increased the size of the catches.

A suitable apparatus for collecting fleas which harbour in dust has been described by Estrade55 as follows.

The apparatus consists of: (a) a large, truncated cone, in the centre of the inside bottom of which a plate with a pin is affixed, and which is filled with a layer of water, flytox, or alcohol before use; (b) a funnel, of hour-glass shape, closely fitting over; (c) a receptacle with a 2-cm-high outer wall and a hole in the centre of its bottom.

The dust to be tested is rapidly filled into the upper mouth of the funnel which, together with the receptacle underneath, is placed into the large container in such a way that its central pin engages with the receptacle. The funnel is then removed, contact thus being established between the dust and the fluid in the container. As soon as this happens, the fleas in the dust begin to jump and land in the fluid where they are drowned.

Most workers resorted to digging operations in order to obtain fleas from the passages and nests of rodent burrows. However, some investigators5, 61, 242 caught wild-rodent fleas with the aid of cotton-wads inserted into the mouth of the burrows, or by trapping. For this purpose, workers in south-east Russia used a trap consisting of a metal box filled with water
and provided with a tube, the open outer end of which was inserted into the opening of the burrow. Pasteboard tubes smeared inside with glue, as used by Traut (quoted by Wu Lien-teh & Pollitzer), seem to have given less satisfactory catches.

Conclusions of the composition of the flea populations examined with the aid of these methods may be reached by establishing the percentage incidence of the various species of loose or burrow fleas found. It is important to note in this connexion that, according to Stewart & Evans, the composition of the flea population at the openings of wild-rodent burrows corresponds quite accurately, though on a lesser scale, to that of the flea material collected from the rodents themselves. Stewart & Evans therefore considered it legitimate to make surveys by studying the fleas obtained from the openings of ten or more burrows. It is not certain, however, whether this conclusion is generally valid.

Some recent workers, particularly Davis and Macchiavello, have recommended that the total or "absolute" flea-index should be determined by considering the results of examinations of the burrows and nests, as well as those of surveys carried out in the usual manner. Macchiavello recommended the application of the formula $\text{AFI} = \frac{\text{RF} + \text{NF}}{\text{TR}}$, where AFI stands for the absolute flea-index, RF for the total flea-population living on the rodents, NF for the absolute number of fleas from flea-breeding or flea harbourage, and TR for the total rat-population of the area concerned. These data were to be obtained "by cross-section surveys of rodent and flea populations, similar to those used in the partial census of human population, and a cross-section counting of nests in a representative limited area".

Desirable as determinations of the absolute flea-indices by this or other methods are, it seems difficult, if not impossible, to make routine use of such procedures.

The results of flea surveys carried out in the usual manner are of great value since they permit an assessment of the plague situation. It is generally accepted that, as has been postulated, first by Hirst and later by Grubbs, even though the infection is present, or is introduced, there is no danger of an epidemic spread as long as the $X. \text{cheopis}$ index remains below 1. Of course, this applies only to areas where $X. \text{cheopis}$ is the sole important vector.

To judge from less detailed information, it would also appear that plague is not liable to assume epidemic proportions as long as the $X. \text{cheopis}$ percentage is below 20%-30%, or as long as the $X. \text{cheopis}$ infestation-rate is below 30%.

\[d\] A further method, used by George & Webster, is described on page 381.
It is, however, of the greatest importance to realize that the seasons during which plague outbreaks occur do not necessarily coincide with the periods during which the incidence of the vector fleas is highest. Kitasato stated in this connexion that, in Japan, the rat fleas were most prevalent during summer, although the plague season occurred in the cold period of the year.

Similarly, it was found by Eskey & Haas that the percentage of plague-infected ground-squirrels in the vicinity of San Francisco Bay was highest during the three summer months when the flea infestation of the animals was lowest.

George & Webster concluded from their observations in the Cumbum Valley of south India that

"the cheopis index and plague incidence are not so closely associated ... as appears at first glance. Thus, in 1931 the decline of plague in February preceded the fall in the cheopis index while in the autumn epidemic the plague incidence increased prior to the increase in fleas. In 1932 the order of events was the same but to a less marked extent. In 1933 the high plague incidence in the spring was not associated with a corresponding rise in the flea-index figure, while the autumn rise in the cheopis index was not accompanied by an increased incidence of plague."

Similar observations were repeatedly made by Pollitzer in central China.

Robie stated that, in Tananarive, Madagascar, there was no definite relation between the cheopis index and the frequency of plague, while Herivaux & Toumanoff found that the cheopis index at Saigon, Indochina, was highest when plague was absent.

Webster & Chitre maintained in general that "the plague season need not correspond with the season of maximum prevalence of rat-fleas although this may happen as a coincidence."

These observations lend strong support to the contention that it is a high incidence of actually infective fleas, and not the frequency of potentially dangerous vector-species, which is of paramount importance in the spread of flea-borne plague.

**Role of Individual Flea-Species**

*Rat-Xenopsylla*

Though the investigations of the Plague Research Commission established the paramount importance of the *Xenopsylla* in the transmission of rat-caused plague, they failed to render full justice to this subject since the members of the Commission laboured under the assumption that *X. cheopis* was the only representative of this genus of flea found on the commensal rats of India. It is to the great merit of Hirst that he was able to prove (through exhaustive studies which were commenced in 1912 and of which the first results were published in 1913) that, in addition to
cheopis, two other Xenopsylla—namely, X. astia and X. brasiliensis—were present on the rats.

Hirst, finding that X. astia was a far less efficient plague-vector than X. cheopis, propounded the idea that the preponderance of this flea in some parts of India offered an explanation of why these localities were free from the infection. This concept was not in accord with the views of the Plague Research Commission and Liston who ascribed the absence of plague from such localities—particularly from Madras City—to climatic conditions unsuitable for the spread of plague. It is not surprising, therefore, that Hirst’s statements were received with a great deal of scepticism at first. Petrie, for instance, maintained as late as 1929 that the question of the relative importance of X. cheopis and X. astia in the spread of plague was still unsettled and that, therefore, further work on the subject was necessary before final conclusions could be drawn.

However, the findings of Hirst, published in a series of studies, were confirmed on the whole by the laboratory investigations of Taylor & Chitre, Goyle, Webster & Chitre, as well as by the results of extensive field-surveys recorded by Cragg and by King & Pandit. General agreement has thus been reached that although X. astia is capable of becoming blocked and of transmitting plague, it is a markedly less efficient vector than X. cheopis or X. brasiliensis. Webster & Chitre stated in this respect that

"... the exact numerical value of the different species cannot yet be laid down. The renewed epizootic in the astia pit provided some information regarding this species. With a flea index of seven the epizootic could be restarted. When the epizootic had ceased the flea index was found to be 3.2. Under the most favourable conditions the necessary astia index may lie between these two figures. In nature a still higher index is probably required. The mixed flea experiments in the plague season, taking both sexes of each species and counting the first transmission in series only, give the relative value as transmitters of cheopis, astia and brasiliensis as 1 : 0.3 : 1.7 respectively."

These conclusions were endorsed by findings made in the course of field surveys. King & Pandit, summarizing the results of a rat-flea survey in Madras Presidency (now Madras State), stated that "the occurrence and severity of plague epidemics are associated with the number of cheopis present and this relation is mainly direct and not just because of the common association of plague and cheopis with climate, and that X. astia, acting as vector in nature without cheopis in south India has produced very few and very small epidemics which did not carry over the off-season."

Hirst, reporting on a rat-flea survey of Ceylon, concluded that:

1. In Colombo, nearly two-thirds of the plague manifestations detected in rats during the period 1918-29 occurred in the main cheopis-area, while the far more extended astia-zones, which contained a few cheopis-infested premises, yielded only one-tenth of the positive rats.
(2) Out of 11 rat epizootics detected in Ceylon outside Colombo, eight occurred in districts with a *cheopis* index of 1.0; in one district, *cheopis* was present, but the index was not determined, while two epizootics occurred in a pure *astia*-area at Galle where the *astia* index was 5.67.

(3) A total of 25 human cases resulted from these two epizootics which did not spread beyond the original focus of infection, and which were separated from each other by a clear interval of seven years' duration.

(4) Evidence from Ceylon and Madras, combined with experimental data, suggested that the critical *astia*-index for independent plague-transmission lay between 6 and 7.

(5) Analysis of the Colombo data suggested that, when an *astia* area is invaded by *cheopis*, plague may become endemic when the *cheopis* index is near unity, the additional *astia* apparently playing a subsidiary part once the epizootic has been initiated by *cheopis*.

Although these findings confirm the paramount importance of highly efficient vector-species in the transmission of plague, they show that less-efficient vectors, if present in sufficiently large numbers, may play a subsidiary, or even, to some extent, an independent, role.

The laboratory observations made by several workers, e.g., Webster & Chitre, Symes, Roberts, and Devignat, have proved that *X. brasiliensis* is an efficient plague-vector; in fact, as already noted, Webster & Chitre found the vector capacity of this flea to be superior to that of *X. cheopis*.

Davis stated that, while no exact laboratory data had been collected on the vector efficiency of the fleas involved in the South African plague outbreaks, *X. philoxera* (*X. eridios auctt.*)—the gerbil flea—and *X. brasiliensis* were regarded, on epidemiological grounds, as equally efficient vectors of the infection to rodents and man. *X. brasiliensis* was probably responsible for the majority of human infections, particularly in the rural areas where it lived closest to man.

Hopkins considered *X. brasiliensis* as the normal initiator of the plague outbreaks in Uganda. In his opinion, *X. cheopis* was very seldom concerned in the initiation of outbreaks, but helped to carry on epidemics which had reached townships.

Dealing with the situation in the Lake Albert plague-focus of the Belgian Congo, Vincke & Devignat stated that though both *X. cheopis* and *X. brasiliensis* had been found infected, the percentage incidence of *X. cheopis* was invariably high (20%-30%), whereas sometimes less than 10% *X. brasiliensis* were found. Even in the zone where *X. brasiliensis* predominated in general, *X. cheopis* was found to be prevalent in some of the plague foci. In the opinion of Vincke & Devignat, *X. cheopis* was a more efficient vector as far as the transmission of plague from rodent to rodent was concerned, whereas *X. brasiliensis* was particularly apt to convey the infection to man.
Therefore, an optimal mixture of both these flea species might lead to the appearance of human plague as well as of epizootics.

According to Riel & Mol,\textsuperscript{213} \textit{X. brasiliensis} was the vector of plague in the Lake Edward plague-focus of the Belgian Congo, from which focus \textit{X. cheopis} was absent.

As stated by Roberts,\textsuperscript{214, 216} \textit{X. brasiliensis} was the principal vector of \textit{P. pestis} in the rural areas of Kenya where plague was endemic in type, whereas \textit{X. cheopis} was mainly, or even solely, involved in the urban manifestations of the infection which were of an epidemic character. Roberts ascribed this different role of the two flea-species to differences in their habitat. \textit{X. cheopis} mainly infested the rodents living underground in the houses and thus had far more easy access to human beings than \textit{X. brasiliensis}; the latter was found mainly on the rodents which sheltered in the thatched roofs of rural buildings but did not actually enter the premises.

Similarly, it was maintained by Sharif & Narasimham\textsuperscript{232} that \textit{X. brasiliensis}, because of its preference for the hilly, woody tracts of the Western Ghats in Bombay State, played a principal role in the plague outbreaks occurring in these localities which showed a marked tendency to spread slowly, but to persist for a long time. \textit{X. cheopis} was probably a more important vector in the low, comparatively warm tablelands of Bombay State where the plague outbreaks were of an explosive nature, but did not last long.

Apparently, \textit{X. brasiliensis}, because it was most prevalent during the plague season, played a more important role than \textit{X. cheopis} in the transmission of the infection in the Salem District of Madras State.\textsuperscript{123}

In addition to playing a predominant role in the above-mentioned areas, \textit{X. brasiliensis} is held to be an important plague-vector in the temperate zone of Brazil, especially in São Paulo.\textsuperscript{15, 207}

Eskey\textsuperscript{50} assumed, primarily on epidemiological grounds, that, in addition to \textit{X. cheopis}, \textit{X. vexabilis hawaiiensis} played an important role in the transmission of plague in Hawaii.

**Rat Ceratophyllinae**

In 1907 the Plague Research Commission recorded two successful transmission-experiments with "\textit{Ceratophyllus}" \textit{fasciatus}, but it was later established that the fleas in question actually belonged to an allied species, now called \textit{Nosopsyllus punjabensis}. However, experiments with \textit{N. fasciatus} were made by McCoy\textsuperscript{166}—who was able to transmit plague with these fleas from rats to ground-squirrels—and, a few years later, by Bacot & Martin.\textsuperscript{8} Although chary of drawing stringent conclusions, Bacot & Martin found that blockage of the proventriculus occurred more readily in \textit{X. cheopis} than in \textit{N. fasciatus}, and gained, in general, the impression that the former could transmit plague more easily than the latter.

In a series of further experiments, Bacot\textsuperscript{7} used batches of 100-300 starved \textit{N. fasciatus} which had been infected by feeding on moribund
plague-mice and were then kept in cages covered with wax-cloth and stored at an average temperature of 7.5°C. By adding healthy mice to these cages from time to time, Bacot was able to prove that plague persisted in the fleas of three cages for 29, 34, and 47 days, respectively.

Comparing the vector capacity of *N. fasciatus* with that of *X. cheopis* through individual feeding-tests, Eskey & Haas, and Burroughs, obtained the following results:

<table>
<thead>
<tr>
<th></th>
<th>Eskey &amp; Haas</th>
<th>Burroughs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>X. cheopis</em></td>
<td><em>N. fasciatus</em></td>
</tr>
<tr>
<td>Average extrinsic incubation-period (days)</td>
<td>21</td>
<td>41</td>
</tr>
<tr>
<td>Length of survival after blockage (days)</td>
<td>2.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Percentage of infected fleas becoming vectors</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Ratio of transmissions to fleas studied</td>
<td>0.42</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Although, according to these observations, *N. fasciatus* is a fairly efficient vector, it is often maintained that it plays a far less important role in the transmission of plague under natural conditions than *X. cheopis*. Hirst, while concluding from observations made in Japan that an epizootic might be “continued by *C. [N.] fasciatus* in the winter months for some time after the *X. cheopis* population has declined”, maintained that there was “no evidence as yet that *C. [N.] fasciatus* or *L. musculi* can either initiate a rat epizootic or maintain it for any length of time in the entire absence of *X. cheopis*”.

Eskey laid stress upon the fact that the extrinsic incubation period was much longer in the case of *N. fasciatus* than in that of *X. cheopis*. In his opinion, plague outbreaks in warm countries, where *X. cheopis* was the vector, ran a rapid course with a high incidence of human infections. In colder climates, where *N. fasciatus* preponderated, the outbreaks were prolonged and the incidence of human plague was low. For instance, in Seattle, Wash., rat plague smouldered for ten years and yet only three human cases were recorded. While assuming that *N. fasciatus* played an important part in carrying over plague, Eskey, like Hirst, disbelieved that this flea could initiate epizootics.

Mohr, dealing with *N. fasciatus*, stated that

“...there has been considerable speculation regarding this flea’s role as a plague vector. To it was attributed the occurrence of plague among domestic rats at Tacoma in 1942... because it was present in great numbers and because no Oriental rat fleas were found on the plague infected rats. The evidence is equally good that the fleas of meadow mice, and possibly white-footed mice with which the Norway rats were associated, caused a secondary epizootic among the rats. No plague was recovered from rats which were far removed from association with the wild species and its fleas.”

These statements deserve great attention in connexion with the question of whether or not *N. fasciatus* played an important role in the transmission of the infection during the past plague-outbreaks in northwestern Europe.
Since, as stated earlier, it is difficult to believe that X. cheopis was rampant there in the past, one is led to believe that N. fasciatus, and possibly P. irritans also, were responsible for the spread of the infection in the historic plague-outbreaks of northwestern Europe.\(^{17, 19}\) No doubt can exist that these pests abounded then, so that their large numbers might have compensated for any shortcomings in their vector capacity. Moreover, there can be little doubt that widespread outbreaks in northwestern Europe like the "Black Death" were due to an invasion of plague from areas in which efficient vector species were present. However, while these considerations speak against the supposed role of X. cheopis in the plague outbreaks in Europe in the past, one should beware of being dogmatic when dealing with a problem, for the solution of which one has to depend on surmise rather than on factual data.

Observations in the Nilgiris district of south India suggested that, N. nilgiriensis, a flea infesting mainly Bandicota bengalensis kok, was associated with X. cheopis in the transmission of plague.\(^{137}\) This was confirmed by George & Timothy \(^{79}\) who found that, out of three N. nilgiriensis which had been fed on an infected mouse, two showed numerous plague-bacilli in their stomachs one week later. Although they did not perform transmission experiments, these workers believed that this flea was "at least a weak vector of plague among rodents". Apparently it did not bite man.

In the opinion of Macchiavello,\(^{159, 162}\) N. londinensis, which took the place of X. cheopis in the highlands of Ecuador, probably played a role in the transmission of plague, but was apparently a poor vector.

Monopsyllus anisus and, to a lesser extent, Paradoxopsyllus (formerly called Ceratophyllus) curvispinus have been considered as vectors of the infection during past plague-outbreaks in Japan.\(^{135}\) It is undecided to what extent M. anisus, which largely replaces N. fasciatus in China, is participating in the transmission of the disease. Wu \(^{251}\) pointed out that, during the epizootics at Shanghai in 1910 and 1911, most rats succumbed between October and April when, by analogy with later surveys, X. cheopis was presumably scarce, but M. anisus and L. segnis were prevalent.

Leptopsyllidae

General agreement exists that L. segnis plays an almost negligible role in the transmission of plague, both because it is a poor vector and because it attacks man with the greatest reluctance. Experimenting with 23 fleas of this species, Eskey & Haas \(^{54}\) noted that five (18 \%) became plague-infected. Apparently, however, these fleas were incapable of transmitting the infection.

Devignat,\(^{44}\) inoculating guinea-pigs with pools of different flea-species, obtained some positive results with L. aethiopica. However, he did not ascribe importance to this flea which infested commensal rats in only a few localities.
Paractenopsyllus kerguisteli is probably of local importance in the spread of plague in Madagascar.²¹⁹

Pygiopsyllidae

In Java, as noted earlier, Stivalius cognatus (formerly thought to be Pygiopsylla ahalae) was found capable of transmitting plague from rat to rat and of biting man.²⁴⁷

George & Timothy ⁷⁰ were likewise able to transmit the infection with the aid of Stivalius (probably a mixture of St. ahalae, St. aperus, and St. ferinus) collected from Bandicota bengalensis kok in the Nilgiris district of south India.

Synosternus pallidus

As noted earlier, Synosternus pallidus was found to be frequent in French West Africa, mainly on the floors of houses, but also to some extent on the commensal rodents. Opinions regarding the role played by this flea in the spread of plague were divided. Advier ¹ found that:

"Synosternus pallidus is, therefore, not the usual parasite of the animals which form the principal reservoir of the plague virus. In consequence of this biological characteristic, the fleas of this species have little opportunity to ingest plague bacilli and, even if they do, they do not seem very capable of transmitting the infection. They do not, therefore, play an important role in the propagation of the disease."

On the contrary, Kartman ¹³⁶ concluded from circumstantial evidence that, in addition to X. cheopis, S. pallidus probably played a role in the transmission of plague from the rats to man, and was also a vector of the infection from man to man. He stated in this connexion that, while the cheopis index was comparatively low (0.96), S. pallidus not only abounded on the floors of the huts, but also infested the rats to some extent.

Synopsyllus fonquerniei

As mentioned earlier, S. fonquerniei, which was formerly rare on the Madagascar high-plateau, has become abundant since 1931.

Dealing with the possible role played by this flea in the transmission of plague, Robic ²¹⁷ pointed out that, contrary to X. cheopis, S. fonquerniei mainly infested the rats living outdoors and was more frequent during the cold seasons. S. fonquerniei had been proved to be a vector as well as a carrier of P. pestis, but since it always occurred in association with X. cheopis it was difficult to assess its role in the spread of plague.

Similarly, it was stated by Girard ⁷⁸ that since plague had spread before S. fonquerniei had become abundant, and since this flea was rarely found inside houses, its importance as a vector was probably not considerable.

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"Synosternus pallidus n’est donc pas un parasite habituel des animaux qui constituent le principal réservoir de virus pesteux. Par suite de ce caractère biologique, les puces de cette espèce ont peu d’occasions d’ingérer des bacilles pesteux et, quand cette éventualité se produit, elles ne paraissent pas très aptes à transmettre leur infection. Elles ne jouent donc qu’un rôle bien peu important dans la propagation de la maladie."
However, it might be capable of playing a role in the perpetuation of the infection among the outdoor rats which were more numerous and in closer contact with one another than those living indoors.

**Wild-rodent fleas**

It is not possible to assess the role played by the wild-rodent fleas in the transmission of plague in as systematic a manner as was done in the case of the commensal-rodent fleas, not only because, as shown by Annex 1, table V (see page 638), a great number of species are involved, but also because their individual vector-capacities have not been uniformly well determined.

Generally speaking, it may be maintained that, as in the case of the rat fleas, marked differences in vector capacity exist between the various wild-rodent fleas found in plague foci. A number of the species studied in the laboratory proved to be almost as capable of transmitting plague as *X. cheopis*; others appeared to be mediocre, or even poor, vectors, while some seemed to be incapable of conveying the infection. However, while it seems permissible to use the positive findings so far obtained for an assessment of the comparative vector capacity of the various species, one should be chary of concluding from laboratory observations, made during limited periods and not infrequently with an inadequate number of fleas, that a given species is actually unable to transmit plague. At least, as has been shown by the observations of Golov & Ioff, practically all the flea species, regardless of whether or not they could act as vectors individually, could convey the infection when attacking susceptible rodents en masse.

It is, however, interesting to note that, in the opinion of Eskey, "it is possible that more wild rodents contract plague from infected flea faeces, cannibalism, and by eating infected fleas than by the bites of fleas". In support of this contention Eskey stated that

"... the fact that it has been possible to obtain a large number of positive guinea pig inoculations from pooled specimens of fleas collected from several different rodent hosts, which showed no evidence of being infected themselves, yet which harbored infected fleas, strongly suggests that these infected insects may feed on their hosts without infecting them".

In evaluating this statement, one should give consideration to what was said in chapter 5 with regard to the diagnostic value of tests using pools of organs or fleas. Although such tests are most accurate in determining the presence of *P. pestis*, they do not permit of conclusions as to how numerous the bacilli were and what reactions they produced. Hence, the tests to which Eskey referred merely indicated that some—or, perhaps, even only one—of the pooled fleas were infected. On the basis of this evidence, one would hardly be justified in drawing conclusions about the ability of the fleas in question to transmit plague when they were infective.
A much-debated question is to what extent the wild-rodent fleas are responsible for the transmission of plague to man. In some of the foci, the wild rodents are hunted for the sake of their fur, meat, or fat and, even in other foci, a study of the case-histories showed that some of the patients had had direct contact with diseased or dead rodents. The preponderance or frequency of axillary buboes noted in the initially affected individuals in some of the foci seemed to furnish proof that infection was often due to direct contact and not to the bites of infected fleas.

Observers of “sylvatic” plague in the USA, e.g., Meyer, Eskey, and Wheeler & Douglas who have recently dealt with this problem, shared the opinion that the wild-rodent fleas were responsible only in part for the conveyance of the infection to man. Thus, Eskey estimated that out of approximately 40 cases of human plague of supposed wild-rodent origin which had been recorded in the western States of the USA up to 1938, less than half had been caused by the bites of infected fleas. Meyer, studying the histories of 50 bubonic-plague patients in the western States of the USA whose infection appeared to have been of wild-rodent origin, found that statements relative to the location of the buboes were available in 39 instances. Of these 39 patients, 17 had primary buboes located in the groin, and 12 had primary buboes in the axilla. Meyer felt convinced that the infection of the patients with groin buboes was the result of flea-bites on the lower limbs, but maintained, with much reason, that the patients with axillary buboes might have contracted infection when handling sick or diseased rodents, or through the bite of infected animals.

As shown by the evidence discussed above, the possibility that man may contract infection by coming into direct contact with plague-affected wild rodents deserves serious attention, and all possible steps should be taken to avert this danger. At the same time, however, it is probable that at least half, or, probably, even a majority, of the patients who had contracted bubonic plague in the wild-rodent foci had been infected through flea-bites.

*Ctenocephalides* species

Both the common cat-flea, *Ct. felis felis*, and the dog flea, *Ct. canis*, proved rather feeble plague-vectors under experimental conditions. Twenty-seven transmission experiments made with *Ct. felis felis* by the Plague Research Commission all gave negative results. Verjbitzki obtained a few positive results when exposing healthy rats to the bites of groups of 10 cat fleas which had fed on infected animals 12-48 hours previously and, also, when making identical tests with groups of 5-10 *Ct. canis*.

Eskey & Haas reported that out of 28 cat fleas which had fed on a plague-affected guinea-pig, two became infected. Apparently, however, it was not possible to test the vector capacity of these fleas.

Wheeler & Douglas obtained positive results when (a) exposing a group of 50 cat fleas immediately after the death of their infected host.
(a mouse) on a healthy guinea-pig, and (b) starving these fleas for two days after the death of the guinea-pig and then again exposing them on a healthy guinea-pig. However, these workers failed to obtain positive results when making individual transmission-tests with *Ct. felis felis*, even though 12 of the 14 fleas used were afterwards proved, by histological examination, to be plague-infected. Wheeler & Douglas expressed the opinion that possibly "a single *C. felis* flea is not anatomically suited to regurgitate a sufficient number of *Past. pestis* to produce an infection. This possibility is supported somewhat by the fact that organisms were never found in the oesophagus of this species ".

Occasional findings of naturally infected *Ct. canis* have been recorded, particularly by Blanc & Baltazard who established, through animal experiments, the presence of *P. pestis* in 50 fleas of this species which had been collected from a plague-infected dog. These workers were also successful when performing an animal experiment with three *Ct. felis felis* found on a plague-infected cat. However, an inoculation test with 20 fleas of this species, collected from a second plague-infected cat which had been sacrificed, gave a negative result. Herivaux & Toumanoff failed to produce infection in experimental animals with both cat and dog fleas which had been collected in plague-infected houses.

In view of this evidence, one ought to share the disbelief of Herivaux & Toumanoff that the common cat- and dog-fleas play a role in the transmission of plague under natural conditions. However, as stated in chapter 6, both cats and dogs may prove dangerous by bringing infected rodent-fleas into houses.

In a report on plague investigations in the Cumbum Valley of south India, the finding of one infected *Ct. felis orientalis* in a plague-infected house was recorded. It was added that since this flea prevails largely on cows and buffaloes—particularly on the calves—and the farmers live in close association with their cattle, it might be potentially dangerous. Some transmission experiments were therefore attempted, but did not give conclusive results.

Devignat, making pooling-tests with various flea species, repeatedly obtained positive results with groups of *Ct. felis strongylus*, a flea replacing *P. irritans* as a human parasite in the Belgian Congo. However, mass transmission-experiments carried out with *Ct. felis strongylus* invariably gave negative results so that its vector capacity appeared to be nil.

*Echidnophaga gallinacea*

That *E. gallinacea* may be capable of playing a role in the transmission of plague was shown by Wheeler et al., who obtained positive results when making pooling-tests with 70 fleas of this species collected from a burrowing owl (*Speotyto cunicularia*), a bird living in contact with the ground-squirrels of California.
The importance of these findings was confirmed by Burroughs, who succeeded in transmitting plague not only with groups of *E. gallinacea*, but also with individual feeding-tests. As far as could be established, the vector capacity of this flea was higher than that of *N. fasciatus*.

**Tunga penetrans**

Devignat stated, without giving further details, that he had obtained positive results when making pooling-tests with "Sarcopsylla" (sic) *Tunga* penetrans.

**Pulex irritans**

As shown by the summaries published by Wu in 1936 and Girard in 1943, as well as by a study of the recent literature, transmission experiments with *P. irritans* have been made by several workers, e.g., the Plague Research Commission, Verjbitzki, Long, Colichon Arbulu & Ramos Díaz (quoted by Ramos Díaz), Blanc & Baltazard, and Burroughs. All these workers were successful only when using numerous, or at least several, *P. irritans* for their tests, the minimum number apparently being five fleas in one of the positive transmission-experiments recorded by Blanc & Baltazard.

Burroughs, who seems to have been the only worker making individual feeding-tests with *P. irritans*, was unable to transmit plague by means of this technique. However, he established the important fact that one of the 57 fleas of this species used for such tests became blocked on the 11th day after infection. As it died one day later, its vector capacity could not be studied. In this connexion, Burroughs maintained that since his *P. irritans* strain had lived on deer for generations, better results might have been obtainable had a strain adapted to rodents been available. Nevertheless, his observation on the occurrence of blockage in this species leaves no room for doubt that *P. irritans* is capable of transmitting plague through its bites.

Findings of naturally infected *P. irritans* in plague houses and on plague patients have been recorded on a few occasions. Thus, the Plague Research Commission established the presence of *P. pestis* in 1 out of 85 *P. irritans* collected in plague houses—a result which stands in marked contrast to the 27 positive findings made in the case of 77 *X. cheopis* which had been obtained simultaneously.

Similarly, as quoted by Girard, out of 142 *P. irritans* collected during a plague outbreak at Yura, Japan, in 1908, seven (4.9%) were found plague-infected, as compared with 22.5% *X. cheopis*, 27% *N. fasciatus*, and 44.6% *L. segnis*.

Wu Lien-teh & Pollitzer were able to infect guinea-pigs with groups of three and two *P. irritans*, respectively, which had been collected during a plague outbreak in the Tung-liao area of south Manchuria in 1928. It is
noteworthy, however, that this epidemic was of a quite unusually severe character. The group of three fleas—which, in contrast to the other group, produced acute plague in the guinea-pig tested—had been collected in an inn used by the villagers as a "plague hospital" where, during his initial survey, Pollitzer found 12 dead bodies besides one patient who eventually recovered. Jettmar, working in the same village in the following year when the plague situation was less serious, found no further evidence incriminating \textit{P. irritans}.

Ramos Diaz obtained a positive result with a pool of 45 \textit{P. irritans} collected from the garments of a plague-affected family at Lambayeque, Peru.

The findings of Blanc & Baltazard in a plague area of Morocco may be summarized as follows:

<table>
<thead>
<tr>
<th>Specimens collected</th>
<th>Number of pools tested</th>
<th>Pools found plague-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{P. irritans}</td>
<td>1,772</td>
<td>53</td>
</tr>
<tr>
<td>\textit{X. cheopis}</td>
<td>580</td>
<td>21</td>
</tr>
<tr>
<td>\textit{Pediculus h. humanus}</td>
<td>2,882</td>
<td>42</td>
</tr>
</tbody>
</table>

Blanc & Baltazard succeeded, in one of several attempts, in infecting guinea-pigs through the bites of numerous \textit{P. irritans} collected in plague houses, and also obtained positive results when inoculating guinea-pigs with fleas of this species which had been collected in a plague-free locality and then fed on moribund plague-patients. Details of these experiments were as follows:

1. Two hundred \textit{P. irritans} were fed on a patient with bubonic plague 3$\frac{1}{2}$, 2, and $\frac{1}{2}$ hours, respectively, before the death of the sufferer. Later, these fleas were fed once daily for nine days on a convalescent, and were then triturated and inoculated into a guinea-pig which succumbed to plague in two days.

2. Two hundred \textit{P. irritans} were fed twice daily for four days on a plague patient with an axillary bubo who died soon after the last exposure of the fleas. On the following day, the 35 fleas which had survived were used for an animal experiment; the guinea-pig inoculated with their triturate succumbed to the infection after seven days.

The question of to what extent \textit{P. irritans} is of actual importance in the transmission of plague has been the subject of much debate.

Plague workers—not only in India, but also in the plague areas to the east of that subcontinent and in Egypt—were mostly inclined to accept the views of the Plague Research Commission, aptly summed up by Petrie as follows:

"Bubonic plague rarely spreads by direct contact, a statement supported by hospital experience and by the circumstance that multiple cases in houses are infrequent, and that when they do occur the attacks are often almost simultaneous, as if they were due to a
common infecting agent. The Commission, taking these considerations into account, and having regard to the slight septicaemia in human plague as compared with that in rats, thought that transmission from man to man by the human flea was a rare occurrence."

Petrie added that observations on *P. irritans* in Upper Egypt by Petrie & Todd, 199 although showing a high infestation during the plague season, did not indicate that these fleas took any share in the spread of the infection.

Further, should fleas or other human parasites play an important role in the spread of plague, one would find a considerable incidence of bubonic cases in outbreaks of pneumonic plague, a form of the disease often characterized by a particularly marked septicaemia. Actually, the incidence of bubonic plague in pneumonic epidemics running their course independently of rodent epizootics is almost negligible. 282

Nevertheless, as recorded by Girard, 79 observers in some plague areas, particularly in Morocco, maintained that *P. irritans* played an important role in the spread of human plague which, in these areas, often involved several members of a family rather than individuals. The importance of this flea, and also of the human louse, was particularly emphasized by Blanc & Baltazard 20 who, although admitting the role of the rodent fleas as initiators of all plague manifestations, reached the conclusion that there were "no epidemics or endemo-epidemics of plague without interhuman transmission in which the principal role is played by human ectoparasites ".

Scrutinizing the postulates of Blanc & Baltazard, Girard 79 expressed the opinion that, in place of the classical assumption that plague invariably spread from the rodents to man and not in the opposite direction as well, a new concept, based on the assumption of a spread of the infection from rodent to man, man to man, and back from man to the rodent, ought to be adopted. He emphasized, however, that, at least as far as his many observations in Madagascar went, free-living *X. cheopis*, rather than *P. irritans*, were responsible for the interhuman spread of plague and its retrocession from man to the rats.

At the same time, Girard laid stress upon the fact that, while the formula "rat-man-man-rat" comprised all epidemiological possibilities, each link of this chain was apt to assume greater or lesser importance, according to the conditions peculiar to each plague area. Therefore, without refuting claims made in regard to a given area, one ought not to consider them generally valid.

One must certainly agree with this conclusion. It is conceivable that, in areas like Morocco, where *P. irritans* occurs abundantly, it might play an important role in the transmission of plague, the high incidence of this species compensating for what it lacks in vector capacity. However, it

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199 Petrie, G. and Todd, A. 199.


250 X. cheopis

279 P. irritans

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"pas d'épidémies ou d'endémique-épidémies de peste sans transmission interhumaine, pour laquelle se placent au premier plan les ectoparasites humains ".
is certain that, in other plague areas, e.g., China, India, and Madagascar, the role of this flea is negligible, the transmission of the infection depending upon the rat fleas, particularly *X. cheopis*.

**Role of Rodent Fleas in Perpetuation of Plague**

*Rat fleas*

As shown by the Plague Research Commission[^202] and other early observers, infected rat-fleas, if kept in the laboratory under suitable climatic conditions, could convey plague after fairly prolonged intervals—43 days after infection in the case of *X. cheopis* (Otten[^199]), and 47 days after infection in the case of *N. fasciatus*.[^7] Nevertheless, the concept that the rat fleas might form a link in the perpetuation of plague, as well as serving as vectors of the infection, received tardy recognition. In fact, evidence proving that fleas may act as “preservers” of plague was brought forward in the case of species infesting wild rodents long before analogous observations were made in the case of the rat fleas.

Studying the problem of plague recrudescence in the Cumbum Valley of south India, George & Webster[^71] commenced their investigations by collecting “wandering” fleas (*X. cheopis* and *X. astia*) in houses or from rat burrows in houses where rat-falls had been observed. These fleas were tested with the aid of culture methods or animal experiments. In 196 tests made in this manner, they found infected fleas in 41 instances.

<table>
<thead>
<tr>
<th>Time interval after the last rat-fall observed in the house</th>
<th>Number of times plague was demonstrated in the trapped fleas *</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 days</td>
<td>33</td>
</tr>
<tr>
<td>3–5 weeks</td>
<td>7</td>
</tr>
<tr>
<td>13 weeks</td>
<td>1</td>
</tr>
</tbody>
</table>

* Pairs of “de-fleaed” guinea-pigs allowed to run loose overnight were used to collect rat fleas in the houses, while burrows were tested by leaving a de-fleaed rat, tethered by a piece of wire 4-5 feet (1.2-1.5 m) long, in the burrow overnight.

Further experiments were carried out at a mean temperature of 79°F (26°C) in specially constructed huts where (a) healthy rats were allowed to burrow; (b) large numbers of *X. cheopis* and *X. astia* were released; (c) infected rats were introduced; and (d) following the death of both the healthy and infected animals, normal rats were introduced after the fleas had been starved for varying lengths of time. It was thus proved that the fleas were capable of transmitting plague after periods of starvation lasting up to 29 days.

Commenting upon these findings, George & Webster pointed out that “whether such periods of starvation are usual in nature is unknown. It seems more probable that wild infected fleas obtain at least an occasional meal, in which case the length of life would presumably be longer and the occasional host would sometimes become infected.”

[^202]: Plague Research Commission
[^199]: Otten
[^7]: *N. fasciatus*
[^71]: George & Webster
[^138]: George & Webster
[^150]: Webster
[^2]: " wandering " fleas
[^316]: " wandering " fleas
[^328]: " wandering " fleas
[^46]: " wandering " fleas
[^7]: *N. fasciatus*
[^90]: *N. fasciatus*
[^204]: " wandering " fleas
Investigations by Girard & Estrade, started simultaneously with, and independently of, the work of George & Webster, and supplemented by the further exhaustive studies of Girard and Robic, also demonstrated the importance of free-living X. cheopis in the perpetuation of plague. These fleas, which found almost ideal conditions for existence in the rice debris accumulated in dark corners of the houses on the Madagascar plateau, were proved to harbour P. pestis in the absence of manifest plague for periods of up to three months.

On the basis of observations made in Brazil, Macchiavello emphasized the important role of X. cheopis when inhabiting the rat-nests. In his opinion, these fleas remained infected after their hosts had succumbed to plague or had become immune and were apt to spread the infection to newly-bred rats or to adult animals which reoccupied the burrows in question. Macchiavello added in a later communication that, according to observations in Peru, X. cheopis could remain infected in empty rat-burrows for periods of up to six months or even longer.

Wild-rodent fleas

Golov & Ioff kept three species of wild-rodent fleas found in south-east Russia (Citellophilus tesquorum, N. setosa, and Ctenophthalmus brevius) at temperatures corresponding to those of the rodent burrows in winter, and established that these fleas remained active and able to feed on hibernating rodents. If infected, they were able to survive for periods of up to 206 days, the plague bacilli in their faeces and stomachs remaining virulent.

Making further exhaustive observations on these and some other flea species infesting the wild rodents of south-east Russia, Golov & Ioff established that these fleas, if given an infective meal, were able to harbour P. pestis for periods of up to 220 days, at temperatures ranging from 14°C to 27°C (57°F to 80°F), and up to 396 days at lower temperatures—0°C to 15°C (32°F to 59°F). If kept within the latter temperature-range, the fleas remained capable of transmitting plague after periods of starvation up to 150 days.

Considering these observations, Golov & Ioff felt certain that the wild-rodent fleas of south-east Russia were not merely plague vectors, but also played an important role as preservers of the infection.

Field investigations, made simultaneously with the work of Golov & Ioff, by Pirie in South Africa proved that the fleas of non-hibernating wild-rodent species were capable of playing an analogous dual role, carrying over plague for periods of two to three months, probably even for four months.

The results obtained by Golov & Ioff were confirmed by several other observers in south-east Russia.
Thus, Tumanski & Poliak,\textsuperscript{262} collecting fleas from rodent nests five months after an epizootic had ceased, found two \textit{N. setosa} and three \textit{Ctenophthalmus pollex} infected with plague.

In order to study this problem experimentally, Evseeva & Firsov\textsuperscript{57} kept a large number of suslik (\textit{Citellus}) fleas underground at a depth corresponding to that of the rodent burrows. Recovering batches of these fleas at different intervals of time, the two workers were able to demonstrate the presence of \textit{P. pestis} in 9 out of 135 fleas kept underground for 7 months and 11 days.

Tiflov & Ioff,\textsuperscript{255} carrying out similar investigations, found \textit{N. setosa} capable of harbouring plague bacilli for at least 180 days.

Observations similar to those described above were made by Barrera\textsuperscript{12, 13} in Mendoza, Argentina. Examining a large number of wild rodents as well as their fleas about three months after an epizootic, he found no evidence of plague in the rodents, but obtained a positive result when making animal experiments with flea pools.

Observations in the western States of the USA also indicated that the wild-rodent fleas were apt to play an important role in the perpetuation of plague. Eskey,\textsuperscript{52} while noting that infected \textit{X. cheopis} survived an average of not more than 16 days with a maximum of 36 days, found that infected wild-rodent fleas could survive for considerably longer periods and, because they were capable of eventually becoming blocked, could thus be instrumental in carrying over the infection during the hibernation period of the rodents.

As has been discussed previously, this conclusion was confirmed by the observations of Eskey & Haas\textsuperscript{54} on the varying length of the extrinsic incubation-period in plague-infected fleas.

Experimenting with hibernating ground-squirrels and their fleas (\textit{D. montanis}), Prince & Wayson\textsuperscript{209} obtained the following remarkable results:

1. Six hibernating \textit{C. richardsoni} were stored for four months at 40°F (4.5°C) in separate nests.

\textit{(a)} Two of these ground-squirrels were inoculated with plague and each was seeded with 100 normal fleas. One squirrel died of acute plague after two weeks, while the second, which continued to hibernate, apparently recovered from the infection. The 23 fleas which were recovered after the end of hibernation were not infected.

\textit{(b)} Two of the squirrels were each seeded with 100 plague-infected \textit{D. montanis}. One of the 14 fleas collected after the squirrels had awakened produced plague when injected, after trituration, into a white mouse. The squirrels themselves did not contract plague.

\textit{(c)} Of the 100 fleas seeded on each of the two control squirrels, 50\% were recovered after 4 months and were able to reproduce.
2. (a) Four *Citellus townsendi* survived four months in hibernation after intracutaneous plague-infection. One sickened on the seventh day after awakening and died one day later of acute plague. The three remaining squirrels proved plague-free. Out of 200 normal fleas which had been placed on the squirrels at the time of their plague inoculation, 42 were recovered at the end of hibernation and proved plague-free.

(b) Fifty infected *D. montanus* were placed on each of four hibernating, normal *C. townsendi* and left on them for four months. Of the 47 fleas which were recovered alive at the end of the hibernation period, 4 were plague-infected. One of the infected fleas was placed on a white rat. This animal sickened 21 days later, and died of acute plague on the 23rd day. Two of the remaining infected fleas died within four days after removal, having failed to feed. The fourth flea fed on each of five white mice during its survival for 10 days, but did not transmit the infection.

These observations leave no doubt that plague may be carried over the hibernation period not only by the rodents themselves, but also by their fleas.

**Conclusions**

In the opinion of some writers, great care ought to be exercised when making conclusions from observations made under artificial conditions upon the role actually played by the fleas in the perpetuation of plague. Thus, Eskey & Haas 54 stated that

"... under natural conditions the average length of life of fleas is probably rather short otherwise animal infestation would be excessive because of the rapidity with which these parasites multiply. Therefore, as the length of the extrinsic incubation is increased the spread of the infection is not only delayed but the chances of the plague-infected fleas surviving the required time for their bites to become infectious are decreased according”.

Similarly, Meyer 176 stressed the necessity for further field-observations so as to ascertain the actual number of infected fleas which survive and act as preservers of plague, because

"... irrespective of the remarkable longevity of fleas under well chosen laboratory conditions which prevent the loss of water by the insects, it is frequently overlooked that the mortality among vectors in abandoned burrows and nests is great, and the life-span correspondingly is short”.

It has to be kept in mind, however, that free-living fleas which have opportunities for subsisting in a suitable environment like rice debris, as well as the fleas which live in inhabited nests with a suitable microclimate, lead a rather sheltered existence and therefore have good chances for prolonged survival. There can be little doubt, therefore, that such fleas do play an important role in the perpetuation of plague.
Role of Flea Transport in Spread of Plague

Although, strictly speaking, the object of the present discussion is to determine to what extent passively transported fleas are apt to spread plague, it seems appropriate to introduce this subject by a short consideration of the movements and transport of fleas in general.

Widespread agreement exists that while the rodent fleas are prone to leave not only their dead hosts, but also deserted burrows, their active movements are restricted to a rather limited range. However, their jumping ability is remarkable.

Experiments undertaken in this respect by the Plague Research Commission showed that guinea-pigs which were allowed to run about on the floor of a flea-infested warehouse, or which were suspended in cages 2 inches (5 cm) above the floor, all contracted plague, whereas another group of these animals, suspended in a cage 2 feet (61 cm) from the floor, remained healthy because they did not become flea-infested.

Further observations have shown that, in general, fleas are capable of making vertical jumps of about 4 inches (10 cm) when starved, and about 3 inches (7.5 cm) when gorged. They can walk up a vertical sheet of glass for about 8 inches (20 cm). The distance they can cover by horizontal jumps is, as a rule, less than 6 inches (15 cm). However, *P. irritans* can jump much higher and further—according to Mitzmain, 7 3/4 inches (19.5 cm) vertically and 13 inches (33 cm) horizontally.

Burroughs found *P. irritans* capable of jumping out of a 12-gallon earthenware crock which had been filled to within 13 inches of the top with dirt. He added that *E. gallinacea*, although not as proficient as *P. irritans*, was also a good jumper, while most of the wild-rodent fleas studied by him were poor jumpers, so that they could be retained safely in an enamel pan about 4 inches (10 cm) high.

Fleas can be passively transported by (a) their hosts; (b) transport vehicles; (c) humans (on the persons, or in the baggage, of travellers); and (d) the movement of goods.

The orbit of the passive transport of fleas on their specific hosts, or on temporary hosts such as beasts and birds of prey, is determined by the range of movements of these animals—a problem which received attention in chapter 6.

The problem of the conveyance of fleas which have sheltered in transport vehicles needs no special attention because such insects are not likely to remain loose for prolonged periods, but will seek a host or hide in goods. Nevertheless, such fleas may prove dangerous in places where plague patients have the possibility of using public conveyances for local transport.

A case in point was observed during the 1920-1 pneumonic outbreak at Harbin where plague sufferers were quite frequently transported in rickshas. The patient in question, a Russian woman, had had no contact with plague patients. 

25
cases, but was certain that she had been bitten by an insect on the right leg while riding in a ricksha. A few days later she developed a right femoral bubo. Since rat plague was altogether absent, there can be little doubt that she was infected through the bite of a human parasite which, having left a plague patient, was lurking in the ricksha.

The problems of a passive transport of plague-infected fleas on the persons, and in the baggage, of travellers, and in goods, deserve serious attention.

Transport by travellers and their baggage

Considering the manner in which plague is spread at distance, the Plague Research Commission laid stress on the results of experiments carried out with bundles of clothing and bedding collected from houses where plague cases had occurred. With the aid of guinea-pigs which were let loose on the opened bundles in a flea-proof warehouse, two rat-fleas and one human-flea were recovered. More important still, 1 of the 26 guinea-pigs successively used for these tests contracted plague, while control experiments with flea-free bedding from a plague hospital gave negative results.

The Commission assumed, therefore, that the spread of the infection at distance was effected by infected fleas carried either on the person, or in the bedding, of travellers who had lived in plague houses or had visited them. In the opinion of the Commission, such fleas were not directly responsible for the causation of human plague (even the travellers carrying flea-infested baggage were able to escape infection), but produced rat plague in the places to which they were transported.

While it is generally admitted that plague-infected fleas may be carried on the persons, or in the baggage, of travellers, opinions vary considerably as to the frequency and, consequently, the epidemiological importance of such a transport.

Petrie & Todd, who made investigations in Upper Egypt similar to those of the Plague Research Commission in India, accepted the view of the Commission. Observers in Madagascar also ascribed importance to a transportation of infected X. cheopis by travellers, while Long maintained that a conveyance of fleas on the persons, or in the effects, of mule-drivers was of importance in the spread of plague in South America.

Hirst, while admitting that “human migration may be a factor in the spread of plague”, postulated that it is “comparatively seldom of primary importance”.

Observers in Java were even more inclined to doubt the importance of a transportation of plague-infected fleas by travellers. Swellengrebel found only two X. cheopis and one Stivalius cognatus (Pygiopsylla ahalae) among the ectoparasites collected from the clothing or baggage of 56,790 travellers who had left the partly plague-affected province of Malang. Even
the clothes of a group of 1,829 persons, among whom there were 393 plague patients, yielded only seven *X. cheopis*. In the opinion of Swellengrebel, the transport of fleas by travellers was, therefore, a minor factor in the spread of plague in Java.

Otten\(^{192}\) incriminated the flea-transport in the produce carried by villagers to the markets rather than travellers in general, whereas Flu\(^{65}\) postulated that the carriage of fleas in rice consignments was responsible for the spread of plague in Java.

Observations by Pollitzer showed that rice transport played the principal role in the dissemination of plague in central and south China. An occasional spread of the infection by travellers was noted but, as was maintained by Sorel\(^ {239}\) in the case of Madagascar, such an importation of plague did not, as a rule, lead to the establishment of foci.

All in all, one ought to agree with the opinion of Hirst\(^ {103}\) that, in general, a spread of flea-borne plague through travellers is of comparatively limited importance.

**Transport in goods**

Most observers\(^{8}\) are agreed that the transport of infected fleas in goods, particularly in grain, raw cotton, gunny bags, rags, and hides, is of great importance in the spread of plague. However, the question of how far the infection can be carried by such fleas has been the subject of much debate.

Hirst,\(^ {103}\) whose investigations have done much to elucidate this problem, maintained that

> "... the available evidence indicates that when an overseas source of infection is but a few days removed an infected flea may be readily transferred directly in grain from the port of origin to the port of entry. Otherwise it may be inferred that a plague epizootic has occurred among the rats on ship-board. The link between the ship epizootic and the shore rats or the rats of the lighters into which cargo is loaded or unloaded may be a plague rat but is much more likely to be a plague flea."

Referring particularly to the importation of plague from Rangoon into the port of Colombo 1,234 miles (1,985 km) away, Hirst stated that

> "the transference of infected fleas all the way from Rangoon to Colombo without their feeding on ship rats *en route* is theoretically possible, but the sea voyage is about two days too long to favour this mode of spread."

In marked contrast to these statements, it was claimed by Long & Mostajo\(^ {152}\) in 1934, and recently by Macchiavello & Mostajo Patrón,\(^ {160}\) that fleas in baled jute-bags from Calcutta were responsible for the reimportation of plague into Peru.

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\(^{8}\) Campbell\(^ {25}\) and Hopkins\(^ {147}\) maintained that the trade in cotton, found by King & Pandit\(^ {137}\) to be of paramount importance in the dispersal of *X. cheopis* and, consequently, in the spread of plague in south India, did not play a role in East Africa. The same observation was made by Roberts\(^ {24}\) in regard to the transport of maize in Kenya.
The statements made by Long & Mostajo were subjected to a painstaking inquiry by de Vogel, who came to the following conclusions:

(1) As far as could be established, rat plague had been absent on the two steamers which had brought the suspected jute-bag cargoes;

(2) A study of the temperatures to which these cargoes had been subjected in transit rendered it altogether unlikely that the fleas supposed to have been enclosed in the jute-bag bales at Calcutta could have survived, or could have remained infective, throughout the journey to Peru.

(3) It was striking that all the plague manifestations supposed to stand in causal connexion with the arrival of the jute bags had been observed in the zone of Peru where outbreaks of the disease used to be frequent, whereas the importation of such consignments into three ports situated outside this zone was not followed by an appearance of plague.

De Vogel felt certain, therefore, that the evidence brought forward by Long & Mostajo was not convincing enough to justify international action in regard to the jute-bag traffic.

The validity of de Vogel's conclusions was contested by Macchiavello & Mostajo Patrón who pointed out, in particular, that the plague manifestations suspected of having been caused by infected fleas enclosed in the jute-bag bales had taken place during a season when there was usually no plague in Peru.

They also maintained that

"... facts observed and described in the past, for which satisfactory explanations were not found, could have been explained in the light of the hypothesis of Long & Mostajo. For example, the events that occurred in 1904 in Antofagasta, Chile, when the steamer 'Gladstone' imported bales of jute bags and there sickened and died certain of the employees charged with the duty of examining the cargo; the finding in 1929, in the same port, of a certain number of fleas identified as X. astia, a flea native to India, the only fleas of this species ever found in the Americas; the occurrence of plague in 1930, after it had been absent for five years; these various incidents could be explained when official documents showed, on each occasion, that there had been imported some millions of kilograms of jute bags sufficiently in advance of the outbreak to account in a simple and complete form for the facts mentioned."

Macchiavello & Mostajo Patrón further claimed that "the reappearance of plague in Perú, in 1944 and 1945, in the rural districts of Chiclayo, even though the increase was not related to circumstances similar to the case of the 'Solafric', revived suspicions of another importation of plague from India due to the active commerce in jute bags which has been maintained despite the war."

Macchiavello & Mostajo Patrón stated that the suspected shipment of 400 bales of jute, consisting of 120,000 bags, had reached the Chiclayo district under conditions which excluded local contamination or infestation with fleas. Likewise, it could be shown that there had been no plague in the locality concerned for a number of years.
Examining ten of the bales, the two workers found 20 *P. irritans* in the external wrappings, and seven specimens of *Xenopsylla*, two of which were alive, in the interior of one of the bales. All the *Xenopsylla* were in a fasting condition. Inoculation of a triturate of the *Xenopsylla* into guinea-pigs "gave, after four passages in series in these animals, a picture of attenuated plague", a result which, in the opinion of Macchiavello & Mostajo Patrón, was compatible with a long persistence of the infection in these insects.

The two workers also had the opportunity of examining a consignment of 5,400 jute bags which had been landed in the port of Huachcho. They stated that during these investigations they were able to collect 24 fleas, 10 of which were *X. cheopis*, 8 being alive. Of these 8 fleas, 6 produced plague in a guinea-pig upon which they were allowed to feed. Animal experiments with triturated fleas also gave positive results.

Macchiavello & Mostajo Patrón concluded that

"...certain bacteriological peculiarities of the strains of germs; the knowledge we have that plague exists in Calcutta; the conditions of humidity and temperature in the holds of the vessels that arrive via the Straits of Magellan; the insanitary conditions that the war has produced in India and which certainly had some effect on the measure of disinsec­tization which at one time were taken with cargos of jute bags; the epidemiology of certain 'out of season' outbreaks of plague that now, as formerly, are again making their appearance contrary to the annual cycle of plague in Perú, and above all, the findings reported herein, do not leave the least doubt but that the hypothesis enunciated by Long & Mostajo more than ten years ago is correct ".

While no doubt can exist that the jute-bag consignments examined by Macchiavello & Mostajo Patrón contained infected, and also, in one instance, infective, *X. cheopis*, it is not easy to share their belief that these fleas had come from Calcutta where, as shown by the following figures, autochthonous plague was absent during the period 1944-6.

<table>
<thead>
<tr>
<th>Year</th>
<th>Calcutta cases</th>
<th>Calcutta deaths</th>
<th>Bengal cases</th>
<th>Bengal deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1944</td>
<td>.</td>
<td>3*</td>
<td>.</td>
<td>3</td>
</tr>
<tr>
<td>1945</td>
<td>.</td>
<td>2*</td>
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<td>0</td>
</tr>
<tr>
<td>1946</td>
<td>.</td>
<td>8*</td>
<td>.</td>
<td>3**</td>
</tr>
</tbody>
</table>

* Importe cases
** West Bengal only

However, even if one could accept the statements of the above-mentioned workers at their face value, it is not likely that a long-distance transportation of infected fleas plays a generally important role in the dissemination of plague. For, if this were the case, instances of a ship-borne transference of this disease would have remained frequent in spite of the measures now universally adopted to reduce, or even to abolish, the rat infestation of sea-going vessels. Actually, instances of a long-distance spread of plague through the maritime traffic have almost ceased to occur. There can be no
doubt, therefore, that infected rats, rather than infected fleas, carried in consignments of goods are responsible for this dissemination of the infection. It is gratifying to note that large-scale studies recently undertaken in the USA, fully support this contention.

In order to assess the potential danger of an importation of plague into the USA through the very considerable jute-imports from India (amounting to approximately 350,000 tons annually), Norris et al.\textsuperscript{187} recently resorted to three methods of investigation, namely:

(1) A search for fleas in random samples of jute materials reaching the ports of San Francisco, California, and San Yuan, P.R. respectively. 

Painstaking examinations of (i) 880 random bolts of Hessian cloth, aggregating 152,000 yards, removed from 179 random bales, and (ii) wrappers of 67 gunny-bag bales as well as 4,994 bags did not lead to the detection of even a single flea.

(2) Test transports of \textit{X. cheopis} in the holds of vessels going from San Francisco to Hawaii or other Pacific ports and back, which gave the following results:

(a) Two cotton bags, each containing 30 \textit{X. cheopis}, were placed at different levels in a jute bale, which was then wrapped, pressed, sewed, and bound with metal strips, and afterwards sent in the cargo-hold of a vessel on a journey to Okinawa and back. Upon return of the bale to San Francisco after 49 days, 57 of the fleas were found dead. It was presumed that the three missing fleas had escaped before the cotton bags had been tied prior to shipment.

(b) Two batches of \textit{X. cheopis} (150 and 100 specimens respectively) were sent in wooden boxes (one of which contained pieces of freshly-cut apples to supply moisture) in the holds of ships to the Hawaiian Islands and back. When the ships returned to San Francisco after round trips lasting a little more than three weeks only, all the 246 fleas recovered were found to be dead. Their microscopic examination did not reveal signs of starvation.

(3) To gauge the effect of pressure, as used in preparing jute materials for export, batches of 25 \textit{X. cheopis} were placed at different levels inside bales of jute wrappers and these were then compressed at about 8,000 pounds (i.e. a pressure lower than that actually used for baling Hessian cloth). At the end of 10 minutes following pressure application, about one third of the fleas were found to be dead, at the end of one hour 50\% were dead, and at the end of 72 hours, 75\%. Norris and his colleagues concluded, therefore, "that the pressure exerted in the baling process is an important factor in reducing chances of survival of fleas trapped within bales of jute products".

Considering the results of these investigations and bearing in mind that fleas in jute bales shipped from India would be subjected, during the first
part of their journey, to even more unfavourable climatic conditions than those prevailing in the holds of ships plying on the Pacific, one may safely state that the danger of a long-distance transport in jute bales of plague-infected fleas (which are more vulnerable than the normal fleas used in the above experiments) is rather remote or presumably even non-existent.\(^h\)

OTHER INSECTS

*Human lice*

Attention to a possible role of human lice (*Pediculus humanus capitis*) seems to have been paid first by the German Plague Commission,\(^{71a}\) who noted a prevalence of cervical buboes in children infested with head-lice and therefore suffering from eczema of the scalp.

Head-lice were also suspected by Herzog (quoted by Dieudonné & Otto\(^46\)), who was able to cultivate *P. pestis* from lice which had been collected from a moribund patient with a cervical bubo, and by de Raadt,\(^{210}\) who succeeded in infecting experimental animals cutaneously or subcutaneously with triturates of head-lice found on a plague patient and on the dead bodies of plague victims.

Identical results were recorded later by Mostajo & Colichón Arbulú\(^{183}\) who worked with nine *P. humanus capitis* collected from the dead body of a plague victim. These workers pointed out that the habit of the Indians in Peru of crushing lice or fleas with their teeth might be responsible for the occurrence of cases of angina pestosa (tonsillar plague).

Long,\(^{151}\) referring to the findings made by Mostajo & Colichón Arbulú, stated that positive results were also obtained when a guinea-pig was inoculated with the triturate of lice collected from healthy persons and then allowed to feed on plague-infected guinea-pigs. However, it was not possible to transmit the infection through live lice.

Further instances of infection in head-lice collected from plague victims have recently been recorded in Madagascar\(^{58}\) and in South Africa.\(^{240}\)

Positive experimental results with *P. humanus* var. *corporis* (often wrongly called *P. vestimentorum*) have been recorded by Swellengrebel & Otten,\(^{247}\) who were able to produce plague in 7 out of 9 guinea-pigs inoculated with batches of 11-250 such lice collected from the garments of plague victims. An identical result was also obtained by Sukneff.\(^{244}\)

Tsurumi et al.,\(^{261}\) working with the same subspecies of louse, were successful after many failures, in transmitting the disease to a guinea-pig on which plague-infected specimens of these parasites had been exposed. Blanc & Baltazard\(^{20}\) obtained the same result in one of ten attempts to transmit the infection to guinea-pigs with the aid of live body-lice found in a plague focus in Morocco. The animal in question succumbed seven

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\(^h\) The problems of flea-borne plague will receive further attention in a future chapter in which the ecology and epidemiology of the disease will be discussed.
days after it had received about 60 bites from body-lice collected from three plague patients and afterwards fed on a convalescent.

Blanc & Baltazard\textsuperscript{20} also recorded the following observations on \textit{P. humanus} var. \textit{corporis}:

(1) Positive results were obtained in guinea-pigs which had been inoculated with triturates of 8-300 lice collected in plague-infected houses, the experimental animals succumbing after 2 to 8 days, i.e., more rapidly than those inoculated with triturated \textit{X. cheopis} or \textit{P. irritans}.

(2) \textit{P. pestis} was found preserved in naturally infected lice for at least 12 days after the last infective meal.

(3) The faeces of naturally infected lice usually contained virulent plague bacilli for periods of up to nine days.

(4) Virulent plague bacilli were found in the carcasses of naturally infected lice for periods of up to 11 days.

(5) It was possible to produce plague in 1 of 3 white mice into the nares or mouths of which a few drops of a suspension of infected triturated lice and their faeces had been instilled. The mouse in question, which had been infected intranasally, died after three days, and showed at autopsy cervical and mediastinal buboes, as well as congestion of a part of the left lung.

As has been noted earlier, Blanc & Baltazard postulated that human fleas and lice played an indispensable role in the epidemic spread of plague, being mainly, if not solely, responsible for the infection of man. It was found impossible, however, to consider their conclusion as generally valid because, as has been shown by observations in most plague areas, including those in China, India, and Madagascar, human vermin played but a minor role in the spread of the infection. At the same time, however, it was admitted that, in some of the areas where it is abundant, \textit{P. irritans} might be of importance in the transmission of plague to man, and this may also be said to hold true of the human louse, mass attacks of which have been found capable of conveying the infection even to experimental animals.

In chapter 1 attention was drawn to the statements of some observers who postulated that human ectoparasites were solely responsible for the spread of plague during the pandemic taking place at the time of Justinian. Since, according to a recently published note by MacArthur,\textsuperscript{155} rats were probably not absent from Europe at that time, the validity of these claims has become questionable. Little doubt can exist, however, that, not only during that pandemic, but also during the historic outbreaks in northwestern Europe in general, human parasites played an important adjuvant role in the spread of bubonic plague because they were then more abundant.
**Bed-bugs** (Cimex lectularius)

Evidence incriminating the bed-bug as a potential or actual vector of plague has been adduced by a considerable number of observers.

In 1897, Iamagiva referred to a plague patient with a left femoral bubo who had apparently been infected by a bed-bug because *P. pestis* could be demonstrated in a swelling on the left knee of the sufferer which had been caused by a bug-bite. A similar, but less convincing, observation made by Calmette & Salimbeni has been recorded in full by Simpson.

Persistence of virulent plague bacilli in bed-bugs collected in plague-infected houses, or infected in the laboratory, has been repeatedly observed, first, by Ogata and Nuttall who noted that the organisms could survive in bed-bugs kept at 68°F (20°C) for 72 hours, but not for 120 hours. Hunter frequently found *P. pestis* in bed-bugs collected from plague-infected houses or the bedding of patients.

Making a thorough study of plague in *C. lectularius*, Verjbitzki found virulent plague bacilli persisting for up to eight days in bed-bugs which had been starved for 4-4½ months before being given an infective meal. Bed-bugs which had been starved for shorter periods remained infected less long. However, *P. pestis* was present for three days in the faeces of such insects which had been starved for one month before infection.

Jordanski & Klodnitski found that the number of plague bacilli in the stomachs of bed-bugs increased from the third to the sixth day after they had been given an infective meal. Involution forms appeared later and the micro-organisms eventually became invisible. Nevertheless, it was possible to obtain positive cultures from plague-infected bed-bugs up to 35 days. The two workers recorded in a second paper that 2 out of 13 bed-bugs fed on a plague-infected mouse survived for 83 days. *P. pestis* could then be demonstrated in these insects five days after they had been fed on a healthy animal.

In contrast to Verjbitzki, Bacot found that for some *C. lectularius*, and, probably, for all newly hatched ones, a meal of septicaemic blood from a mouse dying of plague was fatal, but that those which were not killed by the infecting meal were capable of carrying *P. pestis* for quite prolonged periods. Involution forms were possibly still present in one bed-bug kept at 80°F (27°C) for 60 days after the infective meal.

Cornwall & Menon, infecting bed-bugs either on guinea-pigs or by feeding them through a membrane of rabbit-skin on citrated rabbit-blood mixed with *P. pestis*, also found that, following an infective meal, a large proportion of these insects succumbed within a few days. In some, however, the bacilli multiplied and could remain alive and virulent for periods of up to 38 days.

Positive results were obtained by Wu Lien-teh & Pollitzer when inoculating a guinea-pig with the triturate of three bed-bugs found in an inn which had served as a temporary plague-hospital.
Working with bed-bugs which, after a period of starvation, had been permitted to feed on plague-infected rats or mice, Novikova & Lalazarov demonstrated the presence of *P. pestis* in the faeces of 37% of these insects, the number of the organisms becoming maximal 5-12 days after the infective meal. As shown by the cultivation of bed-bugs which had been triturated and suspended in saline, the infection persisted in them for up to 147 days.

Besides demonstrating that *P. pestis* could persist in *C. lectularius*, Verjbitzki and Bacot were able to transmit plague to healthy laboratory-animals through the bites of infected bed-bugs.

Permitting his infected bed-bugs to feed on the shaved abdomens of 41 guinea-pigs, Verjbitzki was able to transmit plague six times, but he had seven successes in 25 guinea-pigs when he exposed the bed-bugs on the shaved ears of the animals. Verjbitzki established that the minimum number of bed-bugs required to produce plague in a healthy animal through biting was three, and that no conveyance of the infection took place more than five days after the infection of the bed-bugs.

Bacot stated that, in his experience, "only on two occasions did death follow from the bite of infected bugs and there was one doubtful case of infection when a rat fell sick and showed some of the symptoms of an animal suffering from pest, but it subsequently recovered".

As can be gathered from Bacot's records, one of the animals in which fatal plague resulted was a rat, and the other, a mouse. He added that the mouse had been put into a glass jar together with five bed-bugs which had survived for 48 days after infection. On the following morning, only one of these insects could be recovered, the others having probably been eaten by the mouse. After five days the mouse succumbed to typical plague with involvement of the lymph-nodes in the right groin and both axillae.

Successful transmission-experiments were made also by Walker, Novikova & Lalazarov, and Balfour (quoted by Dieudonné & Otto). Walker was able to produce plague in a rat by exposing it to the bites of bed-bugs which had fed on a plague patient. Novikova & Lalazarov succeeded in transmitting plague to a mouse through the bites of a bed-bug which had been infected in the laboratory. According to Dieudonné & Otto, Balfour was able to transmit plague from infected to healthy guinea-pigs through the agency of bed-bugs.

As summarized by Dieudonné & Otto, and by Wu, some observers postulated, on epidemiological grounds, that bed-bugs played an important role in the natural transmission of plague. Walker, in particular, insisted that these parasites had been responsible for the spread of the infection during an outbreak in Burma because they had been plentiful at the time, whereas fleas appeared to be absent. Similarly, Fox postulated that bed-bugs had been responsible for the spread of the infection during a small outbreak of plague at Iloilo in the Philippines where no evidence of an epizootic could be found. However, when it is considered how difficult
it sometimes is to find plague-infected rats or their fleas during an outbreak, little credence can be given to these and similar contentions.

It is significant that the workers who studied the problem of plague in the bed-bugs thoroughly were rather averse to the idea that these parasites played a dangerous role.

Verjbitzki maintained in this connexion that "bugs which have sucked their full complement of blood do not as a rule bite again for a considerable interval".

He showed, however, that bed-bugs which had been interrupted during the act of feeding were inclined to feed again, and were thus able to convey infection from an infected to a healthy host. He also found that "the crushing of infected bugs in situ during the process of biting, occasioned in the majority of cases the infection of the healthy animal with plague".

Bacot, while in accord with Verjbitzki's findings, pointed out that:

1. A transmission of plague through the faeces of infected bugs could be ruled out because these insects do not defaecate during the act of feeding and "hurry after their meal to some nook or cranny to digest at leisure".

2. "The development of B. [P.] pestis within the crop of bugs differs generally from that which takes place in the stomach of the flea in respect of its slower and looser growth, this limitation of activity being accompanied by and possibly due to the preservation of the structural character of the blood for many days after its ingestion into the crop."

3. "The absence of any definite valve between the pump and the crop, together with the looser nature of the growth within the bug, preclude the idea of such mechanical blockage as causes regurgitation and mouth infection by fleas."

Cornwall & Menon were also convinced that plague-infected bed-bugs could not regurgitate their stomach-contents during the act of feeding. They were able to find plague bacilli in the proboscides of bed-bugs up to 46 hours after feeding and therefore considered it possible that infection of a healthy host might result from the washing-out of these bacilli from the proboscis of a bed-bug whose feeding on an infected host had been interrupted. Nevertheless, Cornwall & Menon maintained that plague was not likely to result from the bites of infected bed-bugs.

While this contention deserves attention, it cannot be denied that bed-bugs are capable of transmitting plague. However, there is no reason to assume that they play more than an occasional, or, at most, an adjuvant, role in the conveyance of the infection.

Lice of rodents and other animals

As was established by Simond and confirmed by many subsequent workers, the lice of plague rats are apt to harbour P. pestis. Identical observations were made in the case of Neohaematopinus columbianus—the louse
of the Californian ground-squirrel—by McCoy,\textsuperscript{166} who found that these parasites remained infective for two weeks and were capable of conveying plague to experimental animals.

The presence of \textit{P. pestis} in a louse occurring on tarabagans (\textit{Marmota bobac}) and identified as \textit{Neohaematopinus citelli} ("Linognathoides" citelli) was demonstrated by Sukneff,\textsuperscript{244} who obtained positive results when inoculating guinea-pigs with triturates of such lice collected from either naturally affected or artificially infected Siberian marmots.\textsuperscript{245}

A thorough study of the tarabagan louse was later made by Jettmar.\textsuperscript{127} He established that \textit{P. pestis} rapidly multiplied in the stomachs of these lice, which invariably died 2-3 days after an infective meal. Plague bacilli were also plentiful in the faeces of the infected lice and persisted in their carcasses for about two weeks. Exposure of 40 live specimens of this louse which had been collected from an artificially infected tarabagan produced acute plague in a sisel (\textit{Citellus undulatus}). The tarabagan louse was found capable of biting man, but was apparently unable to survive long when separated from its specific host.

These findings deserve attention because there can be no doubt that the rodent lice play an adjuvant role in the transmission of plague from animal to animal.

Blanc & Baltazard\textsuperscript{20} were able to demonstrate the presence of virulent plague bacilli in \textit{Pedicinus albidus} which had been collected from artificially infected specimens of its specific host—the Barbary ape, \textit{Macaca sylvanus}. They found the louse of the pig (\textit{Haematopinus suis}) not only capable of harbouring \textit{P. pestis}, but also of conveying the infection to guinea-pigs.

\textbf{Mites}

It was maintained by Russo\textsuperscript{222, 223} that free-living mites of the family of Tyroglyphidae, which were apt to feed on the carcasses of plague-affected rodents, could harbour and transmit \textit{P. pestis}. He incriminated, in particular, \textit{Glyciphagus domesticus} and \textit{Tyroglyphus siro}.

Yamada (quoted by Mitsuhori\textsuperscript{180}) established that \textit{Liponyssus nagoyoi} (family of Dermanyssidae), a parasite of the commensal rats—particularly of \textit{R. rattus}—in Japan, could transmit plague from animal to animal. As Mitsuhori added, this mite, which was possibly identical with \textit{Lyponyssus bacoti}, could attack man.

According to a statement made by Jorge,\textsuperscript{135} Bacot & Stanley Hirst succeeded in conveying plague from rat to rat with \textit{Laelaps echidninus}, a mite infesting \textit{R. norvegicus} in many parts of the world.

\textbf{Ticks}

Paying attention to "the ticks infesting rats suffering from plague", Skinner\textsuperscript{236} was able to prove that these parasites could harbour \textit{P. pestis}. 
Findings made in the case of other tick species may be summarized as follows.

**Marmot-ticks.** Skorodumoff \(^{237}\) was able to prove, with the aid of animal experiments, that ticks (\(?\ Rhipicephalus haemaphysaloides\)) collected from naturally plague-affected tarabagans (Siberian marmots) contained virulent \(P.\ pestis\). Identical findings were made by Tikhomirova & Nikanoroff \(^{257}\) and by Borzenkov & Donskov \(^{21}\) in the case of *Ixodes autumnalis*, a tick infesting the marmots of south-east Russia.

**Suslik-ticks.** As summarized by Wu,\(^{251}\) the suslik (*Citellus*) ticks were incriminated by several plague-workers in south-east Russia, particularly by Tikhomirova & Nikanoroff,\(^{257}\) who proved the presence of *P. pestis* in *Rhipicephalus schulzei* with the aid of culture tests and animal experiments, and by Golov & Kniazevski,\(^{86}\) who established that the larvae and nymphs, as well as the adults, of this tick could harbour the infection.

**Argas persicus.** Faddeeva \(^{58}\) proved, by cultivation and animal experiments, that *Argas persicus* (a free-living tick), when fed upon guinea-pigs in the septicaemic stage of plague, could remain infected for periods of up to 110 days.

**Dermacentor silvarum.** Sassuchin \(^{225}\) demonstrated that virulent plague bacilli could persist for up to 35 days in the nymphs of *Dermacentor silvarum*, which during its larval and nymph stages infested susliks or other wild rodents and, when adult, fed on camels, horses, or man. As was afterwards shown by Sassuchin & Tikhomirova,\(^{226}\) the larvae and nymphs, but not the adults, of this tick could harbour *P. pestis*, even though over 60% of them died when given an infective meal. The organisms persisted in the surviving larvae for 2-10 days and in the nymphs for 2-6 days, but invariably disappeared during the moulting stages.

**Hyalomma volgense.** Borzenkov & Donskov \(^{21}\) proved that the larvae and nymphs as well as the adults of the cattle-tick, *Hyalomma volgense*, were apt to harbour *P. pestis* in their gastro-intestinal tracts after they had been fed on plague-infected guinea-pigs, jerboas, or marmots, the organisms remaining viable in the larvae for seven days, in the nymphs for three days, and in the adults for at least 11 days. Borzenkov & Donskov succeeded in conveying plague to healthy rodents through the bites of these ticks, and also demonstrated the presence of virulent *P. pestis* in the faeces of infected specimens.

**Rhipicephalus sanguineus.** Blanc & Baltazard,\(^{20}\) summarizing observations they had made of *Rhipicephalus sanguineus*, a tick principally infesting dogs, stated that "under the conditions of our experiments, it did not transmit plague mechanically when taking its first meal on an infected guinea-pig and continuing to feed after several days on a healthy guinea-pig.
Since, on the other hand, the plague bacilli could not resist the profound changes taking place during the moulting stages of these ticks, one can conclude that, as far as the available evidence goes, *Rhipicephalus sanguineus does not seem to play any role in the natural transmission and preservation of plague*.

Opinions regarding the role played by the ticks in the transmission of plague were sharply divided. Sassuchin & Tikhomirova, like Blanc & Baltazard, came to the conclusion that the species they worked with were of no importance in this respect. On the other hand, as summarized by Wu, Tikhomirova & Nikanoroff asserted that the suslik-ticks, particularly *Rhipicephalus schulzei*, on account of their great prevalence, eagerness to change hosts, capacity for transmitting and preserving other infections, blood-sucking faculty, and hardiness, might, under equal conditions, prove more efficient vectors than other insects, e.g., fleas and lice, and might, on account of their aptitude for changing hosts, be responsible for an extension of the enzootic foci.

In evaluating these divergent opinions, one is led to believe that different tick-species might play different roles in plague so that no hard-and-fast general rule can be laid down. However, while admitting that species like the suslik-ticks are of some importance, one cannot share the opinion of Tikhomirova & Nikanoroff and some other workers in south-east Russia that the ticks play not an adjuvant, but the principal, role in the transmission of plague. No other arthropod vector surpasses or even equals the rodent fleas in this respect.

**Flies**

Observations on the presence of *P. pestis* on or in houseflies (*Musca domestica*) were made by some early workers, e.g., Yersin, Albrecht & Ghon, and Nuttall. While Albrecht & Ghon merely established that flies which had come in touch with plague material were apt to become externally contaminated, Yersin and Nuttall found that *P. pestis* was present in the gastro-intestinal tracts and faeces of these insects.

Nuttall came to the conclusion that plague infection was fatal for the flies, death occurring more rapidly the higher the temperature rose, e.g., after 3 days at 23°-31°C. This opinion was also held by Gosio, who further claimed that fly larvae, which had been fed on plague organs, could develop normally while continuing to harbour *P. pestis*, but that the adult flies developing from them succumbed to the infection in 15-24 hours. However, Hunter and Jettmar were unable to confirm that plague-infected houseflies showed any unusual mortality.

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1 "... n'a pas dans les conditions de nos expériences, transmis la peste mécaniquement en prenant la première moitié de son repas sur cobaye infecté, et en l'achevant en plusieurs jours sur cobaye neut. Comme, d'autre part, le virus ne résiste pas à l'ensemble de modifications profondes qu'est la mue chez ces acariens, on peut conclure de ces brèves expériences que *Rhipicephalus sanguineus ne doit jouer aucun rôle dans la vaction et le maintien naturel de la peste*".
Though, in view of the evidence set forth above, there can be no doubt that houseflies may become carriers of *P. pestis*, it is not at all likely that they then become apt to convey plague.

Wayson,271 working with the stable-fly, *Stomoxys calcitrans*, found that "when ... applied to an animal (guinea pig) acutely ill of this ... disease, or of plague bacteremia, eight bites by one or more flies (not over two have been used simultaneously) will effectively transmit the disease to a healthy animal, if the application is made within an hour after the flies have bitten the affected pig".

In view of this experimental evidence, one cannot exclude the possibility of a mechanical transmission of plague through the stable-fly. Certainly, however, actual instances of such a conveyance of the infection, if occurring at all, are exceptional.

Gosio 86 reported that, when fed on the organs of animals dead from plague, the larvae of necrophilous flies harboured numerous plague bacilli and that these organisms were passed on to the pupae and adults in diminishing numbers. Similar claims were also made by Russo.225 However, interesting as these findings are, they are of no practical importance as far as the transmission of plague is concerned.

Mosquitos

The evidence incriminating the mosquitos, and allied species of blood-sucking insects, as potential vectors of plague may be summarized as follows:

(1) Bonnardiere & Xanthopoulides (quoted by Dieudonné & Otto 46) found plague bacilli in the proboscis and stomach of an insect ("Stech-muecke") which had just bitten a plague patient.

(2) Flu 63, 64 was able to prove that specimens of *Mansonia, Culex pipiens, Anopheles rossi*, and *Aëdes aegypti*, which had been fed on plague-affected laboratory animals, could harbour virulent plague bacilli for some days, but did not transmit the infection through their bites.

(3) Blanc & Baltazard 20 found that *Aëdes aegypti* which had fed on plague-infected guinea-pigs harboured *P. pestis* for ten days after the last infective meal, but were incapable of transmitting the infection.

Many observations made in China during the second World War proved that mosquitos, even though they may become infected, are unable to transmit plague. Although the patients in the emergency hospitals were often covered with mosquito-bites, not a single case of plague occurred which could be ascribed to such bites. With the exception of one medical officer, who was infected during the day through a flea-bite, no instances of bubonic plague were noted among the staff of the hospitals. These findings are in accord with results obtained by Hunter,113 who failed to detect plague bacilli in a group of 20-30 mosquitos which had been caught inside the nets covering plague patients in the Hong Kong Infectious Diseases Hospital.
Triatoma

Mertens explored the possibility that *Triatoma rubrofasciata*, an insect normally feeding on the rats of Java, but occasionally attacking man, might convey plague. He found that guinea-pigs inoculated subcutaneously with specimens of this insect which had been fed on plague-affected animals became fatally infected, and that the insects, if kept starving after an infective meal, could harbour *P. pestis* for at least one month. However, the faeces of infected *Triatoma* were free from plague bacilli. Carrying out transmission experiments with this insect, Mertens obtained only one positive result when using a *Triatoma* which bit a healthy guinea-pig immediately after its meal on an infected animal had been interrupted. *Triatoma rubrofasciata* is, therefore, certainly not a dangerous plague-vector.

Ants

Hankin was able to demonstrate *P. pestis* in ants which had fed on the carcasses of plague rats, and to infect laboratory animals (rats and mice) with triturates of these insects.

In contrast to these findings, Russo maintained that the virulence of plague bacilli harboured by ants was apt to become lost.

Beetles

Early experiments by Cao showed that beetles which had been fed on plague material were apt to excrete virulent *P. pestis*.

Skorodumoff, working in Transbaikalia, was able to produce plague in a laboratory animal inoculated with a triturate of *Necrophorus dauricus* found on a naturally infected tarabagan. However, experiments performed with other specimens of this beetle species, and with *Silpha perforata* collected from infected tarabagans, gave negative results.

Russo found that two species of necrophilous beetles—namely, *Sitophilus* and *Tenebrio molitor*—could harbour *P. pestis* when fed on plague material.

Notwithstanding these positive findings, it is altogether unlikely that beetles can play a role in the transmission of plague.

Cockroaches

Early observations on the presence of *P. pestis* in cockroaches were made by: (a) Cao and Kuester (quoted by Dieudonné & Otto), who found that these insects, when fed on plague material, could void virulent plague bacilli in their faeces; (b) Pound, who ascribed an instance of laboratory infection in a healthy guinea-pig to young cockroaches which had had previous contact with plague-infected animals, and was able to prove this contention by bringing a second guinea-pig into contact with such cockroaches; and (c) Hunter, who demonstrated the presence of *P. pestis* in cockroaches (*Blatta orientalis*) collected in plague houses.
However, further observations indicated that the ability of cockroaches to harbour *P. pestis* was not great. Barber, who injected these organisms into the body-cavities of 61 cockroaches (*Periplaneta americana* and *Rhyparobia maderae*), obtained only one positive result when making animal experiments with these insects. Jettmar found that: (a) as proved by guinea-pig experiments, plague bacilli perished or soon lost their virulence in the alimentary tracts of cockroaches (*Blatta germanica*); (b) it was not possible to infect guinea-pigs with fresh excrement from cockroaches fed on plague material; and (c) inoculation of *P. pestis* into the coxal-stumps of an amputated leg did not produce plague in cockroaches.

It is unlikely, therefore, that cockroaches play any role in the transmission of plague. Certainly, however, it is essential to keep premises where experimental animals are kept free from these pests because, as shown by the observations of Pound and others, young cockroaches have an almost uncanny ability to insinuate themselves into containers which seem impregnable to the inroads of insects.

**Conclusions**

Dealing with the role of insects other than fleas in the transmission of plague, Wu expressed the opinion that “rodent lice and ticks alone have to be taken into serious consideration”, but that “compared, however, to the rodent fleas, especially *X. cheopis*, the role of even these insects is of little import. Indeed, broadly speaking, the terms ‘insect vectors of plague’ and ‘rodent fleas’ may be taken as interchangeable.”

Though, under special conditions, certain insect species other than the rodent fleas may play a comparatively important adjuvant role, in general, one ought to be in agreement with the dictum of Wu.

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Chapter 8

CLINICAL ASPECTS

INCUBATION

The problem of the length of the incubation period of human plague received early and thorough attention on the part of the Indian Plague Commission. As summarized by Simpson, the cases the Commission considered in this respect may be divided into three classes:

Class I, comprising instances in which there was a history of a direct inoculation of infective material as well as such where there was a history of the patient having come into contact with infection on a particular occasion. In both groups there were cases where the incubation period could not have been longer than 24 hours and others in which it extended to five days. The average length of the incubation period was about three days.

Class II, consisting of cases in which there was a history of the patient having been in contact with infection on and after a particular day. The data gathered for this class confirmed those of Class I in placing the period of incubation between one and five days.

Class III, representing cases in which plague developed after removal from infected surroundings. Out of 753 cases noted, 15 (1.9%) developed plague after the 10th day, but it was stated that these patients might have contracted the infection after removal to the camp.

In Simpson’s own opinion:

“The incubation period of whatever type the disease may be varies generally between a few hours and five days, it being rarely longer. Cases have been recorded with longer periods, but it is often difficult to dissociate from them the possible exposure to infected clothes or infected animals at a date later than that which is believed to be the time of infection. Still the evidence at present existing does not exclude the possibility of the period of incubation being prolonged occasionally to 12 or 14 days.”

The plague subcommittee of the Office International d’Hygiène Publique, meeting at Paris in 1926, maintained similarly that the duration of incubation of human plague did not ordinarily exceed 6 days; in the case of direct infection by the respiratory route, the duration of incubation did not generally exceed 3 or 4 days, but it might be as long as 8 days in exceptional cases.
Statements made in some recently-published manuals on plague or tropical diseases regarding the length of the incubation period of plague may thus be summarized:

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Length of incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chun 🅱️​ 20</td>
<td>1936</td>
<td>Usually 3-5 days, but may vary from less than 24 hours to 10 days or, in exceptional cases, even up to 15 days.</td>
</tr>
<tr>
<td>Joyeux 🅱️​ 99</td>
<td>1944</td>
<td>Bubonic plague: 1-5 days, but may be prolonged to 9-10 days. Pneumonic plague: on an average 2-6 days, but may last only a few hours or may be prolonged to 9-10 days.</td>
</tr>
<tr>
<td>McCoy 🅱️​ 119</td>
<td>1944</td>
<td>3-12 days; in most cases 4-5 days.</td>
</tr>
<tr>
<td>Rogers &amp; Megaw 🅱️​ 168</td>
<td>1944</td>
<td>Usually about 3 days, but said to vary from 2 to 10 days; rarely, it may be extended as long as 15 days.</td>
</tr>
<tr>
<td>Strong 🅱️​ 188</td>
<td>1944</td>
<td>Varies generally from 2-10 days, but is usually 3-4 days. Primary pneumonic plague: may not exceed 2-3 days.</td>
</tr>
<tr>
<td>Mackie et al. 🅱️​ 220</td>
<td>1945</td>
<td>Bubonic plague: 2-4 days, less often 10 days. Pneumonic plague: may not exceed 2-3 days.</td>
</tr>
<tr>
<td>Pozzo 🅱️​ 156</td>
<td>1945</td>
<td>Bubonic plague: varies from 2 to 10 days, but is ordinarily 3-4 days. Primary pneumonic plague: from 15 to 20 hours up to a maximum of 2-3 days.</td>
</tr>
<tr>
<td>Napier 🅱️​ 143</td>
<td>1946</td>
<td>2-8 days, rarely longer; average about 4 days.</td>
</tr>
<tr>
<td>Dubois &amp; van den Berghe 🅱️​ 29</td>
<td>1948</td>
<td>2-9 days, most often 3-4 days.</td>
</tr>
<tr>
<td>Mathis &amp; Pons 🅱️​ 127</td>
<td>1948</td>
<td>Bubonic plague: 10 hours to 3 days. Pneumonic plague: 48 hours on an average, but can vary from 1 to 5 days.</td>
</tr>
<tr>
<td>Giordano 🅱️​ 56</td>
<td>1950</td>
<td>2-8 days.</td>
</tr>
<tr>
<td>Manson-Bahr 🅱️​ 121</td>
<td>1950</td>
<td>2-8 days, rarely up to 15 days.</td>
</tr>
<tr>
<td>Macchiavello 🅱️​ 118</td>
<td>1951</td>
<td>Usually 2-5 days, with a maximum of 10-12 days. Incubation periods of only 12-15 hours have been recorded in India.</td>
</tr>
</tbody>
</table>

As will be gathered from the above summary, some disagreement exists regarding the minimal and maximal lengths of the incubation period of plague. However, the various writers are agreed that the length of incubation of bubonic plague is as a rule less than 6 days, which is the period contemplated in the International Sanitary Conventions of 1926/1944 and in the International Sanitary Regulations 🅱️​ 212 adopted by the World Health Organization in May 1951.

Most authorities state that the average length of the incubation period is even shorter in the case of primary pneumonic plague, but it should be noted that Chun, 🅱️​ 20 analysing the experiences made during the 1920-1
Manchurian epidemic at Harbin, found an incubation period of up to 6 days "frequent enough to deserve serious attention from an administrative viewpoint."

Though one ought to share Simpson's opinion\(^{175}\) that observations suggesting abnormally long incubation periods are apt to be fallacious, no doubt can exist that instances of this kind do occur. Reliable observations have in fact been recorded more recently by authors such as Durand & Conseil,\(^{37}\) Fonquernie,\(^{50}\) Calbairac & Seyberlich,\(^{12}\) and Girard,\(^{59, 64}\) who noted—in the case of primary-pneumonic plague in particular—instances of abnormally long incubation, lasting up to 10 days. Consequently, the period during which contacts with pneumonic-plague patients were kept in the isolation camps of Madagascar was prolonged to 10 days. There can be no doubt, however, that nowadays such a prolonged segregation of pneumonic-plague contacts, or possibly even their segregation at all, has become unnecessary in view of the availability of methods of abortive treatment. Application of the now available insecticides certainly renders the segregation of contacts with bubonic patients superfluous.

**SYMPTOMATOLOGY**

**General**

The following clinical features, because commonly met with in all severely-affected plague patients, regardless of the primary localization of the infection, may be dealt with comprehensively, the more so as they are due to an action of circulating plague toxins rather than to that of the causative organisms themselves.

**Onset and prodromal symptoms**

There is general agreement that in the overwhelming majority of severe plague attacks the onset of the disease is sudden so that, as aptly stated by Mathis & Pons,\(^{127}\) one is confronted in 95\% of the cases by the clinical picture of a severe infectious condition of the type of a major septicaemia. Prodromal symptoms, if occurring, may consist of malaise, headache, giddiness, mental apathy or restlessness, nausea, and pains in the limbs or in the lumbar region. Some of these symptoms, particularly headache and nervous disturbances, may persist or, if they were not pre-existent, may appear in the course of the illness.

While, according to some observers, the rapid rise of the temperature in the cases with a sudden onset is initiated merely by some feeling of chilliness or slight shivering, others noted the presence of marked rigors. Macchiavello,\(^{118}\) in particular, stated that plague began commonly "with
a single prolonged intense chill, or with less severe or repeated chills accompanied by trembling or shaking." Such marked rigors were hardly ever observed in the plague patients of China. Rogers & Megaw, who gained their experience in India, also speak merely of chilliness when describing the onset of plague.

In any case, the temperature rises rapidly, often reaching 103°F or 104°F (39.5-40°C) in a few hours or, at most, within a day. The pulse-rate increases at the same time and, as confirmed by observations of the present writer, the pulse may not only soon become more rapid than corresponds to the temperature, but may also commence to be dicrotic, or even intermittent, quite early in the disease.

Generally speaking, the condition of the patient deteriorates incessantly so that the disease may become fully developed within a day if not within a few hours.

**Course**

Many authors, particularly the early writers, maintained that plague patients showed a characteristic facial expression and considered this facies pestica as pathognomonic. These statements cannot be considered to be generally valid, however, because, as pointed out by some observers such as Müller & Pöch, the facial expression and the aspect of the eyes ("Blick") of the plague patients are apt to vary markedly in relation to the state of their sensorium and the pains which they feel. One must also agree with Müller & Pöch that the facial expression noted in some of the sufferers is not characteristic for plague alone, but may be seen in any patient suffering from a disease with "typhous impairment of the sensorium".

However, necessary though it is to bear these restrictions in mind, the general appearance of severely-affected plague patients is often so characteristic that, at least during an outbreak, the presence of the disease may be suspected at a glance. The sufferers appear to be struck down by a most serious illness and, even if they are restless instead of being prostrated, it can be seen that they are in a greatly weakened condition. Except in the case of collapse, the faces of the sufferers are as a rule flushed, and sometimes bloated as well. Early in the disease their countenance often depicts anxiety and distress, but later their facial expression becomes resigned, listless, and apathetic. In patients whose sensorium is not free, the eyes may be staring or may show aimless movements. The conjunctivae are almost invariably injected, often markedly so, the nostrils are dilated, and the lips are dry. As long as fever is present, the skin of the patients is hot and dry. As noted earlier, the pulse is rapid, often more or less dicrotic, becoming in the later stages of the disease feeble, intermittent, and finally imperceptible.
A systematic examination of the patients reveals the following:

**Temperature.** Various types of fever—high continued, remittent, or irregular—may be observed in plague patients according to the form of the disease and the severity of the affection. As aptly described by Simpson, the fever

"... rises in the bubonic form to 103°, 104°F. or may be to 105°F. or to 106°F., [39.5°, 40°, 40.5°, 41°C] and may reach its highest on the evening of the first day and continue at its maximum, but more usually it gradually rises, reaching its maximum on the evening of the second or third and sometimes, but seldom, on the fourth day, an intermission of a degree or more frequently taking place during a part of the day [usually in the morning]. On the third, fourth, or fifth day the temperature usually falls 2 or 3 degrees or more, continues at this low temperature for a few hours or a day and then rises again, reaching nearly the same or a greater height than that of the previous evening... If this stage is successfully passed through, the temperature again falls the next day, and then by successive evening exacerbations and morning remissions steadily comes down by degrees to normal or sub-normal, which may be reached on any morning between the sixth and eleventh day. In simple bubonic cases of a mild character the temperature may fall to normal as early as the second or third day; on the other hand the occurrence of complications or the eruption of buboes may cause great irregularity in the temperature and completely obliterate the more or less typical primary and secondary rise with the apyrexial interval ".

Successful treatment with modern remedies markedly shortens the febrile period, leading to a gradual or sometimes even to a rapid fall of the temperature. However, even if adequate treatment is continued so as to avoid relapses, suppuration of the buboes may lead to the appearance of intermittent fever until the pus is evacuated.

As described by Simpson, in "septicaemic" plague the temperature rises rapidly and, running an irregular course, generally remains high. However, in particularly severe cases "the temperature may not rise above 100°F [37.8°C] or less in the early stage, and it is only if the patient lives long enough for reaction to set in that there is any considerable rise in temperature ".

In the primary pneumonic form of plague, the temperature is high but often runs an irregular course. It may suddenly fall before the death of the patient.

**Circulatory system.** As noted above, an incessant quickening of the pulse, accompanied by a fall in blood pressure and irregularity of the pulse-waves indicates the presence of progressive heart failure. The pulse may finally become so rapid and imperceptible that it can no longer be counted. Subjectively, the patients may suffer from a feeling of oppression in the precordial region; objectively, dilatation of the heart and feebleness of the heart sounds may be noted as the disease progresses. Sudden death from heart failure may take place not only during the acute stage of the
disease but also during convalescence following even slight exertions on the part of the patients.

Most observers are agreed that, as a rule, severely-affected plague patients show a marked polymorphonuclear leukocytosis, according to Bonebakker, with a shift to the left. The number of white blood-corpuscles, though usually not exceeding 20,000-25,000, may reach 50,000 or even more. Wagle & Colah maintained in this connexion that an enumeration of the leukocytes is of prognostic importance, the prognosis being best in patients showing leukocytic counts within normal limits. It is noteworthy, however, that a leukocytosis may also be absent in pneumonic-plague patients and in particularly severe cases of "septicaemic" plague. Leukopenia may be present in this form of the disease and, according to Wagle & Bedarkar, also in some patients with primary pneumonic plague.

Contrary to the findings of other workers, Rogers & Megaw claimed that in half of the plague cases seen by them differential counts showed a high proportion of lymphocytes during the first three days of illness, and ascribed some differential-diagnostic importance to this feature.

It was also stated that in the early stages of plague the number of red blood cells and the percentage of haemoglobin are not rarely increased above normal (Rogers and Castellani, quoted by Strong). After the attacks, particularly if complications are present, a more-or-less marked secondary anaemia may develop.

In severe plague cases the presence of submucous haemorrhages may lead to bleeding from the stomach, the intestines, or the bladder. Bleeding from the nose is not uncommon.

According to what has been stated in chapter 4, an initial bacteraemia is frequent even in apparently uncomplicated cases of bubonic plague, but is of a passing character if the disease tends to run a benign course. As a rule, bacteraemia becomes established in seriously affected patients suffering from any type of plague. It is usually progressive in character, the number of bacilli in the peripheral blood becoming maximal in the agonal period. Observations made in this connexion by the Plague Research Commission (quoted by Petrie and by Girard), showed that out of 74 blood samples taken from 28 plague patients, five of whom recovered, 30 were positive, but that in only six instances was it possible to find the bacilli in smears. In seven of the 23 fatal cases, less than 10 organisms per ml of blood were found. Only five of the 16 other patients showed more than 1,000 organisms per ml of blood, and only two had more than 10,000 P. pestis per ml.

It is important to note that in the experience of subsequent workers, such as Girard and Pollitzer (unpublished observations), the number of plague bacilli in the blood of moribund patients was frequently found to be considerably higher than in most of the sufferers examined by the Plague Research Commission, even blood smears often and quite markedly proving
positive. Still, general agreement exists that, apart from rare exceptions, the degree of bacteraemia in human victims is far below that commonly observed in rats succumbing to acute plague.

The presence of a conspicuous bacteraemia usually renders the prognosis most unfavourable, but it is noteworthy that recovery was observed by the Plague Research Commission even in a patient from whose blood 500-600 colonies of *P. pestis* per ml of blood had been obtained.

On the other hand, as pointed out with much reason by Girard, in man as well as in experimental animals, an overwhelming infection leading to marked toxaemia may result in rapid death before bacteraemia becomes conspicuous.

Devignat recently postulated that bacteraemia was more marked in sufferers infected with *P. pestis* of the variety "amiqua" (i.e., strains acidifying glycerol and reducing nitrates to nitrites) whereas the character of the disease was less serious in epidemics caused by the glycerol-negative "oriental" variety of the plague bacillus. Though, no doubt, the manifestations of human plague observed by Devignat in the Belgian Congo, which were caused by *P. pestis* var. *antiqua*, were of a most serious character, the same held true for many outbreaks caused by the "oriental" variety of the plague bacillus.

Respiratory system. Though involvement of the respiratory tract is not peculiar to the primary pneumonic type but is almost invariably present in severely-affected patients suffering from any form of plague, the physical signs found on examination of the lungs are often rather inconspicuous. This holds true not only in cases where merely some bronchitis or congestion of the lungs is present, but often even for patients where a secondary or primary pneumonic process develops. One might almost say that the marked discrepancy between the slight clinical signs usually found in the respiratory tract of the patients and the often marked lung-involvement seen at autopsy is a characteristic feature of the disease.

While the clinical aspects of plague pneumonia will receive further attention later, it has to be noted here that terminal lung oedema, leading to marked dyspnoea and cyanosis, is practically always present in patients dying from any form of plague, unless they suddenly succumb to heart failure. To judge from the personal experience of the present writer, lung oedema is particularly conspicuous in patients with an apparently primary invasion of the blood-stream.

Nervous system. As maintained by Simpson, next to the local manifestations of the infection the most characteristic symptoms of plague are those connected with the nervous system. Dealing in a masterly manner with the nervous phenomena observed in this disease, he stated that:

"The plague virus evidently produces a progressively intoxicating effect on the nervous system, which displays itself with varying degrees of intensity in different ways on different
constitutions. In some there is insomnia, in others wild delirium, in others stupor, in all more or less loss of coordinating power over the voluntary muscles and dulling of the senses. The staggering gait and the inability to coordinate the movements of the hands are very characteristic symptoms. There is no paralysis of the limbs, but from the physical weakness, vertigo, and toxic impression on the nervous system the voluntary muscles are not completely under the command of the patient. The speech is also peculiarly hesitating, stuttering, thick, lisping, indistinct, and monosyllabic, often like that of a drunken man. The memory is confused, and in answering questions the patient forgets half the sentence or syllable of the word which he began to utter.”

As will be gathered from this description, the nervous reaction of the patients to the plague toxin varies markedly. In fact, some of the sufferers, particularly those with primary pneumonic plague, remain conscious, rational, and with speech unaffected to the last. Sudden transitions may occur, patients who seem apathetic quite unexpectedly leaving their bed and trying to get into the open. Such restlessness is, on the whole, most often seen in the later stages of the disease, apparently because the patients then suffer from “air hunger”.

Possibly, regional differences exist in the nervous manifestations displayed by plague patients. Macchiavello maintained in this connexion that while delirium and stupor are the rule in India and China, in Africa, and especially in South America, these symptoms are lacking in the great majority of cases. No doubt, however, individual differences and different habits of life are also of importance in this respect. For instance, the few soldiers admitted to the plague hospitals of China appeared, as a rule, to be far more restless—or even boisterous—than the patients from the class of farmers or labourers who were usually stuporous or quietly delirious. Nervous excitement was also comparatively less conspicuous in the female patients than in the males, even though the latter were as a rule not addicted to alcohol. Convulsions, which are not often observed in adult plague patients, seem to be more frequent in children affected by the disease.

It is consoling that pari passu with the increased use of the now available remedies, serious nervous disturbances as well as other grave symptoms of plague infection are now far more rarely seen than in the past.

*Digestive system.* Plague patients, while usually having little or even no appetite, almost invariably feel intensely thirsty. They are either constipated or have diarrhoea; according to Mathis & Pons, frequently an initial phase of constipation leading to slight meteorism is followed by a terminal phase with diarrhoea.

Statements as to the frequency of vomiting in plague are not unanimous, some writers merely noting that it may be present, others stating that it is frequent early in, or even throughout, the acute stage of the disease. Macchiavello, who considered nausea followed by vomiting as an early symptom of plague, maintained that usually it was of nervous origin instead of being due to gastro-intestinal causes.
It is unfortunate that even in plague areas where vomiting is not a prominent symptom, like in China, it was quite frequently induced when sulfonamides were administered, thus seriously interfering with treatment by the oral route.

The tongue of a severely-affected plague patient is usually swollen and protruded with difficulty in a tremulous manner. It is at first coated with a creamy-white fur, the tip and edges remaining red. Later the coating becomes dry and glistening and, finally, a yellowish, brownish, or even black coat is formed, similar to that seen in typhus or typhoid. Lips, teeth, and gums may become covered with sordes. If the patients begin to improve, the tongue soon tends to become moist and clean, so that its examination is of some importance for assessing the condition of the sufferers.

**Abdomen.** As a rule, physical examination of the abdomen shows nothing characteristic. The liver and also the spleen are usually moderately enlarged, and the former organ may be tender, particularly if heart failure becomes marked. Occasionally, general tenderness of the abdomen, associated with meteorism and vomiting, was found to be present.

If the deep iliac lymph-nodes are the seat of primary buboes, marked pains and tenderness may be present in the corresponding lower quadrant of the abdomen. If the process is localized on the right side, a syndrome quite similar to that of appendicitis may be produced, and cases are on record where patients showing such a localization of the buboes have been subjected to operation (Macchiavello). It is also noteworthy that, according to some observations of Goldstein, pneumonic-plague patients may complain of pains not in the chest but in the abdomen. He referred, in particular, to one sufferer who “had such rigidity and guarding in the left epigastrium that I suspected an acute abdomen and asked the surgeon to see him. There was nothing in the lungs and pleurae, and it was only after two days that some crepitations at the right base were audible and the patient brought up blood-stained sputum which contained *P. pestis*”.

**Genito-urinary system.** In accordance with the presence of high fever, the urine of severely-affected plague patients is scanty and highly coloured. It often contains traces or moderate amounts of albumin, and if so, hyaline casts are found.

Haemorrhages in the urinary tract, as they frequently occur, may also lead to the presence of erythrocytes in the urine, but marked haematuria, though recorded by some writers, appears to be exceptional.

Chun, who examined the urine of a series of patients suffering from primary pneumonic plague, noted in most instances a diminution of the chlorides, but it is noteworthy that according to Simpson the urine of
plague patients in general is deficient in chlorides as well as in urea and uric acid. Instances of anuria have been described but appear to be exceptional. Retention of the urine in the bladder of unconscious or delirious patients is more common.

It is important to note that the urine as well as the faeces of severely-affected plague patients may contain *P. pestis*.

Unless specific treatment is started early, serious attacks of plague in any form usually lead to abortion or miscarriage in pregnant patients, events which exert a most unfavourable influence on the outcome of the illness.

**Termination**

Unless adequate specific treatment is administered, patients suffering from primary pneumonic plague almost invariably die within a few days, sometimes even within less than a day. The average length of life in 1,128 such victims seen in 1921 at Harbin, Manchuria, was 1.8 days. Similarly, patients suffering from the so-called "primary septicaemic" form succumb, when not energetically treated, almost always within 1-3 days, but sometimes even within a few hours (pestis siderans of the early writers). On the other hand, patients affected by benign forms of bubonic plague without bacteraemia almost invariably recover even if no treatment is given. The average fatality-rate in untreated patients suffering from the usual type of bubonic plague leading to bacteraemia is high, amounting to 60%-90% in India and China.

Though it may take place sooner or later, death during the acute stage of bubonic plague usually occurs within a period of 3-5 days from the onset of illness, so that patients who survive for longer than five days have considerably increased chances of recovery.

In most instances heart failure is the immediate cause of death. Collapse may set in fairly rapidly or the patients may even succumb suddenly when making exertions. In other cases there is a more-or-less prolonged period of agony marked by lung oedema and frequently also by oedema of the brain which leads to low delirium and/or coma.

Occasionally, death during the acute stage of the disease may be due to the development of complications, particularly meningitis. Marked oedema developing round cervical buboes may, in rare cases, lead to death from asphyxia.

Recovery from bubonic plague is usually gradual. The period of convalescence may be long and, as will be described when dealing with the devolution of the buboes, serious or even fatal complications may develop during this stage. As has been noted before, even convalescent patients may succumb suddenly to heart failure.
Bubonic Plague

For various reasons it is impossible to discuss the clinical aspects of bubonic plague as comprehensively as the pathologist is entitled to do when dealing with this form of the disease. Frequently, the morbid process, usually produced through the bite of insect vectors, particularly rodent fleas, leads not only to the appearance of buboes but also to a generalization of the infection, characterized by the serious general symptoms previously described and often also by complications which deserve separate attention.

FIG. 28. INGUINAL BUBO
However, benign cases are met where the infective process remains localized in the primarily invaded lymph-nodes and, owing to the absence of septicaemia, general symptoms, if manifest at all, remain inconspicuous. On the other hand, an invasion of the blood-stream may take place so rapidly that reactions on the part of the regional lymph-nodes fail to become manifest and the clinical picture is that of a most severe generalized infection, often designated as primary septicaemic plague. Though, as stated in chapter 4, this type of the disease has no independent standing from the viewpoint of the pathologist, it presents clinical, particularly diagnostic, features of its own which render its separate discussion desirable.

An entry of the infection through the superficial mucous membranes as well as the appearance of primary skin manifestations at the portal of entry of the infection produce peculiar clinical forms of the disease, which also deserve special consideration.

For these reasons it seems appropriate, for the purposes of the present disquisition, to deal with the different types and varieties of bubonic plague separately.

**Buboes**

*Localization.* General agreement exists that, as a rule, groin buboes are most frequent (about 55%-70%), femoral buboes situated distally from Poupart's ligament being somewhat more frequent than inguinal ones. Axillary buboes (about 20%) and cervical, particularly submaxillary, buboes (about 10%) follow in frequency. Buboes in other locations, such as those in the parotid region, below the clavicle, near the elbow (epitrochlear buboes), and behind the knee (popliteal buboes), are rare. Buboes under the subpectoral muscles are somewhat more frequent. Though the primary buboes usually form at one site only, bilateral and multiple locations have been recorded in a not-inconsiderable minority of cases. The following statistics, which are based on large samples, bear out these statements:

<table>
<thead>
<tr>
<th></th>
<th>Choksy</th>
<th>Jennings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases examined</td>
<td>9,500</td>
<td>16,132</td>
</tr>
<tr>
<td>Percentage incidence of buboes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral</td>
<td>30.87</td>
<td>30.79</td>
</tr>
<tr>
<td>Inguinal</td>
<td>23.25</td>
<td>24.23</td>
</tr>
<tr>
<td>Axillary</td>
<td>21.85</td>
<td>22.18</td>
</tr>
<tr>
<td>Cervical</td>
<td>8.40</td>
<td>9.11*</td>
</tr>
<tr>
<td>Other sites</td>
<td>1.68</td>
<td>-</td>
</tr>
<tr>
<td>Multiple</td>
<td>12.95</td>
<td>13.68</td>
</tr>
</tbody>
</table>

* Comprising besides 6.24% cervical buboes proper, 2.87% of buboes situated in the parotid region.

It is important to note, however, that under certain circumstances axillary buboes, or buboes on the neck, may be more frequent than is
usually the case. Axillary buboes are likely to occur in patients contracting infection in the foci of wild-rodent plague where a transmission of the disease through direct contact with the affected animals is apt to take place. Cervical buboes are apt to be frequent pari passu with a frequent occurrence of an entry of the infection through the faucial mucous membranes leading to tonsillar plague.

Local peculiarities in the dress of the people probably also exert an influence on the distribution of plague buboes. Thus Chun pointed out that whereas in the experience of Choksy femoral buboes were more frequent than inguinal ones, among a series of patients observed in South Manchuria, inguinal buboes were preponderant (40% as against 8.7% femoral buboes). Chun ascribed this discrepancy in part to differences in the style of dress adopted by the Indians and the northern Chinese respectively. In contrast to the Indians, the Chinese not only wore shoes all the year round instead of using sandals or even going bare-footed, but also tied their trouser-ends over close-woven socks. However, since Chun’s observations referred to 80 patients only, no stringent conclusions should be drawn from his as well as from other experiences based upon small samples.

Appearance and development. As is unanimously stated in the literature and has been well borne out by the fairly ample field observations made by the present writer, manifestations on the part of the primarily-affected lymph-nodes appear, as a rule, quite early in the disease, simultaneously with or even before, the onset of the fever. Some writers maintain that in a minority of cases the buboes appear later, sometimes two, three, or even more days after onset of the disease. However, when one considers how inconspicuous the earliest manifestations in the affected lymph-nodes often are, one must wonder whether such an absence of early signs was more apparent than real.

Even when seen within the first hours of illness, as a rule the patients already complain of discomfort or even of some pain at the site of the future buboes. Failing that, it is almost invariably possible to prove that these sites are tender to touch. The affected lymph-nodes are at this stage but slightly enlarged and of normal consistency, but from the first it can be definitely established that they in particular are the seat of the pain or tenderness to touch which the patients feel. If, as is often the case, not one but two or several lymph-nodes become primarily involved, they enlarge at first separately so that at this early stage they can still be delimited by palpation.

The further development of the local morbid process is subject to considerable variation. In the most severe cases which are rapidly fatal, the affected lymph-nodes become but moderately or even slightly enlarged and show no marked periglandular involvement. In the usual moderately
severe type of the disease, enlargement of the lymph-nodes progresses incessantly and development of marked periadenitic changes, followed by infiltration and oedema of the surrounding tissues, leads within the first 12 hours of illness, or even earlier, to the appearance of well-marked buboes which become fully formed within one to five days after onset of the disease. The size of a fully developed bubo usually varies from that of a walnut to that of a hen's egg, but smaller or larger sizes may be observed. The buboes usually have an oval or round shape; their surface may be uneven if they are formed by the fusion of two or more lymph-nodes. Being at first movable, the buboes soon become adherent to the surrounding tissues, the skin becoming involved in this process if superficially situated lymph-nodes become the seat of infection. In such cases the skin loses its normal texture, appears smooth and tense, and may show some degree of reddening, owing to the presence of acute inflammation. Some early writers such as Simpson\textsuperscript{175} state that haemorrhages, carbuncles, or blisters may appear on the skin covering the buboes and that the skin may even become gangrenous, but manifestations of this kind were rare in China and, according to Macchiavello,\textsuperscript{138} also in South America.

Palpation of a fully developed bubo reveals the presence of a doughy or boggy consistency in the outer layers of the swollen mass and a hard consistency in its central part formed by the affected lymph-nodes.

The subjective sensations produced in the patients by the presence of plague buboes may vary considerably, some complaining of constant dull or even stabbing pains, others feeling little or no local discomfort as long as they do not move and keep in a position which lessens pressure on the affected parts of the body. Patients with a groin bubo keep for this purpose the corresponding thigh flexed, those with axillary buboes lie on their back and hold the affected arm away from the trunk, and those with cervical buboes hold their head still and inclined to the affected side. Generally speaking, pain is more marked in the case of smaller buboes than in that of larger ones. Accordingly, it may be present early in the disease but may disappear as the buboes increase in size. Be this as it may, during the acute stage of the disease the buboes are invariably sensitive to touch, often to such a marked degree that even comatose patients react when the affected lymph-nodes are touched. This marked tenderness to touch, which, as a rule, appears in the earliest stage of illness and is noticeable in the case of deep-seated buboes as well as in those lying near the surface of the body, forms one of the most outstanding clinical features of bubonic plague.

Primary buboes of the secondary order, produced by an invasion of the causative organisms through the lymph-channels, may appear in the vicinity of the initially affected lymph-nodes. In particular the intra-abdominally situated iliac lymph-nodes not rarely become involved in this manner. If bacteraemia has become established, lymph-nodes situated
away from the primarily affected ones in other parts of the body may become invaded by way of the blood-stream. As has been noted in chapter 4, the appearance of such secondary buboes does not, as a rule, lead to marked reactions in the surrounding tissues.

**Devolution.** The acute process leading to the appearance of primary plague buboes may subside in various ways. Hand in hand with a gradual improvement of the general condition of the patients, a return to normalcy may take place locally. Often, however, this process is not complete, the affected lymph-nodes remaining somewhat enlarged and indurated; the skin over such lymph-nodes may remain pigmented.

Frequently, suppuration commences in the centre of the bubo early in the second week of illness and an abscess is formed which, barring surgical intervention, is apt to open spontaneously. In favourable cases the fistula thus formed closes after pus has been voided or sloughing has taken place for about two weeks, and a scar is formed which varies in size according to the size of the abscess and the amount of sloughing. However, the process of healing may be delayed and sinuses may form which become secondarily infected. In such cases chronic ulcers may develop and the patients may die some weeks later from sepsis, exhaustion, or amyloid disease (Napier 143). Sad to relate, cases may be observed in which too early or inadequate surgical intervention, or unskilful after-treatment of the wounds, greatly retarded the process of healing or even led to serious consequences.

Early writers referred to instances where excessive sloughing taking place in the course of suppuration led to the formation of large cavernous ulcers laying bare muscles, nerves, and blood-vessels. As pointed out by Simpson,173 these sloughing excavations were particularly dangerous when forming in connexion with iliac buboes in the pelvis, because in such cases haemorrhage from pelvic arteries was apt to result. Instances of this kind are rarely seen nowadays. However, Robic & Minec 165 referred in 1938 to a case where involvement of the iliac lymph-nodes followed by abscess formation caused the death of a plague patient through haemorrhage due to ulceration of a pelvic artery on the 17th day after the appearance of the initial inguinal buboes.

The formation of unusually large scars due to excessive sloughing or retarded healing may lead to chronic regional oedema (Villafañe Lastra & Rodeiro 203) or, in the case of femoral buboes, to chronic oedema of the corresponding lower extremity (Downie; Phillips 151).

Hand in hand with the process of devolution, the causative organisms, often first undergoing involution or becoming the prey of phagocytes, disappear as a rule fairly rapidly from the buboes or from the pus which has formed. However, as will be discussed below, some instances have been
observed in which *P. pestis* continued to be present for weeks or even months.

**Complications**

As Simpson 175 pointed out with much reason, it is an open question whether the secondary lung manifestations which frequently develop in the course of bubonic plague followed by septicaemia should be classed as complications or should be considered merely as extensions of the infective process, so that "from this aspect they form but a part of the disease". It is in accord with this concept that, as stated in chapter 4, "some involvement of the respiratory tract is usually, if not invariably present in bubonic infections followed by bacteraemia, varying in degree from simple catarrh to grave forms of congestion and specific bronchopneumonia".

It is often difficult to recognize the presence of secondary plague pneumonia. Its appearance is apt to lead to a deterioration of the general condition of the patients, but such aggravations may take place for other reasons as well. As noted before, even if bronchopneumonic foci develop, the physical signs found when examining the chest of the sufferers are often inconspicuous. The sputum of such patients may show an admixture of blood or may even assume an aspect similar to that met with in primary pneumonic plague, but in other cases the appearance of the sputum is uncharacteristic. Laboratory examination of the sputum is also not an infallible means of establishing the presence of a secondary plague pneumonia, because plague bacilli may be found in the expectorations of bubonic-plague patients, whose lungs are not seriously or even not at all involved. Nevertheless, abundance of the causative organisms in the sputum suggests the presence of a secondary pneumonia.

Some observers have recorded that a kind of "marasmus" may develop after attacks of bubonic plague and this condition usually leads to death. Such sufferers become emaciated, feeble in body and mind, and unable to take food, and, getting into a typhoid condition, gradually sink. It would appear that the development of this condition is usually secondary to the complications arising in the course of devolution of the buboes which have been referred to above.

The secondary manifestations observed on the skin and mucous membranes of bubonic-plague patients as well as the meningeal complications are described below, together with the primary manifestations of the infection in these organs.

**Skin manifestations**

Primary cutaneous lesions, representing reactions taking place at the portal of entry of the infection, may appear in two different forms:
(1) The morbid process developing at the site of infection may remain restricted to the formation of a usually small vesicle which is filled with a turbid serous fluid containing plague bacilli, and slight local lymphangitis. The vesicles soon break and no further reaction takes place on the part of the underlying or surrounding tissues.

Lesions of this kind are probably more often present than they are noticed, particularly so as they seem to be more frequent in benign than in grave cases of bubonic plague. In fact, some authors described an "ambulant" type of plague in which the morbid process consists solely of the formation of such vesicles at the site of invasion and some local lymphangitis.

(2) The second type of primary skin plague consists of the development of carbuncles at the site of infection. As described by Simpson, this process commences by the formation of ecchymotic or petechial spots which... rapidly increase in size and then rise in the form of blisters with or without umbilication, while the circumference becomes hard, swollen and inflamed. The blisters contain at first a clear, serous fluid, which is later dark, sero-sanguinolent or haemorrhagic; and in the contents are plague bacilli. The blisters soon break and show at their base a moist, bluish-red, inflamed and angry-looking circular or irregular patch, which at this stage may dry up and go no further, or the inflammation may extend to the subcutaneous tissue, causing a circumscribed or diffuse swelling, the centre of which begins in a few hours to necrose, forming a leathery-looking scab. From this centre the necrosis spreads rapidly to the periphery. The result is the formation of indolent ulcers... with hard and red overhanging margins.

To this excellent description it should be added that a ring of vesicles which contain pus and plague bacilli, and which tend to coalesce, often forms on the wall surrounding the plague carbuncles.

Primary plague carbuncles are usually small or moderately-sized, their circumference rarely exceeding one or two inches (2.5-5.0 cm). They may form on different parts of the body according to the site of the infection; comparatively often they are situated on the wrists or ankles of the patients. The appearance of the carbuncles may coincide with, or precede, the formation of the plague buboes. General agreement exists that usually the development of such marked reactions at the site of invasion presages a favourable outcome of the disease.

Generally speaking, primary carbuncles develop only in a small minority of bubonic-plague patients. However, as stated by Macchiavello, they were frequent in some Peruvian outbreaks, though remaining totally absent in others.

Besides the above-described primary lesions, secondary skin manifestations may develop in plague patients suffering from a severe type of the infection. As confirmed by the experiences of França, referred to in chapter 4, skin haemorrhages are frequently found in such cases if properly
looked for. Though sometimes restricted to the vicinity of the buboes, they are apt to be present on other parts of the body as well. The appearance of numerous petechiae or ecchymoses, particularly early in the disease, is an ominous sign.

Carbuncles or necrotic processes developing without preliminary formation of blisters may become manifest in the course of bubonic plague, usually as the result of a metastatic infection through the blood-stream. However, as noted by some observers, carbuncle formation near buboes may be due to a retrograde infection through the lymph-channels. While
usually rare in recent plague epidemics, such marked skin manifestations seem to have been of common occurrence during historical plague outbreaks, particularly the Black Death and the great plague of London in 1665.

Secondary carbuncles or necrotic ulcers of haematogenous origin may develop on all parts of the body. They may reach considerable size, the necrotic process sometimes laying bare muscles, nerves, and blood-vessels; involvement of the last-mentioned may lead to serious haemorrhages. Generally speaking, however, the presence of solitary secondary carbuncles is a prognostically favourable sign.

The appearance of generalized pustular eruptions ("plague pox" or "plague variola") has been recorded by several observers. According to Macchiavello, instances of this kind were frequent in South America, particularly in Ecuador. Martinez Vinueza, observing a series of 227 plague patients, noted the presence of such generalized variolous manifestations in 26 patients; 24 of these sufferers succumbed to the infection.

Meningeal involvement

As summarized by Meyer et al., instances of plague meningitis or, rarely, encephalomeningitis were observed by some of the early workers, such as: the Austrian Plague Commission (one instance of meningitis out of a total of 80 plague cases); the German Plague Commission (3 cases of meningitis and one of encephalomeningitis out of a total of 376 cases); Calmette & Salimbeni (2 cases of meningitis and 1 of encephalomeningitis, a child showing a groin bubo and signs of meningeal involvement recovering under serum treatment); Dürck (primary pneumonic plague complicated by purulent leptomeningitis). No details are available regarding the cases reported by Godinho and Sanhueza.

More recent observations recorded by Meyer et al., Landsborough & Tunnell, and others may thus be summarized:

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Number of observations</th>
<th>Summary of findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crowell</td>
<td>1915</td>
<td>2 out of 75 cases</td>
<td>Died after illnesses of one month and two weeks respectively. At autopsy, purulent meningitis complicating bubonic plague.</td>
</tr>
<tr>
<td>Lafont et al.</td>
<td>1915</td>
<td>1</td>
<td>Meningeal process considered to be primary in nature. Died 24 hours after admission.</td>
</tr>
<tr>
<td>Sheldon</td>
<td>1915</td>
<td>1</td>
<td>Meningeal process considered to be primary in nature. Died after 36 hours. Diagnosed only by smear examination.</td>
</tr>
<tr>
<td>Levy</td>
<td>1920</td>
<td>1</td>
<td>Meningitis following bubonic manifestations in serum-treated patient.</td>
</tr>
<tr>
<td>Author</td>
<td>Date</td>
<td>Number of observations</td>
<td>Summary of findings</td>
</tr>
<tr>
<td>-------------------------</td>
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<td>------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Nogue 146</td>
<td>1923</td>
<td>1</td>
<td>Signs of meningeal involvement developing about three weeks after patient had been admitted with diagnosis of pneumonia. Suspicious bacilli found in lumbar punctate and also in punctate of an inguinal lymph-node. Died a few days later, showing at autopsy <em>P. pestis</em> in smears from the lungs, bronchial lymph-nodes, and liver. Cranium not opened.</td>
</tr>
<tr>
<td>Uriarte 146</td>
<td>1924</td>
<td>1</td>
<td>Bubonic plague complicated by unilateral iridocyclitis and hypopyon. Progressed favourably under serum treatment but suddenly died three weeks later, showing at autopsy abscesses containing <em>P. pestis</em> in the congested meninges.</td>
</tr>
<tr>
<td>Paso 148</td>
<td>1925</td>
<td>1</td>
<td>Serum-treated bubonic-plague patient who later developed signs of meningitis and died on 15th day of illness.</td>
</tr>
<tr>
<td>Williams 299</td>
<td>1934</td>
<td>1</td>
<td>Meningeal process said to be primary in nature.</td>
</tr>
<tr>
<td>Montagne &amp; Rivoalén 139</td>
<td>1936</td>
<td>1</td>
<td>Patient with axillary bubo treated with serum on the fourth, fifth, and sixth days of illness. Meningeal symptoms appeared on the 10th day of illness, the patient dying four days afterwards.</td>
</tr>
<tr>
<td>Meyer et al. 134</td>
<td>1937</td>
<td>1</td>
<td>Chronic relapsing plague meningitis in boy originally suffering from bubonic plague. Died four months after onset of illness. No specific treatment.</td>
</tr>
<tr>
<td>Burton &amp; Hennessey 10</td>
<td>1940</td>
<td>1</td>
<td>Child with skin abscess which healed after incision and two days' sulfapyridine treatment. Meningeal symptoms appeared after two attacks of fever treated with sulfapyridine on the 25th day of illness. Death occurred four days later. Two guinea-pigs inoculated with cerebrospinal fluid died after 11 and 14 days respectively.</td>
</tr>
<tr>
<td>Lewillon et al. 110</td>
<td>1940</td>
<td>1</td>
<td>Admitted with signs of apparently primary plague meningitis on fourth day of illness; died four days later. Plague bacilli found only in cerebrospinal fluid, not in material obtained through puncturing heart, liver, and lungs.</td>
</tr>
<tr>
<td>Villafañe Lastra &amp; Rodeiro 202</td>
<td>1942</td>
<td>4 out of 39 cases</td>
<td>Patients with meningeal involvement complicating bubonic plague who succumbed though given large doses of sulfathiazole.</td>
</tr>
<tr>
<td>Koenigsfeld &amp; Nambiar 99</td>
<td>1946</td>
<td>2</td>
<td>Patients with axillary buboes who improved after initial treatment with sulfonamides but showed signs of meningeal involvement three weeks and one month, respectively, after onset of illness. The first mentioned patient died one day later, the second two days later.</td>
</tr>
</tbody>
</table>
Clinical Aspects

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Number of observations</th>
<th>Summary of findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landsborough &amp; Tunnell</td>
<td>1947</td>
<td>8 out of 203 cases</td>
<td>With exception of one case considered as an instance of primary plague meningitis, meningeal involvement appeared in patients suffering from bubonic plague at the earliest on the 9th and at the latest on the 17th day of illness. Though treated with sulfonamides and serum, all eight patients died, the length of illness varying from 12 to 29 days.</td>
</tr>
<tr>
<td>Videla</td>
<td>1947</td>
<td>1</td>
<td>A patient considered to suffer from primary plague meningitis recovered when given streptomycin intramuscularly as well as intracisternally.</td>
</tr>
<tr>
<td>Fain et al.</td>
<td>1951</td>
<td>1</td>
<td>Child showing on admission fever, headache, and evidence of malaria infection. Initial treatment with quinine, sulfathiazole, and penicillin led after eight days to the disappearance of fever. However, temperature rose again one day later and signs of meningeal involvement appeared 12 days after admission, when blood culture proved positive for P. pestis. Rapid cure through streptomycin administration.</td>
</tr>
</tbody>
</table>

Remarks

(i) No details could be elicited regarding the instances of meningeal involvement recorded by Veintemillas, Mealla, and Kamal et al.

(ii) The recovering patient observed by Singh probably suffered from meningismus and not from meningeal plague.

(iii) Three instances of "cerebral" plague in which symptoms like unconsciousness, delirium, or (once) epileptiform attacks ascribed to the presence of brain oedema were present, were recorded by Wright.

The symptomatology of meningeal involvement caused by P. pestis closely corresponds to that observed in cerebrospinal fever or other acute cases of meningitis. Headache, painful stiffness of the neck, and presence of Kernig’s sign form early and prominent features of the process. Less common symptoms observed by Landsborough & Tunnell in their series of eight cases included convulsions and affections of the cranial nerves (twice respectively) and vestibulo-cerebellar symptoms, deep coma, and vasomotor crisis (once respectively).

As will be gathered from the above tabulation, it was claimed that in a number of instances the meningeal process produced by P. pestis, instead of developing secondarily in patients with bubonic (or exceptionally with pneumonic) plague, was primary in nature. It is conceivable that in contacts of patients suffering from primary pneumonic plague a primary meningeal process might be produced through droplet infection. However, no evidence has been brought forward to prove that in the patients referred to the meningeal process was due to this mode of infection and not to an invasion of the blood-stream by the causative organisms.
This being so, one must view with considerable scepticism the claims that a primary form of plague meningitis exists. It would seem likely that these patients initially suffered from a rapidly progressing type of bubonic infection corresponding to that present in the so-called "primary septicaemic" form where the early invaded lymph-nodes fail to react manifestly because they are rapidly overrun. Moreover, some of the patients claimed to have suffered from primary plague meningitis were seen late in the disease, so that pre-existent manifest buboes might have disappeared before the sufferers came under observation.

Though the appearance of meningeal complications usually led to the death of the patients within a few days, in some of the cases the disease ran a subacute or even chronic course. Two instances of recovery were noted in patients treated with streptomycin.

It will be gathered from the above tabulation that in many of the recently observed cases more-or-less late meningeal involvement became manifest in patients who had received previous specific treatment with serum and/or sulfonamides. Discussing the observations they made in this respect in their eight cases, Landsborough & Tunnell stated that:

"The course of illness in all these patients was longer than is usual in plague, and symptoms of meningitis invariably appeared late in the disease. Bearing in mind that (1) the incidence of meningeal complications was much higher among our patients compared with that in other recent outbreaks in China, and (2) we were able to treat many of our patients more energetically than was possible elsewhere, we wonder whether our therapeutic measures did not facilitate meningeal involvement by prolonging the course of illness in those patients who would otherwise have died earlier".

The two workers produced the following evidence to show that in their series of plague cases the incidence of secondary meningeal involvement had increased pari passu with more intensive treatment:

<table>
<thead>
<tr>
<th>Year</th>
<th>1943</th>
<th>1944</th>
<th>1945</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1943</td>
<td>1944</td>
<td>1945</td>
</tr>
<tr>
<td>Total number of plague cases</td>
<td>88</td>
<td>46</td>
<td>69</td>
</tr>
<tr>
<td>Number of cases with meningeal involvement</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Used per patient:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plague serum (ml)</td>
<td>10</td>
<td>33</td>
<td>92</td>
</tr>
<tr>
<td>sulfathiazole (g)</td>
<td>—</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>sulfapyridine (g)</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

It will be noted that Landsborough & Tunnell ascribed the increased frequency of meningeal involvement merely to a prolongation of the course of the disease through the previous specific treatment. Some other workers, however, were of the opinion that alterations in the virulence of the causative organisms played an important role in the pathogenesis of secondary plague meningitis. Discussing the case of Meyer et al. described earlier, and also a similar observation made in 1943, Jawetz & Meyer stated that in both
"... the original establishment of cerebral foci, undoubtedly occurring during an early bacteremia, did not lead to immediate multiplication of organisms and death, but rather to a persisting of bacilli in a protected location where slow multiplication could be successful. It might be that an infection with a strain of uniformly high virulence could lead to the fulminating meningitis, while strains with a heterogenous population, containing many low virulent organisms, might give rise to the picture seen in California ".

Burton & Hennessey,\textsuperscript{10} considering their observations as well as those of Uriarte\textsuperscript{196} and of Montagne & Rivoalen,\textsuperscript{139} expressed the opinion that in these cases, particularly in their own, the previous specific treatment might have influenced the invasive power of the causative organisms so that the reaction between them and the host might have become altered.

Koenigsfeld & Nambiar\textsuperscript{69} maintained similarly that, in both their cases, "... the meningeval signs occurred in the course of a prolonged illness after some initial improvement. Both patients seemed to respond favourably to sulpha drugs and succumbed only at a comparatively late stage of the disease. Sulphadiazine probably lessened the virulence of the germ to a certain degree, without rendering it entirely harmless, with the effect that the disease took a more chronic course and meningitis had time enough to develop. If this conclusion is correct, more cases of plague-meningitis may be observed in the near future as sulpha drugs are now in general use in the treatment of plague ".

No doubt can exist that insufficient or delayed administration of plague serum or sulfonamides is incapable of preventing the appearance of late meningeal manifestations. Pending further investigations it is difficult to decide whether the mechanism at work is an abatement of the virulence of the invaders or—as seems more likely—their persistence and slow multiplication in a protected location.

Eye involvement

As proved by several well authenticated observations, the eye may serve under natural as well as under experimental conditions as the portal of entry of plague infection. Mizuo,\textsuperscript{137} who, in 1910, made a special study of eye plague entitled "Über die Augenveränderung bei Pest", maintained in this connexion that entry of \textit{P. pestis} into the eye could lead to a generalized infection with or without production of a local reaction. Sharing the opinion reached on experimental grounds by the German Plague Commission,\textsuperscript{54} he also expressed the view that the bacilli entering the eye were carried through the nasolacrimal duct into the nose, and afterwards, gaining access through the nasal or faucial mucosa, reached the lymph-nodes. However, since according to Mizuo's observations plague bacilli were apt to multiply rapidly in the conjunctiva, particularly along the lymph-channels, no doubt can exist that they can reach the regional lymph-nodes directly from the eye.

The following instances of primary eye plague could be found in the available literature.

(1) One case recorded by several workers concerned a nurse working in the Parel Hospital, Bombay, during the 1897 outbreak, who received in the eye a particle of sputum
coughed up by a pneumonic-plague patient. Though the eye was carefully washed, "conjunctivitis set in on the next day, which was followed by a swelling of the parotid, a bubo below the ear on the affected side, and death" (Simpson 175).

(2) According to Simpson 175 a similar case occurred in Hong Kong.

(3) As quoted by Mizuo, Hasegawa observed in 1900 an instance of plague conjunctivitis with symptoms resembling those of conjunctivitis gonorrhoeica; the patient in question died on the day of admission.

(4) Mizuo also referred to the case of a Japanese doctor who, working during an outbreak in 1902 in the Tokyo Plague Hospital, developed a primary dacryocystitis followed by generalized plague infection, with marked cerebral symptoms, and died. According to Mizuo this was the only case of primary plague dacryocystitis on record.

(5) Jorge (quoted by Wu Lien-teh) observed during the 1905 outbreak at Oporto, Portugal, a plague patient who first showed signs of conjunctivitis and then a cervical bubo, but recovered. In this instance also infection seemed to have been due to an entry of pneumonic-plague sputum.

(6) A 2-year-old child, admitted in 1906 to the Osaka Plague Hospital, Japan, with a history of slight trauma (contusion) of the right eye, showed signs of acute plague conjunctivitis, and two days afterwards also swelling of a pre-auricular lymph-node and the parotid gland on the corresponding side. The infection became generalized and led to death on the fifth day after admission (Mizuo 137).

(7) A 14-year-old girl admitted during the 1906 Osaka outbreak with signs of plague conjunctivitis also died on the fifth day after admission—apparently without development of a bubo (Mizuo 137).

It would be rash to claim that the above list is complete. For instance, the present writer was verbally informed that a further case of primary conjunctival plague had been observed during one of the recent epidemics at Foochow, South China. Nevertheless it seems safe to state that primary eye plague is a rare form of the disease.

It is important to note that, on the contrary, secondary eye affections, due to an invasion by the blood-stream, are by no means infrequent. Mizuo who, as stated above, made a special study of this subject noted their presence in 4.3% of his patients, adding that quite frequently both eyes became affected.

As summarized by Simpson, the eye complications met with in plague "may range from a simple inflammatory state to one which is accompanied by ulceration of the cornea, by copious haemorrhages, and in some cases total destruction of the eyesight". In Mizuo's opinion, panophthalmitis and keratitis were comparatively the most frequent complications, followed by ring abscess of the cornea, corneal ulcer, iritis, and conjunctivitis. Pozzo considered a co-existing affection of the iris and the choroid (iridocoroiditis) more common than keratitis or iritis (iridocyclitis) and added that the presence of retinal haemorrhages and optic neuritis had been observed in plague patients.

As had been noted earlier (see page 412), congestion of the conjunctival blood-vessels is a typical sign in severely-affected plague patients. Mizuo maintained that some degree of conjunctival congestion was almost
invariably present, regardless of whether or not the face of the sufferers was flushed. Yamagiwa even upheld that "congestion of the conjunctiva bulbi was one of the three characteristic signs of bubonic plague, the other two cardinal signs being a rapid rise of the temperature and the appearance of painful swellings in peripheral lymph-nodes".  

Referring to the fact that owing to intense congestion the conjunctiva bulbi shows a uniformly red hue in some plague patients, Müller & Pöch 111

\[\text{Das plötzliche Ansteigen der Temperatur, die schmerzhafte Anschwellung der peripherisch gelegenen Lymphdrüsen und die Hyperämie der Conjunctiva bulbi sind die drei charakteristischen Zeichen für die Bubonenpest.}\]
plausibly postulated that this was the reason why early observers often spoke of the "wild look" (wilder Blick) of the sufferers.

**Tonsillar involvement**

As set forth in chapter 4, the tonsils may either become the portal of entry of plague infection or may become secondarily involved in the course of attacks of the disease.

A primary invasion of the faucial organs by *P. pestis* may lead to the formation of tonsillar buboes, but instances of this kind seem to be rather rare. One case was recently mentioned by Kamal et al. In a second group of cases, entry of the infection through the faucial mucosa is followed by a local reaction in the tonsils and almost invariably also by the appearance of cervical buboes. The intensity of these morbid processes may vary considerably. There may be only swelling and more-or-less intense congestion of the tonsils, but in other cases the local signs may be similar to those of follicular angina or they may even be characterized by the formation of ulcers or of membranes similar to those seen in diphtheria. Cases simulating the latter disease were recently recorded by Wright and by Magrou. In Wright's case diphtheria bacilli as well as *P. pestis* were isolated from the tonsils; the patient, who showed no manifest bubo, developed secondary plague pneumonia and died.

The secondary involvement of the cervical glands may sometimes lead to a phlegmonous process similar to that evolving in Ludwig's angina. One of the four cases of "tonsillar" plague described by Wright fell in this category.

Instances of recovery have been reported in the case of patients showing only signs of tonsillar inflammation (de Souza, Wright); as a rule, plague bacilli were rare in the sputum of such patients. Serious tonsillar involvement usually leads to secondary pneumonia and a fatal issue.

Generally speaking, instances of the above-described anginous or "tonsillar" form of plague are rare. However, as reported by Martinez Vinueza and recently by Martinez, cases of this kind, usually followed by secondary pneumonia and death, are quite frequent among the Indians of Ecuador, who are in the habit of killing fleas and lice by catching them with their teeth.

Secondary involvement of the tonsils is, as a rule, the result of a spread of the infection from adjacent cervical buboes through the lymph-channels. Marked oedema, involving the glottis, may develop in such cases and lead to suffocation of the patients.

**Gastro-intestinal involvement**

Discussing the supposed existence of a primary form of gastro-intestinal plague, Dieudonné & Otto aptly stated that
"a primary infection of the gastro-intestinal tract, which Wilm and Galeotti believed to have observed repeatedly in Hong Kong, has not been seen by any of the Commissions working in Bombay; in any case this form is so rare, that great caution must be exerted in accepting its existence. The absence of true primary buboes in the mesenterial lymph nodes, which would be a sine qua non to justify belief in such a form of plague, speaks against the existence of a primary gastro-intestinal type... According to the experiences made in the case of experimental animals, a very massive and direct invasion of the stomach or intestines would be necessary to produce such an infection. However, as shown by the observations made in one case by Trautmann & Lorey [1908] occasionally enormous numbers of P. pestis are found in the intestine of plague patients."

The disbelief of Dieudonné & Otto in the existence of a primary gastro-intestinal form of plague is fully endorsed by the absence of recent reliable observations referring to such a type of the disease. However, as noted before, gastro-intestinal complications, such as vomiting and diarrhoea, are frequently present in severely-affected plague patients and may become conspicuous. The appearance of submucous ecchymoses may lead to haemorrhage into the stomach or intestines, so that a usually slight or moderate amount of blood in the vomits or stools of the patients is not uncommon. Meteorism may be present and, as noted before, may be associated with tenderness of the abdomen.

The presence of jaundice in plague patients has been mentioned by a few recent observers only, such as Williams, Blanchard et al., Bonebakker, and Macchiavello. The last-mentioned author stated that:

"In Brazil, in 1941, we saw an amarillic form [of plague], apt to be confused with yellow fever because of the icterus, the color of the vomitus, and the general symptoms, which were especially violent in the digestive apparatus (hemorrhages, hematemesis, melena, vomiting, and violent diarrhea) with complete absence of buboes."

The unusual plague case with toxic jaundice of the yellow-fever type seen by Williams in East Africa was apparently similar in nature.

Benign forms

Two main types of benign plague caused by an entry of the infection through the skin appear to exist. The morbid process may remain restricted to the site of invasion and its immediate vicinity or regional buboes, and often also a slight or, at most, moderate general reaction may develop.

The first-mentioned type, called "ambulant" plague by Napier, is characterized by the formation of a vesicle at the site of infection and slight local lymphangitis. Constitutional signs remain absent.

Cases of this "ambulant" form of plague appear to be infrequent, but it must be borne in mind that they might be easily overlooked.
The clinical picture present in the second type of benign bubonic plague, which is usually designated pestis minor, may vary considerably. In fact, it is not possible to draw a sharp line of distinction between cases of this kind and those of the usual type of bubonic plague in which bacteraemia does not become established or marked.

In the mildest variety of pestis minor one or a few lymph-nodes (usually those in the groin, but sometimes those on the neck or in the axilla) become somewhat enlarged and tender to touch. Pain in the affected lymph-nodes, if present at all, is not marked. A slight increase of the temperature and malaise may be present for a few days, but the condition is usually not serious enough to induce the patients to lie down. Attacks of this kind are therefore often designated “ambulatory” plague.

In more-marked cases of pestis minor the affected lymph-nodes may be slightly or moderately painful, but signs of marked inflammation and particularly reactions in the surrounding tissues remain absent. Slight or moderate fever, headache, and some prostration are present and there may also be some conjunctival congestion. However, these acute symptoms do not persist for longer than about a week, and even during this period the patients are up and about for most of the time or even are not bedridden at all.

The affected lymph-nodes may apparently become completely healed or may remain indurated or may suppurate.

No agreement exists as to the frequency of pestis minor. While some observers considered this form of plague to be rare, others maintained that cases were frequent, particularly at the onset and at the end of outbreaks. It must be admitted that, since pestis minor patients rarely seek medical relief, attacks of this form of plague may be easily overlooked. On the other hand, one must fully agree with Rogers & Megaw that in localities where plague is expected there might be a tendency to suspect pestis minor whenever instances of gland enlargement are found, even though they might be due to other causes. Rogers & Megaw postulated therefore, with great reason, that in all doubtful cases of pestis minor a laboratory confirmation of the diagnosis is indispensable.

It would appear that pestis minor was more frequent in some plague outbreaks or plague areas than in others. Macchiavello, for instance, maintained that “epidemics of ambulatory plague have been observed in the north-east of Brazil and in Ecuador.” In an earlier report on plague in north-eastern Brazil, he stated that benign plague was called there “ingua de frio” (cold bubo) and also “febre de caroço” (stone fever), though the latter term was originally applied to ordinary bubonic plague. He added that “ingua de frio” appears generally in children under 15, and is characterized by mild and transitory symptoms, monoglandular swellings without much inflammation or pain, and a tendency of the gland swelling to become ligneous, and to reoccur, or to be reabsorbed. It appears
sporadically where plague is endemic and tends to disappear when epidemic. There is no special relationship between cases, though they occasionally appear in small foci. In some of these foci the first cases are severe and even fatal, the later ones becoming increasingly milder. There is a history of previous plague in *rattus*; and it is possible that this type of plague may have some relation to an attenuated virus in fleas”.

It is of interest to add that Kamal, during a plague epidemic in Egypt, also found a number of children who, showing enlarged and sometimes tender lymph-nodes but no other signs, apparently suffered from “ambulatory” plague.

**FIG. 31. CERVICAL BUBO**
Dealing with the problem of pestis minor, Mathis & Pons\textsuperscript{127} pointed out with great reason that "even though a plague patient may be up and about at the onset of the disease, one should not consider the prognosis as favourable. In such cases the appearances are often fallacious and the patient may succumb in a few hours".\textsuperscript{c}

A few observers, such as Ilvento & Mazzitelli,\textsuperscript{86} Leger & Baury,\textsuperscript{106} and Nikanoroff,\textsuperscript{148} reported the presence of plague bacilli in slightly enlarged but not inflamed lymph-nodes of healthy individuals; animal experiments carried out in most of these instances proved the \textit{P. pestis} strains in question to be fully virulent. Whether it is legitimate to consider these persons as healthy carriers of \textit{P. pestis}, as the above-mentioned observers did, seems questionable. True enough, the individuals concerned had no history of a manifest attack, but it is difficult to believe that the invasion of their lymph-nodes by virulent plague bacilli produced no reaction whatsoever. The proposal of Leger\textsuperscript{105} to label such instances as cases of pestis levissima deserves, therefore, serious consideration. It would seem that Sic\textsuperscript{174} actually observed a case of this mildest form of bubonic infection. He concluded that the individual concerned "was not a healthy carrier because of the attenuated reaction in the involved lymph-nodes".\textsuperscript{d}

\textit{Chronic forms}

Instances of chronic bubonic plague have been described by a few authors. Simpson\textsuperscript{175} referred in this connexion to cases "... in which the disease runs a chronic course from the commencement. The patient may walk about notwithstanding a certain amount of indisposition and catarrh and yet succumb later to the disease, and be found the subject of abscesses containing plague bacilli in the lungs, liver, and spleen. This chronic type closely resembles that found in lower animals ".

According to a statement made by Rebagliati\textsuperscript{163} in 1939, atypical cases of plague polyadenitis with a prolonged course and subfebrile temperatures were met with in Peru.

A similar syndrome was discovered in 1941 by Macchiavello\textsuperscript{116} in northeastern Brazil. As he summarized in 1951,\textsuperscript{118} this "multiglandular plague fever" was

"... characterized by a septic fever curve, attenuated plague bacteriemia, and a secondary septicemia caused by nonvirulent microorganisms. The course is prolonged for a month or more, during which time there appear successively from 4 to 14 or more variously situated large buboes. There are alopecia, cachexia, and slow recuperation with a prolonged convalescence; or death may follow ".

Macchiavello\textsuperscript{118} also referred to a chronic benign form of plague in northeastern Brazil, in which

\textsuperscript{c} "... le fait que le malade continue au début de son infection à marcher et à se livrer à ses occupations n'autorise pas à porter un pronostic favorable. Il ne s'agit le plus souvent que d'un aspect trompeur, et le sujet peut être enlevé en quelques heures."

\textsuperscript{d} "... ce n'était pas un porteur sain, étant donné la réaction ganglionnaire atténuée qu'il présentait."
"the buboes, whether suppurated or not, or whether softened or woody, showed periodic inflammatory exacerbations which did not interfere with the patients' occupations, although they caused a certain degree of debility and anemia".

A case of chronic plague recorded by Durand in 1931 appears to have been similar in nature.

It is of importance to add that a few observers, such as Choksy, Dujardin-Beaumetz & Joltrain, Durand & Conseil, Leger & Lhuerre, and Vagedes, found convalescents harbouring \textit{P. pestis} for periods of up to 12 months (one case of Durand & Conseil) in the primarily affected or even in secondary invaded lymph-nodes. In some of these instances, particularly that described by Leger & Lhuerre, it is not easy to decide whether the individuals concerned had actually become convalescent carriers or continued to suffer from benign chronic plague. The line of distinction between these two conditions seems not well defined.

\textit{Fulminant forms}

As has been stated earlier, only a difference in degree and not one in kind seems to exist between (a) instances in which an entrance of the infection through the skin or the mucous membranes leads merely to a slight reaction in the regional lymph-nodes, followed by a rapid entrance of the causative organisms into the blood-stream, and (b) the so-called primary septicaemic form of plague where clinically manifest reactions in the lymph-nodes remain absent, and the fulminantly progressing morbid process seems to produce from the first a most serious generalized infection.

A recent reviewer while accepting the classification of plague in two main forms only, namely (1) bubonic plague including the septicaemic type and (2) primary pneumonic plague, pointed out with much reason that the former name was hardly suitable to designate cases of the disease in which buboes were incapable of detection. He proposed, therefore, the terms "zootic" and "demic" to designate the two above-mentioned forms of plague. However, desirable though it would be to adopt this terminology, it would seem rash to make general use of these startlingly new names in this monograph.

There seems no need to enter at the present juncture into an exhaustive description of the clinical features met with in the fulminant types of bubonic (or, one should rather say, "zootic") plague, because the symptoms and signs of a most severe generalized infection which dominate, or even quite overshadow, the clinical picture in such cases have been fully dealt with earlier. As a rule, high fever is present in the early stage of the disease but, as has been noted, particularly severe and rapidly fatal cases are met with in which the sufferers fail to react even in this respect. The activity of the heart is impaired from the first and deteriorates incessantly as the disease progresses. Lung oedema often becomes marked in the terminal stage. Delirium and/or coma are commonly present.
Skin haemorrhages often become conspicuous and, owing to the presence of submucous haemorrhages, the stools, which as a rule are diarrhoeic, and also the urine may show an admixture of blood.

As a rule, the patients suffering from what is commonly called primary septicaemic plague succumb so rapidly that there is no time for the development of complications. However, some authors, for instance Wright, have stated that such patients can survive long enough to develop pneumonic foci, and it was sometimes claimed that they were then apt to produce primary pneumonic infection in their contacts. Claims of this nature ought to be viewed with scepticism, both because some of the authors seem to have made no clear distinction between secondary and apparently primary plague septicaemia and because the presence of lung oedema rather than that of pneumonic foci might have been responsible for instances of respiratory infection in contacts of such patients. However, in view of the fact that no sharp line of distinction exists between cases with ill-defined buboes and those where no manifest reaction can be detected in the lymph-nodes, one should not be categorical in denying the possibility that lung or other complications might develop in patients apparently suffering from primary septicaemic plague.

Until recently, antibiotics became available for the treatment of plague, only few instances of recovery from the primary septicaemic form have been recorded. Moreover, as aptly stated by Robic during the discussion of a case reported by Le Gall et al., one should not be rash in diagnosing this form of the disease because with the aid of the now-available refined methods of blood cultivation the presence of a few plague bacilli in the blood has been detected. In Robic’s opinion “one is entitled to speak of septicaemia only when the defence mechanisms of the body can no longer prevent the multiplication of the organisms in the blood”.

This consideration ought to be kept in mind when evaluating the statement of Macchiavello that it has recently been possible

“... to confirm the existence of benign or ambulatory cases of plague septicemia (Peru), characterized by the absence of buboes, a feeling of intoxication, oneiric delirium, and at times fever which does not last more than one or two days. The usual diagnosis of these patients is “benign grippe” but blood cultures revealed the presence of very attenuated strains of plague bacilli”.

Primary Pneumonic Plague

Clinically, as well as pathologically, three main types of primary pneumonic (or, one should rather say, pulmonary) plague may be distinguished:

(1) A type characterized by the development of well-marked pneumonic foci (“typical” primary pneumonic plague).

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*e “Il n’y a septicémie que lorsque la défense de l’organisme ne s’oppose plus à la multiplication et à la pullulation des germes dans le sang.”*
(2) A transitory form with slight pneumonic lesions.
(3) A form in which acute congestion and usually lung oedema are marked but where, though plague bacilli abound in the deeper respiratory tract and in the lungs, no consolidation is present in the latter.

The clinical features present in these three types may thus be described:

Typical form

It is important to note that even though typical primary pneumonic plague is characterized by the evolution of more-or-less marked lung involvement, the disease is ushered in by a period lasting 20-24 hours, during which symptoms and signs of a serious generalized infection predominate or are often even the only symptoms present.

The onset of this initial "closed" stage of the disease is usually sudden. High fever appears quickly, often initiated by a feeling of chilliness or slight shivering, far more rarely by marked chills. As in severe attacks of bubonic plague, the pulse soon becomes rapid and often also dicrotic. The tension of the pulse may become lowered even in this early stage of the disease and some arhythmia may become manifest (Durand & Conseil 37). The patients feel prostrated and complain of headache which often becomes intense. However, signs of involvement of the respiratory system are still insignificant or even altogether absent: there is but little or even no cough; expectoration, if present at all, is scanty and uncharacteristic; smear examination of the sputum or saliva shows few, if any, suspicious bacilli.

About 20 to 24 hours after onset of the disease, signs of lung involvement begin to become manifest. Cough appears or gets more frequent and leads to the expectoration of a sputum which may at first be purely mucoid or muco-purulent but soon shows an admixture of specks or streaks of bright-red blood. In typical cases, considerable amounts of a uniformly pink or bright-red coloured sputum, which may show a uniform aspect and a consistency comparable to that of raspberry syrup, or may be foamy, are soon voided. Though sometimes becoming jelly-like or even rather thick and viscid, the sputum of pneumonic-plague patients never reaches the degree of viscosity which is customarily observed in croupous pneumonia (Kasai 65).

Tachypnoea and dyspnoea are as a rule present and become more marked as the disease progresses, but though the patients may complain of pain and a restricted feeling in the chest, they usually seem to suffer far less than one would fear. Summarizing his experiences in the 1920-1 Manchurian epidemic, Chun 20 stated in this connexion that:

"There is not much pain in connection with plague pneumonia in contrast to ordinary pneumonia, though a few exceptions were observed. Nearing death, the patient is very short of breath, mildly delirious, often tries to sit up or struggles to go out into the open air. He is not conscious of much suffering owing to the impaired cerebration".
The physical signs found when examining the chests of the sufferers are often also far less marked than one would expect from a consideration of the postmortem findings. As summarized by Chun:

"The physical signs in the lungs are often slight, even in cases well advanced in the disease. On percussion, dulness is often absent, and the vocal fremitus and resonance unchanged. In a small proportion of cases, however, localized areas of dulness may be distinguished. On auscultation, râles are frequently not heard except before death. When present early in the disease, they are usually of the fine variety. Numerous râles are heard late in the disease, due to the oedematous condition of the lungs. Feeble respiratory sounds or pure tubular respiration over small areas are common."

While it is not uncommon to find a dry pleuritic rub present in pneumonic-plague patients, instances where the presence of a fluid pleuritic exudate could be ascertained through physical examination seem to be exceptional. In one such case, described by Durand & Conseil, marked dullness was found over the right side of the chest. At autopsy, about 500 g of a slightly turbid fluid, which contained fairly numerous plague bacilli, were found in the right pleural cavity besides a fibrinous exudate covering the visceral and parietal pleurae.

The symptoms and signs generally found in patients suffering from primary pneumonic plague are identical with those met with in other severe forms of the disease. Signs of rapidly progressing heart failure and usually also nervous disturbances are marked. Loss of co-ordination of the voluntary muscles, which tended to render the gait of the patients unsteady, was often observed in the Manchurian epidemics.

It is of great importance to realize that even in the group of primary-pneumonic plague cases, called typical for the sake of classification, marked variations of the clinical picture are found.

The following observations deserve notice in this connexion:

(a) Duration of illness. Though as noted before, pneumonic-plague patients, not or inadequately treated, usually survive not longer than a few (2-4) days, a number of instances of longer survival have been recorded. With rare exceptions, these longer illnesses did not exceed 9-10 days, but it is noteworthy that the serum-treated patients observed by Lhuerre & Leger and Uriarte et al. respectively both survived for about a month.

(b) Atypical sputum. Several observers drew attention to the fact that sometimes the sputum of pneumonic-plague patients was of a yellowish or rusty colour similar to that found in croupous pneumonia. As a rule, the sputum did not long retain such an aspect, but occasionally a rusty expectoration continued throughout the illness. In exceptional cases, the sputum, though containing numerous *P. pestis*, remained salivary or mucous throughout the disease (Osborn & Chervenzoff, quoted by Wu Lien-teh).
Wagle & Bedarkar stated that in their experience of pneumonic-plague patients with lobar involvement "the sputum has been found stringy in character, often rusty or mixed with blood and difficult to expectorate".

Similar findings seem to have been made also by Lewin et al. in an atypical case of pneumonic plague with lobar involvement.

A few instances are on record where pneumonic-plague patients had a profuse haemoptysis so that the presence of tuberculosis was thought of (Wu Lien-teh; Goldstein).

Wu Lien-teh adduced evidence to show that the sputum of pneumonic-plague patients tended to lose its characteristic aspects when the sufferers recovered. In exceptional cases, the non-characteristic sputum of convalescents continued to harbour \textit{P. pestis}, in one instance, observed by Gotschlich, up to the 41st day after onset of the illness.

\textit{(c) Frequency of lobar pneumonia.} While, as noted above, generally speaking lobar involvement of the lungs seems to be less frequent in pneumonic-plague patients than lobular foci, Hennessey stressed the fact that 14 of the 19 cases of primary pneumonic plague seen since 1933 at postmortem examinations in Kampala, Uganda, had shown a lobar type and that in those cases "septicaemia was most uncommon." It appeared also that—evidently in contrast to previous observations—a vast majority of the sufferers seen clinically showed lobar pneumonia. Hennessey argued that this change in type was due to a reduction of the virulence of the \textit{P. pestis} strains in Kampala and maintained that:

\textquote{This conclusion is supported by the protracted course of the illness in some cases, durations of 7 to 9 days being occasionally encountered, and by the fall in the plague death-rate in this district since 1935 (712 deaths during the period 1936-39, as against 2,488 deaths during the previous four-year period)  .}

Wright, dealing in 1943 with the recent plague situation in Nairobi, Kenya, also pointed to an increased frequency of lobar involvement of the lungs in pneumonic-plague victims. He maintained that such cases "perhaps are a form that becomes prevalent after the epidemic has begun to wane. It may perhaps be that, as has been shown to be possible experimentally, the organism may become particularly prone to affect the lung".

Interesting as the contentions of Hennessey and Wright are, they are not in accord with the observations made at the end of pneumonic plague outbreaks in China.

\textit{(d) "Spontaneous recovery".} While even in the past occasional success was obtained when patients suffering from primary pneumonic plague were treated with plague serum or—more recently—with sulfonamides, and nowadays spectacular results have been obtained with the aid of antibiotics, instances of recovery of such patients who had not been specifically treated are few and far between. However, a few cases of this kind have
been recorded by Wu Lien-teh\textsuperscript{216} and more recently by Estrade\textsuperscript{39} and Girard.\textsuperscript{60} A further patient, mentioned by Williams,\textsuperscript{209} who showed signs of pneumonia with delayed resolution and \textit{P. pestis} in his uncharacteristic sputum but eventually recovered, was treated with Suramin (a complex sulfonated urea derivative known under various trade names and much used in the treatment of African trypanosomiasis).

The evidence for the existence of mild cases of pneumonic plague is not convincing. No instances of this kind were observed during the Manchurian epidemics of 1910-1 and 1920-1. An individual admitted during the 1921 outbreak at Vladivostock with a history of two weeks' headache and slight cough showed \textit{P. pestis} in his sputum. However, as pointed out by Wu Lien-teh,\textsuperscript{216} only smear examinations seem to have been made and, moreover, it was doubtful whether this man was a plague carrier or was suffering from minor plague. It should be noted in this connexion that nine instances could be found in the available literature in which the presence of virulent \textit{P. pestis} was confirmed in the sputum or fauces of apparently healthy individuals who had been in contact with pneumonic-plague patients (Wu Lien-teh;\textsuperscript{216} Girard;\textsuperscript{61} Suarez;\textsuperscript{189} Tieh et al.\textsuperscript{192}).

It is important to add that occasionally, in pneumonic as well as in bubonic plague, the apparent absence of serious signs may be deceptive, the sufferers soon afterwards succumbing. For instance, Raynaud (quoted by Wu Lien-teh\textsuperscript{216}) referred to one patient who was found attending his customers and was able to stand while being auscultated, yet died the next morning.

\textit{Slight lung involvement}

Practically all workers who saw comparatively large numbers of pneumonic-plague patients observed that a minority of the sufferers, though showing serious general symptoms and signs, had but slight or even almost no cough and expectoration. There can be no doubt that a close relation exists between these clinical findings and the presence of slight pneumonic changes found at autopsy in a minority of primary-pneumonic-plague victims.

In some of the patients with apparently slight lung involvement observed by the present writer in China, spontaneous cough was practically absent so that at first glance these sufferers, who were in a serious general condition, seemed to be victims of "septicaemic" rather than of pneumonic plague. However, they produced typical blood-stained sputum when urged to cough and expectorate.

Similar cases were probably seen by Gale\textsuperscript{53} in South Africa. He stated that cough was present in nearly all his patients, though it was generally very slight and productive of little, if any, sputum. However, "some patients, urged to do so, produced sputum from the lungs much greater in
amount than they had done when left to themselves". Gale added that "physical examination revealed signs of small basal patches of consolidation in several cases which had practically no cough and no sputum, whereas in others with more cough (but scanty sputum) there were practically no pathological signs in the chest". In Gale’s opinion these patients, all but one of whom died,

"... seemed to succumb to toxaemia, possibly septicaemia, which, *inter alia*, robbed them of the normal vigour of the cough reflex. Thus, failing to produce sputum in any great quantity, they were not as infective as pneumonic patients usually are".

Interesting as this hypothesis is, it cannot be considered as generally valid because patients with frank primary pneumonic plague, through often showing signs of marked toxaemia, usually continue to cough and expectorate freely until they die.

Cases probably similar to those of Gale were observed recently by Seal in Calcutta. He noted that, though all his patients showed more-or-less marked signs of lung involvement, cough was "mostly non-productive". This seemed the main reason why many of the contacts escaped infection.

While the observations recorded above leave no doubt as to the occurrence of cases with slight pneumonic manifestations, no sharp lines of demarcation seem to separate this group from that of frank pneumonic plague, on the one hand, and from the type without lung consolidation, on the other hand.

**Absence of lung consolidation**

As summarized by Wu Lien-teh, the occurrence of cases without cough and expectoration had been recorded by some earlier observers of pneumonic plague epidemics, and the occasional absence of lung consolidation had been noted at the autopsy of victims of this form of the disease. However, not much attention was paid to such observations until Wu Lien-teh et al., finding instances of this kind to be apparently frequent at the end of the 1921 outbreak at Harbin, Manchuria, pointed to their epidemiological importance.

Girard, whose investigations have contributed much to the knowledge of "pulmonary" plague, concluded in 1929 that

"these abnormal forms ... develop like septicaemic plague cases. However, they are preceded by a closed pulmonary stage from which the organism passes into the bloodstream but never appears in the expectoration since we could not detect it there by guineapig experiments. The presence of plague bacilli in lung smears, fewer than in typical pneumonic forms, but more numerous than in liver smears (where they are sometimes altogether absent) testifies that the lungs really are involved. These abnormal forms are
therefore pulmonary in their etiology (direct contagion) but septicaemic in their evolution”.

An excellent clinical description of this “pulmonary” form of plague was given by Christie in a report, rendered after the 1910-1 Manchurian epidemic, wherein he stated that “about 10 per cent of all cases in this epidemic seem to have been what is called septicaemic—that is to say without any pneumonic manifestation. The attack is sudden and death rapid; nervous symptoms are prominent; there is giddiness and staggering, the patient often falling by the roadside; great prostration is also present and the patient soon passes into a comatose condition. The face after death is very dark. Few of this type of the disease have died in plague hospitals, as the course of the disease is too rapid.”

Rapid death of patients who, though infected through contact with pneumonic-plague sufferers, showed signs of a generalized infection, but not of pneumonia, was noted by some other observers as well. For instance, Delbreil, apparently referring to six such patients, stated that they died in 2-3 days, whereas the sufferers showing the usual features of pneumonic plague died after 2-6 days.

However, patients suffering from “pulmonary” plague did not invariably succumb rapidly. Two such sufferers, observed by Girard and by Wright respectively, survived for four days. That in exceptional cases “pulmonary” plague patients might survive even longer, seems to be suggested by an observation of Goldstein: the man in question was admitted with a history of four days’ illness on 8 August 1941 and, having shown no physical signs of pneumonia and having produced no sputum died on 11 August. Fever was high but (as in some other plague patients seen at the time) the pulse rate was low, so that a tentative diagnosis of typhoid was made. However, as Goldstein noted without giving details, “plague pneumonia” was found to be present at autopsy.

In Girard’s opinion “the duration of illness in such cases is dependent upon the time at which septicaemia appeared: if this develops rapidly, death occurs quickly; if more slowly, the disease may last four days”.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

As has been emphasized earlier, the diagnosis of plague must rest on the results of laboratory examinations rather than on clinical findings, except when well-marked cases are dealt with during fully confirmed
outbreaks. However, a clinical prima facie diagnosis, always important from the viewpoint of plague prevention, is now also of utmost therapeutical importance because, particularly if sulfonamides only are available, optimal results will be obtained only if treatment is started as soon as the presence of the disease is assumed on clinical grounds. To wait for laboratory confirmation might, particularly under rural conditions, render futile attempts to save the patients. It is unfortunate, therefore, that the presumptive clinical diagnosis of plague is beset with some difficulties.

It would seem at first glance that the history of the illness given by the patients themselves or by their families should be of great value in arriving at a presumptive diagnosis of plague, both by showing that the sufferers fell suddenly ill a short time ago and by referring to characteristic signs such as the presence of painful buboes or the expectoration of blood-stained sputum. Ample experience has shown, however, that the patients themselves and their families often go to almost incredible lengths to mislead the medical staff. It is essential therefore to insist in any case where there is reason to suspect plague, regardless of the history given, upon a thorough examination of the patients, including an inspection of all regions of the body where buboes might be present and, whenever pneumonic plague might come into question, to insist that the patients cough and expectorate.

As shown by many experiences, the danger of misdiagnosing even well-marked plague cases is great in areas where the medical workers are unfamiliar with this disease. This holds true not only of regions where plague is altogether absent, but also of localities where the infection is present among wild rodents but rarely spreads to human beings. The danger to which the latter situation might easily lead is well illustrated by the recent appeal of Link 114 to the physicians in the western States of the USA "to regard all cases of inguinal and axillary adenopathy with a high index of suspicion and institute immediate treatment with streptomycin and sulfadiazine ".

As noted before, the medical workers in plague areas may be prone, on the other hand, to class any patient with enlarged lymph-nodes as suffering from plague. It has been urged, therefore, that in all such cases adequate laboratory investigations be made.

Earlier, emphasis has been laid upon the fact that, even in severely-affected plague patients, general symptoms and signs which are usually present may be conspicuous by their absence. Thus the sufferers, though fatally infected or even not far from death, may not only feel, but even seem, quite well. Fever may be low or absent, and the pulse rate may be normal or in exceptional cases there may even be a bradycardia, suggestive of typhoid in cases where localized signs of plague infection remain absent. Generally speaking, the sole presence of general symptoms and signs renders a clinical differentiation of plague from other acute febrile diseases, particularly from pernicious malaria, difficult.
A differentiation of plague from infections caused by pseudotuberculosis bacilli or other members of the genus *Pasteurella* is not as difficult as it might seem at first glance. Instances of human infection by pastourellae other than *P. pestis* are rare, invariably remain solitary, and so far have been recorded in plague-free rather than plague-affected areas. Moreover, as a rule, the clinical picture produced by these infective agents is markedly different from that in plague.

Infection with *P. pseudotuberculosis* produces in man almost always a typhoid-like syndrome with icterus, a sign usually exceptional in plague (Dujardin-Beaumetz; 31 Topping et al.; 193 Hässig et al.78).

Infections due to other pastourellae according to Tricot & Gauthier 194 never produce a haemorrhagic septicaemia but result in purulent pleurisy, meningitis, myositis, urinary infections, or conjunctivitis. Some instances have also been recorded where the scratches or bites of cats, or the bites of dogs, produced local lesions due to pastourellae.

It will be seen that serious differential-diagnostic difficulties would be likely to arise only in cases of primary conjunctivitis or meningitis. As noted before, instances of primary conjunctival plague or apparently primary plague meningitis are rather rare.

Though the ulceroglandular, oculoglandular, and glandular types of tularaemia (Francis 52) might resemble plague, a differential diagnosis on clinical and epidemiological grounds or through laboratory examinations is not difficult. As summarized by Wilson & Miles: 210

"In the differential diagnosis [of tularaemia] from plague, account should be taken of the absence of pus at the site of infection, the greater variability in size of the granules in the spleen, the rarity with which the lungs are affected, and the failure of the organism to develop on ordinary media."

**Well-Marked Bubonic Plague**

A clinical prima-facie diagnosis of well-developed “typical” bubonic plague is easy because, as has been described earlier, the symptoms and signs present in such cases, particularly the manifestations in the primarily affected lymph-nodes, are rather characteristic.

In contrast with most other conditions which produce an acute inflammation of peripheral lymph-nodes, reactions at the portal of entry of the infection are usually not manifest in bubonic plague. Moreover, if present, the appearance of these local processes is as a rule quite characteristic. This holds true not only in the cases where carbuncles develop but also if pus-filled vesicles form at the site of invasion because their inconspicuousness as contrasted with the marked reactions evolving in the regional lymph-nodes is rather peculiar. Plague bacilli, though sometimes scanty, may invariably be demonstrated in the pus of such vesicles.
Besides usually leading to marked reactions at the site of infection, the manifestations produced by an invasion of other infective agents in the regional lymph-nodes and in the system in general are as a rule less stormy and less rapidly progressive than those caused by *P. pestis*. Commonly, therefore, no differential-diagnostic difficulties arise in cases of well-marked and fully developed bubonic plague. However, should doubts exist, puncture of the affected lymph-nodes should be resorted to, carried out preferably according to the technique recommended by Girard, which renders it easy to use the puncture material for cultivation and/or animal experiments. As noted earlier, cultivation of the material on plain media is essential in plague areas where the simultaneous presence of tularemia might cause differential-diagnostic difficulties.

It is important to add that, according to what has been discussed in chapter 5, bubo puncture, though sometimes necessary, is by no means an ideal method of confirming the diagnosis of bubonic plague. It should be replaced as much as possible by blood cultivation or by serodiagnostic procedures. Adequate examinations of the blood are particularly valuable because it is of great prognostic and therapeutic importance to establish whether or not bacteraemia is present.

As stated before, it is often difficult to decide whether or not secondary pneumatic foci have developed in bubonic-plague patients. Goldstein recommended the method of lung puncture to ascertain the presence of lung consolidation in patients with bubonic or ill-defined pneumatic plague and described his technique thus:

"Choosing preferably a site where there appeared to be some dullness on percussion, I inserted a needle in several directions and made a smear from the fluid obtained by aspiration."

Even if it were possible to consider this method as safe, its reliability seems questionable. It might give false positives in cases where pneumatic foci are absent but bacteraemia is marked. On the other hand, as proved by an observation of Goldstein himself, it is bound to give false negatives in cases where, instead of the consolidated, non-consolidated parts of the lungs have been punctured. It would be preferable, therefore, to resort in such cases to x-ray examinations of the lungs which, as will be shown later, have given most satisfactory results in cases of primary pneumatic plague.

In the usually infrequent cases of tonsillar plague, as well as in the rare cases of primary eye plague, typical buboes as a rule develop in the regional lymph-nodes, thus facilitating a presumptive clinical diagnosis. In view of the fact, however, that an invasion of the faucial mucous membranes or of the conjunctivae by other micro-organisms may produce syndromes similar to those caused by *P. pestis*, thorough laboratory examinations are indicated whenever the presence of these forms of plague is assumed. This
is particularly important in the case of tonsillar plague, the clinical appearances of which may be quite similar to those produced by the diphtheria bacillus.

Exhaustive laboratory examinations are also indicated in the cases where the presence of a primary plague meningitis is assumed, particularly because morbid processes of such a nature may be produced by pasteurellae other than \textit{P. pestis}.

\textbf{Pestis Minor}

As has been described above, the clinical findings in pestis minor are apt to be so ambiguous as to render a definite diagnosis impossible. It is essential, therefore, to confirm the presence of plague through laboratory examinations. Puncture of the affected lymph-nodes, since it causes little discomfort to the patients, may be freely used for this purpose. Unless the condition of the patients takes an unexpected turn for the worse, it is legitimate to await the results of laboratory tests before commencing specific treatment.

\textbf{Fulminant Type of Bubonic Plague}

The difficulties of arriving at a presumptive diagnosis of what is commonly called "primary septicaemic" plague are considerable but should not be overrated, chiefly because:

(1) almost invariably, cases of this type of the disease do not appear independently but in association with bubonic plague cases; and

(2) even a short period of continued observation shows a rapid deterioration of the condition of the patients which is quite uncommon in most of the diseases with which "septicaemic" plague might be confused at first glance.

The present writer for one would not hesitate, therefore, to say that apart from incipient pneumonic plague and from "pulmonary" plague, malaria—which is often endemic in the areas where plague exists—is the only disease really difficult to differentiate from "septicaemic" plague.

It might seem at first glance that examination of suitably stained blood-smears for the presence of malaria parasites ought to be an easy and rapid means of differentiating between this disease and plague. However, it is often difficult to carry out such examinations rapidly enough under rural conditions. Far more important still, for various reasons it is quite often difficult, and sometimes even impossible, to prove the presence of parasites in the blood of patients suffering from the pernicious type of malaria which causes the main differential-diagnostic difficulties. Moreover, as confirmed by observations like that of Le Gall et al.\textsuperscript{103} "septicaemic"
plague may appear in patients who suffer from manifest malaria. Signs of past malaria infection in patients developing "septicaemic" plague may also confound the issue. Thus Yenikomshian recorded the case of a 12-year-old girl seen in a comatose condition who, because she had come from a malarial district, had had malaria in the past, and showed an enlarged spleen, seemed at first glance to suffer from pernicious malaria. However, the presence of "septicaemic" plague was proved through cultivation of the blood and also through spleen puncture performed after death.

As confirmed by observations of the present writer, an adequately prolonged examination of the blood-smears stained for the purpose of detecting malaria parasites occasionally reveals the presence of *P. pestis* even in incipient "septicaemic" plague. Since, however, such positive findings are rather rare and since, moreover, the apparent absence of malaria parasites from the smears is not fully conclusive, a preliminary decision on whether "septicaemic" plague or malaria is present has quite frequently to be reached on clinical grounds. Pending further observation of the patients, or until the results of repeated smear examinations or even of blood cultivation have become available, it is sometimes impossible to form a definite opinion. Whether it is justifiable, under such conditions, to begin plague treatment immediately seems debatable. As far as the present writer can judge, one ought to initiate such treatment under all circumstances as soon as there is reason to suspect the presence of fulminating plague.

**Primary Pneumonic Plague**

*Incipience*

As will be gathered from the description of the clinical features of pneumonic plague given earlier, only general signs of serious illness develop during the initial stage of primary pneumonic plague which lasts 20-24 hours. The difficulties of arriving at a diagnosis during this first stage of the disease are, therefore, similar to those met with in the so-called "primary septicaemic" form of plague. However, the fact that primary pneumonic plague affects exclusively persons who have had recent contact with patients suffering from plague with lung involvement is of utmost diagnostic importance. Indeed, it is a priori likely that any person who falls ill soon after such contact, and shows fever and other general signs suggestive of the presence of an infectious disease, suffers from incipient pneumonic plague.

Since, as has been stated before, smear examination of the sputum or saliva carried out during this stage mostly yields inconclusive or even negative results, until quite recently a prima facie diagnosis of incipient
pneumonic plague had to be made on clinical grounds. Indeed, one might almost say that one could merely suspect but not really diagnose the evolution of this disease.

However, recently Estrade, making a series of x-ray examinations in the case of a pneumonic-plague patient whom he was able to cure, found with the aid of this method evidence of the formation of pneumonic foci quite early in the disease. In his opinion, therefore, "the importance of the x-ray picture at the time of the first rise in temperature leads one to think that systematic x-ray examinations of persons particularly exposed to contagion could be a means of diagnosing incipient pulmonary plague."

As can be gathered from recent publications by Mercier and by Mercier & MacCrumb, this postulation has been confirmed. The value of this new diagnostic method for the treatment and control of pneumonic plague as well as for further studies on the pathogenesis and pathology of this disease can hardly be overrated.

Manifest stage

Owing to the presence of general symptoms and signs of a serious and rapidly progressing illness, associated with often comparatively insignificant signs of lung involvement and the expectoration of a peculiar blood-stained sputum, it is easy to make a presumptive diagnosis during the manifest stage of primary pneumonic plague. Since the sputum teems with plague bacilli, it is also not at all difficult to confirm this diagnosis through laboratory examinations. In view of the fact that, in rare cases, an invasion of the lungs by micro-organisms other than the plague bacillus produced morbid features more or less similar to primary pneumonic plague, adequate culture methods and/or animal experiments should be resorted to in addition to smear examinations. However, one might be less rigid when dealing with quite typical cases during a fully established pneumonic plague epidemic.

If adequate laboratory tests are applied, there is also no difficulty in arriving at a correct diagnosis in cases where unusual features, such as a rusty or otherwise atypical aspect of the sputum or lobar instead of the usual lobular type of lung involvement, are present. It is true that sometimes cases are met which at first glance resemble croupous pneumonia. However, the rapid deterioration of the general condition of the patients, becoming manifest in such atypical cases as well as in the usual type of pneumonic plague, ought to suggest the necessity for thorough laboratory investigations. As has been said before, plague cases will hardly ever be overlooked, if the possibility of their occurrence is kept in mind.

\[h\] "... l'importance de l'image radiographique dès la poussée initiale de température permet de penser que la radiographie systématique des personnes qui ont été particulièrement exposées à la contagion pourrait, le cas échéant, fournir une méthode de diagnostic précoce de la peste pulmonaire."
As previously noted, probably owing to the presence of slight pneumonic changes, spontaneous cough and expectoration may be practically absent in some patients suffering from primary pneumonic plague. In order not to overlook the presence of this form of the disease, one should urge all plague patients with features suggestive of a generalized infection to cough and to expectorate. No doubt x-ray examination would be most helpful in such cases.

"Pulmonary" Plague

Though the information available in regard to the clinical aspects of the form of lung plague in which no pneumonic foci develop is scanty, there can be no doubt that the clinical manifestations present in such cases correspond to those met with in patients suffering from what is called primary septicaemic plague. It follows that the considerations and methods found useful for the diagnosis of primary septicaemic plague will also prove valuable for the recognition of the "pulmonary" form. Comparatively speaking, the latter is less difficult to diagnose, because it develops invariably in persons who have had recent contact with pneumonic-plague patients and usually, or perhaps even always, appears only after the occurrence of typical cases of primary pneumonic plague.

TREATMENT

Though attempts to cure plague may be said to date back as far as the history of this disease, the first milestone on the road to real success was reached only when, in 1896, Yersin began to use a therapeutic serum produced through immunization of horses with P. pestis. From that time onwards until recently, administration of sera prepared either according to Yersin’s original procedure or in other ways remained the only method which, because it gave a modicum of success in the case of the bubonic form, was used on a large scale for plague treatment. The use of sulfonamides, commenced about 14 years ago, greatly improved the therapeutic results in bubonic plague. Quite recently, a most outstanding success in the treatment of all forms of the disease has been attained with the aid of streptomycin and some other antibiotics. Indeed, the results obtained with these therapeutic agents have been so consistently good, even in most severely-affected patients, as to relegate the sulfonamides to the second rank in the treatment of bubonic plague and, as will be discussed later, to render rather questionable the necessity of using the specific sera any more.
Serotherapy

It is difficult to deal comprehensively with the results obtained with serum treatment of plague, not so much because an enormous literature deals with this subject, but mainly because the recorded results are often not comparable. The reasons for this incomparability are as follows:

1. While the observations of some of the workers were based upon sufficiently large samples to show statistically significant results, others reported upon limited numbers of treatments or merely upon a few cases.

2. The serum dosages used by the different workers varied widely and sometimes insufficient doses were used.

3. Marked differences may exist in the efficacy of sera prepared by different methods.

4. The loss in potency which sera undergo in storage may vitiate the results when different lots of the same serum or different sera prepared at different times are compared.

5. Ample observations, recently confirmed by the exact tests of Sokhey et al.\textsuperscript{184} referred to below, have shown that the results of serotherapy, as well as those of other methods of specific plague treatment, depend largely upon the absence or presence of bacteraemia in the subjects treated. Hence the results obtained in countries or in outbreaks where a benign type of plague prevailed are not comparable with those obtained during plague manifestations characterized by a prevalence of serious types of the disease frequently resulting in bacteraemia. One must agree with Sokhey and his colleagues that only the results obtained in patients who show evidence of bacteraemia on admission form standards for assessing the efficacy of different methods of plague treatment. Lack of indications regarding the presence or absence of bacteraemia in the treated patients often renders it impossible properly to evaluate and compare the older records on the serum treatment of plague.

It seems indicated, therefore, that one should assess the results of plague serotherapy in a general manner instead of scrutinizing the records of the individual workers in detail.

General agreement exists that it was but rarely possible to save patients suffering from primary pneumonic plague through serum administration alone. In addition to some more-or-less creditable instances of this kind recorded by Wu Lien-teh\textsuperscript{216} in 1926, recent successes in the serotherapy of pneumonic plague have been reported by: De Moura et al.\textsuperscript{140} (three cures in patients treated early in the disease with average doses of 150-230 ml of plague serum, administered during two days partly intravenously and partly intramuscularly); Gale \textsuperscript{53} (bacteriologically unconfirmed case of a girl, who had been vaccinated against plague five or six
days before she had had contact with a plague patient and who was given 200 ml of plague serum on the fourth day of illness); and Clark & Goldberg 21 (an old woman, who had been given 1 ml of live avirulent vaccine and 50 ml of plague serum prophylactically and received, when falling ill with bacteriologically confirmed pneumonic plague, a total of 400-500 ml of plague serum intramuscularly and subcutaneously).

Only two instances of recovery in serum-treated patients suffering from "primary septicaemic" plague could be found in the available recent literature, and it has to be noted that in the case reported by Le Gall et al. 103 treatment consisted of the production of a fixation abscess, as well as of the administration of 320 ml of serum, whereas the patient referred to by Erzin & Payzin 38 seems to have been treated with sulfonamides as well as with plague serum.

For the reasons stated above, the results obtained by the different workers when using serotherapy in bubonic plague cases, and consequently the opinions they formed regarding the value of this mode of treatment, varied greatly. While the initial results of Yersin 222 and also those with Lustig's serum 1 were most favourable, and equally good experiences were recorded by a few later workers (e.g., by Joltrain 91 during the mild outbreak of plague at Paris in 1920), some observers altogether denied the value of plague serotherapy and many others considered it effective only when administered early in the disease. Choksy (quoted by Simpson 175) who had exceptionally wide experience in the treatment of plague at Bombay, shared the latter belief, stating that:

"Should those who are conversant with the application of the serum in plague be entrusted with 100 persons provided the cases are not septicaemic, they would be able to bring round at least 60, if not more, by the use either of Lustig's or Roux's serum of the strength that has been used in Bombay during the last two epidemics."

As summarized by Dieudonné & Otto, 28 the Indian Plague Commission considered that both Lustig's and Yersin's serum exerted some influence on the clinical course of plague, but that the effect was by no means as marked as in the case of diphtheria serum. Nevertheless, in the opinion of the Commission, attempts to produce a potent serum ought to be continued, since serotherapy was the only method giving some hope for an effective treatment of plague.

As stated in chapter 3, some new types of plague sera have recently become available, two of which—the serum produced with the aid of the avirulent EV strain in Madagascar and the new Haffkine Institute serum—have been used on a fairly large scale with quite satisfactory results. Girard 92 stated in this connexion that with the aid of the new

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1 The various types of plague sera have been described in chapter 3.
Madagascar serum it was possible to cure 60%-65% of the bubonic plague patients, and similar results were also recorded by Le Gall.¹⁰²

As noted before, in their studies on plague treatment Sokhey et al.¹⁸⁴ gave separate consideration to the results obtained in all cases and to those in patients who showed evidence of bacteraemia on admission. They described the simple method they used for this purpose as follows :¹⁸²

"On admission, the diagnosis was first made on clinical grounds. At the same time, before any treatment was given, 0.5 cc. of blood was drawn from a vein and planted in equal quantities on two agar slopes. This was done to determine the presence or absence of plague septicaemia at the commencement of treatment. If the cultures remained sterile after two days’ incubation at room temperature, and there was still doubt about the diagnosis, the bubo was punctured with a syringe and the fluid cultured on agar slopes to confirm the diagnosis.”

The results obtained by Sokhey and his colleagues ¹⁸⁴ from 1941 to 1948 when treating bubonic patients with Haffkine serum may be summarized thus :

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Number of deaths</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>157</td>
<td>37</td>
</tr>
<tr>
<td>Cases with plague bacteraemia on admission</td>
<td>71</td>
<td>36</td>
</tr>
</tbody>
</table>

There was only one death among the 86 patients who showed no bacteraemia at the commencement of treatment as compared with 36 deaths among the 71 sufferers showing evidence of bacteraemia on admission.

It has already been stated that the serum dosages used by the different workers varied considerably. Some utilized doses which cannot be considered as sufficient, while a few, particularly when attempting to save pneumonic-plague patients, administered enormous amounts of serum.

Sokhey et al.¹⁸⁴ recommended administering 40 ml of serum to bubonic plague patients on the first day of treatment, giving half this amount intravenously on admission and the other half subcutaneously six hours later, and giving the same amounts in a similar manner on the following day. While in their experience no further treatment was required in mild cases, it was necessary to administer further doses to severely-affected patients, usually for five days.

As summarized by Le Gall,¹⁰² workers in Madagascar used to give the serum prepared with the aid of the EV strain in average daily doses of 40-60 ml for five days.

Using different sera, some recent workers recommended the use of higher daily doses of up to 100 ml for adults and 50 ml for children (Lobo & Silvetti ¹¹⁵). However, when potent sera are available, daily doses of 40-60 ml may be said to suffice in general.
Sulfonamide Treatment

The history of plague treatment with sulfonamides may be said to go back to the year 1937, when Buttle et al. proved experimentally that sulfanilamide exerted some action on *P. pseudotuberculosis* and *P. septica*. Working with *P. avicida*, Levaditi & Reinie (1938) obtained identical results.

Carman and Vine recorded in 1938 that they had each been able to save three bubonic plague patients through intramuscular injections of Prontosil (*p*-aminophenylsulfonamide), while van Hoof reported in the same year that, in the Belgian Congo, two out of three patients treated with sulfanilamide had been cured. No details could be elicited regarding the statement of Prado Barrientos that, during the 1938 bubonic plague outbreak in the Santa Cruz department of Bolivia, he had obtained fairly satisfactory results with “emergency chemotherapy”.

Important experimental evidence of the efficacy of sulfonamides in plague treatment was furnished in 1939 by Schütze, Durand, and Girard & Girard.

As summarized in 1941 in a *British Medical Journal* editorial, Schütze found that, when sulfapyridine, soluseptasine, and a diaminodiphenylsulfone-glucose compound were given to mice and rats subcutaneously or by mouth, sulfapyridine proved the most efficient drug in both animal species. Finding that the plague serum prepared in the Lister Institute with the aid of Otten’s avirulent strain gave results comparable to those obtained with sulfapyridine, Schütze suggested that a combination of sulfonamide treatment with serotherapy might be advantageous.

Durand feeding mice with daily sulfapyridine doses ranging from 1.25 mg to 2.25 mg per gram of body-weight for periods of 6-7 days after plague infection, found that the animals so treated resisted at least 10,000 *P. pestis*. Two further important facts established in the course of these investigations were that (a) it was often impossible to demonstrate *P. pestis* in sulfapyridine-treated mice which succumbed because they had received too high an infecting dose, and (b) 21 out of 36 sulfapyridine-treated mice which had resisted plague, when challenged 33 days after the original infection, proved to be immune to 2 or even 20 lethal doses of *P. pestis*.

Girard & Girard also obtained satisfactory results when administering sulfapyridine to mice and guinea-pigs even when the treatment had been started at the time of plague infection or, in the case of some of the guinea-pigs, up to two days after infection. Like Schütze, these two authors pointed to the possibility that combined sulfonamide and serum treatment might improve the therapeutic results.

Commenting upon the experiments of Durand and also upon observations made regarding the action of sulfonamides on other micro-organisms,
a reviewer stated in the Bulletin mensuel de l'Office International d'Hygiène Publique \(^9\) that since test animals succumbing to plague infection after insufficient sulfa-medication were often free from \(P. \text{pestis}\) while those surviving infection, because sufficiently treated, were frequently immune, one might assume that

"Dagenan (sulfapyridine) destroys the plague bacillus, leaving the circulating toxin and the antigen intact. Since the degree of immunity is in correlation with the comparatively high dose of microbes used for the first infection, one could conclude that this immunity is produced solely by the preformed toxin, perhaps also by that which can be freed by bacteriolysis". \(^7\)

As shown by Girard, \(^6^2\) sulfapyridine did not protect mice against toxic filtrates of \(P. \text{pestis}\). The observations of several workers that combined administration of sulfonamides and plague serum was apt to give better results than treatment with either remedy alone seem also compatible with the idea that, in contrast to the serum, the sulfonamides exert no antitoxic action (Girard \(^6^5\)). Further evidence is also at hand to render it likely that, as a recent editorial \(^8^7\) puts it, "the sulpha drugs interfered with the growth of the organism (\(P. \text{pestis}\)) but not with the production of the antibodies".

**Bubonic plague**

Large-scale use of sulfonamides for the treatment of bubonic plague was started during the period 1940-1. The early workers had to rely upon sulfapyridine and sulfathiazole, the efficacy of the latter for plague treatment having been proved experimentally by Sokhey & Dikshit \(^1^7^8\) in 1940. Afterwards, however, sulfadiazine and its derivatives were mostly used, not only because these newer drugs were less apt to produce toxic reactions, but also because in the experience of most workers, they were more effective for plague treatment than sulfapyridine and sulfathiazole. However, no full agreement has been reached in this respect, Macchiavello \(^1^1^7\) for instance, considering sulfathiazole to be theoretically best for the treatment of plague.

General agreement has been reached that, since they exert merely a bacteriostatic action, the sulfonamides are most apt to cure plague patients if treatment is started early in the disease. Even then, administration of these drugs, though curbing further progress of the infection, does not as a rule bring about a rapid devolution of the buboes which frequently become suppurated in spite of the treatment.

The superior value of early-commenced sulfonamide treatment of bubonic patients has been well demonstrated by the observations made

\(^J\) "... le Dagenan (sulfapyridine) détruit le bacille pesteux, tout en laissant intacts la toxine circulante et l’antigène. Comme le degré d’immunité est en rapport avec la dose de microbe plus élevée employée pour la première injection infectante, on pourrait en conclure que cette immunité est provoquée uniquement par la toxine préformée, peut-être aussi par celle qui pourrait être mise en liberté par une lyse bactérienne."
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by Sokhey and his collaborators from 1941 to 1948, which, according to their recent summary,\textsuperscript{181} gave the following results:

\textbf{(a) Cases in which treatment was started before the appearance of bacteraemia}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of cases</th>
<th>Number of deaths</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfapyridine</td>
<td>60</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>155</td>
<td>9</td>
<td>5.8</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>62</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>Sulfamerazine</td>
<td>91</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Iodine solution (controls)</td>
<td>74</td>
<td>12</td>
<td>16.2</td>
</tr>
</tbody>
</table>

\textbf{(b) Patients showing bacteraemia at the commencement of treatment}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of cases</th>
<th>Number of deaths</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfapyridine</td>
<td>62</td>
<td>31</td>
<td>50.0</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>119</td>
<td>50</td>
<td>42.0</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>62</td>
<td>13</td>
<td>21.0</td>
</tr>
<tr>
<td>Sulfamerazine</td>
<td>22</td>
<td>7</td>
<td>31.8</td>
</tr>
<tr>
<td>Iodine solution (controls)</td>
<td>75</td>
<td>68</td>
<td>90.7</td>
</tr>
</tbody>
</table>

It will be noted that sulfadiazine gave fairly satisfactory results even when treatment was started late. The number of observations made in this respect with sulfamerazine is too small to permit final conclusions, but, to judge from the available information, this drug was more effective than sulfapyridine and sulfathiazole.

Indications regarding the dosages of the drugs used during these trials were given by Sokhey and his collaborators thus:\textsuperscript{181}

\textit{"Sulphapyridine and Sulphathiazole:} 10 to 14 g were given on the first day and on subsequent days 6 g depending upon the severity of the case for a maximum period of 10 days. The drug was stopped earlier, if the temperature came to normal or the patient showed distinct improvement in general condition. To comatose patients or when the drug produced persistent vomiting, the drug was given intra-muscularly or intravenously.

\textit{Sulphadiazine:} An initial dose of 4 g was followed by 2 g four hours later. Thereafter 1 g was given every 4 hours till the patient’s temperature remained normal for 2 days. This dosage maintained a concentration between 10 to 20 mg per 100 cc of blood.

\textit{Sulphamerazine:} An initial dose of 4 g was followed by 2 g four hours later. Then 1 g was given every 8 hours till the temperature remained normal for 2 days. This dose maintained a concentration between 10 to 20 mg per 100 cc of blood.”

Sulfamethazine (sulfamezathine) has been used for plague treatment in the place of the above-mentioned sulfonamides. Datt Gupta,\textsuperscript{23} comparing the effect of this drug with that of sulfadiazine, recorded the following results:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Cases</th>
<th>Deaths</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadiazine</td>
<td>4 g initially, then 2 g 4-hourly for 4-7 days (per os)</td>
<td>71</td>
<td>7</td>
<td>9.9</td>
</tr>
<tr>
<td>Sulfamezathine</td>
<td>3 g by intravenous injection, then 2 g 4-hourly for 4-7 days (per os)</td>
<td>37</td>
<td>4</td>
<td>10.8</td>
</tr>
</tbody>
</table>

As far as these figures go, sulfamethazine was about as effective as sulfadiazine, but it has to be noted that the latter drug was given solely
by the oral route without an initial intravenous administration of its sodium salt.

Sulfamethazine is amply used in Java where even rather small doses, averaging 3 g per day, have been found satisfactory for the treatment of bubonic plague patients, particularly those who had been previously vaccinated with live avirulent plague bacilli.

That, generally, previous plague vaccination markedly improved the results of sulfonamide treatment in bubonic patients is illustrated by the following results recorded by Patel & Rebello: 149

<table>
<thead>
<tr>
<th>Treated with sulfonamides</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td></td>
</tr>
<tr>
<td>attacks</td>
<td>1,586</td>
</tr>
<tr>
<td>deaths</td>
<td>344</td>
</tr>
<tr>
<td>mortality (%)</td>
<td>21.6</td>
</tr>
<tr>
<td>Uninoculated</td>
<td></td>
</tr>
<tr>
<td>attacks</td>
<td>1,262</td>
</tr>
<tr>
<td>deaths</td>
<td>575</td>
</tr>
<tr>
<td>mortality (%)</td>
<td>45.5</td>
</tr>
<tr>
<td>Total</td>
<td>2,848</td>
</tr>
<tr>
<td>attacks</td>
<td>519</td>
</tr>
<tr>
<td>deaths</td>
<td>919</td>
</tr>
<tr>
<td>mortality (%)</td>
<td>32.2</td>
</tr>
</tbody>
</table>

* Usually sulfathiazole

Since the efficacy of plague treatment with sulfonamides depends upon the establishment and maintenance of a sufficiently high level of the drugs in the blood (10 mg per 100 ml according to Macchiavello 117 in the case of sulfathiazole, sulfadiazine, and sulfamerazine), it is, in the opinion of many observers, advantageous to initiate treatment by administering a sufficiently high dose by the intravenous route instead of giving an adequate initial dose by mouth. Further, though hand in hand with the improvement of the patients the dosage of the sulfonamides should be gradually reduced, it is essential to continue the treatment even during the first few days of convalescence so as to avoid relapses or complications. The recommendations made in these respects by the WHO Expert Committee on Plague, at its second session, held at Bombay in December 1952, 213 were that:

"Early cases of bubonic plague could be effectively treated with sulfonamides, using a dosage of about 10 g on the first day, followed by smaller doses, making a total of at least 50 g during the first week. The first administration of sulfonamides in the dose of 2 g should be made intravenously, preferably in glucose solution. In order to prevent relapses, particularly meningeal plague, the treatment with sulfonamides should be continued for at least 3 days after the temperature becomes normal."

Primary pneumonic plague

Although, as unanimously upheld by all observers, cases of primary pneumonic plague are not as a rule amenable to sulfonamide treatment, the following instances of exceptional success with this therapy could be found in the available literature :

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Number treated</th>
<th>Number cured</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Hoof 83</td>
<td>1939</td>
<td>12</td>
<td>2</td>
<td>Treated with sulfonamide.</td>
</tr>
<tr>
<td>Favarel 48</td>
<td>1945</td>
<td>?</td>
<td>1?</td>
<td>Child with fever, but no pneumonia, in whose saliva P. pestis was found. Cured with sulfapyridine.</td>
</tr>
</tbody>
</table>
### CLINICAL ASPECTS

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Number treated</th>
<th>Number cured</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magrou</td>
<td>1945</td>
<td>1</td>
<td>1</td>
<td>Treated with sulfadiazine.</td>
</tr>
<tr>
<td>Munter</td>
<td>1945</td>
<td>1</td>
<td>1</td>
<td>Previously vaccinated against plague. Treated with sulfadiazine.</td>
</tr>
<tr>
<td>Roux &amp; Mercier</td>
<td>1946</td>
<td>5</td>
<td>3</td>
<td>Treated with sulfathiazole. Two of the recovering patients received also plague serum on the 6th and 8th day of illness respectively, while a fixation abscess was produced in the third.</td>
</tr>
<tr>
<td>Favarel</td>
<td>1947</td>
<td>13</td>
<td>2</td>
<td>Treated with sulfapyridine and thiazomide.</td>
</tr>
<tr>
<td>Than Aung</td>
<td>1947</td>
<td>1</td>
<td>1</td>
<td>Treated with sulfathiazole.</td>
</tr>
<tr>
<td>Favarel et al.</td>
<td>1948</td>
<td>?</td>
<td>3</td>
<td>Treated with sulfathiazole and thiazomide. Two of the patients had been inoculated with live plague vaccine while the third had received 14 g of sulfathiazole prophylactically.</td>
</tr>
<tr>
<td>Tieh et al.</td>
<td>1948</td>
<td>5</td>
<td>3</td>
<td>Treated with sulfadiazine.</td>
</tr>
</tbody>
</table>

**Note**

1. The patient observed by Plum, though considered to have recovered from primary pneumonic plague by some reviewers, had actually bubonic plague with secondary lung involvement.

2. Some further instances of recovery from primary pneumonic plague will be discussed later because the patients in question had received plague serum and/or streptomycin in addition to sulfonamides.

### Combination of serotherapy and sulfonamide treatment

As has been mentioned before, some of the early workers studying the efficacy of the sulfonamides in experimental plague considered the advisability of combining treatment with these drugs with the administration of specific serum.

Actually, almost all workers who gave such combined treatment to bubonic plague patients found the therapeutic results superior to those obtained with either sulfonamides or serum. It has to be noted, however, that the differences found in favour of the combined treatment were not invariably marked, and that Sokhey and Wagle, though establishing that combined treatment with sulfathiazole and plague serum gave better results than medication with sulfathiazole alone (68% cures as against 58.4%), found that sulfadiazine alone gave still better results (79.1% cures).

Besides the above-mentioned statement of Roux and Mercier that they had succeeded in curing two patients with primary pneumonic plague by a combination of sulfathiazole treatment with the late administration
of plague serum, only two records of recovery of pneumonic patients receiving such combined treatment could be found:

(1) The case referred to by Wang 207 was that of a nurse who contracted infection in the isolation hospital at Changteh, China, and who was actually treated by the present author with sulfathiazole and also with plague serum, when her condition became desperate. She suffered possibly, but not certainly, from primary pneumonic plague with an atypically long evolution.

(2) Chang 15 recorded one instance of recovery among eight patients with primary pneumonic plague treated in the isolation hospital at Foochow, China, with sulfadiazine and plague serum.

**Antibiotic Treatment**

**Penicillin**

In vitro tests made by Magrou, 121 as well as animal experiments performed by Witlin and Wilbar, 211 Gupta et al., 76 Herbert, 81 and Sokhey & Habbu, 179 showed that penicillin, even if used in high doses, exerted no, or practically no, action on *P. pestis*. These laboratory findings were confirmed by the unfavourable clinical experiences of observers such as Barreto & Castro, 8 Haddad & Valero, 77 and Link. 114 Nevertheless, a few workers continued to use penicillin in plague cases in addition to specifics like sulfonamides or streptomycin in order to forestall or to control complications, secondary pneumonia (Videla 200) or secondary infections. In the experience of Macchiavello (personal communication to the *Boletin de la Oficina Sanitaria Panamericana* 5) penicillin was not efficacious in warding off plague complications.

**Streptomycin**

Studies on the action of streptomycin on the plague bacillus in vitro and on plague-infected animals were first carried out in 1944 by Meyer and his colleagues (Meyer & Quan 135). Further laboratory studies by several workers, such as Sokhey and collaborators, 179, 181 Hornibrook, 84 Sergiyev, 172 Macchiavello, 117 Wayson & McMahon, 208 Herbert, 81 Quan et al., 159 Girard, 66 Favarel, 46 Néel, 144 and Meyer et al. 136 confirmed the early findings of Meyer and his colleagues that in vivo as well as in vitro streptomycin exerted a powerful action on *P. pestis*, which appeared to be bactericidal rather than bacteriostatic.

In vitro studies carried out in Meyer's laboratory showed, according to Meyer & Quan, 135

“... that some plague bacilli have the same ability to resist streptomycin as have other types of bacteria. It was found that although the ratio of the slightly resistant mutants varied from strain to strain, usually only few highly resistant bacilli were present in a total of several hundred billion organisms.”
The most outstanding result of the administration of streptomycin to plague-infected animals was that pneumonic as well as bubonic affections could be readily cured with the aid of this antibiotic.

In the course of the investigations of Meyer and his collaborators, the bactericidal action of streptomycin in experimental pneumonic plague was fully demonstrated by comparative bacteriological counts of the entire lung tissue of treated and untreated mice, which had been killed at various intervals. As stated by Meyer & Quan: 135

"By the 96th hour after infection, when all untreated mice had died, the lungs and bronchial lymph nodes of treated mice either were sterile or contained only a few thousand plague bacilli in the abscess-like patches of pneumonia. It was impossible to isolate plague bacilli from lung or lymph nodes 100 hours after treatment with streptomycin had been initiated."

Longer periods of treatment (6 days in the case of intranasal infection and 10 days in animals infected by inhalation) were needed to sterilize the lung lesions of experimentally infected guinea-pigs.

Girard, 66 experimenting with guinea-pigs infected with P. pestis by the intratracheal route, was able to cure all 12 animals which had been treated with streptomycin from the 30th or 48th hour after infection, whereas the controls died in 3 days. He ascribed this splendid result to the fact that the antibiotic exerted a bacteriolytic as well as a bacteriostatic action and added that

"the absence of immunity in the cured animals must be ascribed to this double effect which is extremely rapid; such a resistance to a new infection would demonstrate the persistence of the causative organisms in the body, which is the rule in sulfonamide-treated guinea-pigs until they recover."  1

Néel 144 also noted a rapid bactericidal action of streptomycin in intratracheally infected guinea-pigs, the animals becoming free from plague bacilli two days after commencement of treatment. During this time the pathogenic power of the organisms still harboured in the animal body became first lowered and then lost.

Quan et al. 158 showed experimentally that, in contrast to aureomycin, oxytetracycline, 7 and chloramphenicol, streptomycin did not protect mice against the action of plague toxin. This result is in accord with earlier observations of Ramon et al. 101 that culture filtrates of Actinomyces griseus as well as of Penicillium notatum did not materially alter the toxicity of this toxin for mice.

Streptomycin seems to have been first used for the treatment of human plague by Videla 200 in December 1946. Even though only one of the five patients he treated with this antibiotic suffered from typical bubonic

---
1 Oxytetracycline is the non-proprietary name for "Terramycin".

---

"C'est à ce double effet qui est extrêmement rapide qu'il faut attribuer l'absence d'immunité chez les animaux guéris; cette résistance à une nouvelle infection témoignerait de la persistance du germe spécifique dans l'organisme, ce qui est la règle chez les cobayes traités par les sulfamides, lorsqu'ils guérissent."

$k$
plague, while three of the others seem to have had "septicaemic" plague and one showed meningeal involvement, all were cured.

Further results obtained with streptomycin in the treatment of plague may be summarized as follows:

(a) Bubonic plague

<table>
<thead>
<tr>
<th>Author or reference</th>
<th>Date</th>
<th>Number treated</th>
<th>Number cured</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Datt Gupta 23</td>
<td>1948</td>
<td>24</td>
<td>20</td>
<td>&quot;Only severe cases were selected for streptomycin treatment and two died within 12 hours of admission.&quot;</td>
</tr>
<tr>
<td>Haddad &amp; Valero 77</td>
<td>1948</td>
<td>3</td>
<td>3</td>
<td>The patients, who had not responded to treatment with sulfonamides and penicillin, rapidly improved and finally recovered when given streptomycin. Buboes were not influenced when streptomycin treatment was started late.</td>
</tr>
<tr>
<td>Karam Chandani &amp; Sundar Rao 96</td>
<td>1948</td>
<td>5</td>
<td>5</td>
<td>Moribund patients, four of whom had not responded to previous treatment with sulfathiazole and/or serum.</td>
</tr>
<tr>
<td>Wagle 204</td>
<td>1948</td>
<td>32</td>
<td>29</td>
<td>All these patients had bacteraemia when treatment was started.</td>
</tr>
<tr>
<td>Chen 16</td>
<td>1949</td>
<td>3</td>
<td>3</td>
<td>Severely-affected patients, who had not responded to sulfadiazine. Buboes little influenced by streptomycin treatment, had to be incised in two of the cases.</td>
</tr>
<tr>
<td>Estrade 40</td>
<td>1949</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Karam Chandani &amp; Sundar Rao 97</td>
<td>1949</td>
<td>15</td>
<td>12</td>
<td>All patients apparently moribund. The three fatal cases had been admitted on the third day of illness and died within a few hours.</td>
</tr>
<tr>
<td>Robic &amp; Favarel 164</td>
<td>1949</td>
<td>5</td>
<td>5</td>
<td>Received either only streptomycin or also plague serum and sulfonamides.</td>
</tr>
<tr>
<td>Ghosh 55</td>
<td>1950</td>
<td>155</td>
<td>149</td>
<td>Apparently all treated with streptomycin and sulfadiazine.</td>
</tr>
<tr>
<td>Link 114</td>
<td>1950</td>
<td>3</td>
<td>3</td>
<td>Received sulfadiazine, penicillin, and, in two cases, also aureomycin in addition to streptomycin. Two patients treated only with penicillin died.</td>
</tr>
<tr>
<td>Soulage et al. 186</td>
<td>1950</td>
<td>14</td>
<td>14</td>
<td>Treatment with streptomycin only (4 cases) or with streptomycin and afterwards with sulfonamides (8 cases). The two remaining patients had not responded to treatment with plague serum and sulfonamides, but were cured with streptomycin.</td>
</tr>
<tr>
<td>Archives de l'Insti-</td>
<td>1951</td>
<td>13</td>
<td>11</td>
<td>With the exception of two, also treated with sulfonamides.</td>
</tr>
<tr>
<td>tute Pasteur de Tananarive (p. 44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archives de l'Insti-</td>
<td>1952</td>
<td>31</td>
<td>31</td>
<td>With the exception of six, also treated with sulfonamides.</td>
</tr>
<tr>
<td>tute Pasteur de Tananarive (p. 66)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Rao 162 1952 316* 304 (96.2%) The patients were treated with streptomycin and sulfonamides; except in a few serious cases not more than 2 g of streptomycin were used per patient.

Sokhey et al.184 1952 148 142 (95.8%) Four of the deaths occurred in the 37 patients showing bacteremia at the commencement of treatment.

Dubey 28 1953 18 18 (100%) 14 of these patients received only 1 g of streptomycin, while four had to be given a second equally large dose after 24 hours. Buboes subsided without manifest suppuration.

* Sixteen of these patients were said to have suffered from "septicaemic" plague, but no separate statistics were given for them.

(b) "Septicaemic" and meningeal plague

In addition to the observations of Videla 200 and Rao 162 which have been noted above, Fain et al.12 reported success when treating two patients suffering from "primary septicaemic" and primary meningeal plague, respectively, with streptomycin and sulfadiazine.

(c) Primary pneumonic plague

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Number treated</th>
<th>Number cured</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favarel 43</td>
<td>1948</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Huang et al. 85</td>
<td>1948</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Lewin et al.111 **</td>
<td>1948</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Wagle &amp; Bedarkar 205</td>
<td>1948</td>
<td>5</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>Estrade 40, 41</td>
<td>1949, 1951</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Feng 48</td>
<td>1949</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Robic 164 &amp; Favarel</td>
<td>1949</td>
<td>3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Seal 171</td>
<td>1949</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Bouillat 7</td>
<td>1951</td>
<td>3</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Guiller &amp; Jospin 75</td>
<td>1951</td>
<td>5</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Estrade 41</td>
<td>1951</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Mercier 329</td>
<td>1951</td>
<td>10</td>
<td>9 (90%)</td>
<td>—</td>
</tr>
<tr>
<td>Fain et al. 42</td>
<td>1951</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
</tbody>
</table>

** Meyer & Quan 25 referred also to a second pneumonic patient cured in South Africa with streptomycin.
Discussing the results obtained in pneumonic plague treatment by Mercier, Girard stressed that one of Mercier's 10 patients, after having shown signs of temporary improvement, again became feverish and, developing a lung abscess, eventually succumbed. In view of the fact that this secondary lung affection was not caused by *P. pestis* but was obviously due to a secondary infection, Girard urged that, in order to prevent such complications, plague patients should be treated with sulfonamides or penicillin as well as with streptomycin.

Considering this postulation, the WHO Expert Committee on Plague came, in 1952, to the conclusion that

"in pneumonic plague administration of sulfonamides or wide-spectrum antibiotics might be necessary in order to prevent secondary infections or their sequels."

The dosages of streptomycin used by the different workers varied considerably, the more so as some merely initiated treatment with this antibiotic, and then continued it with sulfonamides, while others began to use streptomycin only when the sufferers did not respond to treatment with sulfonamides and/or serum.

In relatively mild cases of bubonic plague, Sokhey and his collaborators used an initial dose of 2/3 g followed by 1/3 g every 4 hours until the temperature had remained normal for 24 hours. In severe cases, they gave 2/3 g of the drug every 4 hours until the temperature had remained normal for 2-3 days.

As summarized by Girard, the dosages of streptomycin administered intramuscularly for the treatment of primary pneumonic plague in Madagascar were 0.50 g every three hours for the first two days, and every four hours on the third day. Doses totalling 2 g per day were then given until the fifth or sixth day of treatment. Girard noted that the two patients treated by Estrade had been given a total of 25 g of streptomycin in six days, but expressed the view that it offered no advantage to give more than a total of 20 g to adults. He added that a child had been cured with a total dose of only 11.5 g.

Girard's opinion was endorsed by the WHO Expert Committee on Plague, which at its second session in 1952 recommended that

"the treatment of pneumonic plague should be carried out with streptomycin in the dosage of about 16 to 20 g administered during a period of from 6 to 7 days. The same treatment should be applied to severe cases of bubonic plague with septicaemia."

*Other antibiotics*

Dealing with the results of antibiotic treatment of mice, which had been infected experimentally with either bubonic or pneumonic plague, Meyer stated that:

"Aureomycin, chloramphenicol and terramycin are good alternatives in plague therapy. Each has been effective in experimental mouse infections when given systematically"
oral or orally. Oral administration gives these agents a distinct advantage over streptomycin in moderately severe cases of plague. They are less bactericidal in vitro than streptomycin against the plague bacillus. Thus far treatment with none of these has resulted in growth of resistant strains as readily as did streptomycin therapy.

Favourable results obtained through treatment of subcutaneously plague-infected mice with aureomycin and chloramphenicol were also reported by Sokhey & Habbu, who found these antibiotics, which were given by mouth, to be as effective as subcutaneously-administered streptomycin. However, in order to obtain identical results, 42 mg of aureomycin or 336 mg of chloramphenicol had to be administered as against 10.4 mg of streptomycin.

Ramchandran recently recorded the results obtained with aureomycin treatment in 12 bubonic and 3 "septicaemic" plague patients. Three of these, including two with "septicaemic" plague, died within a few hours after the onset of treatment. All the others were cured through administrations of aureomycin totalling from 2.5 g to 7.5 g, given in individual doses of 250 mg every two hours. The temperature of the treated became normal after 3-7 days.

Some further satisfactory experiences with aureomycin in the treatment of bubonic plague were recorded by Meyer et al. and by Smadel et al.

A first attempt of Mercier to cure a pneumonic-plague patient with chloramphenicol gave no conclusive results, because it was decided to continue treatment with streptomycin after two one-gram doses of the former antibiotic had been administered subcutaneously.

However, soon afterwards, favourable results were obtained when treating one bubonic and further pneumonic-plague patients with chloramphenicol alone (Girard, Mercier, Mercier & MacCrumb).

Mercier & MacCrumb used the following dosages for the first pneumonic patient whom they treated with chloramphenicol:

1. Initially, 3 intravenous injections of 0.5 g, given at intervals of 3 hours, every time accompanied by oral administration of 0.5 g of chloramphenicol;
2. Afterwards, 3 or 4 doses of 0.5 g each given orally at intervals of 3 hours;
3. Then oral administration of 0.25 g every two hours until the temperature dropped to \(37^\circ-37.5^\circ\)C;
4. Finally, for a few more days, further 0.25-g doses sufficiently frequent to result in the administration of a daily total of 50-60 mg of chloramphenicol per kg of body-weight.

The total dosage amounted thus to 20-25 g.

Mercier & MacCrumb added that they afterwards treated five more pneumonic-plague patients with chloramphenicol, four of whom were cured. The fifth, whose treatment was started late, succumbed. These
two workers also cured two pneumonic-plague patients with oxytetracycline, using somewhat lower initial doses than those given in the case of chloramphenicol but then continuing treatment in a manner similar to that in which the latter antibiotic was administered.

They concluded that

"... Terramycin [oxytetracycline] deserves to take its place side by side with streptomycin and chloromycetin amongst the antibiotics efficacious in human pneumonic plague".  

It is important to add that Smadel et al. 177 effected a rapid cure when instituting treatment with chloramphenicol soon after the onset of fever in a 4 year-old boy suffering apparently from primary septicaemic plague.

Comparative Value of the Methods of Specific Treatment

Returning to the question of whether, in view of the supreme results obtained in the treatment of plague with antibiotics, the use of plague serum might be given up, the following may be stated.

Though general agreement has been reached that the results of treatment with plague serum alone are far inferior to those obtainable with antibiotics and are even inferior to those obtainable with sulfonamides, a combination of the administration of the latter with serotherapy has been advocated by some workers in order to combat toxaemia. Similarly, Girard 68 recently recommended that serum administration should be combined with streptomycin treatment because the specific serum had antitoxic properties while streptomycin, like the sulfonamides, had none. However, in view of the almost miraculous power of this antibiotic to cure even most of the patients suffering from plague in its severest forms, it is impossible to believe that the supposed absence of antitoxic action of streptomycin is of any practical importance in the treatment of human plague. Moreover, other antibiotics found efficacious for plague treatment exert an antitoxic action. It follows that if sufficient suitable antibiotics are available for plague treatment, there no longer exists a need to use specific serum.

In the opinion of the present writer, the same holds true if large-scale use has to be made of sulfonamide treatment. For, even though in the experience of a number of observers combined treatment with plague serum and sulfanamides gave better results than administration of the latter drugs alone, there can be no doubt that far better results can be obtained by using antibiotics in addition to sulfonamides. In other words, the present writer for one believes that the money and effort now spent

\[m\] "... La Terramycine mérite de prendre place désormais aux côtés de la Streptomycine et de la Chloromycétine parmi les antibiotiques efficaces à l'égard de la peste pulmonaire."


in producing plague serum could be used to far better advantage for the production or procurement of antibiotics suitable for plague treatment.

The sulfonamides on the other hand continue to be of importance for the treatment of plague. Owing to lack of funds, and sometimes also of sufficient qualified medical workers to give treatment with antibiotics, so far no full advantage (or in some areas no advantage at all) could be taken of this mode of therapy. Indispensable though it is to remedy this deplorable situation, one should facilitate the efforts made in this direction by using as far as possible sulfonamides in combination with, or in place of, antibiotics. In this connexion, it should be kept in mind that if treatment is started early in bubonic plague, satisfactory results can be obtained with sulfonamides alone, and also that, as shown by ample recent experiences, patients who fail to respond to such initial treat­ment can almost always be saved if antibiotics are resorted to afterwards. Hence, unless fully sufficient amounts of antibiotics are available, it seems legitimate to adopt the following scheme of plague treatment:

1. Every possible effort should be made to detect plague cases early in the disease and to start adequate treatment immediately.
2. Bubonic plague patients detected early, as well as those seen later who suffer from a benign form of the disease, may be treated with sulfonamides.
3. If patients treated with sulfonamides alone take a turn for the worse, antibiotics must be used in addition to these drugs.
4. Treatment with antibiotics must be substituted if patients treated with sulfonamides show signs of toxic reactions due to this medication.
5. Patients suffering from primary pneumonic plague and from “septicaemic” plague, and also sufferers from bubonic plague who, because they have come for treatment late in the disease, are in a serious condition or who show complications such as meningeal involvement, must by all means, from the first, be treated with antibiotics.
6. Constant attention should be given to the possibility of using reduced dosages of antibiotics in combination with sulfonamide treatment or of continuing treatment of patients who have responded well to the initial administration of antibiotics, with sulfonamides.

Whether streptomycin will remain the antibiotic of choice for plague treatment remains to be seen. The possibility that streptomycin treatment with sub-optimal doses might lead to the appearance of drug-resistant plague strains deserves attention in this connexion. However, as pointed out by Meyer and Quan:

“Fortunately, in the treatment of the individual patient this resistance is of minor importance, since it is independent of resistance to sulfonamides. Collateral therapy with sulfadiazine inhibits the streptomycin-resistant strains until the immunity mechanism of the infected host is fully developed. Equally, the epidemiologic importance is insigni-
significant, since drug-resistant strains are, as a rule, confined to closed necrotic lesions, and the infection does not become generalized”.

The necessity of administering streptomycin parenterally is a serious drawback, particularly if qualified medical workers are few and far between. Hence, while it would be rash not to make large-scale use of streptomycin until more is known about the value of other antibiotics in the treatment of human plague, further studies on the efficacy of the latter must be energetically pursued.

**General and Supporting Treatment**

There is no need to enter into an elaborate discussion of the general and supporting treatment to be given to plague patients, because this should be administered according to the methods adopted in cases of serious acute diseases in general. However, apart from the precautions to be taken against a spread of the infection from the sufferers, which will be dealt with in chapter 10, one must give special consideration to the following points when attending plague patients.

**Rest in bed**

It is of vital importance to keep the patients strictly confined to bed during the acute stage of the illness as well as during early convalescence. Failure to abide by this rule may lead to sudden death from heart failure and, moreover, strict rest during the acute stage of illness is essential to prevent or to limit a generalization of the infection. That exertions on the part of the patients are apt to lead to an aggravation of their condition is proved by observations showing that: (a) plague patients who had to walk considerable distances to seek treatment almost invariably became seriously affected; (b) patients who fell ill while travelling were particularly prone to develop secondary lung involvement.

The patients should not even be permitted to get up in order to void urine or stools.

Delirious or comatose patients must be watched with constant care because they may try to escape or may fall out of bed and hurt themselves.

**Diet and fluid intake**

Plague patients ought to be given a nourishing, though adequately light, diet. An ample fluid intake, generally necessary on account of the feverish condition of the patients, is particularly important for those treated with sulfonamides. The urine output of these patients should be watched, preferably measured, and the urine should be examined at least once daily for the presence of albumen and/or blood.
Supporting treatment

Proper supporting treatment, adapted to the individual needs of the patients, is essential. Whenever cardiovascular failure threatens, cardiares such as coramine or, as several recent workers recommended, adrenocortical preparations should be administered. The patients must be watched day and night in order not to overlook a sudden collapse.

Adequate amounts of alkalis must be given to the patients under sulfonamide treatment. Girard stated in this connexion that renal complications due to the administration of these drugs might be avoided if enough alkalis were given to maintain the urine at a pH of about 7.2.

Treatment of the buboes

It is of vital importance to adhere to the following rules for the local treatment of the buboes:

(a) During the acute stage of the disease manipulation of the buboes must be avoided as far as possible. An incision of the affected lymphnodes at that time or the rubbing-in of ointments or other medicaments is strictly contra-indicated.

In the experience of the present writer, it is best to apply to the acutely inflamed buboes ichthyol or ichthyol-belladona ointment spread on gauze pads, because this medication alleviates the pain without irritating the skin.

(b) If pus forms, the buboes should be incised only when fluctuation has become general. Otherwise sinuses may form which may greatly retard the process of healing or even endanger the patients' lives.

(c) Plague workers with little experience in surgery often try to benefit the patients by frequently attending the incision wounds. Actually, these heal best if interfered with as little as possible. In particular, one should change the gauze strips draining the wounds as rarely as feasible.

Abortive treatment

Injections of moderately large amounts of specific serum have been made in the past, and medication with small doses of sulfonamides is used at present, to forestall the appearance of manifest plague in persons definitely known to have been exposed to infection, particularly in contacts with pneumonic-plague patients.

Some authors use the terms "serum prophylaxis" and "chemo-prophylaxis" to designate these procedures. It seems preferable, however, to class them as methods of abortive treatment, so as to indicate that these procedures have little in common with the prophylactic administration of plague vaccines and/or small doses of plague serum to large
groups of individuals considered to be at risk of infection in areas where
plague outbreaks are present or threatening.

**Plague serum**

As can be gathered from *A treatise on pneumonic plague* \(^{216}\) and sub­sequent publications by Wu Lien-teh \(^{217}\) and by Wu Lien-teh & Pollitzer,\(^{219}\) ample use has been made, in the past, of the administration of smaller or larger doses of specific serum to pneumonic-plague contacts. Summariz­ing the observations made up to 1926, Wu Lien-teh \(^{216}\) stated that :

"It has been shown that prophylactic serum often prolongs the incubation period and that patients receiving such preventive treatment are specially benefited by subsequent therapeutic administration. On other occasions anti-plague serum, when given in sufficient doses, seems to prevent the manifestation of plague pneumonia ".

Wu Lien-teh added that though, on account of experimental findings, a dose of 100 ml was recommended for the abortive treatment of pneu­monic plague, dosages of 40 ml to 50 ml might be sufficient, if the serum could be administered early. Failing that, or if there was particularly great danger of infection, doses of 100-200 ml might be advisable. In view of the expense involved and of the necessity to isolate the serum­treated contacts for a longer period than usual, because of a possibly prolonged incubation period, it would be impracticable to use abortive serotherapy during large pneumonic epidemics. However, the method might be practicable and advantageous for the purpose of limiting incipient outbreaks.

Within recent years but little use seems to have been made of abortive serotherapy. It would appear that good experiences were made with administration of 10 or 20 ml of plague immune serum to 47 contacts isolated during a pneumonic plague outbreak in Portuguese India (Figueiredo 49\). On the contrary administration of 60 ml of plague serum to contacts encountered during the 1939 pneumonic outbreak in the Chimborazo province of Ecuador seems to have given no satisfactory results, since some of the persons so treated developed manifest plague (Suárez 189\).

Clark & Goldberg \(^{21}\) gave, in addition to plague vaccine, serum doses — usually of 50 ml in the case of adults and of 25 ml in the case of children— to 35 persons who had been in close contact with pneumonic-plague patients. Only three of the serum-treated contacts developed the disease and one of these was cured through administration of further doses of 400-500 ml of plague serum. It is noteworthy that : (a) one woman who had been given 100 ml of plague serum, because she had attended four pneumonic patients, remained well; (b) three children, who had slept with their mother up to and including the day she died from pneumonic plague and who then each received 50 ml of specific serum, also did not develop the disease.
Pinotti & al.,\textsuperscript{152} as well as Sorol & al.,\textsuperscript{185} reported good success when administering serum to persons who had been in contact with bubonic plague patients.

FIG. 32. PLAGUE CARBUNCLE IN MAN INFECTED THROUGH BITE OF WILD-RODENT FLEA

Though it will be gathered that some success has been obtained with abortive serotherapy, this method has become obsolete because sulfonamides, besides being far more easily applicable and—in contrast to plague serum—not undergoing losses in potency during storage, have also been found to be far more uniformly reliable for forestalling the development of the disease in contacts with plague patients.

\textit{Sulfonamides}

The possibility of using sulfonamides for the abortive treatment of plague seems to have been explored first clinically by Estrade (quoted by Girard\textsuperscript{62}) and by Girard,\textsuperscript{62} and experimentally by Macchiavello.\textsuperscript{117} Pollitzer\textsuperscript{154} who, working in the interior of China, was not aware of the above-mentioned clinical experiences also obtained a most encouraging result during the second World War.

Details of this early work may thus be summarized:

Estrade (quoted by Girard\textsuperscript{62}) started to give daily doses of 2 g of sulfapyridine for three consecutive days to three pneumonic-plague contacts as soon as they showed fever. Their temperature fell within 24 hours and no manifest illness developed in any of these three individuals. Estrade felt convinced that these contacts, who were under observation in an isolation camp, had had incipient pneumonic plague.
Girard\textsuperscript{62} not only obtained an equally good result when treating a pneumonic-plague contact, who clinically showed only some fever, with sulfapyridine (a total of 11 g given during eight consecutive days), but was also able to confirm that the saliva of the woman in question, collected before commencement of treatment, contained virulent plague bacilli.\textsuperscript{n}

As Macchiavello summarized in 1949,\textsuperscript{117} he had been able to establish, through experiments commenced in 1941, that treatment with sulfapyridine, sulfadiazine, and sulfamerazine, started before or within 24 hours after infection, protected 50%-75% of guinea-pigs inoculated with 1,000 virulent plague bacilli—provided that doses of these drugs sufficient to maintain a blood-level of 5 mg per 100 ml were administered. Sulfathiazole used under the same conditions gave even better results (68%-92%) but had to be given in higher doses and was more toxic.

Pollitzer\textsuperscript{154} had full success when, after the death of a pneumonic-plague patient, he gave 3 g of sulfathiazole daily for three days to the two men who had closely attended the sufferer throughout his illness, one of the two even sleeping in the sick chamber.

Further observations made with regard to abortive plague treatment with sulfonamides may be summarized thus:

\textit{Pneumonic plague.} Meyer\textsuperscript{132} established experimentally that guinea-pigs instilled intranasally with \textit{P. pestis} failed to contract pneumonic plague when given sulfadiazine or sulfamerazine at the time of infection.

Though occasional failures have been noted (Le Gall,\textsuperscript{102} Favarel et al.,\textsuperscript{47} Jospin et al.\textsuperscript{92}), actual experience with sulfonamide treatment of pneumonic plague contacts in Madagascar was, in general, most satisfactory. Favarel et al.\textsuperscript{47} stated in this connexion that this method

\begin{quote}
"is used systematically in Madagascar to treat all known pneumonic plague contacts without awaiting the appearance of any pathological manifestation ... Either MB693 (Dagenan) [sulfapyridine] or RP 2090 (Thiazomide) [amino-phenyl-sulfamidothiazol] are used in a daily dose per os of 4 g for adults, 3-2 g for children according to their age ... Dagenan and thiazomide gave, up to now, practically 100\% success in the prevention of pneumonic plague in contacts ."
\end{quote}

Favarel et al.\textsuperscript{47} added that treatment of the contacts was successful even in cases where these individuals had to be left in their houses instead of being taken to isolation camps.

Similarly Girard\textsuperscript{67} stated in 1951 that

\textsuperscript{n} An identical result obtained by Robic (quoted by Favarel et al.\textsuperscript{47}) in the case of a boy found to harbour \textit{P. pestis} in his saliva before abortive treatment with sulfonamides was commenced, serves as corollary to Girard's observation.

\textsuperscript{o} "Elle est appliquée systématiquement à Madagascar depuis plusieurs années chez tous les contacts connus de pesteux pulmonaires et sans attendre chez eux la moindre manifestation pathologique ... On utilise soit le 693 MB (Dagenan), soit le 2090 RF (Thiazomide) à la dose quotidienne per os de 4 g pour les adultes et 3 à 2 g pour les enfants suivant l'âge ... Le Dagénan et la Thiazomide ont pratiquement donné jusqu'ici à Madagascar 100\% de succès dans la prévention de la peste pulmonaire chez les contacts ..."
in contrast to serum-prophylaxis. sulfonamides (Dagenan, Thiazomide) are efficacious in a dose of 3 g per day for 6 days. It is exceptional now to see the development of pneumonia in contacts who have been spotted, isolated, and treated early. This is a considerable progress confirmed by 10 years' experience in Tananarive.

Pollitzer was able to suppress an incipient pneumonic plague outbreak in a rural area near Tsinkiang, China, by giving sulfadiazine, in doses varying according to degree of exposure and age, to 26 contacts. None of these individuals, who were left in their houses, developed the disease.

Results recorded by other observers were as follows:

<table>
<thead>
<tr>
<th>Locality</th>
<th>Reference</th>
<th>Date</th>
<th>Number treated</th>
<th>Drugs and dosage used</th>
<th>Instances of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oran (North Africa)</td>
<td>Roux &amp; Mercier</td>
<td>1946</td>
<td>85</td>
<td>Sulfathiazole (3 g daily, apparently for 7 days).</td>
<td>One (patient recovered)</td>
</tr>
<tr>
<td></td>
<td>Gordon &amp; Knies</td>
<td>1947</td>
<td></td>
<td>4 g sulfadiazine or sulfathiazole per day. Treatment was continued until 2 days after the death of the last victim in the house concerned.</td>
<td>None</td>
</tr>
<tr>
<td>Iranian Kurdistan</td>
<td>Baltazard</td>
<td>1948</td>
<td>?</td>
<td>Sulfadiazine—6 g daily for 1 week to close contacts, 3 g daily for 1 week to those under lesser risk.</td>
<td>None</td>
</tr>
<tr>
<td>Nanking (China)</td>
<td>Huang et al. ^55</td>
<td>1948</td>
<td>64</td>
<td>Sulfadiazine (4-12 g for 4-6 days).</td>
<td>None</td>
</tr>
<tr>
<td>Mukden (China)</td>
<td>Tieh et al. ^102</td>
<td>1948</td>
<td>4 (all found to harbour P. pestis in their throat)</td>
<td>Sulfadiazine (4 g on first day of treatment, then 3 g for 6 days, for adults; half of these doses for children).</td>
<td>None</td>
</tr>
<tr>
<td>Foochow (China)</td>
<td>Feng ^28</td>
<td>1949</td>
<td>33</td>
<td>Sulfadiazine (4 g on first day of treatment, then 3 g for 6 days, for adults; half of these doses for children).</td>
<td>None</td>
</tr>
<tr>
<td>Copshi (Ecuador)</td>
<td>Saenz Vera ^163</td>
<td>1949</td>
<td>40</td>
<td>&quot;Adequate doses of sulfathiazole&quot; (apparently 3 g daily).</td>
<td>None</td>
</tr>
</tbody>
</table>

Note. A failure of abortive treatment with sulfapyridine was reported by Plum in Nairobi, Kenya.

^P "Comparant avec l'échec de la séroprophylaxie, les sulphasides (Dagenan, thiazomide) sont efficaces à la dose de 3 g par jour pendant 6 jours. Il est maintenant exceptionnel de voir éclater un cas de peste pulmonaire chez des contacts repérés, isolés et traités précocement. C'est un progrès considérable, que confirme une expérience de 10 années à Tananarive."
As will be noted, the dosages used by the different workers, and the number of days for which they administered the sulfonamides chosen by them, varied considerably. The standard recommendation made in this respect by the WHO Expert Committee on Plague in 1952 was the administration of daily doses of 3 g of a suitable sulfonamide over a period of 6 days.

_Bubonic plague._ As recorded by some workers, such as Gordon & Knies, Lewis et al., Macchiavello, and Saenz Vera, administration of sulfonamides to contacts with bubonic plague patients was effective. Erzin & Payzin had good results when giving plague serum as well as sulfonamides to such contacts.

However, as pointed out by Pollitzer, it would not be advisable to make large-scale use of abortive sulfonamide treatment when dealing with bubonic outbreaks, because

"... during these the danger of human infection is much less limited as to space and time than is the case in lung pest where familial explosions are the rule. In rat-caused and flea-borne plague on the contrary, where as a rule merely off-chances for human infection exist, the cases are usually spaced out and in the great majority of instances we find but one victim per house. To administer chemo-prophylaxis (scilicet abortive treatment) to the contacts of patients suffering from bubonic plague without lung complications, seems therefore as a rule not indicated. To use the method on a settlement- or precinct-wide scale would be quite expensive and, seeing that the protection thus conferred is of a rather ephemeral nature, the value of the procedure would be questionable."

Certainly, however, it is imperative to administer abortive treatment with sulfonamides to contacts of bubonic patients with lung involvement. One should also not hesitate to use this method for the purpose of warding off bubonic plague attacks if special conditions, such as the occurrence of laboratory or postmortem accidents, or the occurrence of plague on a ship or in a port hitherto free of plague, warrant this. It must be realized, however, that, in general, other methods are of prime importance for the control of bubonic plague.

To administer antibiotics, instead of sulfonamides, for abortive plague treatment would be inadvisable, if for no other reason than that the danger of producing drug-resistant strains would be far greater when limited amounts of the antibiotics were used for this purpose than when large doses were given for the treatment of manifest plague attacks. One should realize, therefore, that while the antibiotics are of supreme importance for plague treatment in general, sulfonamides are the drugs of choice for the special purpose of abortive treatment.
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Chapter 9

Epidemiology

Introduction

In dealing with the epidemiology of plague, as will be gathered from chapters 4 and 8, two fundamentally different forms of the disease should be considered:

1. bubonic plague, produced, as a rule, by the bite of plague-infected insect vectors, mainly rodent fleas;

2. primary pneumonic plague, due to a spread of the infection from man to man.

Even though "free-living" infected rodent-fleas may be temporarily important in the causation of bubonic plague in man, the continued existence of this form of the disease depends in the long run upon the persistence of the infection in the rodents. The name of "zootic" plague recently recommended for this form of the disease is therefore most appropriate.

Though initially dependent upon a spread of the infection from bubonic plague patients with secondary lung involvement to members of their families or other contacts, primary pneumonic plague is apt to spread, regardless of whether or not infected rodents and/or infective fleas continue to be present in the localities in question. In fact, purely pneumonic plague epidemics, due to the arrival of patients with secondary or primary lung involvement, have quite often been observed in localities where the rat populations were originally free from plague and where they remained entirely so even when the disease became rampant in man.

No doubt, therefore, primary pneumonic plague well deserves the recently proposed designation of "demic" plague—the more so because, just as in the so-called bubonic outbreaks, cases without apparent buboes are met with, so in pneumonic epidemics, pulmonary cases without manifest lung involvement occur and rare bubonic cases may be produced through
contact infection or, exceptionally, through the bite of infected human ecto-parasites.

**BUBONIC (ZOOTIC) PLAGUE**

**Cause of the Outbreaks**

As noted in chapter 6, the Plague Research Commission was able, through a series of well-planned experiments, to disprove that infection through the air, through direct contact, or through contaminated inanimate objects was of any importance in the transmission of plague from rat to rat, and to establish, on the other hand, that plague was principally an insect-borne—and, particularly, a flea-borne—infection.

At the same time, the Commission adduced convincing proof that what had been established in regard to the spread of the infection among the rats, also held true for the conveyance of bubonic infection to man. As summarized by Lamb,⁴⁰, ⁴¹ the Commission found, in this respect in particular, that:

(1) A direct spread of the infection from bubonic patients was most unlikely, because (a) their excreta as well as those of the rats were found to be non-infectious when tested in the laboratory under conditions analogous to those in nature, and (b) the pus of healing buboes contained few, if any, virulent *Pasteurella pestis*.

(2) There was no convincing evidence to show that the human flea played an important role in the conveyance of the infection to man.

(3) Those attending bubonic plague patients remained singularly free from infection, the plague hospitals being in fact the safest places during the outbreaks.

(4) The contacts of patients who developed bubonic plague after arrival in a hitherto unaffected locality invariably remained well.

(5) The great majority of the patients whose history was accurately known had had no contact with previous cases before falling ill.

(6) If bubonic plague appeared in a settlement, in the great majority of instances not more than one case occurred per house.

(7) If multiple cases occurred in any house, they often appeared simultaneously, as if infected from a common source.

(8) If successive cases appeared in a house, invariably there was evidence of a higher rat-mortality than that found in houses yielding single plague cases.

The Plague Research Commission also established that close relationships existed between *Rattus rattus* epizootics and bubonic epidemics in time, in place, and in quantity.
As summarized by Wu Lien-teh, in Bombay City, the average time-interval between the epizootics and the appearance of the epidemics was 10-14 days, and in a Punjab village, about a week—the difference being due to the fact that the Bombay figures were based on plague deaths, and those for the village on attacks.

The period of 10-14 days observed in Bombay comprised: (a) three days which, according to laboratory observations, had to elapse before the fleas, coming from the dead rats, were willing to attack man; (b) the average incubation period of human plague (three days); and (c) the average length of illness ($5\frac{1}{2}$ days).

The observations of the Commission also left no doubt that the places of infection in Bombay were the same in the case of R. rattus and in that of man. The same held true for the Punjab villages, but there account had to be taken of the close aggregation of the houses and the intercommunication of rat burrows between neighbouring habitations.

However, the Commission laid stress upon the fact that, evidently because many plague rats died in their burrows, infected fleas could be trapped in houses where no rat-falls had been noted.

A close relation in quantity was found to exist between the rattus epizootics and bubonic epidemics. If no rats with acute plague were found during the off-seasons, human cases were also absent. During the seasons the epidemic curve closely followed that of the rattus epizootics. An intimate relationship also existed between the number of plague rats found in a house and the appearance of human plague. Thus, in one of the villages studied, human cases occurred in only $3\%-4\%$ of the houses in which single plague rats had been found, whereas they occurred in $28\%$ of the houses in which more than one plague rat had been found.

Generally speaking, the conclusions reached by the Plague Research Commission have remained unchallenged up to the present. In particular, it has been confirmed through ample observations in various plague areas that, in contrast to the "demic" form of plague, the plague cases appearing during "zootic" outbreaks in individual houses usually remain single.

As has been discussed in chapter 7, some subsequent observers have maintained that in addition to, or even instead of, rodent fleas, human ectoparasites, particularly Pulex irritans, are of importance in the transmission of bubonic plague. While admitting that in places where these ectoparasites abounded, they might play a role in this respect, it was stressed that in most plague areas, particularly those in China, India, and Madagascar, the part taken by human parasites in the conveyance of the infection was negligible, the transmission of plague to man depending upon the rat fleas. It was admitted, however, that the importance of wild-rodent fleas in the conveyance of the disease to man was not as universal, human infections in the "sylvatic" plague foci being partly due to direct contact with diseased rodents.
Attention was also drawn to observations showing that cases of tonsillar plague were found to be frequent among the Indians of Ecuador, who were in the habit of killing fleas and lice caught by them with their teeth.

A few observers, such as Durand & Conseil and Nikanoroff, conceived the idea that human convalescent or healthy carriers might be of importance in the perpetuation and spread of bubonic plague, the former two authors postulating that individuals harbouring \( P. \text{pestis} \) might develop plague bacteraemia if they became the prey of intercurrent diseases such as influenza or measles. However, no definite evidence has ever been brought forward to support these assumptions and there seems no reason, therefore, to revise the opinion expressed by Wu Lien-teh that "like rodents with chronic plague, human carriers represent, so to speak, a sidetrack of the infection which ends blindly".

While it is generally admitted that even though plague-infected rats are found in individual houses or compounds, human cases do not invariably follow in them, the opinion, held by most workers, that rat plague may exist in a community without leading to the appearance of the disease in man has not been universally shared. Petrie, in particular, seems to have been sceptical in this respect. Discussing the discovery of "clandestine" foci of rat plague in Java by Swellengrebel & Hoesen, he pointed out that the absence of human cases in these localities might have been merely apparent, because the methods available for case-finding were far less exact than the pooling tests used to detect the presence of \( P. \text{pestis} \) in the rats and their fleas.

There can be no doubt, however, that—as proved by recent experiences—rat epizootics may run their course without leading to human plague. The paucity, or even absence, of human cases in foci of wild-rodent plague also deserves great attention in this connexion.

**Trend of Epidemics**

In correlation with the slow evolution of the causative epizootics and the consequent restriction of the number of flea vectors initially available for human infection, the onset of bubonic epidemics is, as a rule, rather gradual. Hand in hand with an increasing rodent mortality and a corresponding increase of the "infection quantum" represented by infective rodent fleas, the outbreaks spread and reach the stage of their full development. This period of maximal morbidity and mortality is followed by a third stage of gradual decline of the epidemics (Dieudonné & Otto). The evolution of bubonic outbreaks is thus markedly different from that of pneumonic epidemics, which develop far more rapidly. However, in contrast to the latter which, though apt to become rampant, are usually of an episodic nature, bubonic manifestations, because they serve, so to speak,
merely as an index of a persisting rodent infection, tend to recur—often over prolonged periods—whenever conditions for a transition to man become suitable. From what has been discussed in chapters 6 and 7, it will be gathered that this periodic or, one should say more accurately, "seasonal" incidence of bubonic plague outbreaks is governed by changes in the climatic conditions which, mainly by altering the infectivity of the flea vectors, exert a powerful influence upon the trend of the epizootics and, consequently, a most marked, though indirect, influence upon that of the epidemics.

The data furnished in chapter 1 regarding the seasonal incidence of plague in the recently or currently affected areas may thus be supplemented and summarized:

<table>
<thead>
<tr>
<th>Area</th>
<th>Plague seasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>North China. Outbreaks started in late summer or autumn, sometimes extending into the cold season (Wu Lien-teh 98). Fukien Province. Commencement in spring (usually between April and June). Epidemics lasting until early autumn, sporadic cases occurring as late as October and November (Park 59). Kwang-tung Province. February-June (Park 59). Hainan Island. February-April (Landauer 42).</td>
</tr>
<tr>
<td>Burma</td>
<td>The usual plague season falls in the period November to April, with a peak in February in Upper Burma, and in March in Lower Burma, where there may be a secondary rise in July (Park; 59 Wilcocks 95).</td>
</tr>
<tr>
<td>Viet Nam (South)</td>
<td>The first three quarters of the year, with a peak in April-May (Park; 59 Herivaux &amp; Toumanoff 32).</td>
</tr>
<tr>
<td>Thailand</td>
<td>According to Bangxang, 3 case incidence begins to increase in September, reaching its highest point in February and March. Low level from May to September, particularly during the wet months of June and July.</td>
</tr>
<tr>
<td>Java</td>
<td>Seasonal incidence not pronounced because, despite the presence of a dry and a wet monsoon, the temperature remains practically uniform throughout the year. Still, plague mortality begins to increase in the third quarter of the year, reaching its maximum in December or January (middle of wet season), commencing to decrease in February, and continuing to decrease in May and June (dry season) (Park 59).</td>
</tr>
<tr>
<td>India</td>
<td>Dealing merely with the areas which were chiefly affected within recent times, Park 58 stated that: “Although plague is at a minimum during the hot and dry summer months, which in general all over India are from March to May, the incidence during the colder months varies in the different provinces owing to the varying rainy season, which in turn controls both the lowering of temperature and the rise in humidity ... The South-west Monsoon breaks on the west coast of India early in June, and provides suitable climatic conditions to permit in Bombay Presidency a peak incidence in October. Thereafter, the infection maintains a fairly high level till the end of the year, only to decline in the heat of March-April of</td>
</tr>
</tbody>
</table>
the following year. Madras Presidency is subject to both the South­west and the North-east Monsoons and its seasonal incidence is thus more complicated, but on the whole it provides a peak incidence much later than Bombay in January, in common with the neigh­bouring States of Hyderabad and Mysore. The North Indian Provinces of United Provinces and Bihar are seen to have their peak incidence in March, and in common with the rest of India, the incidence declines sharply with the dry heat of the second quarter."

Egypt
As stated by Kamal 37 (see also Wakil, quoted by Pollitzer 63), the months of maximal plague prevalence are: in northern Egypt (which has a Mediterranean climate), June and July; in middle Egypt, May; in upper Egypt (which has a hot and dry climate tempered by northern winds), March and April.

Tunisia
Outbreak at Ferryville observed by Magrou 48 lasted from August 1944 until March 1945. Percentage incidence of Xenopsylla cheopis was, according to Meunier, 51 highest in summer.

Algeria
Incidence highest in August (mean temperature 80°F (27°C)) plague then declining with the increased rainfall and falling tem­peratures from September onwards (Davis 13).

Morocco
According to Davis 13 it could be noted "that plague is most pre­valent at Casablanca in May and at Marrakesh in June and that in both areas there are two peaks during the year—namely, in May­June and October at Casablanca and in June-July and November at Marrakesh. The hot weather of midsummer seems to have a depressing effect on plague incidence in both areas ".

Senegal
Davis 13 stated that: "The most prominent feature of the seasonal epidemiology of plague in Senegal is the sharp rise after the dry period January-February (when the dry harmattan winds blow) to a peak in June before the onset of the rainy season during July­September. There is a noticeable recession in the two hottest months of the year—namely, September and October. The rise in tempera­ture and relative humidity before the rains seems to favour human­plague transmission, which is checked by high temperatures at the end of the rainy season (October)."

East Africa
Dealing with the three following East African plague regions, Davis 13 stated that the equatorial climate at relatively high eleva­tions with rather slight variations in mean temperature throughout the year appears to favour a uniform distribution of human plague without marked seasonal peaks.
Belgian Congo. Case incidence lower between December and April, higher between May and November with a slight recession in August and September; the periods of lower incidence correspond to the drier times of the year and the first months of the long rainy season which follow the dry season of December and January (Davis 13).
Uganda. Seasonal plague incidence similar to that in the Belgian Congo. According to Hopkins, 65 all endemic foci are situated in areas with a rainfall above 45 inches (1,140 mm).
Kenya. Though cases were recorded in all months, Roberts 70 found during an outbreak at Keruguya a rise in the case incidence at the time of the harvest, the incidence becoming highest between the two rainy seasons.
As emphasized by Davis, in contrast to the fairly uniform seasonal distribution of plague incidence in the above-mentioned East African regions, there is a well-marked plague season in Tanganyika which, lasting from February to April, corresponds to the single rainy season. The tropical climate prevailing during the rest of the year obviously prevents an incidence of human plague.

According to Robic, the climatic conditions prevailing in the hot and damp coastal areas are unsuitable for the persistence of plague, while the relatively cool and moist weather on the high plateau creates a most favourable environment for its continued existence. Though cases occurred there throughout the year, plague regularly assumed epidemic features during the period of October to March, when, as recently summarized by Girard, free-living X. cheopis abounded in the houses where plague cases had occurred, and were frequently infective. During the cool and rainless winter months, when the fleas appeared to be inactive, the incidence of bubonic plague was lowest. However, as stated by Le Gall and by Girard, pneumonic plague cases were comparatively more frequent in winter.

As stated by Davis, in the Union of South Africa, plague epizootics may be in progress at all times of the year. Likewise the secondary infections of commensal rodents, which are directly responsible for human manifestations of the disease, may take place at any time. Nevertheless, human attacks were most frequent in summer. The seasonal incidence of human plague in Basutoland, in South-West Africa, in the Bechuanaland Protectorate, and in Northern Rhodesia corresponded to that in the Union of South Africa (Davis).

According to Meyer, epizootics among the ground-squirrels, which led to the appearance of sporadic plague cases in man, began early in spring, rose in intensity during the summer months, and slowly declined during autumn to disappear entirely during the winter in regions where the animals hibernated. However, in some localities young ground-squirrels, which were apt neither to aestivate nor to hibernate, could be found plague-infected in December and January.

As summarized by Mohr, the cheopis infestation of the Honolulu Norway rats was lowest in October and highest in January. June also appeared to be a high month, but May and July showed low infestation-rates. Mohr added that, since temperature and humidity at Honolulu were high, but not too high for X. cheopis, infestation of the rats with this flea was marked.

As noted in chapter 1, the 1943 plague outbreak in Aragua State appeared in July, i.e., one month after an increased incidence of X. brasilienensis had been noted.

From chapter 1, it may be gathered that bubonic plague was most frequent at São Paulo in the south of the country in summer with a peak in January. In the north-east of Brazil, on the contrary, the incidence of the disease was comparatively highest from July to October, while the onset of the epidemic season in Ceará, which is still farther north, was in May and June. Dealing with the epidemiology of plague in Brazil in general, Barreto & Castro noted an increase of the case incidence in spring.
or autumn when, following periods of heaviest rainfall, the temperature ranged from 19°C to 26°C (66°-79°F) and the relative humidity from 66%-83%.

Argentina

While, as maintained by Villafañe Lastra et al. the plague incidence was formerly highest in summer, the recent rural outbreaks occurred mainly in winter (Sussini; Barrera 4).

Bolivia

According to Siles, both summer and winter outbreaks of plague were observed.

Peru

Generally speaking, the annual plague epidemics in Peru tended to reach their peak during the summer months, but in areas where the winter months were warm, the plague seasons fell into an earlier period than in localities with a colder climate (Eskey 19).

Ecuador

Plague was generally most rampant in December, of low incidence in June. However, the outbreaks in Loja Province occurred mainly during the dry season from May to December (Moll & O'Leary 54).

At first glance, the data assembled in the above tabulation appear to be rather contradictory, because it will be noted that in some of the plague areas the case incidence was highest during the warm seasons and in others during the cool seasons, and that the prevalence of rain sometimes favoured and sometimes cut short the spread of the infection to man. However, whenever it is possible to correlate the statements made in regard to the seasonal incidence of bubonic plague with adequate meteorological data, it will be found that invariably the epidemics occurred during seasons in which the temperature was moderately high and the saturation deficiency of the air low. Though some exceptions appear to exist, as a rule bubonic plague epidemics occurred at times when the prevailing climatic conditions were within the limits found suitable by Brooks for the occurrence of such outbreaks in India.

**Spread and Persistence of Outbreaks**

The consequences of plague invasion of the various recently or currently affected countries which, as can be gathered from chapter I, was due mainly to importations by the sea-route varied according to local conditions. Whenever the climate prevailing at the time of the invasion or throughout the year was unsuitable, the infection failed to entrench itself. It is noteworthy, however, that even though, owing to such unfavourable conditions, plague was unable to gain a permanent foothold in the coastal regions of Madagascar, it still managed to penetrate into the interior of the island where almost ideal conditions for its persistence existed.

The prevalence, or sole presence, of comparatively inefficient vector fleas was also apt to impede the spread of plague, particularly its transition to man. Thus, as pointed out by Eskey, in Seattle, Wash., USA, where *Nosopsyllus fasciatus* was the vector, plague smouldered among the rats for
ten years, and yet only three human cases were recorded throughout this period.

However, instances such as those referred to above were exceptions rather than the rule. Usually, the invasion of a sea-port by the maritime route (or, as shown in the case of Hong Kong and Canton, even by the land route) was followed not only by an often prolonged, though gradually decreasing, local occurrence of plague, but also almost invariably by a spread of the infection to the hinterland. Practically without exception this led first to the involvement of inland towns connected with the ports by major traffic routes—particularly railways and rivers—and secondly to a further spread of the infection to rural areas which, because it usually depended upon a transport of infected fleas by primitive means of communication, was apt to take place in a rather haphazard manner. For reasons which will be discussed later, plague, while tending gradually to disappear, or to become minimal, in the urban centres of the hinterland as well as in the coastal towns, showed a most marked tendency to remain endemic in rural areas. Quite often, the infection, sallying forth from these strongholds, produced epidemics in adjacent areas which no longer permanently suffered from the disease.

Although it is legitimate to consider the above-described mode of plague propagation, which has been observed regularly in widely distant countries, as typical, many local variations of the process have been found to exist, owing principally to differences in the rodent species which served as reservoir of the infection. While in some of the countries commensal rodents continued to harbour the infection when rural areas became involved, in others the infection spread to wild-rodent species. Foci of true "sylvatic" plague could thus be formed, dangerous to man only when he penetrated into the remote haunts of the species concerned. In other cases, however, the wild rodents involved lived so near to man that their fleas could cause human infections within the settlements or could at least infect commensal rodents which in their turn brought the disease into the houses.

Under these circumstances, it is not surprising to find that different authors proposed different schemes for classifying the various stages of plague. Thus Devignat distinguished three levels ("plans") on which plague could develop—the "domestic-murine" ("murin domestique"), the "sylvatic murine" ("murin selvatique"), and the "human" ("humain")—which could be independent of one another or could be brought into relation by different modes of transmission.

In a valuable study, Macchiavello distinguished four main stages in the propagation of plague in South America, namely: (a) invasion of the ports; (b) spread by railways and other means of communication to the towns of the hinterland; (c) subsequent involvement of adjacent rural areas; and (d) transition of the infection to wild rodents.
Important though the latter classification is for historical surveys, it suffices for the purposes of the present disquisition to give separate consideration only to: (a) urban manifestations; (b) rural manifestations, including under this heading, besides rat-caused outbreaks, those of a "peridomestic" origin, due to an interchange of the infection between commensal and wild rodents or to proximity of the latter to human habitations; and (c) wild-rodent plague in the strict sense of the term. These three main categories will now be dealt with seriatim.

**Urban manifestations**

As stated before, for various reasons, particularly owing to untoward climatic conditions or to the lack of efficient flea vectors, plague may fail to gain a foothold, or at least a permanent foothold, in the urban centres into which the infection has been introduced. However, as shown by many examples, given a suitable climate, a sufficiently large and susceptible rodent population, and the presence of a sufficient number of capable vectors, plague often became firmly entrenched among the rats of the affected cities or major towns, and persisted in the rodent populations for at least a number of years, not rarely for many years. If, as was often the case, the prevailing climatic conditions did not favour perennial epizootics, plague assumed epizootic proportions whenever suitable seasonal changes of the weather took place. The high mortality of the rats during these seasons led to the liberation of infective rat fleas and, consequently, to the appearance of human plague manifestations, which, at least during the period immediately following the introduction of the infection, practically always assumed epidemic proportions.

During the off-seasons, when, as a rule, the surviving rats were capable of attracting the fleas which had left the few animals that succumbed to plague, human cases, if occurring at all, were few and far between.

Several reasons have been adduced to explain the gradual diminution and ultimate disappearance of the infection, which are characteristic for urban zootic plague. Thus some authors, such as, recently, Macchiavello, stressed that a decimation of the rats through severe epizootics might greatly diminish or even abolish the chances of infection among the scattered survivors. However, the importance of this factor is limited, because urban plague often spreads in an irregular manner so that, even in severely-affected precincts, groups of rats may remain unscathed. Moreover, unless the reduction of the rat population becomes extreme, an increased fertility of the survivors is apt to lead soon to a re-establishment of the former population level or even to an increase in population. As maintained in chapter 6, a vicious circle may exist in this respect, a decrease of the rat population through an epizootic leading to more frequent births during the following off-season, which in their turn help to promote the next epizootic.
One must fear that the procedures employed hitherto for rat destruction play an ambiguous role in the control of urban plague, since they also often act as a stimulus for a high compensatory fertility among the survivors. That, generally speaking, one should not think too highly of the routine methods formerly used for plague control, is exemplified by the observations of Uttley in Hong Kong. He concluded that the decrease of plague in that port, and its final disappearance in 1923, could not be ascribed to the measures taken, because simultaneously the incidence of the disease decreased in the adjacent parts of south China, where no, or at least no intensive, control work had been done.

Even at best, the influence of the factors considered above is of limited importance compared to that exerted by the gradual extinction of plague-susceptible rats which, taking place in the course of the successive epizootics, leads to an increasing prevalence of plague-resistant rat strains and finally to the exclusive presence of rats refractory to the infection. The evidence adduced in this respect in chapter 6 leaves no room for doubt that during the present pandemic, as well as in historical times, this gradual replacement of susceptible rats by resistant rats exerted a profound influence upon the trend of the urban zootic plague outbreaks, and finally led to their disappearance.

As has been stated in chapter 6, not much information is available as to how long this herd resistance was apt to continue in the absence of autochthonous plague. It stands to reason that, in such cases, a gradual multiplication of a few susceptible rats, which had been spared during the past epizootics, might take place, and that, likewise, susceptible imported rats might have a chance to multiply. As was shown in chapter 6, such an evolution actually took place in Bombay. As also observed there, a change in the situation may likewise be effected by a multiplication of rodents other than the common rats, which, because formerly less numerous, have suffered little in the past rat-epizootics and, therefore, have not lost their herd susceptibility to plague.

That under such circumstances a reimportation of plague may create a dangerous situation for man, has been proved by recent experiences in Bombay, which may be recorded thus:

<table>
<thead>
<tr>
<th>Year</th>
<th>Plague-infected rodents found</th>
<th>Plague cases among residents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R. rattus</td>
<td>Gunomys kok</td>
</tr>
<tr>
<td>1948</td>
<td>2</td>
<td>42</td>
</tr>
<tr>
<td>1949</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1950</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1951</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1952</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

It will be noted that, owing no doubt to a reimportation of the infection from Bombay State, an enzootic was present among Bandicota bengalensis kok (Gunomys kok) and, to an apparently lesser extent, also among R. rattus in 1948 and 1949, and was obviously responsible for a number of plague
cases among residents of Bombay who had never left the city. Though no infected rodents were found afterwards, the epizootic probably continued, because further plague cases among residents were recorded in 1950 and 1952 respectively. It should be noted in this connexion that so far no pooling tests have been used for rat examination in Bombay.

In assessing the importance of these observations, one must admit that (a) an unusually favourable situation for the reappearance of plague has been created in Bombay through the recent multiplication of the fully susceptible *B. bengalensis kok*, and (b) as proved by the almost all-yearly occurrence of imported human cases, chances for reimportation of plague into the city were considerable.

Nevertheless, though they are probably exceptional, these findings show that zootic plague may become re-established in an urban community comparatively soon after it has become extinct.

The extent to which plague-affected urban communities serve as distributing centres of the infection may vary considerably. Apart from the degree to which the cities and towns are affected, which often shows seasonal variations and is apt to decrease in due course, the role which these centres play in the distribution of plague depends, in the first place, on their commercial importance and on the means of communication they possess. If railways or steamers are available for traffic, a spread of plague from seriously-affected urban centres, particularly from those of great commercial importance, is practically inevitable and is apt to lead to an importation of the infection into even quite distant towns, which then become secondary plague-distributing centres. Vice versa, the extent to which these secondary centres spread the infection decreases pari passu with their lessened importance for commerce and traffic. If they possess only primitive means of communication, they may distribute the infection merely in their immediate environs. Naturally, such a spread to surrounding rural districts is bound to take place also in the case of important plague-infected centres, in addition to a long-distance spread of the infection by modern means of communication ("peste urbano-rural" of Macchiavello 47).

**Rural manifestations**

In contrast to purely "sylvatic" plague, the epidemiology of which will be dealt with separately, the rural manifestations of zootic plague—due to a presence of the infection in commensal and also in "peridomestic" wild rodents—have no independent standing in so far as, practically always, their initial appearance, and sometimes even their recurrence, is due to an importation of the infection from some plague-affected urban centre. Greenwood, 31 studying the outbreaks in the Punjab, maintained in this connexion that such an importation of the infection was most likely to occur in the case of villages which were large and situated near major lines of communication. The proportion of small villages which remained
free from infection when plague became epidemic in an area was therefore much higher than that of larger villages or towns.

The problem of the persistence of plague in rural areas has been studied by numerous workers. Confirming and supplementing observations made in this respect by the Plague Research Commission and other early investigators, Kunhardt, in a paper read in 1912 at the Second All-India Sanitary Conference, held at Simla, India, drew a distinction between “incomplete” and “complete” rural plague outbreaks. If the infection was introduced late in the season into a large settlement, an “incomplete” outbreak was apt to result and plague was likely to be carried over into the next season. On the contrary, “complete” outbreaks, as they occurred in small villages or in large communities which had been affected early in the plague season, were not carried over, apparently because the rat population had been decimated to such a degree that a further spread of the infection among the scattered survivors had become impossible. Kunhardt had actually found that during the period 1899-1904 only 17 villages in the Poona district (Bombay State) had carried over the infection during the off-seasons, none of them more than once. He disbelieved, therefore, that plague was endemic in the rural parts of the district.

Browning-Smith, in a paper read at the same conference, was not in agreement with Kunhardt, stating that, in the Punjab at least, the factors determining the local appearance of plague seemed to be very complex. He admitted, however, that plague rarely appeared in autumn in a place which had had a “complete” epidemic early in the year. Turkhud, who also made a report during the 1912 conference, concluded from careful observations made in the Satara district (Bombay State) that, during each off-season, plague was carried over only in one village, whence the infection spread to the other villages which became involved in the same year. A different village was found to be the fountainhead of the infection in each subsequent year.

Acting upon the recommendation, made in 1910 by the Punjab Plague Committee, that in plague control work attention ought to be concentrated on places in which “incomplete” outbreaks had occurred, Kunhardt & Chitre worked out a scheme for predicting the carrying-over of epidemics, based upon (1) the size of the communities in question, and (2) the month when the first autochthonous human-plague case had occurred. As summarized by Wu Lien-teh, the two workers found that a carry-over of plague was possible under the following conditions.

<table>
<thead>
<tr>
<th>Population of more than</th>
<th>Date of first autochthonous plague case</th>
</tr>
</thead>
<tbody>
<tr>
<td>25,000</td>
<td>November</td>
</tr>
<tr>
<td>10,000</td>
<td>December</td>
</tr>
<tr>
<td>4,000</td>
<td>January</td>
</tr>
<tr>
<td>1,800</td>
<td>February</td>
</tr>
<tr>
<td>800</td>
<td>March</td>
</tr>
</tbody>
</table>
Strickland,\textsuperscript{83} while agreeing in principle with Kunhardt & Chitre, pointed out with much reason that the figures arrived at by these two workers were not generally valid, but that, on the contrary, a separate formula had to be worked out for each plague district. Thus in his experience in the Belgaum and Dharwar districts (Bombay State), a carry-over took place in settlements which were much smaller in size and had been infected earlier than had been found by Kunhardt in the near-by Poona area. Strickland also suggested that the transportation of grain after the harvests, because it facilitated the transportation of rats and fleas, might lead to an increased number of village infections.

According to the observations of George & Webster,\textsuperscript{24} the villages of the Cumbum valley in southern India also did not suffer from plague "equally from year to year ". It was, no doubt, due to this inconstancy of the plague manifestations that, as established by these two workers, the rats of the Cumbum valley were almost invariably susceptible to cutaneous or subcutaneous inoculations with \textit{P. pestis} (97.5\% positive results in 320 rats tested).

Observations similar to those recorded above were also made by workers in China. Landauer,\textsuperscript{42} discussing the plague situation in Hainan, came to the conclusion that, in that island,

\begin{quote}
the infection of the villages represents a distinct phase in the epidemiology of plague. The towns are infected first, but the disease has a tendency to become extinct after a series of more-or-less violent eruptions. The second stage (which does not follow necessarily) consists of an invasion of the country, where the disease assumes a different character. The small number of rats implicated leads to an uninterrupted chain of sporadic cases or of small epidemics, comprising at most 10 persons per locality. Once the disease has reached this stage, it rarely shows a tendency to disappear spontaneously from the regions thus affected and very often towns which had been free from plague for a number of years are reinfected from the villages \footnote{... l'infection villageoise représente une phase distincte dans l'épidémiologie de la peste. Les villes sont infectées les premières mais la maladie a tendance à s'étendre après une série d'éruptions plus ou moins violentes. La seconde étape (qui ne doit pas nécessairement faire suite à la première) consiste dans l'invasion de la campagne, où la maladie prend un caractère différent. Le petit nombre de rats impliqué conduit à une chaîne ininterrompue de cas sporadiques ou de petites épidémies, comprenant au plus 10 personnes par endroit. Arrivé à ce stade, il est extrêmement rare de voir la maladie s'étendre spontanément dans les régions ainsi infectées et très souvent des villes qui ont été exemptes de peste pendant un certain nombre d'années sont réinfectées par les villages.}.\footnote{... l'infection villageoise représente une phase distincte dans l'épidémiologie de la peste. Les villes sont infectées les premières mais la maladie a tendance à s'étendre après une série d'éruptions plus ou moins violentes. La seconde étape (qui ne doit pas nécessairement faire suite à la première) consiste dans l'invasion de la campagne, où la maladie prend un caractère différent. Le petit nombre de rats impliqué conduit à une chaîne ininterrompue de cas sporadiques ou de petites épidémies, comprenant au plus 10 personnes par endroit. Arrivé à ce stade, il est extrêmement rare de voir la maladie s'étendre spontanément dans les régions ainsi infectées et très souvent des villes qui ont été exemptes de peste pendant un certain nombre d'années sont réinfectées par les villages.}
\end{quote}

Similar conclusions were reached by Yang et al.\textsuperscript{101} who worked in the Fukien Province. They maintained that

\begin{quote}
... scattered cases typical for the offseason are never found in towns. They sometimes occur in the outskirts of towns, especially among the hut population, but are most frequent in isolated farms or small hamlets. Due to their isolation, number and small size, such farms rarely become infected twice. Their rat populations have had no chance to become immune and may be likened to small piles of firewood scattered over the whole region. Sparks in the form of infected fleas or rodents set them on fire and when the large fire in the town has burnt out long ago for lack of combustible matter, there is always a new pile of fuel available in the country side ".
\end{quote}
The excellent descriptions of the trend of rural plague outbreaks given by Landauer and by Yang et al. are all the more important in view of the opinion held by some observers that the appearance of the rural manifestations is invariably the result of an importation of the infection from a plague-affected urban centre. Thus Sharif & Narasimham, recently studying the ecology of plague in two districts of Bombay State, maintained that the idea that plague is more a rural problem is fallacious. In their opinion, the big grain centres received the infection from some infected village through fleas imported with grain, and caused a dissemination of plague, mainly through grain, to other villages. Usually, the grain centres themselves did not become seriously involved in this progress of the infection because, owing to past epizootics, a large proportion of their rats was plague-resistant.

It is undeniable that at a late stage of their infection plague-affected towns, as it were, serve as relay stations for the spread of the disease from one rural area to another without markedly suffering themselves. However, as proved by the above-quoted observations in China, rural plague may persist and spread after the infection has become extinct in the adjacent towns, and may be responsible, in due course, for a reinfection of the latter.

Pollitzer, who during the second World War had fairly ample opportunities of observing the evolution of plague in hitherto unaffected districts of south China ab initio, found that if a new county became invaded, as a rule rat plague, followed in due course by human infection, first became manifest in the county ("hsien") capital or some other centre like a market town. Occasionally, however, the disease first appeared in some village situated close to a previously infected district. If so, the "hsien" capitals or other important towns almost invariably became involved in their turn and then served as distributing centres. As in other countries, plague in the towns gradually decreased and then disappeared, but occasionally the infection was reintroduced from adjacent rural foci.

Plague was as a rule shortlived in the numerous villages attacked. Appearance of the infection early in the season led to a "complete" outbreak with numerous rat-falls and human victims, the marked reduction of the susceptible rat population often resulting in a total disappearance of the disease. If importation took place late in the season, limited epizootics, with little or even no human plague, developed. Rat-falls became few and far between after the end of the season, but almost inevitably such "incomplete" outbreaks led to a carry-over of the infection and thus to conspicuous outbreaks in the following season.

The local reappearance of plague in more than one season was rare but, though not persisting for long, the infection was often carried from the affected to hitherto unaffected villages, usually through the transport of infected fleas in rice cargoes.
The conclusion reached by Pollitzer was that, while in the rural areas a local endemicity was exceptional,

"the great tendency of the infection to spread to hitherto plague-free localities creates a condition of what might be termed *area-wide* endemicity, characterized by marked changes in the localization and extent of the individual outbreaks from season to season".

Several workers besides those in India and China apparently referred to the existence of such an "area-wide" endemicity. Thus, as stated by observers such as Robic and Sorel, plague in an "endemo-epidemic" form is widely spread on the Madagascan plateau where, as stated by Girard at the second session of the WHO Expert Committee on Plague, even in the city of Tananarive the rats continue to be susceptible to the infection. Discussing the peculiarities of plague in the Belgian Congo, Devignat upheld that in the Ituri region the infection was not immobilized but constantly moved from place to place in a fairly large territory, causing only isolated human cases, which were unconnected with one another in time and space.

To judge from statements made by Villafañe Lastra et al. and by Macchiavello, an area-wide endemicity existed also in some of the South American plague foci.

In a valuable study on the spread of plague in the southern and central divisions of Bombay State, Sharif reached the important conclusion that two types of epizootic could be observed in these regions. In the warm tablelands and plains the infection was often severe, leading to a heavy rat mortality and, consequently, to the disappearance of the disease within a short time. On the other hand, in the cooler regions, comprising the watersheds of the Western Ghats, plague spread slowly but, owing to a lower rat mortality, persisted for a long time. In the opinion of Sharif, these areas, which have a moderately moist and cool climate throughout most of the year, and also the hilly part of Hyderabad State, were endemic centres responsible for the occasional appearance of epidemic plague in the other parts of the affected area of Bombay State.

Since, according to the observations of previous workers who have been quoted above, an area-wide endemicity seems to have existed in districts of Bombay State now found by Sharif to suffer from occasionally imported epidemics only, one might postulate that the establishment of endemic foci in the hilly parts of the State represents the ultimate stage in the progress of the infection from the coast to the hinterland.

Be this as it may, it is certain that, in marked contrast to urban plague, rural plague, because it is usually unable to cause a gradual extinction of the susceptible host populations, is not a self-limiting disease but is apt to last for very long periods. It is important to stress this point, particularly because some observers, such as, recently, Baltazard et al., believe that, in contrast to plague manifestations caused by certain wild-rodent species, rat-caused plague manifestations are invariably of a rather ephemeral
character. It may be claimed that the evidence adduced above does not support this view.

As stated earlier, wild rodents of peridomestic habits may take part in the causation of rural plague manifestations as well as the commensal rats. Macchiavello distinguished accordingly between two types of rural plague in South America, namely:

1. “Pure” or “campestral” rural plague, in which R. rattus and X. cheopis alone played a role; and

2. “Agrestial” rural plague (“peste rural agreste”), in which epizootics among peridomestic wild rodents were added, “as a temporary and transitory epiphenomenon”, to those among R. rattus.

Outbreaks of the former type were far more persistent than the manifestations of “agrestial” plague, because the fleas of the peridomestic rodents were inefficient vectors and the climatic conditions in the open fields were favourable for X. cheopis at certain times only. However, as observed in Argentina for instance, the presence of “agrestial” plague was apt to lead to an entrenchment of the infection among “sylvatic” rodent species.

As has been discussed in chapter 7, Roberts found that X. brasilienensis was the principal vector of P. pestis in the rural areas of Kenya where plague was endemic in type, whereas X. cheopis was mainly, or even solely, involved in the urban manifestations of the disease, which were of an epidemic character. Similarly, it was maintained by Sharif & Narasimham that X. brasilienensis was the main vector in the endemic areas situated in the Western Ghats of Bombay State, where plague showed a marked tendency to spread slowly but to persist. On the contrary, X. cheopis appeared to play the principal role in the low and warm tablelands of the State, where the outbreaks were of an explosive nature but did not last long.

In view of the fact that X. brasilienensis is at least as efficient a plague vector as X. cheopis, these different roles of the two flea species cannot be ascribed to differences in their vector capacity. As pointed out by Roberts, X. brasilienensis, because it infested mainly the rats which sheltered in the thatched roofs of houses, was bound to prevail in the rural areas of Kenya, whereas X. cheopis, which mainly infested the rats living underground, found a suitable habitat in the towns. There can be no doubt as well that the climatic conditions in the hilly districts of Bombay State were more favourable to X. brasilienensis than those in the tablelands, whereas the more adaptable X. cheopis could thrive in the latter.

“Sylvatic” plague

As aptly stated by writers such as Lobo & Silvetti, the fundamental epidemiological difference between rat-caused and wild-rodent plague
is that the presence of the infection among the rats is apt to lead to the appearance of collective human cases in settlements, whereas wild-rodent plague in the strict sense is, as a rule, responsible merely for the occurrence of sporadic cases in persons who have entered the haunts of the species concerned. Nevertheless, in view of the often enormous extent of the wild-rodent plague foci, the aggregate number of human infections contracted in them may be considerable, and the case-mortality is apt to be high since the patients often receive no adequate treatment, either because they live away from centres of civilization or because, owing to its sporadic incidence, the presence of the disease is not recognized. It must also be kept in mind that importations of the infection from the wild-rodent plague foci in Transbaikalia or Mongolia into hitherto unaffected areas of China have led to the most disastrous pneumonic plague outbreaks on record in modern times.

Periodicity of Outbreaks

Cyclical periodicity

Since the severity of zootic plague outbreaks in a given locality depends largely on the number of susceptible rodents available, one would expect that a marked depletion of this fuel for the spread of the infection through a severe initial epizootic would lead to a lesser incidence of the disease until the rodents had become numerous once more. In other words, one might assume a priori that, unless particularly suitable conditions for a rapid re-establishment of the rodent population exist (as may be the case in urban communities), zootic plague would show a cyclical periodicity, years in which a severe outbreak occurred being followed by a period of one or two years during which the infection causes less havoc.

In the course of an investigation on plague in the Punjab, Greenwood found that this surmise was only in part justified by statistical evidence. There was no marked regularity in the succession of severe and mild outbreaks and, therefore, "it would not be safe to predict that a province seriously ravaged in one year will escape lightly in the following season". He admitted, nevertheless, that an exhaustion of susceptible rats exerted some, possibly a considerable, influence on the trend of plague, while climatic changes played a less, probably a much less, important role in this respect. Still, in Greenwood's opinion, there remained "a tertium quid which is not apt to be placed in evidence by statistical inquiries based on existing data".

However, observations made by subsequent workers have shown that in India, as well as in other areas, plague outbreaks may show a marked cyclical periodicity. Thus Sharif stated that:

"In the non-endemic areas [of Bombay State], such as are found in the Sholapur District, few localities get infected in one plague season, and then a very large number
of them in the next one or two plague seasons. In the third or fourth plague season, only a few of them suffer, and then plague disappears for about a year or two."

Hence, as maintained by Sharif, "the peak year in the Sholapur District appears to recur about every five years, with complete absence of plague for a year or two".

Dealing with the epidemiology of plague in north-east Brazil, Macchiavello stated:

"It is believed that an area in which plague has died out because of a lack of susceptible material may be reinfected if the rodent population happens to become sufficiently great at the same time that an opportunity for reintroduction of the plague organism occurs, such as the migration of infected rats. If this happens, however, at a time when one of the periodic nonplague epizootics has practically wiped out the rodent population, reinfection will not occur, but may skip several years until another opportunity arrives. This would explain the reappearance of plague in 5- or 10-year cycles in certain areas."

Silva, studying the situation in Ceará State in particular, found that a reactivation of plague foci which had been dormant for two to five years was not infrequent.

Plague manifestations caused by wild rodents in Argentina showed, according to Barrera, a cyclical periodicity of two years.

In South Africa, Davis found:

"A periodicity of 5-6 years in the incidence of human plague points to the existence of a general periodicity in the fluctuations in numbers of the wild-rodent population in the Union as a whole; this shows signs of breaking down as human outbreaks become more and more associated with certain limited hyperendemic areas . . ."

Quite possibly, the seriousness of the plague situation existing in the Punjab at the time during which Greenwood made his statistical investigations was, in essential respects, comparable to that present in the hyperendemic areas of South Africa.

Secular periodicity

Ample evidence has been furnished in chapter 1 to prove that the plague pandemics, following the same laws as individual outbreaks of the disease, showed a well-marked "secular" periodicity: a stage of gradual rise and spread was followed by a period of full evolution during which the disease, because it appeared mainly in the form of major epidemics, exacted a grievous toll in lives; and this period was followed in turn by a stage of gradual decline. The information supplied in that chapter also leaves no room for doubt that the present plague pandemic has reached the period of decline.

However, even though plague, which but a few decades ago ranked high among the diseases decimating mankind, now occupies a rather inconspicuous place in the fatality lists, it would be wrong to assume that this infection has altogether lost its sting.
Indeed, reconsidering this problem in the light of what has been discussed in the foregoing pages, one is led to ascribe the great reduction in the incidence of plague largely to the fact that this disease, which earlier in the present pandemic raged in urban communities, has now almost disappeared from these centres of population, and mainly occurs in rural areas where, as a rule, the case incidence is rather low. It follows that the spectacular decrease in the incidence of human plague is not by any means accompanied by a corresponding restriction of the areas in which the infection is present among the rodents. On the contrary, it must be realized that the extent of the areas in which the rodents are affected has become greatly enlarged during the present pandemic, because, progressing almost invariably from newly invaded coastal regions to the hinterland, plague has become entrenched among the rodent populations, and not rarely among the wild-rodent populations, of vast areas which had been unaffected before onset of the pandemic. This is a situation which cannot be viewed with equanimity, the less so because there is reason to believe that, in some areas, plague among rural rodents, particularly among the wild species, has not yet reached the limits of its possible regional extent, and because it is certain that some of the foci of wild-rodent plague are much larger than is usually assumed.

Forecasting of Epidemics

Attempts to forecast the appearance of bubonic plague epidemics have been made in various ways. As noted previously, Kunhardt & Chitre proposed a scheme for predicting the reappearance of outbreaks due to a carry-over of the infection from the preceding plague season. However, Strickland, while approving of this method, emphasized that the formulas worked out for this purpose had only local validity. Forster (quoted by Wu Lien-teh), while admitting that in the Punjab a drop in the plague mortality during November and December indicated a slight, or at most a moderate, incidence in the following spring, stressed also the importance of climatic factors. Prolonged periods without rain during autumn and winter, because they adversely affected the reproduction of fleas, were apt to reduce the plague mortality in the following spring. The absence of rain during November in particular seemed of great importance in this respect. Comparing the plague statistics for different areas of India with the corresponding meteorological data, Rogers found that seasonal variations in mean temperature and saturation deficiency exerted an important influence on the seasonal plague incidence, obviously by acting on the vector fleas. Rogers established, in particular, that the climatic factors of the previous year influencing the incidence of the disease were (a) the mean
temperature during the hot weather and monsoon seasons, and (b) the saturation deficiencies in both these seasons as well as in November and December. If these values were low, plague incidence was favoured. However, as was to be expected, Rogers noted that the effect of favourable climatic influences was less marked after years of high plague incidence than after a period of low incidence of the disease.

Rogers found it possible to use such meteorological observations as a basis for forecasting plague epidemics in certain parts of India and obtained, as stated by Wu Lien-teh, satisfactorily results during the period 1930-2. However, no further advantage seems to have been taken of this method. Convenient though it would be, it is not possible to base forecasts of plague epidemics upon observations of seasonal changes in the incidence of the vector fleas. It is true that, as stated in chapter 7, in regions where X. cheopis is the sole important vector, plague does not assume epidemic proportions as long as the cheopis index remains below one, and that in localities where the index is constantly above this critical level, seasonal changes in the frequency of X. cheopis are often observable, which may be of value in assessing the plague situation. It had to be stated, however, that periods during which plague epidemics occur need not necessarily coincide with those during which the cheopis incidence is highest and that indeed it is a high incidence of actually infective fleas and not the frequency of potentially dangerous vector species which is of paramount importance in the spread of flea-borne plague.

As discussed in chapter 4, Shih & Pollitzer found that observations on the frequency of rats showing signs suggestive of plague at autopsy and/or marked bacteriological evidence of the infection were apt to serve as a yard-stick for assessing the seriousness of the plague situation in the localities in question. The value of this simple method, which gave satisfactory results even when only a limited number of animals was available for examination, should not be underrated.

Race, Age, Sex, and Occupational Incidence

Most workers are agreed that differences in the race and sex incidence of bubonic plague cases, as well as the occasionally observed increased incidence of the disease among certain occupational groups (e.g., dock workers handling grain cargoes), are due merely to differences in the degree of exposure of the various groups to the infection and not to intrinsic causes. The validity of this opinion is well illustrated by observations on the sex incidence of the disease. In some plague areas, for instance according to Norman White in India, females were found to be more frequently affected; in others, in South Manchuria for example, males were more frequently affected, while in a third group of foci the incidence of disease
in the two sexes was about the same. Landauer found that while this was the case in the towns of Hainan, in the rural areas of that island more females than males fell victims to the infection.

Gill maintained that the often observed rarity, or even absence, of bubonic plague in young children was due to a state of resistance to the infection, engendered through the secretion of an endocrine gland which ceased to function when the children reached the age of about five years. However, the fact that sometimes cases among young children were comparatively not rare speaks against this assumption. For instance, Favarel, in Madagascar, found 104 cases in children up to two years of age out of a total of 2,994 bubonic and septicaemic cases (3.1%).

In the experience of most observers, including Favarel, the incidence of bubonic plague was highest in adolescents and in adults up to the age of about 45 years. That this rule is not invariable, however, is shown by the observations of Barreto & Castro who found that out of 746 plague patients, 724 of whom had bubonic plague, 25.7% were in the age-group of 0-9 years and 28.2% in that of 10-19 years.

There can be no doubt that this unusual age incidence of the disease in Brazil was due to peculiar extrinsic conditions and not to intrinsic causes.

**PRIMARY PNEUMONIC (DEMIC) PLAGUE**

In opposition to the usual opinion, some workers postulated that primary pneumonic plague might arise de novo, man contracting the infection more or less directly from plague-affected rodents without the intervention of human cases of zootic plague with secondary lung-involvement.

Thus, it was suggested by Simond and by Zabolotny that an introduction of the infection into the mouth by means of fingers soiled either by the faeces of plague-infected fleas or by handling plague-affected wild rodents might lead to pneumonic plague. Connal & Paisley and Nikanoroff maintained that dust contaminated with the faeces of plague-infected rodents might also produce a primary lung-infection.

Scrutinizing the above-mentioned and other statements made regarding the existence of “original” cases of primary pneumonic plague, Wu Lien-teh and Wu Lien-teh & Pollitzer found that, as a rule, such claims had not been substantiated by sufficiently accurate observations. The diagnosis was often based merely upon the history of the patients; complete autopsies were exceptional and thorough histological investigations had never been made in such cases. As proved by observations on “tonsillar” plague, the fact that oral infection of man with P. pestis led as a rule not to primary pneumonic plague, but to bubonic plague often followed by secondary lung-involvement, also deserved great attention.
Nevertheless, a number of instances were found which deserved the benefit of the doubt, while the "originally" pneumonic character of a few such cases—due almost invariably to infections contracted in the laboratory—could be taken for granted.

As has been stated in chapter 8, one should not be categorical in denying the possibility that patients with so-called primary septicaemic plague may occasionally be instrumental in passing respiratory infection to persons coming in contact with them, and the same might hold true exceptionally for patients with uncomplicated "tonsillar" plague or even for apparently healthy individuals harbouring *P. pestis* in their sputum or fauces. There can be no doubt, however, that in most instances primary pneumonic plague infection is due to contact with patients who suffer from bubonic plague with secondary lung-involvement.

**Rise of Epidemics**

The problem of why pneumonic plague epidemics arise received early attention on the part of the Indian Plague Commission, who noted that there are difficulties in the way of assuming a simple interrelation between the inhalation of plague bacteria in the lungs and the supervention of primary plague pneumonia.

Endeavouring to substitute a more adequate theory, the Commission suggested:

"(a) that there may be something, either in the form or in the manner in which the infectious material escapes from the body, which favours the conveyance of the infection into the lungs of persons in attendance on cases of plague pneumonia; (b) that there may be something specific in the infective material which conditions the supervention of plague pneumonia when the material is introduced into the lungs."

The Commission emphasized that there was no specific difference between the bacilli causing bubonic and pneumonic plague respectively. There might be a difference in virulence, but the evidence regarding this was contradictory. The Commission stressed, however, that the plague bacillus may, in the case of infective material derived from a pneumonic case, be associated with some other bacillus which favours its growth and contributes to the production of plague pneumonia.

The opinion of the Indian Plague Commission that intrinsic causes are responsible for the rise of pneumonic epidemics has been endorsed by many other workers, whose views may be presented as follows.

**Specific character of the causative organisms**

Practically all later observers agreed with the Indian Plague Commission that, as far as is known, no specific difference exists between strains causing
zootic and pneumonic (demic) plague respectively. Wu Lien-teh aptly stated in this connexion that

“it is as easy to cause primary pneumonic plague in suitable animals by inhaling them with a strain from a purely bubonic human case, as to produce bubonic plague in laboratory animals by percutaneous or subcutaneous infection with freshly isolated pneumonic strains or even directly with sputum or material obtained at the postmortem of lung victims”.

This opinion was endorsed by Girard, who stated in a recent summary that the oneness (“l’unicité”) of the plague bacillus causing pneumonic, as well as bubonic, plague manifestations could no longer be contested.

Special virulence of pneumonic strains

The question of whether or not the \( P. pestis \) strains causing primary pneumonic plague are endowed with a particularly high virulence has been answered differently by different workers. As will be discussed later, some observers are of the opinion that the virulence of the causative organisms increases during the outbreaks but, as rightly stated by Wu Lien-teh, such an exaltation of the virulence, gradually taking place in the course of the epidemics, could not explain their rise.

Mixed infection

The opinion of the Indian Plague Commission, that primary pneumonic plague might be the result of a mixed infection, has been shared by some subsequent workers.

Thus Norman White, on the basis of theoretical considerations, conceived the idea that

“the plague bacillus alone does not, and cannot cause widespread epidemics of pneumonic plague . . . and that it seems more than probable that there is an additional organism at work—in other words, the plague bacillus in symbiosis with another organism is responsible for epidemic manifestations of pneumonic plague, which is a disease sui generis”.

Adopting this idea, Nicolle & Gobert expressed the opinion that an association of the influenza virus with \( P. pestis \) might be of importance in the rise of pneumonic plague epidemics. A similar conclusion was recently reached by Sokhey, who stated that pneumonic plague was a combined virus and bacterial infection, and that the virus factor made the disease highly infectious, whereas plague pneumonias, as they occurred as complications to bubonic infection, were not infectious.

Dealing with the statement of Norman White, Wu Lien-teh pointed out with much reason that “no line of distinction can be drawn between sporadic and epidemic manifestations of pneumonic plague . . . Whether pneumo-pest spreads or not depends upon extrinsic and not upon intrinsic factors.”
In regard to the claim of Nicolle & Gobert, who maintained that in Tunis a close connexion existed between outbreaks of influenza and pneumonic plague, Wu Lien-teh stated that:

"Influenza is not always prevalent at the time of pneumonic epidemics. In fact only very few instances are on record where a simultaneous existence of both diseases was noted. In many others, where special attention was paid to a possible co-existence of influenza during pneumonic epidemics, the former disease was conspicuous by its absence."

This opinion was endorsed by Girard, who emphasized that in Madagascar the seasonal occurrence of influenza did not provoke an increased incidence of pulmonary complications in the course of plague outbreaks.

Contrary to the opinion of Sokhey, it is impossible to consider only patients suffering from primary pneumonic plague as infectious, in view of the fact that, as a rule, epidemics of this form of the disease have been caused by bubonic patients with secondary pneumonia. It is not surprising that a spread of the infection by the latter patients was less frequent in India than in countries or plague areas with a cooler climate.

\textit{Pneumotropismus of Pasteurella pestis}

As summarized by Wu Lien-teh and by Girard, some observers tried to explain the peculiarities of pneumonic plague by the assumption that \textit{P. pestis} could become specially adapted to the lungs, with the result that strains of this nature would produce lung involvement even when entering the body through the skin. However, the experimental evidence presented in this respect is not convincing, and it is also noteworthy that secondary lung-manifestations were by no means particularly conspicuous in the few patients who contracted bubonic plague during pneumonic epidemics through contact with the sputum of the sufferers or in other ways, e.g., through direct contact or through the bite of infected human parasites.

Moreover, it is clear that this supposed pneumotropismus of \textit{P. pestis}, which could develop only through repeated passage from lung to lung, could not be responsible for the rise of pneumonic plague outbreaks.

\textit{Role of the rodent species involved}

Claims were made by some workers that human infections derived from certain wild-rodent species, especially the Siberian marmot, were particularly apt to result in secondary lung-involvement and that this peculiarity might account for the frequent appearance of primary pneumonic plague manifestations in the areas in question. However, the evidence available in this respect is by no means convincing. Thus, as can be gathered from the
information collected by Wu-Lien-teh,\textsuperscript{96} the incidence of secondary pneumonic plague was not particularly high in Transbaikalia, where the tarabagan was the main reservoir of the infection and commensal rats played no role in the causation of the disease. Among 280 plague patients in that area, whose histories could be studied, only 10 might have suffered from primary pneumonic plague.

More important still, pneumonic plague manifestations have been found to be frequent in countries where ordinary rats alone formed the reservoir of the infection, for instance in Madagascar.

\textit{Role of the flea species involved}

No evidence exists to show that the species of vector fleas involved exerted any influence upon the appearance of pneumonic plague manifestations in man. \textit{X. cheopis} is the only vector of the infection in Madagascar where pneumonic plague is frequent.

It is of interest to note, in this connexion, that according to Eskey\textsuperscript{20} lung involvement was particularly marked in experimental animals which had been inoculated with pooled fleas, collected in the USA for the purpose of plague diagnosis, or with fleas infected with \textit{P. pestis} in the Plague Laboratory of the US Public Health Service (San Francisco). It would not seem, however, that this peculiar feature was of any practical importance. As noted in chapter 1, pneumonic plague was not unusually conspicuous in the USA, and most of the cases of wild-rodent origin occurred singly.

As will be gathered from the statements made in the foregoing pages, it has not been possible to ascribe the rise of pneumonic plague epidemics to any peculiar property of the causative organisms or to a mixed infection. There is also no convincing evidence to show that the presence of the infection in any particular rodent or flea species is of special importance in the causation of such outbreaks. Since, however, as aptly stated by Wu Lien-teh,\textsuperscript{98}

\begin{quote}
"apart from rare instances, primary pneumonic plague is not passed directly from rodents to man, but arises from human cases with secondary lung involvement, it becomes clear that factors which help to mould such secondary pneumonic features deserve our serious consideration."
\end{quote}

Discussing this problem, Wu Lien-teh\textsuperscript{98} stressed the fact that, as a rule, pneumonic plague outbreaks could be traced back to a single patient—or, at most, a few patients—suffering from bubonic plague with secondary pneumonia; quite often outbreaks were traced to a traveller who developed such manifestations while proceeding from an infected to a hitherto uninfected locality. Clearly, therefore, the rise of pneumonic plague epidemics depends not so much upon the frequency with which any kind of lung-involvement is present in the bubonic patients in general, as upon
the appearance of well-marked secondary pneumonia, leading to frequent
cough and a copious expectoration of *P. pestis* in individual sufferers.

It has been maintained, in this connexion, that an unusual susceptibility
to respiratory infections in general was apt to influence not only the
frequency, but also the severity, of secondary pneumonia in bubonic
plague patients, and consequently the rise of primary pneumonic outbreaks.
Thus Girard 27 expressed the opinion that differences in the susceptibility
to pneumococcal infections helped to explain why pneumonic plague was
rare in the coastal areas of Madagascar and rampant on the high plateau.
Similarly, Wakil 92 believed that an increased susceptibility of the dark­
skinned inhabitants of Upper Egypt to lung affections in general was partly
responsible for the high incidence of pneumonic plague in that region.

It has to be noted, however, that this factor, although apparently
exerting an influence in some plague areas, was found to be of no importance
in others. Thus, it is noteworthy that the Chinese in Manchuria, while apt
to fall an easy prey to pneumonic plague, were rather resistant to pneumo­
coccal infections (Wu Lien-teh 98).

Factors lessening the resistance to plague infection are, no doubt, of
universal and great importance for the evolution of marked secondary
lung-involvement in bubonic patients.

Attention was paid to this point by some early workers such as Simond 80
who maintained that plague pneumonia might develop in individuals
whose lymph-nodes were unable to hold back the causative organisms
introduced by the bite of infected fleas. A similar view was also put forward
in 1905 by Elliot (quoted by Wu Lien-teh 98).

Far more important than such rather remote possibilities is the well­
established fact that travellers who fall ill with bubonic plague before
leaving an infected locality, or en route, are particularly prone to develop
marked secondary lung-involvement. Petrie & Todd 61 maintained that the
appearance of these lung manifestations was due to the muscular efforts
made by such people which, causing the detachment of infected thrombi
from blood-vessels round the buboes, led to lung embolism. No doubt,
however, extrinsic factors, such as cold or rainy weather and defective
nutrition during the journey, may also be of importance in the development
of marked secondary pneumonia in travellers.

Petrie & Todd 61 postulated that, generally speaking, malnutrition,
particularly vitamin deficiencies, might enhance the susceptibility to plague
infection. They assumed that these factors might have been partly respon­
sible for the frequency of pneumonic plague during the pandemic known
as the Black Death.

Seyfarth (quoted by Wu Lien-teh 98) drew attention to the fact that
not only travellers, but also patients suffering from "ambulatory" plague,
might develop lung complications when their resistance was impaired in
some way, e.g., when they caught colds.
An elaborate hypothesis of Petrie & Todd was that a high saturation deficiency of the air, because it caused an excessive evaporation of moisture from the pulmonary mucous membranes, might lead to the development of secondary pneumonia.

Whether differences in racial susceptibility to plague infection might account for the rise of pneumonic plague epidemics, as has been claimed by some observers, seems rather doubtful. Presumably, the differences ascribed to this cause were really due to extraneous influences, such as differences in nutrition or other standards of life, or to differences in susceptibility to respiratory infections in general.

While, as shown above, an increased susceptibility to respiratory infections in general, or a lessened resistance to plague infection, is apt to exert an influence on the rise of pneumonic plague epidemics, the appearance of such outbreaks is also greatly promoted by extrinsic factors, such as adverse weather conditions or unfavourable standards of life, which lead to close contact between patients suffering from bubonic plague with marked lung-involvement and their families and friends.

Factors Influencing the Spread of Pneumonic Plague

Infectivity of the patients

Being caused by an entry of \( P. pestis \) through the lower parts of the respiratory tract, primary pneumonic plague is almost invariably contracted only by persons coming within close range of the patients, and usually results from the spraying of the infective material by the cough of the sufferers. Though persons who were near the patients for but a short time occasionally contracted the disease, as a rule infection was found to take place in those individuals who had had prolonged contact with the sufferers.

Since the frequency of the cough and the quantity of the plague bacilli sprayed by it may show most marked differences, depending upon a greater or lesser severity of the respiratory involvement, it is not surprising to find that the opinions held by different observers in regard to the infectivity of primary pneumonic plague vary greatly. It is true that patients in the early stage of the illness, i.e., the stage during which cough and \( P. pestis \) in the expectoration are rare or even absent, and similarly patients suffering from "pulmonary" plague without lung consolidation, are practically innocuous and that those suffering from slight pneumonic plague, in which cough is often inconspicuous and plague bacilli may be scanty in the sputum, are not highly dangerous. However, the reverse holds true for patients suffering from the typical form of the disease, in which cough is frequent and the sputum teems with \( P. pestis \). Still, it is noteworthy that even the contacts of such patients may escape infection when,
Woman wrapping her dead daughter in raffia matting. On her back can be seen her grandchild who was found playing near the body of his mother.

Consciously or unconsciously, they adopt precautions which, as Wu Lien-teh put it, may seem useless from a theoretical point of view.

**Immunity**

While, as noted in chapter 3, there can be little doubt that instances of natural resistance to pneumonic plague infection exist, these are of such rare occurrence as to be of no practical importance. The same holds true for the few apparently healthy persons found to harbour virulent *P. pestis* in their sputum or fauces who, one must suppose, have acquired an immunity against the infection. How long persons who had been cured of primary pneumonic plague remain immune is still unknown.

It has been claimed by some observers that certain races, particularly European ones, are not liable to contract pneumonic plague. That such an apparent resistance to the infection is due merely to extrinsic causes, however, is well illustrated by the fact that during the 1920-1 epidemic hundreds of Russians living under unfavourable conditions fell victims to the disease.

There can also be no doubt that the comparative rarity of pneumonic plague in children and in aged people, as well as the sometimes observed
absence of the disease in other groups of the population, are due merely to less close contact, or to absence of contact, with patients.

_Climatic conditions_

Though it is generally agreed that the spread of pneumonic plague is most markedly influenced by the prevailing climatic conditions, some differences of opinion exist as to the manner in which these factors exert their influence.

Fraser and Jennings maintained in this connexion that an excess of carbon dioxide, likely to be present in overcrowded and ill-ventilated houses, might favour the spread of the disease because, as had been shown by Marsh, this gas exerted in vitro a favourable influence on the growth and virulence of _P. pestis._

According to Manaud lung changes were more marked in plague-infected laboratory animals kept at lower temperatures (15°-18°C) than in those kept at about 30°C. He also established that guinea-pigs which had been made to inhale frozen particles of _P. pestis_ suspensions were apt to develop primary pneumonic plague, whereas those which had been made to inhale fluid suspensions of the organism at room temperature developed cervical buboes. Manaud postulated, therefore, that human infections during the 1910-11 Manchurian outbreak had been effected by the inhalation of frozen sputum particles which, he contended, remained more easily suspended in the air and penetrated more deeply into the respiratory tract than liquid particles.

Teague & Barber concluded from inhalation experiments made at different temperatures and under varying degrees of humidity that a lower water deficit of the atmosphere, such as is present during cold weather, caused the sputum droplets to float longer in the air—a feature which, in the opinion of the two workers, favoured the spread of pneumonic plague. Teague maintained in this connexion that the water deficit in the badly heated houses of Manchuria was not higher than that of the outside air.

In the opinion of Chabaneix, the 1910-11 epidemic was probably stopped by the dry weather prevailing in March and April.

Scrutinizing this evidence, Wu Lien-teh pointed out with much reason that pneumonic plague epidemics had occurred not only in cold weather but also in countries with a warmer or even a hot climate where the weather conditions considered as essential by Teague & Barber did not exist, and that—far more important still—a mediate infection, as presupposed by these two workers and also by Manaud, was of rather limited, if any, importance as compared to direct infection contracted in the immediate vicinity of the patients.

An influence of bad ventilation, combined possibly with that of an excess of carbon dioxide in the air, deserves attention but it is not easy to separate
the influence of these factors from that of overcrowding, brought about by absolutely or comparatively unfavourable weather conditions, such as cool or rainy seasons, or marked differences between day and night temperatures.

Social conditions

Unfavourable economic conditions, or other factors of a social nature, which lead to overcrowding, play side by side with, or sometimes even in place of, adverse weather conditions a most important role in the spread of pneumonic plague. The people's habit of congregating round the sick and holding much-attended and prolonged burial ceremonies—a common practice in many countries—also exerts an ominous influence in this respect.

That adverse social influences may promote the spread of pneumonic plague, even in localities with a warm climate, is well proved by the observations of Wakil in Upper Egypt. He noted that, owing to the system used for irrigating the fields in the southern areas of Upper Egypt,

"during the month of March, April and May, when plague is most prevalent in Southern Egypt, as well as in the subsequent months, great numbers of inhabitants are obliged to remain idle in their badly ventilated houses. There is thus a greater risk of the propagation both of bubonic and pneumonic plague than in other parts of Egypt."

This overcrowding of the houses was all the more dangerous because people hailing from the regions concerned often went to work in the then infected ports of Egypt, but almost invariably went back to their native places when they contracted a disease. If they had become plague-infected, they often arrived "with pneumonic plague complications caused by the long and tedious journey from the extreme north to the extreme south of the country."

Control measures

One must fully agree with the statement of Wu Lien-teh that "there is probably no infectious disease which, theoretically, is so easy to suppress as lung plague". Indeed, even in the past, when no effective means for abortive treatment existed, isolation of the patients, combined with segregation and careful observation of their contacts, was bound to cut short the outbreaks. Unfortunately, however, the people, resenting the hospitalization of the patients and still more their own segregation, often did all they could to hide the presence of the disease.

It has to be noted, on the other hand, that in certain remote regions (e.g., Mongolia), the people themselves often devised surprisingly adequate methods of protection against pneumonic plague.

Concluding remarks

As will be gathered from the foregoing pages, various factors influence the spread of pneumonic plague, the comparative importance of them
being apt to vary from locality to locality. If adequate facilities for control work are available, outbreaks of this disease may be quickly terminated or may even be nipped in the bud. Failing such facilities, the infection is apt to spread, particularly if—or as long as—cases of a highly infectious character prevail and if untoward weather conditions or adverse social conditions or, as is frequently the case, a combination of both these factors lead to overcrowding.

Local and Extramural Spread

The local spread of pneumonic plague is usually of a familial character. Appearing first in one or, at most, a few households, the infection is carried by visiting relatives or friends to other houses in the same community which are then apt to become subsidiary centres for the spread of the disease. A transport of the infection per saltum often takes place simultaneously, effected by persons who are incubating pneumonic plague or who are already actually ill. As is well illustrated by a comparative study of the pneumonic plague epidemics in Mongolia and China, the intensity and rapidity of this extramural spread of the infection depend upon the density of the population and the kind of communications available. In Mongolia, which was sparsely populated, and in Shansi, where the means of communication were primitive,
a slow spread took place. A much more rapid spread, not rarely characterized by long-distance sprints of the infection, could be observed in Manchuria which possessed a railway system.

**Rat Plague during Pneumonic Outbreaks**

Dealing with plague in the Punjab, Gill stated that the mode of spread of the pneumonic type "is direct from man to man, but, owing to the readiness with which rats become infected, it is liable to give rise to a rat epizootic, which in turn gives rise to a bubonic plague epidemic".

The validity of this statement, which stands in marked contrast to the opinion reached by most other workers, appears to be rather questionable if it is considered that (a) at the time when Gill made his observations, rat-caused bubonic, as well as pneumonic, epidemics occurred in the Punjab, and (b) it is often rather difficult to prove the persistence of rat-plague during the off-seasons. It seems most likely, therefore, that the appearance of epizootics during pneumonic outbreaks, as observed by Gill, was due to the recrudescence of pre-existing enzootics and not to a recent spread of the infection from pneumonic patients to the rats.

For similar reasons, no credence can be given to the claim of Allain that the rat epizootic leading to the 1920-1 plague outbreak in Madagascar was due to the infection of a few rats in the vicinity of a hospital where some earlier pneumonic-plague patients had been confined. Girard was rather disinclined to agree with Allain's contention.

As proved by observations made during the 1910-11 and the 1920-21 epidemics in Harbin and in Vladivostock respectively, in very rare instances rats may contract plague during pneumonic epidemics. It must be emphasized, however, that this transition of the infection never led to the appearance of epizootics. It seems altogether unlikely, therefore, that rats, even if they should become infected during such epidemics, could prove dangerous.

**Occurrence of Bubonic Cases in Pneumonic Plague Epidemics**

As noted before, rare bubonic cases which were definitely not due to an infection derived from plague-affected rodents, have been observed in the course of pneumonic epidemics. According to their pathogenesis, or probable pathogenesis, these cases may be classified as follows:

(a) *Infection through the bite of a patient.* This unique instance, recorded by Leumann, concerned a hospital employee in India who had been bitten in the thumb by a delirious pneumonic-plague patient. The employee developed an axillary bubo on the corresponding side, but recovered.

(b) *Skin infection through pneumonic plague sputum.* One instance of this kind observed by Jettmar concerned a woman who, having wiped
away the sputum of a pneumonic-plague patient, developed a cubital bubo, and died.

(c) Entry of plague sputum into the eye, as referred to in the eighth of these studies.\(^68\)

(d) Contact infection through the skin. Two cases of this kind are on record, concerning patients who had kissed pneumonic-plague victims and, developing plague carbuncles on the face, succumbed to the infection (Wu Lien-teh\(^96\)).

(e) Contact infection through the oral or faucial mucosa. The three patients whose records Wu Lien-teh\(^96\) was able to obtain showed "tonsillar" plague and cervical buboes. One of them recovered.

Reference has also to be made to a case observed by Fimayer.\(^22\) The patient in question, who had contracted infection through contact with pneumonic-plague patients, developed a submaxillary bubo. It was considered uncertain whether the infection had entered through the oral or faucial mucosa, or through the eye.

(f) Infections apparently due to the bites of human parasites. One must agree with Wu Lien-teh\(^96\) that the few patients with groin buboes observed during the Manchurian pneumonic epidemics had been infected through the bite of human parasites. As stated by this author,\(^98\) the rarity of such cases proved "that rat-fleas alone are of practical importance in the transmission of bubonic plague".

Age, Sex, and Occupational Incidence

Statistics collected during the 1920-1 epidemic at Harbin\(^10\) showed the following age incidence in 1,252 patients suffering from primary pneumonic plague:

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>21</td>
</tr>
<tr>
<td>11-20</td>
<td>83</td>
</tr>
<tr>
<td>21-30</td>
<td>593</td>
</tr>
<tr>
<td>31-40</td>
<td>385</td>
</tr>
<tr>
<td>41-50</td>
<td>130</td>
</tr>
<tr>
<td>51-60</td>
<td>33</td>
</tr>
<tr>
<td>61-70</td>
<td>6</td>
</tr>
<tr>
<td>71-80</td>
<td>1</td>
</tr>
</tbody>
</table>

Total 1,252

It should be noted that the incidence of the disease was highest (78.1\%) in the age-group of from 21 to 40 years. Cases among young children and aged people were rare but, as noted before, it is certain that this was due merely to lessened chances of contact and not to intrinsic causes.
All but 67 of the 1,252 patients admitted to the Harbin Plague Hospital were males, and 1,139 of them were labourers. However, this peculiar sex—and occupational—incidence of the disease is explained by the fact that the infection spread mainly among members of the labouring class who, leaving their wives and children behind, had come to Harbin in search of work, and lived there under most unhygienic conditions in overcrowded shelters. Experience elsewhere showed that pneumonic plague has no predilection for either sex.

Decline of the Outbreaks

There can be no doubt that extrinsic factors exert a most marked influence upon the decline as well as upon the spread of pneumonic plague outbreaks. If good facilities for control work are available, the outbreaks are bound to terminate rapidly, while less well controlled, or uncontrolled, outbreaks are apt to last much longer. It is also obvious that epidemics which started or gained impetus at a time when the weather was inclement will decline and eventually terminate when the climatic conditions become favourable, because then the contact between the people will be less intimate than when cold or rainy weather compels them to crowd together in the often rather narrow space of their closely shut habitations. Thus it was often held that the major pneumonic outbreaks in China, which had invariably become widespread in winter, declined as soon as the temperature began to be warmer in spring, and terminated when the weather had become good.

The question of whether, in addition to these extrinsic factors, intrinsic causes for the decline and termination of pneumonic plague epidemics also exist, is a most interesting one. A few observers believed that a termination of the outbreaks might be brought about by a gradually decreasing virulence of the causative organisms. There can be no doubt, however, that, if such a decrease in virulence took place at all, it occurred only in exceptional instances. Generally speaking, the virulence of *P. pestis* remained high throughout the epidemics or, as some workers, such as Wu Lien-teh,96 Girard,27 and Duffau & Lallement,17 maintained, even gradually increased.

It would seem impossible, at first glance, that under these circumstances a spontaneous decline of pneumonic plague epidemics could take place. However, observations made at the end of the 1921 epidemic in Harbin and also in Vladivostock showed nevertheless that pneumonic plague outbreaks might decline spontaneously. As noted in chapter 8, it was found that, whereas the victims on whom autopsies were performed during the course of the outbreak at Harbin showed evidence of lung consolidation, most of the autopsies carried out at the end of the epidemic revealed no such signs, but only congestion of the lungs and the deeper parts of the respiratory tract, in which, however, the blood-stained exudate, usually met with in the cases with pneumonic foci, was absent. One patient, afterwards
found to have succumbed to this pulmonary form of the disease, had actually had no cough or expectoration.

As summarized by Wu Lien-teh et al. in 1924, similar observations had also been made during the pneumonic plague outbreak in 1921 at Vladivostock. There the incidence of "septicaemic" plague cases, as the Russian workers called cases showing no evidence of lung consolidation, was not high during the course of the epidemic, attaining a possible maximum of 15.2%. However, except for a few bubonic patients and some doubtful ones supposed to have suffered from intestinal plague, practically all victims seen at the end of the outbreak showed features of "pulmonary" plague.

Since the patients suffering from this type of the disease were obviously little capable or incapable of producing respiratory infection in their contacts, Wu Lien-teh and his colleagues felt entitled to assume that the prevalence of such cases at the end of the outbreaks at Harbin and Vladivostock was at least partly responsible for the termination of these epidemics.

That pneumonic plague epidemics may be really self-limiting was proved by a further observation of Pollitzer & Li in Hunan Province, China, where such an outbreak was seen to end before any control measures had been taken—obviously because the last patients in each of the two first-affected households, and all patients in the subsequently affected families, had no sputum, and in four out of nine instances also no cough. The spread of the infection in this epidemic, which was due to an importation of the disease by a traveller, may be illustrated thus:

<table>
<thead>
<tr>
<th>Source of infection</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with patients having cough and bloody sputum</td>
<td>13</td>
</tr>
<tr>
<td>Contact with patients having only cough but no sputum</td>
<td>2</td>
</tr>
<tr>
<td>Contact with patients having neither cough nor sputum</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>

The reason why such "pulmonary" cases become prevalent at the end of the outbreaks is not clear. In view of the rapidity with which the change from the pneumonic to the "pulmonary" form took place in the epidemic observed by Pollitzer & Li, it is difficult to believe that this transition is due to an increased virulence of the causative organisms, produced through their direct passage from man to man. It is hoped that further work will throw light upon this interesting and important problem.

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Chapter 10

CONTROL AND PREVENTION

ANTI-RODENT MEASURES

Control of Commensal Rodents

Killing by mechanical means

The method of killing rats with the aid of sticks or clubs may be advantageous under special circumstances, for instance in order to deal with animals met with when digging out burrows, or when otherwise carrying out harbourage demolition. However, since numerous rats are sometimes met with in the course of such operations, some of the animals are bound to escape unless many helpers are available, or unless—as was sometimes successfully carried out in China in the case of smaller structures—the buildings in question are temporarily surrounded by a fence made of corrugated iron sheets.

Shooting rats—those prowling about at night on garbage dumps for example—has been occasionally resorted to, but, as the handbook entitled Rat-borne disease: prevention and control aptly points out, this method, though providing good sport, does not materially reduce the number of animals. Quite satisfactory results may be obtained when means of flooding rat-burrows are available, the rats either drowning or being forced to come out and be killed. However, while this or other methods of destroying rats by simple means may prove advantageous under suitable conditions, such procedures are of little, if any, value in large-scale anti-rat campaigns. It is significant that reference to extensive killing of rats with the aid of sticks seems to have been made in but one recent report (Palestine, 1941).

Wholesale killing of rats by the people during plague outbreaks should not be encouraged. If they meet with sick rodents at such times, they should kill the animals by burning them or by drowning them in some large vessel filled with water. Such rodents as well as those found dead during the outbreaks should not be touched directly but should be handled with tongs.
or similar implements, which are often available for tending the kitchen fires. Pending delivery to the staff, the carcasses should be kept in covered containers.

Whether or not the people should be urged to kill rats during the off-seasons or in plague-free localities in general, depends upon the local conditions. Good results have sometimes been obtained in this direction when premiums have been offered for each rat delivered. However, it is often difficult to pay such rewards for prolonged periods. Generally speaking, it is preferable, therefore, to obtain the voluntary co-operation of the people in anti-rat activities through public-health education and propaganda. However, as will be discussed later, in such educational and propaganda work the attention of the people should be drawn to other methods of rat control rather than to the killing of the animals by mechanical means which often cannot be done on a really effective scale.

Use of cats or other predators

Cats

Most modern observers are of the opinion that cats, while useful in keeping the houses free from commensal mice, are of questionable value for the purposes of rat control, particularly for that of *Rattus norvegicus*. In order to determine the predatory relation of Baltimore alley cats to Norway rats, Jackson examined 500 specimens of cat faeces and found that rat-remains were present in but 6.7% of the specimens, garbage or table scraps evidently constituting the staple food of the cats. In his opinion "the predation of these cats constituted only about 20 per cent. of the annual post-weaning mortality necessary to keep the rat population stationary". However, Storer thought that cats, though in general of minor value in rat control, might be capable of capturing rats which immigrated into premises previously disinfested by other means. In the opinion of Barnett, cats like other predators were of some importance in localities where little or no systematic rat-control work was done.

Dogs

Some, though not all, breeds of dogs, particularly terriers, are capable of dealing successfully even with Norway rats. Storer maintained in this connexion that rat terriers were particularly useful in dealing with rats which had been "blocked" (see page 527), or with those trying to escape when stored goods were moved.

The large-scale use of dogs for rat control was recommended recently by Silva who suggested that suitable breeds should be reared in colonies. It is certainly an advantage that, in contrast to cats, dogs are little susceptible to plague. They, like the cats, do not attack shrews (Herivaux & Toumanoff).
**Ferrets**

Domesticated ferrets have been used for the purposes of rat control in several areas. The females of this species are particularly useful in this respect because, in contrast to the bigger males, they are able to follow the rats into their burrows. However, as noted in chapter 6, the presence of plague has been confirmed in ferrets used for rat-catching in South Africa.

**Mongoose**

Doty stated that mongoose (*Herpestes* sp.), introduced into Hawaii in 1883, were considered to be of fairly high value for rat-control work. However, as noted in chapter 6, instances of plague have been observed in such animals. The fact that they are unable to climb and are apt to destroy poultry as well as rats also detracts from their value in plague control.

**Trapping**

The traps used for commensal rodents usually belong to two of the three categories distinguished by Mason: either “enclosing” traps, which imprison the victims without injuring them, or “killing” traps. However, professional rat eradicators, in the USA in particular, also make some use of “arresting” traps, supposed to seize the animals without killing them.

The various patterns of “enclosing” traps utilized for catching rodents belong either to the class of basket traps, similar in shape and construction to those used for catching fish, or to the trap-door type, their doors shutting when a lever, upon which the bait is hooked, is moved.

Trap-door traps destined for the capture of individual rats should be made of sufficiently stout wire and should measure about 13 by 6 by 5½ inches (33 by 15 by 13 cm). Basket-type traps are often of a larger size so that several rats may be caught successively. Usually, they are also made of wire, but large box-like wooden models have been devised, by Richter & Emlen for instance, and—for the capture of cotton rats—by Scott. Suitably small-sized “enclosing” traps must be used for the capture of mice.

The generally used patterns of “killing” traps, commonly called “snap” or “break-back” traps, have a solid, flat, and usually wooden base, and kill the animals by the action of a frame made of heavy wire, which is actuated by a coil spring and released by a trigger. The size of such traps should be about 3½ by 7 inches (9 by 18 cm) for rats, and 2 by 4 inches (5 by 10 cm) for mice. They can be used unbaited, if the trigger surface is enlarged with the aid of a square of cardboard or fly-screen so as to provide a platform on which the rodents can step.

The “arresting” steel traps are non-baited traps with a platform trigger and two steel jaws which are snapped together by means of a single
A. Wooden snap or breakback rat trap; the same type in smaller sizes is used for mice. B. Steel jump-back trap, size 0 (the leather padded jaws are not necessary). C. Schuyler trap (made in both large and small sizes). D. All-steel quickset mouse trap. E. 4-hole choker-loop mouse trap. F. Wire-cage trap (two sizes available).
flat spring. The size suitable for rats (No. 0) has a jaw-spread of about 3½ inches (9 cm). As a rule, half or more of the rats are caught alive in such traps.

In addition to the above-described usual patterns (shown in fig. 35), other kinds of traps have been devised, including those which kill the rodents by electrocution or with the aid of lethal gases (Nicholson & Vetter).

It is sometimes possible to trap many rats infesting a building with the aid of a "blocking" method. The rats must be given food in one of the rooms or cellars of the structure in question, which is blocked off so as to possess only one opening communicating with the outside. Arrangements must be made by which this opening can be closed from some other part of the building. When the rats have become accustomed to frequenting the blocked-off room, the opening is closed at a time when they are assembled. The animals may then be killed with the aid of dogs or by clubbing, or—if this can be done safely—a lethal gas may be pumped in.

Dealing with the technique of capturing commensal rodents with the traps ordinarily utilized, some workers stressed that these ought to be smoked, or dipped into boiling water, or scrubbed after rodents had been caught in them, because otherwise the animals would fear to approach the traps. However, most recent observers are not in favour of such views, Storer, for instance, stating in this connexion that "contrary to popular opinion, human odour from handling traps will not keep rats or mice away; it is not necessary to wear gloves or to boil, wash or smoke the traps".

Scalding the traps with boiling water is advisable when rats have been left to decay in them—incidents which should not be permitted to occur. If blood or entrails of freshly killed rats adhere to the traps, it suffices merely to scrape them off.

It is, however, of decisive importance to keep the traps in good repair and in perfect working condition. Those provided with springs must be set on "hair-trigger", so that the least touch will release the spring. As stated in the handbook on rat-borne disease, a good test of sensitivity is to touch the trigger with a flat piece of writing-paper. If this is not sufficient to spring the trap, the setting is improper or the trap needs adjustment.

In order to maintain steel traps in good working condition, it is essential to keep them free from rust. The technique recommended for this purpose by Wiley is thus summarized in Rat-borne disease: prevention and control:

"Pour 1 gallon of water into a crock and then add 2 pounds [1 kg] of phosphoric acid (85 percent H₃PO₄ syrup obtainable from chemical supply companies). Hold rusty traps by their chains and drop them into the solution. After about 2 to 3 minutes, remove traps and wipe off or hang up to drain."

As added in the handbook, this procedure removes all rust and provides a protective coating. However, badly rusted traps or those treated in pre-
viously used phosphoric acid solutions must be soaked for a longer time.

Even traps functioning properly will not prove successful unless they are used in a skilful manner. The directions of prime importance in this respect may be set forth thus:

1. Commensal rodents will not be attracted by baited traps if other food supplies are available in their vicinity.

2. Results of capturing rats may be considerably improved by exposing the baited but unset traps for 3-5 nights so as to give the animals a chance to overcome their fear of new objects. Then the traps should be freshly baited and the catches set. When trapping mice, it is not necessary to follow this procedure.

3. It is also advantageous to camouflage the traps with the aid of pieces of paper, sacking, straw or other materials, taking care, however, not to interfere with the action of the trigger. Satisfactory camouflage can also be effected by placing a board or a box over the traps in such a position that the rats have space to enter and the action of the spring is not impeded.

4. If break-back traps are exposed on earth floors, they should be dug in so that the top of their base is flush with the ground. Some workers also recommend fastening traps exposed on hard floors to some nearby object by means of a cord or light wire about 2-feet (0.5 m) long. Break-back traps may be fixed to pipes with the aid of hose clamps or may be suspended on overhead beams which are used as runs by the rats. As stated in *Rat-borne disease: prevention and control* for this purpose

   “a 1/8-inch [3 mm] hole may be drilled one-half inch from the front center of the trap, a small finishing nail driven into the beam at the point of setting, and the trap hooked over this nail. A string from the back of the trap is tied to a nail in the beam below. Thus, when a rat is caught, the trap is jarred off the nail and the rat and trap hang down by the string. Traps can be solidly nailed to the beam if desired, but these will not permit other rats to travel the same runs so as to be caught in other traps”.

5. It is of the utmost importance to put the traps in proper positions. As far as possible, traps destined for the capture of rats should not be placed on, but near, the runways, because the animals may be wary of obstructions in their path and also because they like to make little detours in search of food. However, unbaited snap-traps must be placed directly in the runs, since they offer no food to lure the animals away. In distributing the traps, care must be taken to place cage-traps with their longitudinal axis parallel to the direction of the runs, and break-back traps at right angles to them.

The position of the traps should be changed at frequent intervals, particularly if no, or no more, captures are made.

Traps for mice should be placed round their holes or, in the case of a general infestation, round stacks of goods or along walls, at regular intervals (Barnett 7).
6. Proper baiting of the traps is as important as their proper placement. The opinion that some special sorts of bait (cheese, in the case of mice for instance) are particularly attractive to the rodents has sometimes been expressed. Actually, however, almost any kind of human food may be used for baiting the traps, in many places the rodents showing a preference for the most readily available foodstuffs rather than for choice titbits. It is advisable, however, to vary the baits every few days.

7. In order to obtain good results with trapping, the traps must be carefully serviced as well as adequately placed and properly baited. It is best to distribute them or to set those left in the houses or godowns (warehouses) as late as possible in the afternoon and to inspect them again each morning, in order not only to collect the captured rodents but also to reset the traps sprung by rodents which have not been caught. Great attention must be paid at the same time to the replacement of baits which have been consumed or have become stale, mouldy, or rancid. Even if the baits have not visibly deteriorated, it is usually best to replace them not later than every third day.

Since one worker cannot adequately attend to more than 200 traps, a considerable staff is necessary for the proper conduct of large-scale trapping operations.

The views held by modern workers regarding the purposes for which trapping of commensal rodents should be carried out may be set forth thus:

(1) General agreement exists that trapping is indispensable for procuring rats and their fleas for laboratory examination in areas invaded or threatened by plague.

Most workers recommended the use of "enclosing" traps for this purpose. However, Gross & Bonnet recently postulated that snap-traps, because they were considerably more efficient in retrieving rodents, were preferable to cage-traps. They admitted that the rats and mice caught in snap-traps lost an appreciable part of their fleas, but pointed out that since 2.7 times as many infested rodents were obtained with such traps, the total number of fleas collected from these animals was higher than that obtained from the rodents caught in cage-traps. Since DDT was regularly applied in all human habitations of the plague area in question (Hamakua, Hawaii), Gross & Bonnet considered the liberation of fleas from the rodents caught in snap-traps as not dangerous. Still, while admitting that it might be permissible to use such traps in place of cage-traps in localities where ample facilities for DDT application are available, generally speaking, it seems preferable to use the latter for the purpose of obtaining rodents and their fleas for laboratory examination.

(2) It is generally held that trapping carried out with a sufficiently large number of traps is usually an effective means of dealing with mouse
infestations, the more so because, in contrast to the rats, mice do not become "trapwise" but continue to be attracted by the traps.

(3) Many workers deny that large rat infestations can be successfully dealt with by trapping procedures. In their opinion rat-trapping is merely a method of supplementary value, useful particularly for dealing with small groups of rats left over after poisoning or gassing campaigns or in cases where it is impossible to use poisons or lethal gases.

This opinion cannot be considered as absolutely valid because, as shown by some recent experiences, e.g., those of Morgan and his collaborators,\footnote{Morgan and his collaborators, 143} it is possible by waging a kind of "Blitzkrieg" with very numerous traps to reduce even large rat infestations to proportions manageable by subsequent poison administration. It must be admitted, however, that rat-trapping campaigns carried out with the usually available resources, though often continued for a great many years, frequently do not lead to a permanent reduction of the rat populations because the numerous survivors, enjoying an increased food supply, multiply freely and thus keep the population level stationary even if many hundreds or a few thousands of these animals are captured every day. Hence, unless it should prove possible to break this vicious circle by increased trapping, other methods must be adopted permanently to reduce the rat populations.

Poisoning

The following poisons are used for the eradication of commensal rodents:

*Arsenic*

Arsenic is commonly used for the purposes of rodent poisoning in the form of arsenic trioxide (As$_2$O$_3$), often called white arsenic or arsenious oxide, sometimes also called arsenious anhydride or arsenious acid. It is a white crystalline powder which is rather insoluble in water. As a rule, therefore, it is added to solid baits for the purposes of rodent eradication. The toxicity of arsenic trioxide varies according to the size of its particles, its acceptibility to the rodents varies according to its purity. Therefore, as stated by Barnett,\footnote{Barnett} it is necessary to use "a standard, fine, refined material which has been tested in the field and found to be satisfactory". Kalmbach\footnote{Kalmbach} recommended that arsenic trioxide used for rat poisoning should be pulverized to the point where it would pass through a 200-mesh screen.

Statements regarding the concentration at which arsenic trioxide should be added to the bait material vary considerably. Workers in the USA\footnote{Workers in the USA} recommended a concentration of 3\% by weight only, but Dieke\footnote{Dieke} recently found that a concentration of 4\% was necessary to obtain complete kills of *R. norvegicus* under laboratory conditions, and baits containing 15\% of the poison have been widely used in South America, prepared according to Long\footnote{Long} as follows:
CONTROL AND PREVENTION

(a) Poison * packets

\[\frac{\text{Coarsely ground corn meal}}{\text{Cheap wheat flour}} = 35\%\]
\[\frac{\text{Grated cheese, ground dried fish or meat, dried blood, or finely ground peanuts}}{\text{Commercial arsenic}} = 15\%\]
* Put into quantities of one teaspoonful (sufficient to kill one rat) in paper packets.

(b) Fish baits **

\[\frac{\text{Cheap fresh fish without bones}}{\text{Commercial arsenic}} = 85\%\]
** Spread as paste on bread, paper, shavings, or banana leaves, and placed near rat-runs.

(c) Fresh blood poison ***

\[\frac{\text{Fresh blood from slaughter-house, boiled down to jelly}}{\text{Flour, meal, or ground salt fish}} = 60\%\]
\[\frac{\text{Commercial arsenic}}{\text{Commercial arsenic}} = 25\%\]
*** Used like the fish baits.

The practice in Great Britain (see Barnett) is to mix white arsenic with damp sausage-rusk, bread mash, or soaked wheat in a proportion of 10% per weight (1 part of arsenic in 10 parts of bait) or with sugar-meal in the proportion of 15% by weight (1 part of arsenic in 7 parts of bait).

The approximate doses of arsenic trioxide which killed 50% of the animals (LD50), expressed in mg per kg of body-weight, were, according to the handbook on rat-borne disease, 50-150 in the case of R. norvegicus and 100-120 in the case of albino rats, while doses corresponding to 65 mg per kg of body-weight killed all R. rattus tested (LD100). The poison was also found effective for mice. The lethal dose for man is estimated at 1.5-15 mg per kg of body-weight so that with a concentration of 3% arsenic trioxide the lethal dose for a man weighing 150 pounds (68 kg) is contained in approximately \(\frac{1}{10}\) ounce to 1 ounce (3-30 g) of the poison-baits (Ward).

Arsenic trioxide has the advantage of being inexpensive. As maintained by Long, the slow action of this chemical is also advantageous because the poisoned animals, succumbing not sooner than 24 hours after consumption of the baits, are able to seek refuge in their burrows instead of dying in the open and thus scattering their fleas.

On the other hand, the fact that arsenic is a highly dangerous poison for domestic animals and man, as well as for the rats, deserves most serious attention. Still, the use of 17,691 pounds (8,070 kg) of packets containing arsenic trioxide in Guayaquil, Ecuador, caused, according to Long, comparatively few fatalities in domestic animals (mainly in chickens, sometimes in dogs or cats, occasionally in donkeys), and but one death in man—namely of a boy who had partaken of 10 poison packets. The brother of this child, who ate 6 of the packets, survived. The distribution of over 40,000

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\[a\] Consisting of 9 parts by weight of national flour and 1 part of castor or fine sugar.
pounds (18,000 kg) of arsenic-containing fish baits caused no accidents in man or in domestic animals, the latter not being attracted by this type of bait.

To minimize accidents, tartar emetic may be added to the poison baits in the proportions indicated in table XIX.

**TABLE XIX. PROPORTIONS OF TARTAR EMETIC TO USE WITH RODENTICIDES**

<table>
<thead>
<tr>
<th>Rodenticide</th>
<th>Small amounts</th>
<th>Large amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>poison</td>
<td>tartar</td>
</tr>
<tr>
<td>arsenic trioxide</td>
<td>60 g</td>
<td>22.5 g</td>
</tr>
<tr>
<td>thallium sulfate</td>
<td>10 g</td>
<td>6 g</td>
</tr>
<tr>
<td>zinc phosphide</td>
<td>20 g</td>
<td>7.5 g</td>
</tr>
<tr>
<td>alphanaphthylthiourea</td>
<td>60 g</td>
<td>60 g</td>
</tr>
</tbody>
</table>

* As recommended in *Rat-borne disease: prevention and control* (metric weights have been given in brackets to facilitate comparison).

Persons poisoned with arsenic trioxide (or with other arsenic-containing substances) ought to be treated by stomach lavage and administration of magnesium sulfate as a cathartic. If they are seen immediately after they have swallowed the poison, the officinal antidote, consisting of a freshly prepared solution of ferric hydroxide and magnesium oxide, may be administered first.

**Sodium arsenite**

Although sodium arsenite (Na₂HAsO₃) according to Paranjothy in a concentration of 10% is as effective as arsenic trioxide, it has never been much utilized as a rat poison. It deserves attention, however, that, being more soluble in water than white arsenic, it can be used not only in bait form but also as a spray. According to the handbook on rat-borne disease, sodium arsenite solutions prepared for rodent poisoning should have a concentration not exceeding 3%.

**Barium carbonate**

Barium carbonate (BaCO₃), a heavy, fine, white powder, has been found useful as a rat poison only if added to the bait material in high concentrations—according to most workers in a proportion of 20% by weight. Its LD₉₀ was found to be 750 mg per kg of body-weight in the case of albino rats (Barnett), and 1,480 ± 340 mg/kg body-weight (Dieke & Richter) in the case of wild Norway rats, most of which succumbed within 24 hours after
administration of the poison. The lethal dose for man is, according to
Ward, $229 \times 800$ mg per kg of body-weight, so that with a poison concentration of 20\% 9.9 ounces (280 g) of the bait mass are fatal for a man of 150 pounds (68 kg).

Barium carbonate is relatively innocuous to domestic animals except to
cats which seem to be particularly susceptible to this poison. This rodenticide also possesses the advantage of being as inexpensive to use as arsenic trioxide (Paranjothy $^{158}$). Nevertheless, in the opinion of most recent workers, in view of the availability of more-efficient rodenticides barium carbonate should not be used any more. Though this holds true in general, one should not overlook the fairly satisfactory results obtained with this poison by Harrison & Woodville $^{79}$ during a recent plague outbreak in the Bahan precinct of Rangoon, Burma.

The procedure adopted by the two workers was:

(a) to lay out at suitable points from 4 to 8 ounces (100-200 g) of boiled rice;
(b) to renew this rice daily for 3 more days of prebaiting;
(c) to replace on the fifth day the baits by a similar amount of boiled rice which contained about 16\% (1 part in 5 by weight) of barium carbonate;
(d) to collect and destroy excess poison baits and dead rodents on the sixth day.

While, before poisoning, the mean number of rodents trapped was 22.4 per night with a standard error of 1.5, after poisoning, the mean number trapped was 8.3 ± 2.2, indicating a reduction of about two-thirds. The incidence of plague in the area treated dropped accordingly, only three cases being recorded after the poisoning campaign. It is noteworthy that the two rodent species most frequently met with at the time were $R. \text{exulans} \text{ concolor}$ (45\%) and $B. \text{bengalensis}$ (31\%).

As far as the present writer is aware, the distribution of baits containing barium carbonate never led to fatal accidents in man. Care has to be taken, however, to keep this poison separately and not in the drug stores so as to avoid its being issued instead of barium sulfate which unlike BaCO$_3$ is insoluble in water and can be ingested with impunity.

In cases of barium carbonate poisoning, the stomach must be emptied with the aid of an emetic or a stomach tube, preferably by lavage with water containing one ounce (28 g) of magnesium sulfate per gallon. Sodium sulfate or magnesium sulfate (well diluted) should then be administered as often as necessary in one-ounce doses in addition to symptomatic treatment.

**Phosphorus**

While but rather limited use has been made of phosphorus-containing baits for large-scale anti-rodent campaigns, this highly dangerous chemical forms the base of widely sold rat and mouse poisons to be used by laymen in the form of spreads on bread or other foodstuffs. This is a practice which should be vigorously discouraged or, better still, altogether prohibited.
Should there be any need for rodent-baiting by laymen, the now available red-squill preparations or, better still, the inoffensive substances mentioned below ought to be preferred.

There is also no more reason for continuing the use of phosphorus for general anti-rodent campaigns. It seems unnecessary, therefore, to give directions here for the utilization of this poison.

**Red squill**

The preparations of squill used for rat poisoning are derived from the dried and ground bulbs of the red variety of *Urginea maritima*, a plant native to the countries around the Mediterranean. Their action depends upon the presence of a rodent-toxic glycoside, scilliroside, which was isolated by Stoll & Renz in 1941.

Red squill is no doubt the oldest rodenticide still in use, having been recommended for the eradication of mice by an Arabian author writing early in the 13th century (Stoll & Renz). Shepard even maintained that reference to the rodenticidal properties of red squill had been made on stone tablets dating from the pre-Christian era.

Red squill also occupies a unique position among the usual rodent poisons in so far as it is rather safe to use because most animals other than rodents refuse to consume baits containing this poison and because it possesses marked emetic properties. Consequently it is apt to prove fatal only to those animals which are unable to vomit, particularly the rodents, and not to man or most domestic animals.

Desirable as it is, therefore, to make large-scale use of red squill for the purpose of rodent control, this is difficult in so far as the originally available preparations are apt to vary widely in potency and often prove rather ineffective. In order to obtain satisfactory results, it is indispensable, therefore, to use exclusively brands of red squill of which the efficacy has been ascertained through methods of biological standardization. It is of great importance in this connexion that, as found by Crabtree et al., the potency of low-grade red-squill powders may be brought to a satisfactory standard by extracting the active rat-killing principle from them and adding adequate amounts of these extracts to the raw powders to be “fortified”.

Depending upon the kind of rats used for the biological standardization tests, the standards recommended by different workers vary. A satisfactory recommendation made by Barnett was that red-squill preparations should have an LD₅₀ of 500 mg/kg for wild Norway rats. Similarly a toxicity standard of 600 mg/kg is considered as minimal in *Rat-borne disease: prevention and control*. As stated there, the products available in the USA often proved effective against rats in lower concentrations than the 10% by weight recommended for 500-600 mg/kg red-squill. Nevertheless, it was found desirable to re-test each batch before actually purchasing it, because such tests on locally-obtained rats were bound to indicate not only
the desired concentration of the poison, but also the acceptability of the baits. The procedure recommended for this purpose was as follows:

"Rats in separate cages should be given small baits (90 to the pound [0.45 kg]) with proportions of squill to baits of 1:9, 1:11, 1:13, 1:15, 1:17, 1:19, 1:21, and 1:23 by weight. If the baits containing 1:21 and stronger kill the rats but the 1:23 does not, it is recommended that the next stronger mixture be used in the field, that is, the 1:19 mixture. The kills during tests should occur in about 12 to 24 hours."

As maintained by observers such as Doty and Meunier & Rouffia, R. rattus was found to be more resistant to red squill than the Norway rats. Meunier & Rouffia noted in this connexion not only that higher doses were necessary to kill R. rattus rattus and R. r. alexandrinus, but that these animals survived for at least 24 hours after poisoning.

It is generally held that red-squill is unsuitable for poisoning domestic mice. However, Dybing et al. ascribed these failures to a poor acceptance of the baits containing the poison by these animals and not to their lessened susceptibility to red squill. Actually, the red-squill preparations tested experimentally by Dybing et al. proved more toxic to mice than to rats.

Most observers found that male rats were considerably more resistant to red-squill preparations than the females. This difference seems to be due to hormonal influences because, as shown by Crabtree et al., castrated male rats were as susceptible as the females but became once more resistant when given testosterone preparations. The fact that, according to Dybing et al., the resistance of young male rats to scilliroside was lower than that of animals weighing over 100 g may serve as corollary to these observations.

As stated in the handbook on rat-borne disease, red squill can be used successfully with all food baits which can be ground finely and mixed thoroughly with the rodenticide. Cubed fruit or vegetables are unsatisfactory because they will not hold enough squill to kill the rats. Unless lower dosages are indicated by locally-made standardization tests, the poison should be admixed with the bait material in a proportion of 1/9.

Large-scale tests with a red-squill concentrate containing 0.7% scilliroside were carried out by Barnett et al. who found that:

(a) this preparation was effective in the field against R. norvegicus but not against R. rattus;

(b) assayed against white rats, it had an LD₅₀ of about 25 mg/kg for males and about 5 mg/kg for females;

(c) the LD₅₀ for adult fowls was more than 400 mg/kg, while pigs, dogs, and cats given doses of up to 16 mg/kg did not succumb;

(d) the preparation was unpalatable to pigs, dogs, and cats, but was consumed by fowls if admixed with mash in a concentration of 1%.

Since red squill is extremely irritating to the skin, great care must be taken not to handle or even to touch it with bare hands. Preferably, rubber
gloves should be worn during bait preparation. The baits must be distributed or collected with the aid of implements such as forceps, tongs, or chopsticks.

Though otherwise red squill is the safest among the rat poisons of the usual type, it is nevertheless necessary to prevent access of children and domestic animals to the baits. Particular care is indicated in the case of sheep and goats, said to be unable to vomit. In cases of squill poisoning the stomach should be emptied unless copious vomiting has occurred, and symptomatic treatment should be administered to prevent collapse.

Strychnine

Most modern workers, if enumerating strychnine at all among the rodent poisons, are agreed that in view of its poor acceptance by commensal rats it is unsuitable for the control of these species. This was, for instance, the opinion of Emlen & Stokes\textsuperscript{53} who recorded that strychnine sulfate given in 1% concentration to \textit{R. norvegicus} killed but 32% of the animals tested. Dieke,\textsuperscript{42} using this poison in lower strengths, found that only 6 out of 20 Norway rats were killed, the lethal dose (LD\textsubscript{50}) being 4.8±0.4 mg/kg body-weight.

However, some workers recommended baiting with alkaloid strychnine or with strychnine sulfate for the control of commensal mice and, as will be discussed later, as well as for that of certain wild-rodent species. Thus Moore,\textsuperscript{140} though considering trapping the method of choice for the control of commensal mice, stated that they might be poisoned with a mixture made from (a) 1 ounce (28 g) of powdered strychnine alkaloid; (b) 2 ounces (56 g) of common borax; and (c) 8 pounds (3.6 kg) of large-flaked rolled oats, distributed in teaspoonful quantities or exhibited in little boxes, so as to provide a permanent poison supply available to the mice at all times.

Storer,\textsuperscript{212} while admitting that baiting with strychnine-coated wheat was useful for mouse control in outbuildings, stated that trapping these animals was the “better practice” in residences. Indeed, since arsenic trioxide and zinc phosphide are also effective for the control of commensal mice, it seems rather questionable whether strychnine should be used at all to poison these animals.

The treatment indicated in the case of strychnine poisoning is described in the 1952 edition of \textit{The Extra Pharmacopoeia}\textsuperscript{127} thus:

"The main object of therapy in strychnine poisoning is the prevention of convulsions and the immediate treatment is the intravenous injection of a short-acting barbiturate such as thiopentene sodium. Light chloroform anesthesia may be used temporarily to control convulsions if a barbiturate is not immediately available. Gastric lavage may then be carried out, using a solution of potassium permanganate 60 gr. in 2 gal. of water; or a 2% solution of tannic acid may be employed. The patient should be kept lying down in a darkened room. If respiratory depression occurs artificial respiration, or inhalation of oxygen with 5% carbon dioxide, should be employed."
**Thallium sulfate**

Thallium sulfate (Tl₂SO₄) is a heavy, white, crystalline powder which is fairly soluble in water. It has been found fully effective against Norway rats as well as against *R. rattus*, the lethal dose for the former (LD₅₀) being 15.1-16.7 mg per kg of body-weight, that for *R. rattus* (LD₁₀₀) being 35 mg/kg.²¹⁷ Its action is slow; the killing time for Norway rats varying, in the experience of Dieke & Richter,⁴³ from 1.5 to 6 days.

Unfortunately, thallium sulfate, though one of the most potent rodenticides, is also one of the most dangerous, the more so as it possesses no distinctive odour or taste, is readily absorbable through the unbroken skin, and is apt to cause secondary poisoning in predators like cats and mongoose, which devour the carcasses of poisoned rodents (Doty ⁴⁷). Dogs are also quite susceptible to this poison (LD₅₀ less than 16 mg/kg ²¹⁷).

The lethal dose for man is, according to Danzel, ⁵⁴ about 0.02 g (20 mg) per kg of body-weight, i.e., 1.2-1.5 g for an adult, 0.5-0.6 g for a child. Numerous instances of thallium poisoning, including cases of persons consuming bait materials mixed with this chemical, are on record (see Munch et al.;¹⁴⁸ Chodzko ²⁸). One must, therefore, fully agree with the now generally accepted opinion that thallium sulfate, which is also comparatively expensive, should not be used for the purposes of general rat control, at least not unless tartar emetic has been added to the bait material (see table XIX, page 532). Indeed, the present writer for one fails to see why it should be used at all.

Thallium sulfate was formerly admixed with the bait materials in proportions of 1.5% or even 2.0% by weight but, as shown by the experience of Doty ⁴⁷ and confirmed by recent tests in the USA,²¹⁷ a concentration of 0.5% is sufficient. It may be incorporated in the usual solid bait materials or distributed in aqueous solution. The use of rubber gloves is indispensable for the preparation of solid or fluid baits, and contact with these materials during their distribution or collection must be rigidly avoided.

As stated in *The Extra Pharmacopoeia* :¹²⁷

"Washing out the stomach and use of laxatives are important in acute poisoning [with thallium]. Injection of histamine, followed by renewed rinsing of the stomach and the use of diuretics is also advisable. To render inactive any thallium circulating in the blood 10 ml. of a 10% solution of sodium thiosulphate should be given intravenously. This can be repeated several times a day and should be continued over a period of time."

Blood transfusions and administration of large doses of vitamin B₁ and vitamin B₂ may also be advisable (Prick et al.¹⁷₅).

**Zinc phosphide**

Zinc phosphide (Zn₃P₂), a black powder slightly soluble in water, often possesses a garlic-like odour due to the slow release of phosphine gas
(PH₈) which, while apt to be repulsive to man and domestic animals, seems to attract rather than to repel the rodents.

Zinc phosphide is generally considered to be an efficacious poison for rats and mice, the handbook on rat-borne disease ²¹⁷ for instance considering it more effective against commensal rats than arsenic trioxide or red squill, though less so than sodium fluoroacetate and thallium sulfate (see table XX).

**TABLE XX. RELATIVE EFFECTIVENESS AND SAFETY OF THE MOST COMMON RODENTICIDES**

<table>
<thead>
<tr>
<th>Rodenticide</th>
<th>Recommended concentration in baits by weight (%)</th>
<th>Relative effectiveness against rats</th>
<th>Relative safety to man and domestic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium fluoroacetate **</td>
<td>0.32</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Thallium sulfate</td>
<td>0.5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Zinc phosphide</td>
<td>1.0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Alphanaphthylthiourea</td>
<td>2-3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Red squill (fortified or extract)</td>
<td>5-10</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

* Data obtained from Rat-borne disease: prevention and control.²¹⁷

** Recommended only for use in water.

The lethal dose of zinc phosphide (LD₅₀) for Norway rats ranges, according to Dieke & Richter,⁴³ between 37.6 and 43.4 mg per kg. The LD₅₀ for R. rattus is 42.5 mg per kg as compared to 40 mg/kg in the case of dogs and presumably also in that of man.²¹⁷ Generally speaking, zinc phosphide acts fairly rapidly, killing the rodents in less than 24 hours, according to the handbook on rat-borne disease ²¹⁷ usually in 6-12 hours. Nevertheless, Morgan ¹⁴¹ found that after baiting with this poison the majority of the rats did not die out in the open but under floorboards or behind casings and partitions. However, Middleton ¹³⁹ stated that on farms many rats poisoned with zinc phosphide died in the open.

Opinions on to what extent zinc phosphide can be used safely for the control of commensal rodents vary considerably. In the handbook on rat-borne disease ²¹⁷ it is admitted that this poison “is safer than other highly toxic rodenticides because both its odor and color are objectionable to man and pets”. Nevertheless the view is expressed that, except “in those business establishments where a toxic poison will be safe”, zinc phosphide should be distributed only in baits containing tartar emetic as well (see table XIX, page 532). Sharing this opinion, Emlen & Stokes,⁵⁸ during a rat-poisoning campaign in Baltimore, used exclusively zinc phosphide baits to which
tartar emetic had been added in a proportion of 1%. However, these baits, which were considered as "essentially harmless", gave poor results.

It must be noted, on the other hand, that large-scale use of zinc phosphide baits, to which no tartar emetic had been added, has been made outside the USA, for instance in Hawaii (Doty 47), in Great Britain (Barnett 7, 9), and in Italy (Martorana 128), apparently without any serious mishap. Doty, who used zinc phosphide in a concentration of 0.5%, considered it less dangerous for domestic animals than thallium sulfate and maintained in particular that, in contrast to the latter, zinc phosphide did not produce secondary poisoning in predators which had devoured poisoned rodents. However, as was pointed out in a review of Doty's article in the Bulletin of Hygiene,20 in Great Britain, where higher concentrations of zinc phosphide were used, fatalities had been noted in cats after they had fed upon rats killed by this poison. According to Middleton's 139 experiences in farms, zinc phosphide was dangerous for poultry as well as for cats.

As will be gathered from the statements made above, different concentrations of zinc phosphide have been used for rat eradication. The recommendation in the handbook on rat-borne disease 217 was to add this poison to the bait material in a proportion of 1% by weight. Lesser proportions were used by some workers such as Doty 47 (0.5%) and Johnson 99 (0.75%), higher ones by Emlen & Stokes 53 (3%) and Martorana 128 (5%). The official British recommendation 7 was to add zinc phosphide to wet bait bases (such as damp sausage-rusk, bread mash, or soaked wheat) in a proportion of 2.5% by weight (i.e., 1 part in 40 parts of bait) or to admix it with sugar meal in a proportion of 5% by weight (1 part in 20 parts of bait). It should be noted in this connexion that, with a concentration of 2% in the baits, the lethal dose of zinc phosphide for a man of 150 pounds (68 kg) is contained in 4.9 ounces (138.9 g) of the bait material (see table XXI).

### TABLE XXI. TOXICITY TO MAN OF RODENT BAITS PREPARED WITH COMMON POISONS

<table>
<thead>
<tr>
<th>Poison</th>
<th>Acute lethal dose to man (mg/kg)</th>
<th>Concentration used in baits</th>
<th>Weight of bait containing a lethal dose for a 150-lb (68-kg) man (ounces)</th>
<th>(grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic trioxide</td>
<td>1.5-15.0</td>
<td>3% (1.56)</td>
<td>0.12-1.22</td>
<td>3.4-34.0</td>
</tr>
<tr>
<td>Barium carbonate</td>
<td>0.002</td>
<td>20% (1.5)</td>
<td>0.9</td>
<td>26.9</td>
</tr>
<tr>
<td>Strychnine</td>
<td>0.1</td>
<td>0.3% (1/320)</td>
<td>0.8</td>
<td>22.7</td>
</tr>
<tr>
<td>Thallium sulfate</td>
<td>2.0</td>
<td>1.5% (1.35)</td>
<td>3.2</td>
<td>90.6</td>
</tr>
<tr>
<td>Zinc phosphide</td>
<td>4.0</td>
<td>2.9% (1.95)</td>
<td>4.9</td>
<td>138.9</td>
</tr>
<tr>
<td>Alphanaphthythiocarbide</td>
<td>unknown</td>
<td>5.0% (1.20)</td>
<td>Probably very large</td>
<td></td>
</tr>
<tr>
<td>Sodium fluoroacetate</td>
<td>5.0</td>
<td>0.4% (1.256)</td>
<td>3.15</td>
<td>89.2</td>
</tr>
</tbody>
</table>

* After Ward.229
Cubed fresh fruit or vegetables may be used as bait bases for zinc phosphide. According to *Rat-borne disease: prevention and control* such baits may be prepared by placing cubed materials and zinc phosphide, after each is carefully weighed, in an ordinary bucket. The bucket then is given a rotary motion until all cubes are evenly coated, and no loose poison remains in the pail.

However, the usually-employed bait bases are advantageous in so far as fats or oils may be incorporated in them, increasing the absorption of the poison in the body. Storer's recommendation was to use for this purpose corn oil, mineral oil, or glycerin, at 12 to 24 fluid ounces per 100 pounds (7.5-15 ml/kg) of bait, first mixing the zinc phosphide and the bait base until the former was evenly distributed, and then slowly stirring in the well-warmed oil or fat. It seems significant that both Doty and Johnson, who obtained good results with low zinc-phosphide concentrations, used baits enriched with vegetable oil. This is a point which deserves attention but it must be realized that such enriched baits will be more poisonous to man and domestic animals as well as to rodents.

Murray, while obtaining satisfactory results with zinc phosphide baiting when the weather was dry, noted that during wet spells the baits became unpalatable and lost their toxicity. To avoid such a deterioration of the baits, it has been recommended to wrap them in waxed paper. As a rule, however, it is preferable to remove the baits after they have been exposed for one night and to replace them when necessary by fresh baits.

To avoid liberation of phosphine during storage, zinc phosphide must be kept dry in well-closed containers. Though as a rule the presence of this gas can be detected by odour before dangerous concentrations have developed, it is well to prepare zinc phosphide baits in the open or in well-ventilated rooms.

It is indispensable to wear gloves during bait preparation. The finished baits must never be touched with bare hands but must be distributed with the aid of suitable instruments.

The treatment recommended in the case of poisoning with zinc phosphide or other phosphorus compounds is as follows:  

"Wash out stomach thoroughly with 1% potassium permanganate solution, using stomach tube. Alternatively, give 5 gr. [grains] [0.3 g] of copper sulphate in water, repeating the dose, first as emetic then as antidote; or use stomach tube with dilute solution of copper sulphate, 15 gr. [grains] [0.9 g] to 2 gal. of water. Give medicinal charcoal with ½ oz. [14 g] of magnesium sulphate, repeating the charcoal frequently. Alkaline drinks and dextrose, but not oils, fats or white of egg."

*Alphanaphthylthiourea*

This rodenticide, better known under its abbreviated name "ANTU", was discovered by Richter (see Richter, 1945) in the course of studies on the self selection of diets by rats, during which it was found that these animals invariably died when tested with phenylthiourea (phenylthiocarbamide), a bitter substance generally considered to be non-toxic. A search
was started accordingly for a thiourea derivative which would have the
same high toxicity for rats as phenylthiourea without its bitter taste.
As a result of these screening tests alphanaphthylthiourea, a fine, grey,
water-insoluble powder with very little odour or taste was chosen. It also
possessed the advantages of being non-irritating to the skin and of keeping
well when stored dry.

Exhaustive studies on the action of ANTU on rats and other animals,
undertaken by Richter and his collaborators as well as by other workers
in the laboratory and in the field, have shown that:

1. ANTU exerts a particularly fatal action on adult Norway rats, its
LD₅₀ for these animals varying, according to Ward,²²⁹ from 6 to 8 mg per
kg of body-weight.

2. Young R. norvegicus, found to succumb to ANTU doses ranging
from 20 to 50 mg per kg body-weight, and albino rats (LD₅₀ 24-40 mg/kg)
seem comparatively more resistant to this poison.

3. R. rattus and commensal mice were found to be far more resistant
to ANTU than the Norway rats. The LD₅₀, expressed in mg per kg of body­
weight, was 250 or more for R. rattus,²¹⁷ while that for albino mice was
70 (Munch ¹⁴⁵). In the opinion of almost all workers the use of ANTU
should be restricted, therefore, to the control of R. norvegicus.

4. The susceptibility of other animal species to ANTU is shown by the
following data culled from the handbook on rat-borne disease²¹⁷ (lethal
doses expressed in mg per kg of body-weight):

<table>
<thead>
<tr>
<th>Animal</th>
<th>LD₅₀ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>16 (LD₁₀₀)</td>
</tr>
<tr>
<td>Pig</td>
<td>50 (LD₁₀₀)</td>
</tr>
<tr>
<td>Cat</td>
<td>100 (LD₁₀₀)</td>
</tr>
<tr>
<td>Chicken</td>
<td>5,000 (LD₁₀₀)</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>3,500-5,000 (LD₅₀)</td>
</tr>
</tbody>
</table>

Though, as indicated by these figures, cats were comparatively resistant
to ANTU in laboratory tests, actually they as well as dogs and pigs are
endangered during the poisoning campaigns. Baby chicks were found to
be quite susceptible to ANTU (Anderson & Richter ²).

5. The lethal dose of ANTU for man is estimated to be about as high
as that for monkeys (? 4,000 mg per kg body-weight). It is important to note
in this connexion that, as stated in 1947 by Emlen,⁵² in Baltimore "ten
children have been reported to have eaten ANTU baits since 1943; all but
one had their stomachs emptied by stomach pump, and none showed any
ill effects ".

As established by Richter ¹⁷⁷,¹⁷⁸ and confirmed by other observers,
Norway rats fed in the laboratory with sublethal doses of ANTU were
apt to develop a tolerance and/or refusal response for baits to which this
poison had been added. Emlen ⁵² ascribed great practical importance
to the latter factor, stating that in Baltimore "the repetition of Antu-corn
campaigns at yearly intervals has been successful, but repeat campaigns at
shorter intervals have given some poor results, apparently as a result of
bait refusal”. The validity of Emlen’s experiences was confirmed through recent laboratory studies of Gaines & Hayes, who found that under simulated field conditions bait shyness could be demonstrated for at least four months after the last exposure to ANTU, i.e., much longer than had been formerly assumed.

Norway rats which have consumed sufficient doses of ANTU as a rule die fairly rapidly, the survival time ranging, according to Dieke & Richter, from 16 to 30 hours. Rats of this species as well as other animals which succumb to acute ANTU poisoning show, at autopsy, marked pulmonary oedema or pleural effusion, or a combination of both. Landgrebe & Morgan maintained in this connexion that pulmonary oedema was massive in rats dying within four hours after ANTU poisoning, while those surviving longer showed pleural effusion. As noted by McClosky & Smith, rats which had been killed by large doses of ANTU after they had become tolerant to the poison showed no pleural effusion even when they died within 24 hours, but usually their lungs were oedematous and haemorrhagic. The liver of some of these animals and of all rats surviving ANTU poisoning for more than 72 hours showed fatty degeneration.

Rats succumbing to ANTU poisoning are apt to die in the open, presumably because they suffer from air hunger (Violet & Rinaudo).

ANTU may be admixed with all sorts of bait materials, including cubed fruits or vegetables. However, as pointed out with much reason in the handbook on rat-borne disease, under urban conditions, finely ground corn or other sorts of grain, because not usually eaten by cats or dogs, form the safest vehicle for the poison. The concentration at which ANTU was added to the bait material by the different workers varied from 1% to 3.5%; a concentration of 2%-3%, as recommended in the handbook on rat-borne disease, is, generally speaking, most satisfactory. Violet & Rinaudo, when recently using ANTU for a large-scale poisoning campaign in Lyons, France, wrapped their baits, which contained 3.5% of the poison, in quantities of 4-5 g in newspaper. Though they distributed 20-60 of these packets per building, each packet containing 140-175 mg of ANTU, they did not encounter any untoward incident.

Besides being used in the usual form of baits, ANTU (in a concentration of 20% in pyrophyllite or some other inert material) has been distributed in the form of copious patches on rat-runs, in rat-burrows, or in other harboursages in order to kill the rodents which, after having walked through these patches, consume the poison by licking it off from their paws and fur. One must, however, agree with the handbook on rat-borne disease that the use of this rather expensive procedure should be restricted to localities where it would be inadvisable to distribute ANTU in ordinary baits.

A modification of this method was to utilize a mixture containing 8% DDT as well as 20% ANTU and 72% of an inert powder, so as to kill the rat-fleas as well as the rats coming in contact with the patches. However,
Wiley found that in buildings so treated only 41% of the Norway rats were killed. Deaths among the commensal mice amounted to 70% but there can be little doubt that this better result was due to the action of the DDT rather than to that of the ANTU.

So far, no antidote to ANTU poisoning in man has been found. However, as pointed out by Richter, the marked insolubility of this compound makes prompt voiding of the stomach, preferably by lavage, an effective counter measure. Cathartics and alkalis should not be used in cases of ANTU poisoning. Should oedema of the lungs develop, one should stop the intake of fluids and administer oxygen.

Sodium fluoroacetate

Often called "1080" according to the catalogue number of the originally tested sample, sodium fluoroacetate was singled out as a most effective rodenticide in the course of large-scale screening tests carried out in 1943 and 1944 in the USA (Kalmbach, 1945, 1948). Most interestingly, it was afterwards learnt that monofluoroacetic acid was the toxic principle of a plant (Chailletia toxicaria) used to poison rats in Sierra Leone (Klingsmith) and also of Dichapetalum (Chailletia) cymosum, commonly called "gifblaar", feared as a stock poison in South Africa (Marais).

Sodium fluoroacetate is a light, white, crystalline compound, which is tasteless and possesses no, or at least no marked, odour. It is easily soluble in water, but practically insoluble in oils. As shown by the figures quoted below, this poison is a most effective rodenticide but, unfortunately, is at the same time also most dangerous for other animal species and for man.

As can be seen in table XXII dogs, cats, pigs, and goats are particularly susceptible to 1080. As pointed out in this connexion in the handbook on rat-borne disease, a dose of 2.5 mg, capable of killing 50% of Norway rats weighing about one pound (0.45 kg), would also kill a dog weighing 25 pounds (11 kg) or a cat weighing 10 pounds (4.5 kg). As far as it is permissible to judge from the experience made in monkeys, a 15-pound (7.5-kg) child might be killed by consuming 35 mg of 1080, i.e., less than the amount (50 mg) contained in a 1/2-ounce (14 g) cup-full of 1080 solution as used to poison rats.

Symptoms in rats poisoned with sodium fluoroacetate begin to appear after 20 minutes, and the animals succumb after 1-8 hours or even sooner (according to Dieke & Richter after 45-240 minutes as compared to 12-90 minutes in the case of strychnine sulfate). The rats, obviously because they are rapidly overcome by the action of the poison, usually die in the open, quite frequently near the places where the poison baits or cups have been exhibited. Hence, unless stringent precautions are taken, instances of secondary poisoning in cats, dogs, or pigs, which devour or even merely gnaw the carcasses of 1080-poisoned rodents or birds, are bound to occur.
the more so because the carcasses of the poisoned animals remain dangerous even if they have decayed or have become dry. The death-toll recorded by Gilcreas in an instance where 1080-baits had been placed deep in the rat-burrows of a village dump was about 20 dogs and cats.

**TABLE XXII. LD₅₀ OF SODIUM FLUOROACETATE EXPRESSED IN MG PER KG BODY-WEIGHT**

<table>
<thead>
<tr>
<th>Animal</th>
<th>LD₅₀ (mg/kg)</th>
<th>Animal</th>
<th>LD₅₀ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norway rat</td>
<td>3.7 (or less)</td>
<td>Horse</td>
<td>1.0</td>
</tr>
<tr>
<td>R. rattus</td>
<td>1.4</td>
<td>Pig</td>
<td>0.3</td>
</tr>
<tr>
<td>Albino rat</td>
<td>2.5-7</td>
<td>Chicken</td>
<td>6-30</td>
</tr>
<tr>
<td>Commensal mouse</td>
<td>8-10</td>
<td>Mourning dove (Zenaidura macroura)</td>
<td>10, 10, c</td>
</tr>
<tr>
<td>Cat</td>
<td>0.35-0.50</td>
<td>Sparrow (Passer domesticus)</td>
<td>2.7, 4</td>
</tr>
<tr>
<td>Dog</td>
<td>0.07-0.20</td>
<td>Rhesus monkey</td>
<td>5-7.5</td>
</tr>
<tr>
<td>Goat</td>
<td>0.3-0.7</td>
<td>Man (estimated)</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Data obtained from Rat-borne disease: prevention and control.

a The LD₅₀ in 55 starving Norway rats poisoned by Dieke & Richter with the aid of a stomach-tube was 0.22 ± 0.01.

b According to Hughes.

c Only 33% of these birds were killed.

d LD₅₀.

It is important to note in this connexion that, as proved by Gratch et al., (a) sodium fluoroacetate does not reach the liver and spleen of rats killed by this poison in sufficient amounts to cause secondary poisoning in guinea-pigs inoculated with such tissues for diagnostic purposes, and (b) 1080 in the concentration used for rat-poisoning does not exert a bacteriostatic action on Pasteurella pestis.

As shown by studies of Chenoweth & Gilman, the causes of death in animals poisoned with methyl fluoroacetate were different in different species. In the case of rabbits, goats, horses, and spider-monkeys, the heart became primarily affected and death was due to ventricular fibrillations. In the case of the dog and guinea-pig, the central nervous system was primarily affected and death was caused by cessation of the respiratory activity. In cats, pigs, and rhesus monkeys both the heart and the central nervous system became involved. Rats as well as hamsters developed changes in which depression and delayed bradycardia were prominent, but did not usually exhibit ventricular fibrillation. Death in these two species, if occurring early (4-6 hours after), appeared to be due entirely to respiratory depression.

As stated in the handbook on rat-borne disease, rats surviving the administration of sublethal doses of the poison "show neither aversion to
nor serious tolerance of 1080". However, Barnett & Spencer\textsuperscript{12} found that, in five out of six field tests made by them, “populations of \textit{R. norvegicus} which had survived baiting with 1080 showed shyness [refusal] of the poison when it was given in a new bait base”. The general conclusion reached by these two workers was that “although 1080 is probably more effective in direct poisoning than other poisons used in the past, it does not give as consistent results as the standard poisons do after prebaiting.”

Though 1080 may be easily admixed with all sorts of solid bait materials, the workers in the USA urged with great reason that this poison should be distributed preferably in fluid form. It must be kept in mind, in this connexion, that the rodents, instead of immediately consuming solid poison-baits, may carry them away to places where they cannot be recovered.

As has been shown by Nicholson et al.,\textsuperscript{150} a concentration of 12 g of sodium fluoroacetate per gallon of water is fully effective in killing Norway rats. Addition of 7 g of 1080 per gallon of water suffices to kill \textit{R. rattus} and mice.

Though sometimes advantage has been taken of watering-fountains as used in chicken yards to make 1080 solutions available to the rodents, as a rule, shallow cups, able to contain no more than 3/4 of an ounce (21 g), and filled to only half their capacity, are utilized for this purpose.

Wiley\textsuperscript{232} recommended the use of cups made of waxed paper, on which were stamped, by means of red waterproof ink, the word “Poison” and suitable symbols (e.g., a skull and cross-bones). He also advocated that the cups should be dyed a light-brown colour so as to make them less conspicuous to children.

As a further precaution it was recommended that a solution of nigrosine be added to the 1080-containing fluids so as to give them a warning black colour. The acceptance of the solutions by the rats seemed not to be materially reduced by the addition of this dye.\textsuperscript{217}

Concentrations of 1080 varying from 0.25\% to 1\% have been used by different workers for the preparation of solid baits. The standard recommendation of Ward\textsuperscript{229} was to mix one gram of the poison with each pound (0.45 kg) of the bait material, thus using 1 ounce (28 g) for 28 pounds (12.7 kg) of bait.

Rubber gloves must be worn by the staff members preparing 1080 solutions or baits. The latter must not be touched with bare hands but must be distributed and collected with suitable instruments. Unused baits as well as poisoned rodents, which must be collected as soon as possible, should be disposed of by thorough incineration or by burial at a depth of at least 2 feet (0.75 m). The latter method should also be used when it is impossible or inadvisable to dispose of unused 1080-poisoned water by flushing it down a sewer. Preferably, the cups used for the distribution of the solutions
should not be utilized again but should be burnt or deeply buried as soon as they have been collected.

Large-scale use of sodium fluoroacetate has been made by some workers with success and, as has been shown by Macchiavello and Macchiavello et al., the community-wide distribution of this poison in combination with DDT application is an effective means of cutting-short plague outbreaks. Nevertheless, in the opinion of most workers, 1080 should not be utilized for general anti-rat campaigns but should be employed only if possibilities for the primary or secondary poisoning of domestic animals, as well as danger for human beings, can be fully excluded. Certainly, however, one should not hesitate to utilize this most effective poison in locations where it is safe to do so, for instance, during weekends in factories, godowns, schools, and offices, or in other buildings which are not frequented at the time. In emergencies, possibilities for the temporary evacuation and locking-up of the premises to be dealt with ought to be given consideration.

It deserves great attention that, as shown by the work of Hughes, quite satisfactory results may be obtained when using 1080 in place of fumigation procedures to free ships from rats. As this worker summarized in 1950, the ratio of rats killed by 1080 to that of rats estimated to be aboard was 85.5% in the case of the 96 vessels baited in 1946 and 1947, and 91.8% in the case of 283 vessels baited afterwards. The value of this procedure, which could be applied easily in the case of vessels of even smaller size plying on inland waterways as well as in seaports, is certainly considerable.

So far no specific antidote for treating 1080 poisoning in man is known. The symptomatic treatment to be used in such cases is outlined by Hughes thus:

"Ten-eighty is absorbed readily by the gastrointestinal tract and must, therefore, be removed immediately if harmful effects are to be prevented. The patient should be made to vomit at once by sticking a finger in the throat or [the stomach should be voided] by other means. Give a dose of magnesium sulfate (Epsom salt) or other cathartic as a purge.

In the event of nervous system excitation the careful use of barbiturates of medium duration of action, such as sodium amytal, intravenously if necessary, is suggested. Other than complete rest and adequate sedation, little can be done to prevent progression of cardiac symptoms. Should ventricular fibrillation occur, intracardiac injection of 5 cc. of 1-percent solution of procaine hydrochloride might be attempted to restore an organized heartbeat. Although symptoms of 1080 intoxication will usually subside within 1 day, the patient should be kept quiet for a period of 3 days if there is any sign of action on the heart."

It is interesting to note that, as shown by Tourtelotte & Coon, dogs poisoned orally with approximately \(2 \times \text{LD}_{50}\) of 1080 were invariably saved when barbiturate treatment was started half an hour or 3 hours after poisoning. 80% of dogs which had received \(4 \times \text{LD}_{50}\) of 1080 were saved when such treatment was started half an hour after poisoning, only 17%
of those poisoned with the same dose were saved when the treatment was commenced after 3 hours. In the case of dogs poisoned with 6×LD$_{50}$ of 1080, barbiturate treatment was ineffective even if started half an hour after poisoning. Sodium acetate and ethanol had some antidotal effect when given to dogs immediately after they had been poisoned with 1080, but these agents were of no value as adjuncts to barbiturate treatment when given half an hour after poisoning.

**Anti-coagulants**

The mode of action of the anti-coagulants used for the purposes of rodent control is fundamentally different from that of the above-described rodenticides. The efficacy of the latter depends upon their administration in single doses adequate to kill the animals. Single doses of the anti-coagulants, unless excessively large, are incapable of exerting a lethal action. However, if repeatedly ingested in smaller or even quite small doses, the anti-coagulants, because they inhibit the formation of prothrombin, are apt eventually to prove fatal to the poisoned animals by producing haemorrhages in the tissues of the body, and occasionally also external bleeding. It is clear that this peculiar "residual" action of the anti-coagulants can take place only if their admixture with the bait material is not recognized by the rodents, because otherwise the animals would cease to consume the poison-baits for sufficiently long periods.

O'Connor, who seems to have been the first worker to use anti-coagulants for rat-extermination, as well as Diagne et al., stated to have obtained satisfactory results with dicoumarol (3:3'-methylene-bis-4-hydroxycoumarin), but, as shown by Armour & Barnett, the consumption of this compound is apt to produce acquired bait refusal (bait shyness) in the rats. However, general agreement has been reached that 3-(α-acetonylbenzyl)-4-hydroxycoumarin, recommended as a potential rodenticide by Link and his co-workers (see Scheel et al.), is free from this drawback and may be used therefore for the gradual eradication of rodent populations.

This anti-coagulant, afterwards called warfarin, is a stable, colourless, crystalline solid, free from odour and taste not only for man but apparently also for the rodents. It is available in a concentration of 0.5% in corn-starch powder and may thus be easily admixed with dry bait materials. It can be used in solution as well, but, as stated by Upholt, "ordinarily is not recommended for use in this form since one of its attractive features is the fact that it can be left in permanent bait stations with only infrequent checking. Dry baits are obviously desirable from this standpoint ".

The concentrations at which warfarin ought to be used vary according to the species of rodent to be dealt with. As shown by Hayes & Gaines and accepted in recent recommendations for the use of this anti-coagulant (US Public Health Reports, 1952), generally speaking a concentration of 0.05 mg/g of bait material (0.005%) is effective for the control of Norway
rats, but under certain conditions these rodents may be controlled “a little more rapidly, though no more surely, by use of bait containing 0.100 mg of warfarin per gram of bait”.

Mice, though showing more individual variation, generally react in the same way as *R. norvegicus*, but a higher warfarin concentration (0.25 mg/g of bait material or 0.025%) is necessary for the dependable control of *R. rattus*. Doty 48 concluded from cage tests that *Rattus rattus alexandrinus* were comparatively most resistant to warfarin used in a concentration of 1/4,000 because they succumbed after having consumed 43.4% of their body-weight of poisoned rolled oats as against 39.1% in the case of *R. r. rattus* and 23% in the case of the Norway rats.

As established by field tests made by Gross et al., 74 baiting with rolled oats to which warfarin had been added in a concentration of 0.925% by weight, though satisfactory for the control of the common rats (particularly *R. r. alexandrinus* and *R. norvegicus*) and also for *Mus musculus*, did not reduce the incidence of *R. hawaiiensis*.

Though, in order to be effective, warfarin-containing baits must be ingested by the rodents several times, consumption need not necessarily take place on consecutive days. Crabtree, 31 who experimented with albino rats, noted in this connexion that results remained satisfactory if the poison-baits were offered on 4-5 occasions at intervals of 1-4 days. However, Link & Mohr 114 postulated in a recent summary that the intervals between warfarin feedings should not be longer than 48 hours.

Laboratory investigations as well as field observations have shown that, if warfarin is continually offered to susceptible rodents, the mortality becomes considerable after 4-5 days and most of the animals succumb within a week, practically all within two weeks. This is well illustrated by the experiences of Doty 48 who found that in cage tests 78.7% of the warfarin-fed rats (*R. norvegicus* and *R. rattus*) were dead seven days after the first ingestion of the poison and 95.4% after nine days, the few remaining animals surviving for 10 or 11 days. Since, in his opinion, in actual practice four days had to be allowed for the average rat in the field to find a bait station and to start consuming the poison, Doty concluded that “to kill 80 per cent. would require a total of 11 days, 95-96 per cent. would require 13 days and positive 100 per cent. kill would require 16 days”, provided that no rats immigrated. He proposed, therefore, that warfarin-treated oats should be exposed in the field stations for at least 17 days or longer if there was active migration. Hence, though the use of warfarin obviated the necessity of prebaiting with unpoisoned baits, the period of its application was considerably longer than that required to prebait and then to expose the ordinary rat-poisons—operations lasting a total of 9-10 days.

Though warfarin may be admixed with all sorts of foodstuffs, cereals form the most suitable bait bases for this poison because they facilitate a long exposure of the baits and reduce the danger of their consumption by cats
and dogs. As noted above, Doty 48 and also Gross and his collaborators 74 used rolled oats to prepare warfarin baits while workers in the USA considered yellow corn meal of a cooking grade as the bait of choice. Since, as stated above, warfarin is used in rather low concentrations, thorough mixing of the poison with the bait material is essential. If it is proposed to leave the baits exposed instead of replacing them at frequent intervals, the addition of 0.25% - 0.4% of p-nitrophenol is advisable so as to prevent the formation of moulds.

In order to derive maximal benefit from the residual action of warfarin, many workers recommended not distributing the poison-baits in the usual manner but exposing the material in bait boxes. This procedure, which will be dealt with in a general manner later (see page 560), has the double advantage of making the poisoned material permanently available to the rodents and preventing its consumption by larger domestic animals or by children.

Like ANTU, the anti-coagulants may be used as tracking powders laid down in sufficiently large quantities at strategic points such as the entrance of rat-burrows and rat-runs. One proprietary brand in particular, containing a coumarin derivative (Reiff & Wiesmann 176) in a concentration of 1%, has given excellent results when distributed in this manner.

As shown by these experiments as well as by those made when using anti-coagulants in bait form, advantage may be taken of these substances for the purposes of "initial" rodent control. However, it must be realized that, if used in this manner, the anti-coagulants not only act considerably more slowly than the usual rodent poisons but are also much more expensive to use. Doty 48 noted in the latter connexion that "a study of comparative cost of materials for poisoning rats under our field conditions showed warfarin to be 50.7 per cent. higher than thallium sulphate and 78.3 per cent. higher than zinc phosphide".

While it may be necessary, therefore, to utilize other rodenticides for initial anti-rodent campaigns, the anti-coagulants, because they can be employed continually, are of eminent value in keeping the premises in which other procedures had been used to decimate the rodent populations permanently free from these animals. Indeed, on account of their residual action, warfarin and similar compounds offer "a sort of chemical ratproofing in places where a conventional ratproofing is impractical for economic or other reasons." 23

It also deserves great attention that, as pointed out by Hayes & Gaines, 81 warfarin appears to be most suitable for keeping ships permanently free from rats.

As generally held, the danger of direct poisoning of domestic animals through ingestion of bait materials containing anti-coagulants is not considerable and, as noted above, this danger may be practically eliminated by exhibiting these materials in bait boxes. However, instances of secondary
poisoning in cats and dogs which consumed large numbers of warfarin-poisoned rodents have been observed. To counteract this danger as far as possible, prompt collection of the poisoned rodents must be insisted upon, particularly during the period immediately following the commencement of “initial” anti-rodent campaigns.

As far as is known, the distribution of bait materials containing warfarin or similar compounds has never led to untoward incidents in man.

It is curious to note in this connexion the case of a young man who, in order to commit suicide, ingested during a period of 6 days a total of 4 ounces (113 g) of a warfarin preparation containing 567 mg of the anti-coagulant. He showed on admission four days after consumption of the last dose a petechial rash and a brownish-red discharge from the nostrils. There was laboratory evidence of hypoprothrombinaemia. Red blood-corpuscles were found in the urine which afterwards showed temporarily gross admixture of blood. The stools were of normal colour but one bowel movement was followed by voiding of blood. However, administration of vitamin K and blood transfusions, as recommended for the treatment of poisoning with dicoumarol and related substances, effected recovery within less than a week (Holmes & Love 87).

Other rodenticides

In addition to the above-mentioned widely known rodenticides the following substances found to be poisonous for rodents deserve attention even though some of them have not yet been utilized for actual control work.

1. 2-chloro-4-dimethylamino-6-methylpyrimidine, “Castrix”, was developed during the second World War by German scientists so as to replace rodenticides which had become unavailable. Du Bois et al. 49 reported in 1948 that this compound produced in all animal species studied symptoms typical of central nervous system stimulants, particularly convulsions, which began to appear 15-45 minutes after either oral or intraperitoneal administration. Castrix was found to be more toxic to laboratory rats than either ANTU or 1080, the lethal dose (LD_{50}) being 1.25 ± 0.10 mg per kg body-weight when given orally, and 1.00 ± 0.06 when administered intraperitoneally. The LD_{50} for mice given the poison intraperitoneally was 0.42 ± 0.05 mg per kg, the corresponding figure for dogs was about 0.50. Rats first given sublethal doses of Castrix developed no tolerance to the poison. They appeared to be more susceptible to it in summer than in winter.

It is important to note that sodium pentobarbital was found to be an effective antidote against at least 10 LD_{50} of this poison in rats and dogs even if administered after convulsions had appeared. Kalmbach 101 maintained, therefore, that “since it can be antidoted, it may prove to be of
advantage to use Castrix as a raticide rather than sodium fluoroacetate (1080) in many instances. So far, however, this advice does not seem to have been followed.

2. Guillaume obtained satisfactory results in rat control with glucochloral, admixed with solid bait materials, particularly grain, or with bouillon in a proportion of 20%. The use of this substance did not seem to lead to instances of secondary poisoning in domestic animals.

To judge from limited experiences made by Tara, glucho­chloral was effective against commensal mice even if used in a concentration of 1/1,000.

3. As established by Karel, the phenylhydrazine derivative of fluoroacetic acid, "Fanyline", was readily accepted by albino rats and mice when offered in corn meal in concentrations of 1% or 2%. The oral median lethal doses of this compound, expressed in mg per kg body-weight, may thus be compared with the corresponding values obtained with 1080:

<table>
<thead>
<tr>
<th>Species</th>
<th>Fanyline</th>
<th>1080</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albino rat</td>
<td>9.1 ± 2.7</td>
<td>2.5-7</td>
</tr>
<tr>
<td>Albino mouse</td>
<td>44.9 ± 2.9</td>
<td>8-10 (commensal mouse)</td>
</tr>
<tr>
<td>Cat</td>
<td>greater than 0.25</td>
<td>0.35-0.50</td>
</tr>
<tr>
<td></td>
<td>less than 0.5</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>0.1-0.25</td>
<td>0.07-0.20</td>
</tr>
</tbody>
</table>

As will be noted, Fanyline, while less poisonous for albino rats and markedly less poisonous for mice than 1080, was but slightly less dangerous for dogs than the latter. The toxicity of Fanyline for cats was quite similar to that of sodium fluoroacetate.

Karel expressed the opinion that the lethal action of Fanyline was due to the fluoroacetate moiety of the molecule. He considered as an advantage the fact that, in contrast to 1080, Fanyline was but slightly soluble in water.

4. Studying the properties of the nitro-dyes (colorants nitrés) and possibilities for their use, Pastac found that certain of these substances, such as Martius' yellow (2,4-dinitro-a-naphthol) and Victoria yellow (2,4-dinitro-o-cresol), because physiologically active and, at the same time, colourless and tasteless, appeared to be suitable for the eradication of rats or other harmful animals. He ascribed the toxic action of these substances to a catalytic activation of the intracellular respiration which, because it rendered the absorption of a sufficient amount of oxygen impossible, led to death from asphyxia.

These nitro-dyes, the lethal dose of which was 0.01 g per kg of body-weight, could be admixed with all usual solid bait bases.

During the 1946 anti-rat campaign at Marseilles, Bestieu actually used dinitro-o-cresol in premises where a consumption of this poison by domestic animals could be prevented. Baits were prepared by impregnating...
small pieces of bread, which had been previously coloured with methylene-blue, with a 2% solution of the poison in a proportion of 500 ml per kg of bait. The poisoned rats were found to succumb within two hours, their carcasses showing evidence of desiccation.

5. As stated by Kalmbach, some of the organic phosphates designed originally as insecticides, such as hexamethyltetrathosphate, tetraethylpyrophosphate, and parathion ("Thiophos"), were found to be highly toxic to laboratory rats. Kalmbach was careful to state, however, that these highly toxic substances were particularly dangerous because they were readily absorbable through the unbroken skin.

Kalmbach added that attention was paid to the possibility of using thiosemicarbazide, which had given satisfactory results with laboratory rats, against orchard mice.

It may be conveniently added that, besides poisons, inoffensive substances such as plaster of Paris have been recommended for the purposes of rodent eradication with the idea of producing, in this way, a mechanical obstruction of the gastro-intestinal tract of the animals. Cors used a mixture consisting of 6 parts of plaster of Paris with 2 parts of wheat flour and 1 part of sugar for this purpose.

Mixtures containing unslaked lime instead of inoffensive substances have been recommended as well so as to produce burns of the stomach mucosa when the lime becomes slaked through the action of the gastric juice. Using this method in grain-stores and in fields, Ivanow & Djarewa were very successful in the case of M. musculus, but obtained only 60%-70% success with R. norvegicus.

Rules for conducting poisoning campaigns

The methods of conducting poisoning campaigns against the commensal rodents, particularly against the rats which are most amenable to this mode of control, have been greatly improved during recent years, not only owing to the introduction of more efficacious rodenticides, but also on account of much progress in the knowledge of the biology, habits, and reactions of these animals. The procedures now adopted for the conduct of the campaigns may be set forth thus:

General principles

While, in the past, poisoning of commensal rodents was often done in a piecemeal manner by dealing only with single houses or small groups of houses, it is now realized that, in order to prevent a rapid re-infestation of the treated premises, the poisoning campaigns must be conducted on a large scale. Whenever possible, the work should be carried out at one and the same time on a community-wide scale; failing that it ought to embrace whole precincts or at least blocks of buildings.
It has likewise been realized that poisoning of the commensal rodents must be done in an intensive as well as in an extensive manner by dealing with all foci of infestation within the areas concerned. It is essential, therefore, to pay attention not only to all infested buildings, including — besides residences — outhouses and godowns, but also to other rodent locations such as garbage dumps and sewers. Recent experiences in Great Britain have proved the great importance of such a "vertical" system of rodent control including the exposure of poison-baits in the sewers as well as in the buildings above them (Barnett 9).

Initial surveys

Though, as summarized by writers such as Dice 11 and Emlen et al.,54 various methods have been recommended for determining the numbers of small mammals such as the commensal rodents, only some of these procedures are of practical value for assessing the degree of infestation before poisoning campaigns. These are: (a) questioning the people regarding the presence and frequency of the rodents; (b) estimating, by means of counting, the number of rodent-burrows or tracks present or the number of rodents seen; (c) systematically searching for droppings and other signs of infestation; and (d) determining the consumption of unpoisoned baits offered to the animals.

Unless the number of rats or mice is small, the people are, as a rule, able (though not invariably willing) to tell whether or not these animals are present in their houses, outhouses, or godowns. Usually, however, not much credence can be given to statements made by the people in regard to the degree of rodent-infestations.

Counting the rodent-burrows gives, as a rule, no reliable results, because in buildings their number is seldom related to the density of the rodents (Chitty 26). Likewise, an inspection of the tracks made by the animals in dust, mud, or snow or in deposits of plaster of Paris or similar materials laid down for this purpose (fig. 36 and 37), while showing the presence of rodents, will give little indication as to their number (Chitty; 26 Morgan 142).

Gunderson 78 maintained that on ships in particular it was possible to determine the degree of rat-infestation by counting the number of animals seen. However, Chitty 26 stated that, owing to the nocturnal habits of the rats, no representative fraction of them was likely to be seen in daytime except when methods such as harbourage destruction or bolting the animals with gas or ferrets were used. Emlen et al. 54 found the counting method of some usefulness in places such as public markets where the presence of long corridors and continuous artificial illumination created unusually good facilities for observation. However, in the opinion of these workers "indices so obtained should be based on repeated counts combined with a careful analysis of factors in the local situation. Rarely can they be expected to provide more than a crude index."
The kinds and amounts of droppings and other signs useful for an estimation of the degree of rat-infestation are shown by the following tabulation culled from Rat-borne disease: prevention and control: 

<table>
<thead>
<tr>
<th>Signs observed</th>
<th>Degree of infestation (number of rats present)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light ((1-20))</td>
</tr>
<tr>
<td>Fresh droppings</td>
<td>None or few groups; generally all of same size.</td>
</tr>
<tr>
<td>Active runs and smears</td>
<td>None or few and relatively indistinct.</td>
</tr>
<tr>
<td>Fresh gnawings</td>
<td>None to few nightly.</td>
</tr>
</tbody>
</table>

On board ship in particular, it is often possible, by paying close attention to these signs of rat-infestation, particularly to the droppings, to arrive at a fairly accurate estimation not merely of the degree of infestation but of the number of rats present. However, great experience is necessary to make such estimations reliable. Moreover, as recently pointed out by Morgan,\(^{142}\) a number of extrinsic factors may render a correct interpretation of the results difficult or even impossible. Morgan upheld in particular “that no accurate estimation of the rat population can be made with the cargo in situ”.

More-reliable estimations of the number of rats present in buildings or other locations on land as well as on ships can be obtained by offering an ample weighed amount of plain bait material to the animals, determining the consumption of this food by weighings repeated at intervals of 24 hours, and dividing the amounts thus found by the approximate quantity of food eaten by one rat per day (25-30 g according to Chitty & Shorten \(^{27}\) in the case of \(R. norvegicus\)). However, such determinations show merely the probable minimum population of the rodents, because young animals are apt to eat less than the adults and also because the latter do not necessarily consume their full quota (Morgan \(^{142}\)). It has to be noted, however, that the rodents, if offered the unpoisoned bait material for several days, become accustomed to partaking of it freely.

Public-health propaganda

It is essential for the proper conduct of rodent-poisoning campaigns to obtain the co-operation of the public by the use of all available methods of public-health propaganda. In particular, it is of great importance to enlist the help of the people in preventing access of children and of domestic animals to the poison-baits and the consumption of poisoned rodents by the latter. At the same time, the people must be urged to promote bait-consumption by the rodents through making other food materials, as far
FIG. 36. RAT TRACKS AND TAIL MARKS IN THE MAKING

FIG. 37. RAT TRACKS IN DUST
as possible, inaccessible to them. While, generally speaking, the people should also fight the commensal rodents by reducing possibilities of harbour-age, such clean-up operations should not be undertaken immediately before poisoning campaigns, so as not to disturb the animals.

**Choice of poisons**

The choice of rodenticides to be used during individual poisoning campaigns depends upon several variable factors, particularly the species of commensal rodents present, the nature of the sites to be dealt with, the attitude of the people, and the available funds and facilities. Of great importance are also the reasons for which the campaigns are undertaken. Thus, in emergencies created by the presence or threat of plague outbreaks, rapidly acting poisons should be given preference, while in plague-free times or localities, the use of anti-coagulants ought to be considered.

Since commensal rodents which have consumed sublethal doses of a rodenticide may become poison-shy, refusing to consume further baits containing the same poison, it is not advisable, in general, to use several rodenticides during the same campaign, because then no suitable poison might be available for follow-up treatment or campaigns to be repeated at short intervals. However, it may be advantageous to use, in addition to the general distribution of less dangerous poisons, 1080 in sites inaccessible to children or domestic animals.

**Choice of bait bases and bait preparation**

The appropriateness of bait bases depends not only upon their compatibility with the poisons chosen but also upon their acceptability for the rodents to be dealt with. It is of utmost importance to realize in this connexion that bait materials found fully acceptable in one locality may prove quite unsatisfactory in another. Hence, before definitely choosing the bait base or bases to be used in a poisoning campaign, one must never omit to ascertain their acceptability by means of test-baiting, i.e., by distributing small quantities of the unpoisoned bait materials in the form in which it is proposed to use them, and then observing how well these materials are consumed by the rodents.

It is often maintained that different bait bases should be chosen to cater for the different food preferences of (a) the Norway rats, more inclined to be meat and fish eaters, and (b) *R. rattus*, said to prefer vegetables and fruit. Actually, however, simple bait-bases such as grain or sausage-rusk (a biscuit meal used as filler for sausages) are often fully acceptable to both rat species and to the mice as well. To use such plain bait bases is desirable for the sake of economy and expediency of bait preparation. Moreover, plain baits are less attractive to cats and dogs than those containing meat or fish.
The various ingredients used for the preparation of poison-baits, particularly the rodenticides themselves, must be accurately weighed. Thorough mixing is essential so as to distribute the poisons evenly.

Workers in the USA often recommend the use of several (2-4) different types of bait base during one and the same poisoning campaign. However, elsewhere, fully satisfactory results have been obtained with the use of only one type of bait base. It is, however, indispensable to employ different bait bases in follow-up treatments or for quickly repeated campaigns, because rats which have not succumbed after ingestion of a poison-bait may refuse to partake of the same bait material even if a different poison has been admixed with it. In fact, as stated by Barnett,9 "on the whole, shyness develops more readily to the bait base (e.g., flour) than to the poison, although physiologically it is the poison that is responsible for it". Consequently, the routine procedure used in Great Britain for dealing with surface rat-infestations was to distribute initially 2.5% zinc phosphide in sausage-rusk, to bait three weeks later with sugar meal containing 15% arsenious oxide, and, if a third treatment was necessary, to use 10% red squill admixed with bread mash.7

As maintained by Barnett,7 "mice do not develop bait base shyness, although they seem to show a general shyness of food after sublethal doses of poison. There is therefore no need to change the bait base for the second treatment. The general shyness can be overcome by prebaiting: 4 days will usually be sufficient, but sometimes up to 10 days is needed".

While, as noted before, it is preferable to use 1080 in the form of watery solutions, as a rule the other rodenticides are admixed with different solid bait bases. Materials such as rolled oats or other sorts of grain, flour, or meal may be distributed loose or wrapped up in waxed paper. Water or some kind of oil may be used to prepare a dough or paste with the bait material to which the poison is added, and the bait mass is then distributed in the form of balls or small cakes which may be dipped in hot oil to make them firmer and more attractive to the rodents. As already described, diced 1/2-inch (1.3-cm) cubes of vegetables or fruit may be coated with the rodenticides. To distribute the poisoned bait material in loose form is advantageous because the rodents are not apt to carry it away as they often do in the case of wrapped-up or piece baits. Moreover, such loose bait material, if distributed in small quantities (5 g corresponding to one level teaspoonful), is little apt to attract the attention of cats and dogs. On the other hand, wrapped-up or piece baits, which should also be used in 5-g doses, can easily be inserted into sheltered sites such as rat-burrows. In actual practice, it may be well, therefore, to prepare both loose and wrapped-up or piece baits so as to be able to deal adequately with the different sites to be baited.

As mentioned before, p-nitrophenol may be used to prevent mould formation in warfarin-containing grain. Other methods recommended in
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the handbook on rat-borne disease for preventing spoilage of poison-baits are (a) to use vegetable oils instead of water or milk to prepare solid baits; and (b) to coat the baits with a tasteless material such as glycerol. However, the use of such preservatives is not indicated in the case of highly toxic poison-baits which must be promptly collected and discarded, if not rapidly consumed.

The precautions to be taken when handling poisons absorbable by, or irritating to, the skin, for the purpose of bait preparation, have been referred to already when dealing with the individual rodenticides.

Prebaiting

As summarized by Barnett in a valuable report on the principles of rodent control, on account of their reaction to new objects, rats may at first completely avoid partaking of an unfamiliar food offered to them or may even avoid food to which they are accustomed, if it is offered at a new place. After this initial stage the rats tend to go through a phase of tentative nibbling. It follows that if poisoned bait materials are offered to the rats straight away, the latter may consume these baits only in sublethal doses, if at all, and may, therefore, become poison-shy and/or shy to the bait base used. To overcome this impasse, the procedure recommended is to distribute, first, unpoisoned bait materials, and to use poison-baits, prepared with exactly the same foodstuffs, only after the latter have been available to the rats for some (usually four) days, so as to accustom them to the consumption of the given food at the given places.

This method of prebaiting seems to have been used first by Pemberton (1925) and by Doty (1938) in Hawaii, but—as stated by Martorana—early mention of it was also made by Schauder & Gotzer (1930).

Prebaiting may be done in two different ways, either by offering to the rats a surplus of bait-material or by exhibiting amounts smaller than the quantity the animals could consume (token-baiting). A kind of combination of these two methods was recommended by Storer thus:

“A given weight of prebait—say 2 ounces [60 g], which may be measured by bulk with a suitable spoon—is put out at each station. On succeeding days, if much is eaten, the station is refilled, doubling the amount each time (4, 8, 16 ounces, etc. [120, 240, 480 g]), and this practice is continued until the amount taken “levels off,” when a maximum number of rats will be eating at the station.”

The method proposed by Storer, though time-consuming, is advantageous if one wishes to use prebaiting procedures not only to overcome the new-object reactions of the rats but also for purposes of census-taking, because it gives more reliable results in the latter respect than short-term surplus baiting.

Though, as shown by Barnett et al., one may arrive at an estimate of the number of rats by comparing the consumption-rate of token-baits at different feeding-points, results obtained in this way did not always
tally with those obtained through surplus baiting. However, token-baiting is fully satisfactory if one wishes merely to accustom the rats to the bait material and feeding places. Indeed, in the opinion of Chitty & Shorten token-baiting was more satisfactory for this purpose than surplus baiting. Certainly it is less expensive than the latter procedure, because, as stated in the British instructions, it is sufficient, when dealing with surface infestation, to prebait with 1, 2, or 4 ounces (30, 60 or 120 g) on each of four consecutive days, and to use prebaits of 4 ounces (120 g) daily for two days in the case of sewers.

While prebaiting has become a standard practice in Great Britain and is also amply used elsewhere, particularly in Hawaii, some workers in the USA do not consider this procedure indispensable for successful rat-poisoning. According to the handbook on rat-borne diseases, "prebaiting is recommended in difficult poisoning cases, but not in a routine poisoning program".

Since the commensal mice display little or no new-object reactions (Southern, quoted by Barnett, 1948), there is certainly no need to use prebaiting procedures in their case. Prebaiting is also unnecessary before poisoning campaigns with warfarin or similar compounds, the action of which depends upon the repeated ingestion of bait materials containing these substances.

**Distribution of poison-baits**

In order to poison commensal rodents, baits may be laid (a) in holes, (b) on the surface; and (c) in containers.

Hole-baiting is a satisfactory method, the more so because one can easily ascertain the results of poisoning by blocking all the openings of the burrows in question after the rodenticides have been inserted: any rodents which survive will have to re-open one or some of the holes.

Unfortunately, however, the scale on which hole-baiting can be done is apt to be limited, the rats often living in other locations instead of in burrows.

Surface-baiting, while the least safe of the three above-mentioned procedures, is quite effective if care is taken to deposit the baits so that the rats are intercepted between their nesting sites and their usual sources of food (Barnett 1947). Therefore, whenever possible, the poison-baits should be laid near the mouths of burrows or near the rat-runs but not directly on the latter because, as the handbook on rat-borne disease justly says, "rats prefer to investigate rather than stumble over baits". Generally speaking, the baits should be deposited in dark, secluded corners or sheltered locations so that, as stated in the above-mentioned handbook, "an untrained individual should not see many of the baits after a trained man has placed them properly".
Frontal access to baits deposited near walls may be prevented by leaning pieces of boards against the walls to cover the baits or by placing boxes or similar objects before them.

Some workers recommend that baits be placed not only on the ground or floor but also on elevated surfaces, particularly ledges or beams on which the rats run. However, other authorities are strictly against this practice. In particular it has been stressed that baits should not be laid on boxes, packages, or bags containing human food or animal feed (Storer 212).

As has been stated before, bait-containers (bait-boxes) possess the double advantage of preventing access of children and larger domestic animals to the baits and of facilitating the establishment of permanent feeding stations (fig. 38). Their use is particularly indicated if operations are conducted in the open, so as to protect the bait material against inclemencies of the weather.

A disadvantage of the bait-boxes is that some extra time must be allowed before prebaiting and/or poison-baiting can be commenced, so as to accustom the rats to these new objects. In the opinion of Barnett, an interval of at least 10 days has to be left between installation of the bait-containers and the commencement of operations.

Feeding stations protected against the access of children and larger domestic animals may be improvised, for instance by leaning a piece of board over baits deposited near a wall, as mentioned before, or by inverting an open box, provided with suitable openings, over the baits. Generally speaking, however, it is advisable to work with permanent types of bait-boxes which, though initially expensive, are economical in the long run because they are repeatedly usable. A great advantage of solidly constructed bait-boxes is also that pending use they can be firmly affixed to the ground or floor, thus preventing spilling of their contents.

Many patterns of bait-boxes made either from wood or from metal have been recommended, ranging from simple contrivances to the elaborate "protected poison points" (P₉) devised by Elton and his collaborators and briefly described by Storer 212 thus:

"Special box of wood, 3/8-1/2-inch [7-12 mm] thick (or of galvanized iron); removable cover fits over top; box when placed against a wall forms a tunnel without floor; rat must travel in 3 directions to reach bait inside on bottom, below small ledge; large enough for several rats to feed at one time; poultry or pets cannot reach into bait compartment; lid may be fastened by inside spring to prevent opening by children, and box may be bolted to floor or wall; bait not easily spilled when box is overturned, yet prebait can be removed easily."

The usual patterns of bait-boxes, permitting direct entrance of the rodents, ought to be provided with openings not larger than 2 inches (50 mm) by 3 inches (75 mm) or even 2 inches (50 mm) by 2 inches (50 mm).

For outside work it is advantageous to use bait-containers made of pieces of some hollow circular material, e.g., of drain pipes, 15 inches
FIG. 38. CONTAINERS FOR SAFE EXPOSURE OF BAITS USED TO POISON RATS

A. Leaning board with a tunnel under which rats may eat. B. Box shelter for either bait or trap. C. Sheet metal hood to protect bait from rain. D. and E. Wooden bait boxes. F. Metal bait box. G. Drain pipe or sewer pipe for garbage dumps. H. Special wooden box (protected poison point).
(375 mm) long and with an internal diameter of 3 inches (75 mm), which have been blocked at one end. As stated by Barnett,7 poultry can reach inside these pipes, but some protection may be achieved by placing them with the open end 3 inches (75 mm) from an object such as a wall or a brick.

Special techniques have to be used for the baiting of garbage and refuse dumps and of sewers.

Since, as stated by Schuler,188 the distribution of red-squill baits on garbage and refuse dumps gave unsatisfactory results, and calcium cyanide dust could not be used there on account of the presence of numerous openings, highly toxic poisons had to be employed. Pending their exposure it was necessary to have guards on constant duty or to fence the dumps safely. It was preferable, however, to use bait-boxes for prebaiting and baiting. As an alternative, baits which became non-toxic in a few days could be distributed. Schuler188 recommended for this purpose the addition of two parts of zinc phosphide and one part of magnesium carbonate to 16 parts of rolled oats and 48 parts of ground horse meat or fresh fish. He maintained that, if placed under cover on rat-trails or in burrows, these baits became decomposed to a non-toxic residue after several showers of rain.

For poisoning work in sewers, Barnett7 recommended special bait trays and bait depositors which were lowered through the manholes. Poisoning was first to be done, after two days prebaiting, with zinc phosphide, and four weeks later with arsenious oxide in a different bait base. Whenever necessary, single maintenance treatments were to be carried out at six-monthly intervals. It was permissible to use the previously employed poisons and bait bases for such repeat-treatment because there was no evidence to show that bait-shyness persisted in wild rat-populations for as long as six months (Barnett, 19478).

Whatever modification of rodent-poisoning methods is used, one must always realize that, except in the case of the anti-coagulants, the success of the poisoning campaigns depends upon a large initial kill. Hence it is of utmost importance to avoid "underbaiting" by offering an amount of poison-bait more than sufficient for the rodents estimated to be present. As stated in this connexion in Rat-borne disease: prevention and control:217

"A major fault in unsuccessful poisoning programs is the use of too few baits. Many times an individual will report great success because every bait was taken. This may, however, simply mean that there were many more rats than baits. One hundred to two hundred baits of each type is not considered excessive for a heavily infested business establishment. Since there are about 80 to 90 individual baits per pound, this would not be expensive treatment ".

Similarly, bait-boxes must be kept constantly supplied with an amount of poison-bait sufficient to feed all rats coming to them. It should be noted, however, that the amount of poison-bait consumed may be considerably lower than that of prebait eaten at the same feeding stations. Storer 212
maintained in this connexion that "a guess as to the number killed can be made, on the basis of 1/3 ounce (10 g) per rat . . . ".

For the sake of safety it is best to distribute the poison-baits as late as possible in the afternoon and to collect those which have not been consumed, as well as the dead rodents, as early as is feasible on the next morning. This procedure is also advantageous in so far as it permits of preparing the baits on the day on which they are laid.

To facilitate the collection of unconsumed baits, the places in which baits are deposited should be marked with chalk or by affixing small labels bearing the inscription "Poison—do not touch". During bait distribution a final warning must be given to the people to safeguard children and domestic animals.

If it is quite safe to do so, poison-baits may be left in position for one or two more nights. Even then, however, the baited places must be visited every morning in order to collect the dead rodents.

**Disposal of unconsumed baits and poisoned rodents**

As has been stated already, unconsumed baits as well as the poisoned rodents must be destroyed by incineration or must be buried to a depth of at least 2 feet (60 cm). No other method of disposal is safe.

A serious nuisance may be created during poisoning campaigns by rodents dying and decomposing in places which cannot be reached without major damage to the structures in question, e.g., within double walls. For such cases, the blowing-in of deodorants such as powdered activated charcoal, chloride of lime, or solutions of formaldehyde, lead acetate, or lead nitrate has been recommended (Storer 212). Should this be impossible, deodorants such as pine oil or wintergreen oil should be sprayed as near as possible to the source of the odour. A mixture of naphtha flakes in white gasoline is particularly effective for this purpose but cannot be used if there is a fire hazard.217

**Assessment of results of campaigns**

It is generally agreed that the number of carcasses recovered after the distribution of poison-baits does not indicate the success obtained, because, even if most of the rats or mice have been killed, not many or even only a few of them may be found dead. Stress has to be laid, therefore, upon ascertaining the number of survivors rather than upon counting or otherwise assessing the number of casualties.

Some workers used trapping procedures to assess the number of surviving animals, but the reliability of this method was doubted by other observers. Two methods generally considered as valuable for appreciation of the results of poisoning campaigns are the following:

1. Close inspection by experienced observers for the signs of rodent-infestation mentioned earlier. If, owing to a decimation of the rodents,
signs of infestation are insignificant, it may be helpful to sprinkle plaster of Paris or other dusts near the shelters or on the runs of the animals and to study the tracks made by them.

(2) Post-baiting, done by distributing unpoisoned bait materials different from those used during the campaigns and watching the consumption rate of these foodstuffs. It is generally agreed that post-baiting should not be commenced immediately after poisoning but only after an interval of one or preferably two weeks. As maintained by Chitty & Shorten, a surplus of material should be used for post-baiting.

If the use of this method indicates the necessity for further poisoning work, it is legitimate to carry this out with the aid of the bait base utilized for the purpose of post-baiting. Accordingly, the British instructions recommended for the fourth week of poisoning campaigns:

"Lay test baits of sugar meal at each point baited in week 1. Where bait is taken by 3rd day, continue to bait at these points; poison on 5th day with 15% arsenious oxide in sugar meal."

Virus

The various brands of "virus" used for the purposes of rodent control are actually bacterial strains, mostly those belonging to the Salmonella enteritidis group (see Leslie), distributed with the idea of producing fatal infections not only in the rodents which directly consume these materials but also in those which come in contact with, or devour the carcasses of, the original victims.

Opinions regarding the efficacy of this method and the advisability of using it are sharply divided. It was warmly recommended by French workers in particular, more recently by Petit (1936, 1943) and Auvray (1942) for instance, and is still widely used in France.

With very few exceptions, recent observers in other countries consider the method of using virus not only as ineffective but also as rather dangerous for human beings. In fact, numerous cases of human gastro-enteritis due to the distribution of virus preparations for the purposes of rat-control are on record. Leslie, who summarized the evidence available in this respect up to 1942, added that "there are, also, reasonable grounds for believing that these bacterial types may be pathogenic for a number of domestic animals, including some poultry".

The unfavourable opinions held by most plague workers in regard to the above-described method have been endorsed by a resolution passed by the WHO Expert Committee on Plague in 1952, which stated that "the experts were definitely against the use of virus preparations as rodenticides".
Fumigants

Lethal vapours or gases produced from the following substances have been found useful for killing commensal rodents and in part also their ectoparasites:

**Carbon disulfide**

Carbon disulfide (CS$_2$) is a colourless liquid with a boiling-point of 46.3°C. It is readily volatilized at ordinary room temperature, the vapour being 2.63 times as heavy as air. The vapour, which has an unpleasant odour, is extremely inflammable and apt to be explosive when mixed with air, for instance when coming in contact with hot surfaces such as those of steam pipes (Frear 56).

Though mainly important as an insecticide, carbon disulfide has been used on a fairly large scale for the destruction of wild rodents in California, South Africa, and south-east Russia (see section on the control of wild rodents, page 582), and of free-living commensal rats in Hawaii. The usual technique was to introduce absorbent materials soaked with carbon disulfide into the burrows, and then to plug the burrows.

As will be gathered, carbon disulfide has to be handled with extreme care. It is far too dangerous to be used in buildings.

**Carbon dioxide**

Carbon dioxide (CO$_2$) in solid form ("carbonic-acid snow"), which is widely employed as a refrigerant, has occasionally been used to kill rats (Pieniazek & Christophers 165) and mice (Barnett 9). It does not seem to act as a poison, but, when evaporating and thus displacing the oxygen in the air, merely suffocates the rodents. Carbonic-acid snow must be handled with spoons made of horn or plastic, and not with the bare hands on which it may produce burns.

**Carbon monoxide**

In the past ample use has been made of the mixture of carbon monoxide (CO) and CO$_2$, generated with the aid of a complicated apparatus usually installed on a barge, for the purposes of ship fumigation. However, little, if any, use is now being made of this method, mainly on account of its inability to destroy the rat-fleas.

According to Kalmbach, 102 in the USA some use has been made of carbon monoxide, which may be obtained in crude form from the exhausts of motor-cars, to deal with rodent infestations in outdoor storage installations. As stated in the handbook on rat-borne disease, 217 rat-burrows in particular may be treated in this way by attaching "a flexible hose to the exhaust pipe of an automobile, running the tube into the burrow and operating the motor on a 'rich' gasoline mixture with the choke pulled out". It is unlikely, however, that this procedure, which on account of the
lethal action of the exhaust gases on man must be used with great precaution, exerts a reliable action on the rat-fleas, because, as established by De Raadt, dry heat has to be applied in combination with carbon monoxide to kill these insects. De Raadt found that in this way Xenopsylla cheopis were killed after exposure to carbon monoxide at a temperature of 50°C for 45 minutes.

Chloropicrin

Chloropicrin or trichloronitromethane (CCl₃NO₂) is a slowly volatilizing colourless fluid, the vapours of which produce, even at relatively low concentrations, intense irritation of the eyes—a property which renders chloropicrin useful as a forewarning agent in hydrocyanic-acid fumigation. Besides being used as an insecticide, chloropicrin has occasionally been utilized for the purposes of rat destruction, for instance by Lewis et al., who, during a plague outbreak at Dakar, poured the fluid on the floor of huts and then covered these for 24 hours with tarpaulins. It should be noted in this connexion that, according to Sherrard, the minimum lethal concentration of chloropicrin vapours for Norway rats was 1 ounce per 1,000 cubic feet at a temperature of 63°F (1 g per m³ at 17.2°C) and with an exposure for 4 hours.

Frear stated that chloropicrin “is superior to certain other fumigants in its complete freedom from fire and explosion hazards, ability to penetrate bulk commodities, non-reactivity with metals, fabrics and colors under fumigating conditions, and has a pronounced odor and lachrymatory effect, so that no ‘warning gas’ need be added”. In the experience of Sherrard, it also exerted no deleterious or toxic action on foodstuffs, which could be consumed without ill effect after exposure. However, the action of chloropicrin is slower than that of hydrocyanic acid, it is relatively toxic to living plants and seeds, and its odour is apt to persist for days (Frear; Martorana 129).

Hydrocyanic acid

Hydrocyanic acid (hydrogen cyanide—HCN) in the pure state is a colourless liquid below its boiling-point (26°C or 78.8°F) and is a colourless gas above this temperature. It has a distinctive odour which, however, is not marked enough to warn inexperienced persons and causes no discomfort even if the gas is present in lethal concentrations (Williams 294). If used for purposes of fumigation, HCN—one of the most rapidly fatal poisons known—is apt to cause death not only when inhaled but also because it easily penetrates ordinary clothing and is then readily absorbed by the skin. In the opinion of Williams, workers provided with efficient gas-masks might remain for half an hour in air containing 2 ounces of HCN per 1,000 cubic feet (2 g per m³) without experiencing signs of poisoning, but should not stay longer than 5 minutes if the concentration of the gas amounts to 8 ounces per 1,000 cubic feet (8 g per m³).
Generally speaking, hydrocyanic acid gas possesses most marked
penetrating powers but, as aptly stated by Williams,\textsuperscript{234} "penetration being
merely one feature of diffusion, it is not surprising to find that the gas also
rapidly passes out of materials it has penetrated". Hence, unless excessively
heavy HCN concentrations have been used, and as long as the weather
is dry, a comparatively short airing will remove most of the gas from
fumigated articles, so that, for example, an hour's airing will render a
mattress treated with the gas safe to sleep on. However, since water absorbs
HCN and holds it, specially when the weather is cold, moist articles must
be aired longer than dry ones.

As a rule, the gas absorbed by water is given off so slowly as not to
prove dangerous, but this may not hold true if absorption of a comparatively
large amount occurs on a cold day and warm weather follows quickly.

In the concentrations generally used for fumigation in rooms, HCN is
not absorbed by foodstuffs in dangerous concentrations so that it is sufficient
to air these articles for two or three hours before they are consumed.
However, food subjected to higher concentrations (10-20 ounces per 1,000
cubic feet or 10-20 g per m\textsuperscript{3}), as used in fumigation chambers, must be
aired for 24 hours at least (Williams \textsuperscript{234}).

If applied in these higher concentrations, HCN fumigation is injurious
to delicate vegetables, such as lettuce, and probably also to bananas, interfer­ing with their ripening. Further, as pointed out by Williams,\textsuperscript{234} eggs
do not, as a rule, hatch, because their shell is penetrated by the gas. Other­wise HCN fumigation is not injurious to commercial commodities, even
including fragrant articles such as tea and tobacco. Its application is
cheap, the amount of 2 ounces of HCN per 1,000 cubic feet (2 g per m\textsuperscript{3}),
as used for rat destruction, costing, according to Williams (1931),\textsuperscript{234}
12\textsuperscript{1}/\textsubscript{2} cents.

Hydrocyanic acid may be used for the purposes of rodent—as well as
of insect—control in the following ways:

1. It may be generated on the premises to be treated (a) through the
action of 50\% sulfuric acid on sodium cyanide, or (b) by adding sodium
cyanide as well as sodium chlorate to 50\% hydrochloric acid.

2. Rapid evaporation of the gas may be effected by (a) releasing
liquid HCN which has been prefabricated and filled into heavy steel
cylinders, or (b) distributing porous inert materials, such as fuller's earth,
paper or wood-pulp discs, in which liquid HCN has been absorbed, in
the premises to be dealt with.

3. Dusting with calcium cyanide, from which HCN is released less
rapidly under the action of the air moisture.

Little use is at present made of the tedious generation methods mentioned
in (1). The procedures mentioned in (2) are amply used for the purposes
of ship fumigation, and also to some extent for the disinfection of ware-
houses and of establishments such as mills. Since, however, they can be applied efficiently and safely by specially trained workers only, they are not suitable for anti-plague work in general. The use of calcium cyanide, on the contrary, is of importance for the latter and will, therefore, be dealt with in a detailed manner.

**Calcium cyanide**

Calcium cyanide (Ca(CN)$_2$) is available in the form of a fine greyish-white powder suitable for pumping operations and in different coarser granulations to be distributed or scattered in sites where a slower HCN evolution is desired. One commercial brand has been supplied in the form of tablets together with a special pump which first grinds the tablets and then blows out the resulting fine powder.

The calcium cyanide and HCN contents of the various brands available vary considerably, as shown by the following findings made by Sokhey et al.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Percentage of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca(CN)$_2$</td>
</tr>
<tr>
<td>A ... .......</td>
<td>42.30</td>
</tr>
<tr>
<td>B (tablets)</td>
<td>.... 84.94</td>
</tr>
<tr>
<td>C ... .......</td>
<td>... 19.78</td>
</tr>
</tbody>
</table>

Measuring the yield and the rate of evolution of HCN from these three preparations, Sokhey et al. found that products A and B, on coming in contact with the air, gave up their total HCN content, the maximum yield being reached in the case of B in 15 minutes, and in the case of A in 30 minutes. Preparation C yielded only about half its HCN content, and it took 90 minutes to reach even this low concentration. It should be noted, in this connexion, that in the experience of Metzger calcium cyanide, when spread in extremely thin layers, yielded about three-quarters of its HCN content in half a minute and practically all of it in one minute. This gas evolution took place even if the air humidity was 25% or less.

It is generally held that the chemical reaction produced by the action of moist air on calcium cyanide is as follows:

$$\text{Ca(CN)}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{HCN} + \text{Ca(OH)}_2$$

As usually stated, the residue of calcium hydroxide, left after HCN has been liberated, is harmless. However, in the opinion of Williams this is not quite the case because after liberation some of the HCN is absorbed by the calcium hydroxide and changed to calcium cyanide and water. Under any circumstances there is always some calcium cyanide left in the residue which, therefore, must be gathered up and safely disposed off.

Williams added, however, that

"one method of getting around this is to take advantage of the fine powder form of the material and to blow it into the air, from which it settles as a fine dust. There appears to be no great objection to this, in the absence of foods, from a safety standpoint, it
being inconceivable that anyone would sweep up this and eat it; but where foods are fumigated, they become inseparably mixed with the fumigant. If calcium cyanide is left for several days airing, the cyanide content finally becomes so low as to be negligible.

Opinions on to what extent calcium cyanide may be used for the destruction of rodents and their fleas vary considerably. As maintained in the British instructions, "against infestations in the open, and where rat holes are accessible and easily identified, dusting with powders that liberate a poisonous gas can be useful". According to the handbook on rat-borne disease, calcium cyanide should be used in rat-control to fumigate burrows and, under certain circumstances, small enclosed spaces or harbours ("spot-fumigation"). However, as will be shown later, in other areas much more liberal use has been made of the application of calcium cyanide.

Calcium cyanide treatment of rat-burrows may be done either by pumping in fine dust or by placing heaps of coarser granulations, or brands which give off HCN slowly, about 6 inches (15 cm) down each hole. The treated holes must then be promptly blocked.

To get good results with the first-mentioned method, sturdy and powerful pumps must be used, preferably those provided with a "cut-out" device which renders it possible to blow in air after the required amount of dust has been delivered. The pumps should be so adjusted that they deliver a known amount of the dust with a given number of strokes, for instance, as recommended by some workers, 1 ounce (28 g) with 30 strokes. The amounts delivered with a given number of strokes may be ascertained by weighing either the filled pumps or their contents before and after a counted number of strokes has been made.

Before actual pumping operations are started, the extent of the rat-burrows to be treated and their often considerable ramifications must be ascertained. It is of great importance to realize in this connexion that a large burrow may have openings in two adjoining rooms or even in two adjacent houses. Accordingly, unless care has been taken to block, before commencement of pumping, all openings of the burrows to be treated except that to be used for the introduction of the dust, a watch must be kept for the escaping of dust and also the bolting of rats not only in the rooms where pumping is carried out but, as far as necessary, also in adjoining rooms or houses. As recommended by Yang et al., a mixture of portland cement, sand, and broken glass should be used for sealing burrows in lime mortar floors and good rubble walls. Holes in mud floors and poorly-built walls may be sealed with moistened clay or with pieces of sod.

In order to prevent a back-flow of the dust, materials such as clay, sod, rags, or paper must be firmly packed round the nozzle of the pump after its introduction in the hole to be treated. After the required amount of dust, followed by several blasts of air made with the aid of the "cut-out" device, has been pumped in, the nozzle is quickly withdrawn and the hole is sealed in the above-described manner.
The amounts of calcium cyanide used by different workers per rat-hole were at variance. Thus, Roberts considered it necessary to administer 1 ounce (28 g) of the dust to an average burrow and 2 ounces (56 g) to a burrow with many ramifications. Yang et al. recommended pumping in 4 g of calcium cyanide when dealing with a rat-hole 2 feet (60 cm) in length, and increasing the dosage by 1 g for each additional foot (30 cm) of length. Workers in India often found that 1 pound (0.45 kg) of calcium cyanide was sufficient to treat about 60 average burrows or 100 small burrows.

In addition to burrows, cracks in walls leading to rat-harbourages may be dealt with in the above-described manner. Thornton recommended applying the nozzle at the lowest point of the cracks and then sealing the cracks with clay as the dust made its appearance.

To deal safely with loosely piled stone walls, Thornton advised plastering these on both sides with clay and subsequently pumping calcium cyanide into the holes made in the latter by the rodents.

As pointed out in the handbook on rat-borne disease, small enclosed spaces inside buildings, such as the boxed-in bases of shelving, counters, and lockers may be "spot fumigated" if there is no danger of the dust going into living-quarters or other occupied rooms. It was recommended that no place larger than about 100 cubic feet (3 m³) in volume be treated in this way and that small hand-bulb dusters be used instead of powerful pumps. Thornton upheld that the latter could be used to treat underfloor spaces with calcium cyanide at the rate of about 2 ounces per 120 square feet (5 g per m²) of floor space. To reach such spaces, holes could be drilled in the floor which, after completion of dusting, could be plugged with corks cut flush with the floor level.

Williams established that calcium cyanide could be used safely and efficiently to deal with enclosed rat harbourages, such as pipe-casings on ships as a preliminary step to general fumigation of the vessels. Since the vast majority of such harbourages had a capacity of not more than 10 cubic feet (0.3 m³) or even considerably less, 4 strokes with a pump delivering 1/12 of an ounce (2.4 g) per stroke were sufficient to dust them adequately.

As proved by experiments made in Africa and to a lesser extent also in India, whole huts or small houses may be treated safely with calcium cyanide dust. Thornton recommended the following procedure for this purpose:

1. Remove all occupants (people and domestic animals) as well as all foodstuffs.
2. Look for and dust all rodent-burrows.
3. Hang all clothes, bedding, and similar articles on rafters.
4. Spray floor lightly with kerosene emulsion.
5. Cover hut completely with canvas sheets.
6. Pump calcium cyanide into room and thatch, using about 1.5-2 pounds per 1,000 cubic feet (28-35 g per m³), holding pump in inverted position to do this quickly.
(7) If possible, hold hut under fumigation for at least four hours (or pump in excess of dust).
(8) Remove canvas, open doors and windows, wait a little before entering.
(9) Air clothing, bedding, etc. in the open.
(10) If possible keep occupants away until next morning. If this is impossible, have floor swept before they enter.

Hopkins advised applying calcium cyanide only to the rat-holes and the thatch of huts in which plague had occurred or was likely to occur, surrounding the huts during this operation with men provided with sticks to kill bolting rats. The thatch was then removed and spread out in the sun, but the people were permitted to use it for re-thatching. Roberts recommended the use of pumps provided with long handles to deal with thatched roofs, to which the rats in the huts in question were mainly confined. Calcium cyanide was pumped in at a rate of 2 pounds (1 kg) per roof.

George, studying possibilities for the disinfestation of thatched roofs in India, found it sufficient to apply calcium cyanide to the interior of the houses, using 12-16 ounces of the dust per 1,000 cubic feet (12-16 g per m³) with an exposure for 3-4 hours. It was necessary, however, to close tightly doors and windows, to seal all small openings with wet mud, and to close gaps between the walls and roofs with rolls of gunny bags before pumping was started. If the dust was applied in this way, hydrocyanic acid gas diffused through the thatch and killed rats and fleas therein.

Calcium cyanide has also been used to deal with rodent and flea infestations in other sites, e.g., with grain and haystacks and with railway trucks carrying produce. Its use for the purposes of grain disinfestation in general will be discussed later in connexion with the problem of flea control.

Since HCN is slightly lighter than air (specific gravity, 0.9348), it is well to deposit calcium cyanide at the base of the objects to be disinfested or, when treating objects such as cracks in walls or grain and haystacks, to start pumping at the lowest point and gradually to move the nozzle of the pump upwards.

Though, generally speaking, it is not necessary to adopt elaborate precautions as indicated in the case of HCN fumigation, when working with calcium cyanide, the following safety rules must be strictly followed:

(1) The tins containing calcium cyanide must be tightly closed as soon as the necessary amount of dust has been withdrawn and must be stored in a dry and safe place.

(2) Preparations for calcium cyanide application (filling and testing the pumps, etc.) must be made in the open or in open sheds. Unless the latter offer protection, operations should be suspended if there is a high wind. In a slight wind, the operators should stand to windward of the tins and pumps so that any dust blown out is carried away from them.
(3) It is inadvisable to dust in wet weather or to deal with burrows in the open when the ground is wet.

(4) Calcium cyanide application should be made by teams of at least two workers so that, should one inhale HCN, the other, or others, can take him into the open and assist him.

(5) Generally, it is not necessary to use gas-masks when handling and distributing calcium cyanide but they should be worn preferably for spot fumigation in rooms. Also, as suggested by Hopkins, it is well if each team is provided with one gas-mask to serve in emergencies.

(6) Before operations are commenced in buildings, all inmates including domestic animals must be sent out and must be kept away until it is safe for them to re-enter the premises.

(7) All foodstuffs and, as far as possible, all water-supplies ought to be removed from the premises to be treated.

Opinions as to how long the people and domestic animals ought to be kept away from premises treated with calcium cyanide vary considerably. Some workers considered a few hours’ ventilation sufficient, while others advised that the premises ought not to be re-occupied for 1-3 days after application of the dust. Generally speaking, as long as good facilities for ventilation and airing of objects dusted exist, an evacuation until the morning following treatment ought to be ample. As recommended by Yang et al., one might in emergencies spray chloropicrin at the rate of 50 ml per 1,000 cubic feet (28 m³) in the endangered rooms so as to make sure that the people do keep away.

Since after calcium cyanide application the rodents often succumb in their burrows, not rarely decomposition of the carcasses of these animals produces bad odours in the houses treated. The methods mentioned earlier in this study may be used in such cases. To seal all cracks in the walls of simply-constructed houses may be helpful as well.

The following methods for treating victims to poisoning with cyanides are given in *Rat-borne disease: prevention and control*:

"Any case of cyanide poisoning must be treated rapidly. Persons using this fumigant should always carry amyl nitrite ampoules, a sodium nitrite solution of 0.3 grams in 10 cubic centimeters of water, a sodium thiosulfate solution of 25 grams in 50 cubic centimeters of water, and a sterile 10-cubic centimeter syringe and needle. Until the nitrite solution can be injected INTRAVENOUSLY by a physician the patient may be tided over by holding a broken amyl nitrite ampoule under his nose for 30 seconds in every 2 minutes. The 10-cubic centimeter sodium nitrite solution should be injected by a physician as soon as possible, the injection being slow and taking 3 to 4 minutes. Follow this injection by another slow INTRAVENOUS injection of the entire 50-cubic centimeters of sodium thiosulfate solution over a period of 10 minutes. Call a physician at once but do not wait for him to administer the amyl nitrite. If breathing stops, apply the prone pressure method of artificial respiration immediately."

...
The results obtainable with calcium cyanide may be evaluated as follows:

1. It would seem at first glance that application of calcium cyanide is particularly advantageous because this chemical is endowed with pulicidal as well as rodenticidal properties. Actually, however, reductions in the incidence of either rodents or fleas obtainable through single calcium cyanide applications are not drastic enough to be of lasting value. A considerable minority, sometimes even about half of the rats in the localities treated in this way, remains unscathed, with the result that increased breeding of these survivors rapidly restores the former population level of the animals. The reduction in the rodent-flea incidence effected through calcium cyanide dusting is even more temporary, mainly because it is impossible as a rule to deal effectively with the aid of this procedure with the flea nurseries in deeply situated rodent-nests (Ganapathy 59).

It follows that the only way in which it is possible to keep the rats and their fleas at a permanently low level through application of calcium cyanide alone, is to repeat the campaigns at short intervals, as it is usually recommended, every three months. Quite possibly, by continuing such quarterly campaigns for some years one might bring about a very marked reduction of the rats or might even well-nigh eradicate them. However, the effort and expenditure involved would render this impracticable.

Even single applications of calcium cyanide have been found to be of definite value in so far as they were apt to limit or, if commenced early in the outbreaks, even to cut short the spread of plague. However, results obtainable with single applications of DDT are markedly better than those with regularly repeated calcium cyanide applications. Thus comparative tests made in the Nilgiris District of Madras State, India, in 1948-9 showed, as summarized by Wagle & Seal, 227

"that DDT is very effective in keeping the general flea index below 1 for nearly 16 months after a single application, while with repeated cyanogas [calcium cyanide] fumigations in Coonoor town the monthly flea index remained quite high. Again, while plague occurred in 45 out of 460 village units regularly fumigated with cyanogas every 3 or 4 months, all the 90 village units treated with DDT escaped infection during the same period."

While, therefore, there can be not the least doubt that DDT rather than calcium cyanide must be used for plague control in general, the latter remains of some value for certain specific purposes, particularly for grain disinestation.

**Methyl bromide**

Methyl bromide (CH\_3Br), having a boiling-point of 3.56°C, is at ordinary temperatures a colourless gas which is heavier than air (specific gravity, according to Frear 56 3.20 at 20°C.). In the concentrations used for fumigation, the gas is not inflammable. It is easily liquified and is, therefore, usually distributed in this form in cylinders.
Though not necessarily harmful even in relatively high concentrations, methyl bromide is apt to exert a delayed toxic action on man. Workers handling this chemical should, therefore, wear suitable masks, the more so because the sweetish odour of the gas is not pronounced.

As recently stated by Gracie, methyl bromide was used successfully in Great Britain to deal with considerable mouse infestations in hangars, where bagged grain was stored. For this purpose the piles of bags were sealed under balloon fabric and methyl bromide was injected. The whole operation (sheeting, fumigating, and ventilating) took three days.

**Sulfur dioxide**

Sulfur dioxide \((\text{SO}_2)\), generated by combustion of sulfur in pots or with the aid of a special apparatus (Clayton machine), or released from cylinders containing the chemical in liquid form, has been amply used in the past for ship fumigation and is still utilized for this purpose to quite some extent in ports where it has not been found possible thus far to apply more satisfactory methods.

Fumigation of rat-burrows or other enclosed spaces with sulfur compounds has also been practised in some countries. In India, for instance, sulfur-containing candles, called “neem-battis” or “bhoosa-battis” were utilized to fumigate rat-burrows. As was to be expected, Yacob found these candles markedly less effective than calcium cyanide.

Concerning the fumigation of ships, one must agree with Williams that \(\text{SO}_2\) is the only nearly-safe fumigant known because it causes severe irritation if present in the air in concentrations far below the fatal level. However, as rightly pointed out by Hirst, this property is a drawback as well as an advantage, because the rats as well as man get early warning of the presence of the gas and have, therefore, time to seek refuge in enclosed spaces such as covered bilges, into which the gas could only penetrate with difficulty.

A still greater drawback is that \(\text{SO}_2\), being a very heavy gas (specific gravity, 2.26), has slow powers of penetration, so that it has to act for 6 hours at least to be effective. To make matters worse, \(\text{SO}_2\), if used for this length of time and in the required concentration of 3\%, tarnishes metals and damages cereals and other goods so that it can be used in empty holds only (Hirst; Wu).

It will be gathered that the method of fumigating ships with sulfur dioxide has, indeed, little to recommend it and one must wish that it will be discarded as soon as possible.

**Other methods**

In addition to the above-described methods the following procedures used for the destruction of commensal rodents deserve mention:
Electrocution

Besides electricity-charged traps, other means have been used to electrocute rats, e.g., fixing live cables in sewers or suspending baits between electric wires from which the insulation has been removed for a short distance (Rucker, quoted by Wu 238).

Use of sticky substances

Flat boards coated with highly sticky substances have been placed in the runs of rats or mice in order to entangle the animals. Flat trays filled with a mixture of linseed oil and resins and provided with baits placed in the centre of the trays on pieces of cardboard have been used in the same way. However, as pointed out in the handbook on rat-borne disease,217 the sticky substances tend to run in hot weather and to solidify in cold weather. Moreover, these procedures are rather expensive to use and, though fairly effective against mice, are relatively inefficient in the case of the rats.

Khan 104 claimed to have obtained good results when placing in the openings of rat-burrows or on rat-runs sticky materials, such as molasses, which had been lightly sprinkled with dried and powdered datura-belladonna leaves and copper sulfate, or with sodium antimony tartrate powder.

Flame throwers

Donovan & Hopkins 45 found that flame-throwers (fire-torches), hitherto used only in industry and in war, were efficient for the purposes of rodent and flea control. They recommended, in particular, one type of torch with a four-gallon tank connected with a burner coil by a flexible rubber hose. Though the flame had a very high temperature, Donovan & Hopkins claimed that the torch could be used on wood, bamboo, or cane surfaces without setting fire to them.

As stated by these two workers, the flame-throwers were found effective "for burning off vegetations from ditch banks; killing rats and fleas in burrows; burning abandoned rat nests with their possibly infected fleas, in mud walls, stone fences, adobe walls and other shelters; killing fleas within infected houses; killing rats, fleas, spiders, bed bugs, ticks, cockroaches, lice and other vermin in cracks and crevices of walls and floors, in routine sanitation work in dwellings, restaurants, warehouses and other premises; killing rats in sewers; and igniting brush fences (a favorite rat harbor), garbage and rubbish piles".

The value of flame-throwers for plague-control work was confirmed by Sáenz Vera (1943) 183 who, however, stated that it could be used only in sites where no fire hazard existed—an opinion shared in the handbook on rat-borne disease.217 Barreto & Castro 13 considered the use of flame-throwers less effective and more dangerous than that of calcium cyanide. It seems also significant that Sáenz Vera in a further publication 184 speaks only of the use of the latter and not of that of flame-throwers.
Rat-proofing

The following methods may be used for the purposes of rat-proofing which, as broadly defined by writers such as Bowdoin & Boston,\textsuperscript{19} aims at "the separation of rat and man".

Exclusion

Exclusion methods, by which the ingress of rodents into houses or other structures is prevented, while being the most effective of all rat-proofing procedures, are also those most difficult to apply. Their implementation requires not only the availability of adequate materials and funds but also a high degree of specialized technical knowledge. And even if all these facilities are available, it may be difficult or even impossible to apply the exclusion methods effectively in the case of huts or houses of primitive construction. Still, one cannot help feeling that the defeatist attitude, the presence of which in rodent-control work in general is deplored by Barnett,\textsuperscript{9} is particularly conspicuous in the field of rodent exclusion. The expenditure for work of this nature is not rarely overrated. Moreover, though it may be impossible for financial or technical reasons to rat-proof all existing buildings, this ought not to detract attention from possibilities of dealing effectively with some of them, particularly those in which grain or other foodstuffs are accumulated, or from possibilities of making new buildings impervious to the rodents—a task which can often be achieved satisfactorily at a moderate increase of the building cost.

Essential measures for rendering buildings inaccessible to rodents, particularly to rats, are the following:

1. To provide solid floors, preferably concrete floors, and/or to install L-shaped curtain walls, the lip of which should lie at least 2 feet (60 cm) deep and should protrude outwards for one foot (30 cm) or better 2 feet (60 cm). As stated by Porges,\textsuperscript{172} such curtain walls, which may be built around existing buildings, protect these against the rats in the absence of foundation walls or of insufficiently deep foundations. The curtain walls are also useful in the case of structures built on supports not high enough to prevent rats from gaining entrance.

2. To use what Bowdoin & Boston\textsuperscript{19} call "vent stoppage", i.e., the blocking of all openings \(\frac{1}{2}\)-inch (50 mm) wide or wider through which the rodents can gain entrance into the buildings. For this purpose a careful search must be instituted for holes made by the rodents, and these must be sealed, preferably with cement containing pieces of broken glass. Man-made openings, such as those through which pipes or conduits pass, must be closed with the aid of cement or metal sheathing. Wooden sills and doors at ground level should be protected against gnawing with the aid of sheet metal. Windows less than 4 feet (120 cm) off the ground must be screened with adequately strong wire netting. If \textit{R. rattus} are present,
the same ought to be done in the case of the windows in upper storeys, unless rat-guards have been placed on all conduits and pipes so as to prevent the animals from climbing up. Attention must also be paid to the possibility of rats jumping into such windows from poles or trees standing too near the houses.

Great attention must also be paid to the openings of sewers which must be adequately protected with solid grids. The improved buildings must be periodically inspected so as to ensure that the barriers created against the rodents are kept in good condition.

Smaller buildings may be effectively protected against the access of rodents by placing them on supports at least 18 inches (45 cm) high and provided with rat-guards (metal collars) extending at least 9 inches (23 cm) from the supports. Buildings protected in this manner, or even covered platforms placed on higher poles provided with rat-guards, are suitable for the storage of foodstuffs, particularly of grain.

Rat-guards placed on cables mooring ships to the shore are an important means of protecting vessels against the access of rats, provided that the rat-guards are of a sufficiently large size and carefully adjusted. Unfortunately, however, it is not easy to fulfil these two desiderata. Denney, carrying out experiments with various patterns, found that the *R. r. alexandrinus* used for this purpose was finally able to pass over guards with a diameter of 35 inches (90 cm) as used in the Panama Canal Zone. As shown by recent tests recorded by Stock, the electrically charged rat-guards recommended by some workers may also prove unsatisfactory.

Whole settlements as well as individual houses or blocks of houses may be protected against the ingress of rodents. Thus Vincke & Devignat were successful in keeping villages in the Belgian Congo, in which deratization had been carried out, free from re-infestation by surrounding these settlements with trenches 18 inches (45 cm) wide and about 40 inches (1 m) deep with vertical sides. Rats which fell into these trenches could not jump out but occasionally escaped through drainage channels. However, care was taken to have these run in directions away from the villages.

**Abolition of shelterage**

Methods of abolition of shelterage, or of "internal" rat-proofing as these procedures are sometimes called, are of great importance in so far as (a) their application is less tedious and less expensive than that of exclusion methods, and (b) they can be successfully used in buildings of a primitive construction from which it would be difficult, or even impossible, to exclude the rodents.

The question of whether "internal" rat-proofing should precede or should be followed by eradicative procedures has been differently answered

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*Footnote: Adequate details for the implementation of these procedures will be found in pamphlets published by Holsendorf and Silver et al. as well as in *Rat-borne disease: prevention and control.*
by different workers, but a majority is in favour of first reducing the rodent populations by the application of eradication methods and then resorting to rat-proofing. Calhoun was not, in general, in favour of this policy because, in his opinion, “sanitation procedures which involve the limitation of availability of food or harborage for rats are unlikely to increase more than temporarily the number of rats in adjoining areas”. He admitted, however, that, when dealing with groups of rodents known to harbour human diseases, killing techniques had to be applied first in order to avoid “a wide and rapid dissemination of the disease carrying rats”.

The methods to be used for internal rat-proofing must be adapted to the species of rodents present. If Norway rats alone are found, main attention must be paid to the abolition of shelterages situated underground and on the ground level of the buildings. If R. rattus are present, great care must also be taken to deal with the dead spaces created by double walls and false ceilings and to replace, whenever possible, thatched roofs by those made from solid materials.

If, as was the case in Java, bamboo poles are used for the purposes of house construction, these must be properly sealed to prevent the rats from sheltering in these hollow fixtures. However, at present wooden beams are used for house construction or house improvement in Java.1

It is essential to deal with the above-mentioned and similar “structural” rodent harbourages in as radical manner as possible, making in particular determined efforts to reach and destroy the rodent nests. At the same time, however, due attention must be paid to the feelings and needs of the people during the performance of drastic operations of this kind. For instance, they often insist upon having double walls and false ceilings not merely for the sake of attractiveness but as a means of protecting themselves against cold or heat. These needs for insulation should not be disregarded, therefore, but should be met with in an adequate manner. Thus, insulating materials such as boards or mats formerly used for the creation of double walls should be nailed directly to the main walls of the houses. If it is necessary to retain false ceilings, sufficiently large openings should be provided in them to prevent the rodents frequenting the spaces above such partitions being unobserved and also in order to make it possible to inspect and clean these spaces.

Articles of furniture, such as cabinets and chests of drawers, should be placed on supports and should preferably not be placed directly against the walls so that the spaces under and behind them can be inspected and cleaned.

Great attention must be paid to the abolishment of temporary harbourages created by accumulations of useless materials and by the storage of fuel or other bulk supplies. The former, which often offer excellent nesting

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1 See section on rat-harbourage in chapter 6.
facilities to the rodents, must be removed and burnt. Necessary bulk supplies should be staked on supports high enough to prevent the rodents from sheltering under them and to facilitate house-cleaning.

Abolishment of shelterage possibilities for rats on board ship, instituted through the pioneer work of American experts, particularly of Grubbs & Holsendorf, has given the most excellent results. The systematic use of these procedures renders it possible to keep the vessels free from rats to such a degree that they can be exempted from periodical fumigation.

**Food protection**

It seems legitimate to deal with the methods used for the protection of food supplies from commensal rodents in the course of the present disquisition because, as they constitute an important means of separating these animals from man, they form part and parcel of the methods of rat-proofing as defined above.

Food supplies for human consumption kept in individual houses can be safeguarded by keeping them in solid covered containers, for instance, in earthenware vessels—as they are often available in rural areas—which may be covered simply by stone plates. Another satisfactory method is to use baskets or other suitable means to suspend the food supplies from the ceilings in such a manner that the rodents cannot reach them.

Even if adequate care is taken of the stored food supplies, the commensal rodents are often able to find ample nourishment in containers used for the preparation and consumption of meals, such as pots, bowls, or plates, which have not been promptly cleaned after the food has been partaken. Indeed, in less affluent households, in which stored food supplies may be scanty, the food remnants in uncleaned containers, left standing during the night, often form the main source of food supply the rodents find in the houses. It is, therefore, of great importance to remedy this situation by cleaning all food containers soon after the meals, particularly after the evening meals, and by safely disposing of the food residues.

Great care must also be taken to prevent access of the commensal rodents to food supplies, particularly to cereals, kept for feeding the livestock. If not bulky, such supplies may be safeguarded in the same way as those for human consumption. Large amounts should be kept in solid bins or in cribs or on platforms raised on supports which are provided with rat-guards.

The supplies in small food-shops may be protected against the access of commensal rodents in the same manner as those kept in residences. Establishments, in which foodstuffs are stored or handled in bulk, should be protected with the aid of exclusion methods as well as those of internal rat-proofing.
Sanitation

Adequate sanitation methods, implemented within the houses and compounds as well as on a community-wide scale, are of great importance for the proper control of the commensal rodents. All household refuse must be deposited in solid covered receptacles. Back yards and alleys must be kept clean and free from accumulations of waste products which, if considerable, are apt to offer shelter as well as food to the rodents. When dealing with emergencies in settlements having no, or no adequate, social services, the plague workers must also make temporary arrangements for the proper collection and disposal of the refuse. It is better under such circumstances to burn the latter or to bury it at a depth of at least three feet (1 m).

Public-health propaganda and education

It is no exaggeration to say that full success in the fight against the commensal rodents can be obtained only when it is possible to make the people conscious of the harm caused to them by these animals and thus to enlist their sympathy for, and help in, the work.

Though the choice of the methods to be used for this purpose depends upon the local conditions, it is advisable under all circumstances that lessons dealing with the damage caused by the rodents and the procedures for their control be made part of the school curricula.

When trying to reach the adults, emphasis ought to be laid upon publicity through illustrated posters, newspapers, and broadcasts rather than upon the distribution of leaflets or pamphlets. Illustrated lectures, particularly the demonstration of films, may have a still greater appeal. Temporary or permanent exhibits, particularly if held in model houses showing the various means of rat-proofing, are also of considerable value. It may likewise be useful to interest the people in anti-rodent work by arranging for “cleanliness” days or weeks, during which volunteers are set to work under supervision to deal with accidental rat-harbourages and insanitary conditions in general.

Perhaps more important still than these propaganda methods are informal talks given to the inhabitants of houses visited for the purposes of surveys and control work. The periodical inspections of the buildings, which must be made in all localities where anti-rodent programmes have been implemented, offer good opportunities of keeping the people interested in the work.

To what extent the confidence and co-operation of the population can be obtained is well illustrated by experiences in Java, where it has been possible to continue the house-improvement work, instituted on a large scale under Government auspices in 1914, through the voluntary efforts of the people.
Administrative action

In rodent-control work as in anti-plague work in general it is preferable to use methods of persuasion rather than administrative action to obtain the goodwill and help of the public. It is, however, advisable to make two exceptions to this rule:

(1) After a careful consideration of the local conditions and the easily available building materials, regulations for the rat-proof construction of new structures ought to be promulgated and building permits ought to be issued only if the plans have been drawn up in accordance with these specifications.

(2) The implementation of approved rat-proofing methods ought to be made obligatory for the establishments in which goods attractive to the commensal rodents, particularly foodstuffs, are stored or handled in bulk. However, all possible efforts ought to be made to render the execution of such work as easy as feasible, for instance by supplying the necessary materials at cost-price from the stocks of the rodent-control or anti-plague service.

Planning and co-ordination

Disappointing though it often is that no or but limited use can be made of drastic rat-proofing procedures, even less satisfactory methods, if applied not at the spur of emergencies but according to a well-considered long-range plan, are apt to give good results. It is of great importance in this connexion to link up the anti-rodent work carried out for the purposes of plague control with that of other agencies which are, or ought to be, interested in the fight against these animals. Indeed, though the plague workers must be ready to take the action dictated by emergencies, by rights, rodent-control work should form a permanent part of the programmes adopted for the improvement of environmental sanitation in general.

Comparative value of the methods of commensal-rodent control

Unanimous agreement has been reached that the methods of “building-out” the rodents through the application of rodent-proofing procedures are by far preferable to killing campaigns, which may give rapid results but, in order to prove effective in the long run, have to be carried out periodically, or—in the case of the anti-coagulants—have to be continued. An emphatic resolution to this effect was adopted by the WHO Expert Committee on Plague at its second session in 1952.237

Nevertheless, the methods of directly attacking the rats are not only indispensable for the fight against plague but are also of great auxiliary value in so far as (a) owing to the nature of the structures to be dealt with or for financial or administrative reasons it is often difficult or even impos-
possible to take large-scale advantage of rodent-proofing methods, and 
(b) even if the latter can be used, it is necessary, as a rule, to decimate the 
rodent populations before or, as some workers advocate, immediately after 
exclusion or internal rat-proofing procedures have been implemented. It is, 
therefore, often indicated to utilize a judicious combination of proofing 
and eradicative methods for the fight against the commensal rodents.

Control of Wild Rodents

As described by Wu Lien-teh\textsuperscript{239} and by Wu Lien-teh & Pollitzer\textsuperscript{240, 241} 
large-scale campaigns aiming either at the destruction of wild rodents 
round plague-threatened settlements or at eradication of these animals in 
more extended areas have been carried out in South Africa, south-east 
Russia, and the western parts of the USA, particularly California. Besides 
shooting the wild rodents—a procedure found successful in the USA—the 
methods mainly used against these animals were:

(a) Poisoning with strychnine or sometimes with other rodenticides, especially arsenic.

(b) Fumigating the rodent-burrows with carbon disulfide, and, in south-east Russia, 
also with chlorine or chloropicrin. As claimed by Mamontov & Kolpakova,\textsuperscript{125} the in-
troduction of calcium cyanide into the burrows of the ssel, \textit{Citellus pygmaeus}, also 
gave satisfactory results.

In South Africa, ample use was also made of destroying the wild-rodent 
burrows with the aid of agricultural dynamite. Distribution of “virus”, ex-
perimented with there, as well as in south-east Russia, proved unsatisfactory.

As claimed by Ioff\textsuperscript{96} in 1941, widespread anti-plague operations, includ-
ing campaigns against the \textit{Citellus} populations, led to a liquidation of the 
epizootics in the Caucasian steppes where in 1937, despite thorough 
and widespread investigations, no plague foci could be discovered. Since 
no further information on these areas is available, it is not possible to say 
whether or not this success was really permanent. Meyer,\textsuperscript{135} commenting 
on Ioff’s report, professed scepticism, pointing out that optimistic claims 
made in 1914 in regard to the eradication of plague in California had been 
disproved in a most disappointing manner by further experience. In fact, 
as has been stated in chapter 1, plague not only persisted there but gradually 
became entrenched among wild-rodent populations in neighbouring 
states. Recently reviewing this situation, Link\textsuperscript{113} came to the conclusion 
that “the vastness of the area involved makes it impracticable even to 
think about the eradication of plague in these rodents even if one could 
forget the illuminating story connected with attempts to eradicate plague 
in the California ground squirrel ”. Consequently, while closely watching 
the plague situation in the wild rodents, workers in the USA now lay 
emphasis upon protecting urban settlements against inroads of the infection 
through the control of the commensal rodents in these places. However,
as pointed out by Meyer,\textsuperscript{135} satisfactory though this policy is as far as it goes, work of this kind is unable to “liberate the rural communities from a constant hazard which is thus far, unfortunately, unrecognizable and immeasurable”.

An identical policy has now been adopted in South Africa where, according to Davis,\textsuperscript{37} “the main principle of control is ... to keep the sylvatic foci under surveillance and to eliminate them on a small scale when practicable, but to concentrate upon preventing the infection from becoming established in close proximity to man”.

**VECTOR CONTROL**

**DDT**

2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane, commonly called DDT in abbreviation of its generic name, dichlorodiphenyltrichloroethane, is a comparatively slow-acting but most effective insecticide because (a) mere contact with minute amounts of this compound is apt to kill insects such as lice, mosquitos, and fleas inexorably though gradually, and (b) surfaces on which DDT has been deposited retain this killing power for considerable lengths of time, not rarely for many months. This property of prolonged “residual” action makes DDT particularly useful for the control of plague and flea-borne typhus as well as of other insect-borne diseases.

As shown by ample experiences, DDT is fully effective for the control of the common rat-flea species (*Xenopsylla cheopis*, *Nosopsyllus fasciatus*, and *Leptopsylla segnis*), and, as shown by Sharif,\textsuperscript{195} also for that of *X. astia*. Observations in the southern part of the USA suggested that *Echidnophaga gallinacea* was less amenable to control with DDT than the above-mentioned species and this was confirmed by Macchiavello in Tumbes, Peru. However, as pointed out with much reason by Davis,\textsuperscript{35} these sticktight fleas possibly escape the action of DDT merely because they are wont to bury their head and thorax into the skin of the rats and are thus little exposed to the insecticide. Hill \& Morlan\textsuperscript{84} ascribed the erratic results they obtained with DDT dusting in the case of these fleas to the care they took in distributing the insecticide only in places where chicken could not come in contact with it. In fact, Eads\textsuperscript{51} obtained satisfactory control of *E. gallinacea*, when freely applying 10\% DDT dust to a large hen-house.

Boiron\textsuperscript{16, 17} noted that *Pulex irritans*, which had recently replaced *Synosternus pallidus* in the houses of Dakar, Senegal, was not, in contrast with the latter flea, amenable to control with DDT, and therefore raised the question of whether a resistance to this insecticide might have enabled
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*P. irritans* to become predominant. However, experiences made by other workers, by Davis and Macchiavello for example, showed this flea to be fully sensitive to the action of DDT.

General agreement exists that the control obtainable with DDT dusting in the case of the tropical rat-mite, *Lyponyssus bacoti*, and the rat-louse, *Polyplax spinulosa*, is more or less incomplete. Nicholson et al., applying, for the purposes of combined typhus and malaria control, DDT in spray form to the walls and ceilings of the houses as well as to the floors and rat-burrows, obtained more encouraging results with *L. bacoti* and believed, therefore, that this mite was not affected by DDT dust on account of its climbing habits.

According to Wiley & Fritz, DDT dusting gave satisfactory results in the case of two other rat-mites—*Echinolaelaps echidninus* and *Laelaps nuttalli*.

It is distressing to note that recently some evidence has become available to show that flea populations as well as groups of certain other insect species may acquire a resistance to DDT after repeated applications of this insecticide in the localities in question. Kilpatrick & Fay recorded in this connexion that in 1949 several reports had been received of the persistence of numerous *Ctenocephalides felis* after as many as three applications of 5% DDT dust in the infested premises. The two workers made experiments, therefore, to see whether *X. cheopis* stocks reared in the laboratory could be made resistant to DDT by continued exposure of the survivors of earlier tests to further doses of the insecticide. It was possible to show in this way an increased DDT resistance of the fleas through the F₃ generation, the mortality decreasing from 65% in the parent generation to 32% in the F₃. Since no further increase in resistance was shown in further tests through the F₇ generation, Kilpatrick & Fay did not ascribe practical importance to these experimental findings. Good (quoted by Hess), who had found some evidence of the development of a DDT resistance by *X. cheopis* under field conditions, likewise assumed that "such resistance as may have developed is not sufficient seriously to affect operational programs where 10 per cent DDT dusts are used to control this species". However, Sáenz Vera (Ecuador) in a report submitted in 1952 to the WHO Expert Committee on Plague stated that:

"Unfortunately, since 1950, we have noticed that in certain areas, considered as plague foci and systematically treated with DDT, a number of fleas survive the application of the insecticide ."

Application of a new batch of DDT in these areas gave no better results, while the formerly used batch was found to be fully effective in a hitherto untreated area.

When visiting Brazil in 1952, Sáenz Vera was informed that, in rural areas of the Pernambuco State as well, new applications of DDT had
little effect. He added in a further (personal) communication that the fleas involved were *X. cheopis*, *P. irritans*, *N. londinensis*, and *Polygenis* species.

Action has been initiated by WHO to study further this matter.

It was noted by some observers that dusting with DDT for the purposes of plague or typhus control exerted a lethal action not only on the fleas but also to some extent on the rodents. Thus Machiavello,\textsuperscript{119} describing his work in Tumbes, mentioned that rats (*R. rattus*) dusted with a heavy dose of DDT (1-2 g of a 10\% mixture) may be poisoned and die because they regularly and carefully lick their fur. Referring again to this matter in 1949,\textsuperscript{121} he stated that the average lethal dose of DDT dust for these rats equalled 0.5 g of the pure substance but that the range of toxicity varied from 0.05-1 g. The action of the insecticide did not seem sufficiently reliable to replace the usual rodenticides in the field.

However, the use of 5\% DDT dust for the control of mice and that of 50\% dust for the control of rats and *Meriones* was recommended by Sergent & Sergent.\textsuperscript{191, 192} Likewise, Wanson & Camphyn\textsuperscript{228} suggested that 50\% DDT dust might be used for the destruction of rats and gerbils.

It is generally agreed that the distribution of 5\% or 10\% DDT dust may be used with advantage for the destruction of commensal mice. It seems not advisable, however, to use 50\% DDT to destroy larger rodents which may be dealt with more effectively and economically by other methods.

*Technique of application*

DDT-containing dusts or fluids are used for the control of plague and flea-borne typhus either singly or in combination.

To apply DDT by dusting, it is necessary to obtain it from the producers ready-made in 10\% strength, because it is not possible to prepare such formulations from concentrates without special machinery. If it is desired to use 5\% dust, the 10\% formulations may be mixed as needed with equal parts of inert materials such as talcum powder or kaolin. Flour has also been utilized for this purpose. Care must be taken that the inert material added has an adequately fine particle size.

Claims have been made by some workers that 5\% DDT dust gives as satisfactory results in flea control as the originally used 10\% formulation. However, Good\textsuperscript{68} stated that in his experience 5\% DDT dust was definitely less satisfactory for *cheopis* control than 10\% dust. This opinion was endorsed by a statement made in 1951,\textsuperscript{24} according to which “on 5 percent DDT dust projects, rats from premises dusted more than 1 year previously showed a high percentage of infestation, and an average per rat examined of 0.7 as compared to 0.3 for comparable periods of 10 percent DDT dust projects”. As noted in a 1952 report,\textsuperscript{174} “most operators have preferred to use the 10 per cent formulation, particularly where it was desirable to control other ectoparasites more resistant to DDT, for example the cat flea.”
For these reasons as well as in view of the recent observations on DDT resistance quoted earlier, the present writer is definitely of the opinion that, in order to work with adequately high concentrations of the insecticide, the use of 5% dust formulations should be discontinued. Essential though it is to be economical in plague work, one must beware of false economies as they are sometimes practised in DDT application in particular.

FIG. 39. HAND DUSTER WITH LONG METAL NOZZLE

In addition to the hand dusters with long metal nozzles (see fig. 39) supplied by the producers, in distributing DDT dust it is necessary to use: (a) foot pumps of the pattern used for calcium cyanide distribution, provided preferably with an additional dust reservoir tank of 5-pound (2.25 kg) capacity; (b) hand-operated rotary blower dusters; and (c) hand-shaker dusters—both large ones, consisting of a box made of sheet metal with a removable wire-gauze net or a perforated removable metal screen at one short side and a stout handle on top, and small ones (capacity $\frac{3}{4}$ pound or 0.33 kg), cylindrical in shape, with a removable perforated lid and provided with a handle 3 to 6 feet (1-2 m) long. If no shaker dusters are available, these can be improvised by punching holes into the lid of a tin can, preferably one with a screwed-on lid.

DDT solutions can be simply prepared by dissolving technical grade DDT in kerosene at the rate of 5 g to 100 ml of the solvent or by taking 6.7 ounces of technical DDT per gallon of kerosene. Other solvents, for instance camphor oil, may be substituted for kerosene.\(^d\)

\(^d\) A specification for technical DDT has been established by the WHO Expert Committee on Insecticides (Insecticides : manual of specifications for insecticides and for spraying and dusting apparatus, specification WHO/SITECH/1, p. 1).
DDT emulsions may be made by dissolving technical DDT in a good solvent and then adding soap or another emulsifier so as to render further dilution of the fluid with water possible. However, to obviate the tedious process of preparing emulsions in the field, the fluid DDT concentrates supplied by the producers—which merely require addition of water to obtain a 5% formulation—may be utilized. Dry "wettable" DDT products, containing an emulsifier in powder form, have recently become available; from these, suspensions can be made by simply adding enough water to obtain a 5% strength of the insecticide.

Various models of sprayers, ranging from small hand-driven patterns to large pressure sprayers, have been recommended for the distribution of fluid DDT preparations. Whatever kind of sprayer is used, it is essential to apply the material as a coarse spray and not as a fine mist. Nozzles which give a fan-shaped spray are preferable (Bishopp 15).

Generally speaking, DDT emulsions and suspensions, because they leave a more massive deposit, are preferable to the solutions for flea-control work. The inhabitants of well-appointed houses may object to these deposits. On the other hand, however, the smell of the solutions is objectionable unless an odourless solvent is available, and a fire hazard may be created by their distribution.

A further important reason why, for the purposes of flea-control, DDT in dust form is preferable to fluid preparations is that the sprayed materials remain fixed to the surfaces on which they have been spread, while dust deposits are apt to become transferred to the fur of the rodents and to be carried by the latter to their burrows and nests. The only drawback of dusting is that the deposits on the floors may be swept away by the people. Nicholson et al. 149 suggested for this reason that such vulnerable sites should be sprayed with fluid DDT preparations while dust ought to be used in the other parts of the premises to be treated.

It is generally recommended that fluid DDT preparations be used in a strength of 5% and applied at the ratio of about 40 ml per square metre or of one gallon per 1,000 square feet so as to obtain deposits of 200 mg of concentrated DDT per square foot. In sites needing less intensive treatment, half of these dosages may suffice.

Though larger or lesser dosages of 10% DDT dust have been used by some workers, it is usually considered adequate to apply the dust at the ratio of 2-3 g/m² or 200-300 g (7-10 ounces) per 1,000 square feet. It is, however, important to note that Davis 35 and most other workers in the USA maintain that the quantity of DDT used ought to depend upon the degree of rat-infestation rather than upon the size of the sites to be treated.

In order to apply DDT efficiently for the purposes of rodent-flea control, it is essential to make preliminary inspections of the premises to be treated so as to ascertain what species of rodents are present and to
assess the degree of infestation. Whenever possible, preliminary rat-trapping ought to be done in the localities to be dealt with so as to establish the flea indices and/or the percentages of flea-infestation.

Most workers insist that, in order to obtain fully satisfactory results, it is necessary to apply DDT not only to all rat-runs on the floors as well as in elevated positions (e.g., on rafters), but also to the rodent-burrows and other enclosed or semi-enclosed harbourages, such as dead spaces under floors or between double walls, which may be rat- and/or flea-infested.

A sound principle now adopted in the USA is that, when applying DDT to the rat-runs, stress ought to be laid upon “patch-dusting” rather than upon uniform distribution of the dust in thin layers, so as to increase the chances that the rodents, after having come in contact with the deposits, spread the dust over their fur when preening themselves and carry it into their burrows.

Patches of DDT dust ought to be placed with the aid of shaker-cans \((a)\) in the first line round the entrances of rodent-burrows or other holes made by the rodents on level surfaces, and \((b)\) on rat-runs both on the floors and on rafters or other elevated surfaces. The quantity of the dust to be used for individual patches depends upon the degree of rodent-infestation present. As stated in *Rat-borne disease: prevention and control*:\(^217\)

“When infestation is light, only a relatively thin layer, enough to be seen, is needed. For a moderate amount of rat evidence a patch \(\frac{1}{32}\)-inch [0.8 mm] thick is sufficient. Heavy infestations demand patches of \(\frac{1}{4}\) of an inch [6.4 mm] or more”.

The rings of DDT dust made round rat-holes ought to have a diameter of 6-12 inches (15-30 cm). DDT patches on rat-runs, which should be placed at intervals of 10-15 feet (3-4.5 m), preferably in narrow parts of the runs, should be 6 inches (15 cm) wide by 18 inches (45 cm) long on an average.

In addition to surface dusting, particularly patch-dusting, the rodent-burrows as well as all other enclosed harbourages (e.g., those below double floors, between double walls, or above false ceilings) must be thoroughly dusted with the aid of appropriately powerful pumps, so as to cover as far as possible the entire inside of these enclosures with a light film. As stated in the handbook on rat-borne disease,\(^217\) the foot-pumps as used for calcium cyanide distribution are satisfactory for treating burrows and enclosures of less than 50 cubic feet \((1.4 \text{ m}^3)\) in volume. Rotary or knapsack dusters ought to be used for larger enclosed or partially-enclosed spaces. When dealing with rodent holes in vertical surfaces it is well to place a patch of DDT dust within their entrances after pumping has been done in the above-described manner.

In places where plague is present, and particularly in the case of houses where rat or human plague has become manifest, it may be advisable to apply, in addition to the above-described methods, more intensive pro-
procedures of DDT dusting. Attention ought to be paid in this connexion to the beds and clothes of the people and to the supplies and stores, and possibly also to articles of furniture such as easy chairs, settees, cupboards, and cabinets. Care must be taken, however, that DDT is not spread on foodstuffs destined for consumption by man or domestic animals or on eating, drinking, and cooking utensils.

Some workers applied DDT dust to the inside of the clothes which were worn by people living in plague-affected houses or settlements in the same manner as this insecticide is used for the control of louse-borne typhus. Ordinarily, however, there is no need to use this tedious procedure in plague-control work.

It is most desirable to evaluate the results of DDT-dusting campaigns by trapping rats in the treated localities and ascertaining the flea indices and/or the percentages of flea-infestation of the rodents. Particularly reliable results will be obtained if these values can be compared with those ascertained under analogous conditions before DDT dusting had been started. Whenever possible, such rodent-flea surveys should be continued or repeated at suitable, e.g., monthly, intervals, so as to get indications for the necessity of re-applying the insecticide.

The use of DDT for the purposes of flea control is without danger for man or domestic animals if the following precautions are adopted:

1. On account of its similarity to flour, supplies of DDT dust must be conspicuously labelled and kept in safe custody.

2. Though, in contrast to the solutions and the emulsions, DDT dust is absorbed by the skin only when this is greasy or oily, it should not be allowed to remain on the skin. Workers should wash their hands frequently, particularly before eating.

3. Workers should wear gauze-cotton masks when dusting DDT. Dust respirators are preferable when powerful pumps are used.

4. As noted, DDT dust must not be allowed to come in contact with foodstuffs. Particularly, as stated in this connexion in the handbook on rat-borne disease,\textsuperscript{217} it should not be applied to sacks of flour, salt, sugar, cornmeal, or stored grain. Grain sweepings from treated establishments should not be used to feed animals (Ludwig & Nicholson\textsuperscript{118}). Eating, drinking, and cooking utensils must also be safeguarded.

5. Dogs may be disinfested with DDT dust by placing it on their necks where it cannot be licked off. Cats should not be dusted with DDT.

If DDT is swallowed, vomiting should be induced immediately and the stomach should be washed as soon as possible. A saline cathartic should then be administered. If tremors or other nervous symptoms appear, phenobarbital should be given. Intravenous administration of 10\% calcium gluconate, found effective by Vaz et al.\textsuperscript{220} in DDT-poisoned dogs, might be considered.
**Results**

The outstanding value of DDT application in rodent-flea control is exemplified by the following observations:

Excellent results have been obtained with DDT dusting in the control of *cheopis*-borne typhus in the southern parts of the USA. Thus Morlan & Hines \(^{144}\) (see also Hill et al.\(^{85}\)) reported that in two counties, where patch dusting had been done five times during the period of April 1946 to September 1947, effective control of this disease was obtained and maintained without further effort for about three years after completion of the last dusting cycle.

In the experience of Macchiavello,\(^{119,120}\) DDT dusting gave most satisfactory results in the control of various plague manifestations in Peru. Thus, reporting on the use of this method in the 1945 outbreak at Tumbes, he stated that:

"The effectiveness of the application of DDT can be appreciated by: (a) the stopping of the epidemic 4 days after finishing the first application of DDT; (b) the 81.6 per cent lowering of the flea-infestation of the rats and the 87.9 per cent diminution in the numbers of fleas found in rat nests, after the first application of DDT. There was a final reduction in the number of fleas in the epizootic foci of over 90 per cent. Rat plague was reduced 75.6 per cent after the first application of DDT and 100 per cent after the second."

As recently reported by Mercier,\(^{132}\) systematic use of house dusting in the town of Tananarive, Madagascar, carried out twice yearly with mixtures consisting of equal parts of 5% or 10% DDT and 5% or 10% benzene hexachloride immediately after mixtures of these insecticides had been sprayed for the purposes of malaria control, gave most gratifying results. Human cases in Tananarive, which had become markedly less frequent since partial use of DDT had been made from 1947 onwards, remained altogether absent after the systematic campaigns had been started in 1949. Whereas previously 6-8 fleas had been found on each trapped rat, the infestation-rate fell to 0.25 (1 flea per 4 rats) after systematic disinsectization had been started. The *cheopis* index was after that time 0.05 as against at least 1.50 previously.

Satisfactory results with DDT in the control of plague were also obtained in South Africa. Thus, as stated by Davis,\(^{38}\) systematic dusting of some 6,000 huts, carried out every 4 months in Ngamiland with 1/2 pound (225 g) of the dust per hut, led to a most marked reduction of the flea incidence. After dusting had been done for the 7th time, only 5 fleas were found in one of the 500 huts sampled.

Wagle & Seal,\(^{227}\) in a report submitted in 1952 to the WHO Expert Committee on Plague, adduced ample evidence that in India also application of DDT had usually given gratifying results, so that:

"Most of the public health workers agree that DDT has been able to keep down plague infection in their States since its introduction on a reasonably large scale and they feel that the results obtained give every promise of a possibility of eradication of plague through its concerted use."

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The methods of DDT application mainly utilized in India for the purposes of plague control were (a) indoor residual spraying with emulsions containing 5% of the insecticide, and/or (b) treatment ("insufflation") of the rat-burrows with 10% DDT dust. On the basis of the experiences of Viswanathan & Rao, Wagle & Seal stressed the advisability of combining these two methods. In their opinion it was best to use both procedures simultaneously, but if funds were limited, it seemed permissible to apply DDT in the form of sprays during plague outbreaks and to dust the burrows during the interepidemic periods. Though lesser dosages were often used, better results were obtained when sufficient amounts of the emulsions were applied to produce deposits of 70-75 mg of pure DDT per square foot (7-7.5 mg/m²).

It is of great importance to note that application of DDT was found to be not only far more effective but also more economical than that of calcium cyanide. Thus in the Nilgiris district of Madras State, India, the cost of the 2,000 pounds (907 kg) of 10% DDT dust needed to treat 90 village units once yearly was Rs 1,750/- . Should calcium cyanide have been used instead, three applications instead of one would have been required and the cost of the 3,000 pounds (1,360 kg) of the material needed for this purpose would have been Rs 4,500/- .

Benzene Hexachloride (BHC)

Among the five known isomers of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane; empirical formula C₆H₆Cl₆) the gamma-isomer has been found to possess marked insecticidal powers. It is available in the form of a light, buff-to tan-coloured powder, in the form of solutions made with organic solvents, of emulsions, and also of pellets to be used for fumigation. As summarized by Andrews & Simmons, "the toxic action of BHC against insects may be as a contact poison, as a stomach poison, as a fumigant, or as a combination of the three". On account of this wider range of action BHC kills insects more rapidly than DDT does but, because it is volatile, its residual action is considerably less long-lasting than that of the latter insecticide.

The superior pulicidal effect of DDT was well demonstrated through comparative experiments carried out by Sharif in the Haffkine Institute, Bombay, India. He established that DDT admixed with sand in a ratio of 1/200,000 exerted a residual action for as long as 238 days, whereas BHC, if used under the same conditions in a concentration of 1/5,000, retained a residual action for only 48 days. Analogous differences were found in tests conducted by pumping DDT and BHC respectively into artificial rat-burrows.
As recorded by Wagle & Seal,\textsuperscript{227} field trials in India also showed that the action of BHC on the rat-fleas was less marked and persistent than that of DDT.

As shown experimentally (Furman;\textsuperscript{57} Vashkov & Serebryakova;\textsuperscript{219} van Someren\textsuperscript{206}), benzene hexachloride could exert a lethal action on rats and mice. Van Someren\textsuperscript{206} maintained in this connexion that application of BHC dust at the rate of about 2 g (60 mg gamma) on floors, or dusting of rat runs and burrows, or poisoning with baits containing this chemical might be of value for the control of \textit{R. rattus}, \textit{R. natalensis}, and—to a lesser extent—mice. \textit{Arvicanthis}, which seemed more resistant to BHC than the above species, was thought to be amenable to higher doses, particularly if given in bait form.

**Other Insecticides**

It may be claimed that there is no need to enter into a detailed discussion of the formerly used methods of flea-control, such as spraying infested premises with kerosene-soap emulsions, because these time-honoured but little effective procedures have become obsolete since DDT and, to a lesser extent, BHC have proved incomparably more efficient. However, the question of to what extent other modern insecticides exert a pulicidal action is of importance in so far as their use might come in question in areas where the flea populations have become DDT-resistant. The following findings have been recorded in this respect:

**Chlorinated benzenes**

As established by Ernst & Meijers,\textsuperscript{55} 1-hydroxy-symmetric-polychlorine benzene as a 5% powder appeared as effective against fleas as 5% DDT preparations. In the doses used for the disinfestation of cats and dogs, it was not toxic for these animals.

As far as can be judged from limited experiences made by Laurans,\textsuperscript{140} another chlorinated benzene compound, polychlorocyclane sulfide, was effective against dog-fleas (\textit{Ctenocephalides canis}) if used as a 4.5% powder.

**Chlordane**

Chlordane, like DDT a chlorinated hydrocarbon, with the empirical formula $C_{10}H_6Cl_8$, is available in the form of powders, solutions in oils, and emulsions, and stands in regard to its volatility and residual action between DDT and gamma benzene hexachloride. Like the latter it exerts a fumigant action besides being apt to kill various insects by contact action. It is compatible with DDT (Gray\textsuperscript{72}).

As stated in a recent report,\textsuperscript{174} "a 10% chlordane dust is recommended for controlling soil infestations of cat and dog fleas. A single application of this material has given effective control of infestations, whereas repeated applications of DDT would have been required ".

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Similarly, it was stated by Kilpatrick & Fay\textsuperscript{106} that \textit{Ctenocephalides felis} infestations, which could not be effectively dealt with by three applications of 5% DDT dust, were much reduced or even eliminated through the use of 5% chlordane. To explore the action of this insecticide on DDT-resistant rodent-fleas would be of considerable interest.

\textit{Organic phosphates}

Because, as noted before, organic phosphates are extremely toxic to man and because they possess very limited residual action (Upholt\textsuperscript{218}), they should not be used for the purposes of flea control.

\textit{Organic sulfur compounds}

As stated by Velbinger,\textsuperscript{221} phenothiazine (C\textsubscript{12}H\textsubscript{9}NS), when applied in the form of dust or washes to dogs, exerted a lethal action on \textit{Ctenocephalides canis}.

According to Frear,\textsuperscript{56} phenothiazine, which is widely used as an anthelminthic in veterinary medicine, has many desirable characteristics as an insecticide but is apt to show a variable action and occasionally causes dermatitis in man.

Organic thiocyanogen compounds, known under various proprietary names, exert, according to Kerr,\textsuperscript{105} but little action on fleas as compared to that of DDT. As stated by Upholt,\textsuperscript{218} these compounds have been used in combination with pyrethrum in order to ensure that insects knocked down by the latter would be killed instead of recovering. Since, however, the thiocyanates are presumably more toxic than other substances exerting a synergistic action when used with pyrethrum, they have to be utilized with caution.

\textit{Piperonyl compounds}

Andrews & Simmons\textsuperscript{3} noted that piperonyl cyclonene, because it exerted a synergistic action on pyrethrum, appeared to be promising for flea control, if used in combination with the latter, but lacked the desired residual toxicity for rat-fleas in particular.

According to Gray,\textsuperscript{72} piperonyl butoxide, which is also used in combination with pyrethrum, had residual properties and, because it was free from hazards to warm-blooded animals, could be freely used around foodstuffs. As noted below, Smith\textsuperscript{204} found that piperonyl butoxide in combination with pyrethrum extract exerted within 24 hours a toxic action on \textit{X. cheopis} and on \textit{Ctenocephalides felis}.

It should be noted, in this connexion, that recently attention has been paid in India to the possibility of using the locally available pyrethrum instead of imported synthetic insecticides, particularly DDT. Experiments in this direction were carried out in the Haffkine Institute with powders
containing pyrethrins in a concentration of 0.2% on mixed populations of *X. cheopis*, *X. astia*, and *X. brasiliensis*. Weekly observations made in order to test the residual effect of this powder showed that, though 100% of the fleas exposed for 15 minutes were knocked down on the first day, the knock-down effect was only 75% after seven days, 50% after two weeks, and nil after four weeks.\(^\text{82}\)

One must fear that under natural conditions the residual effect of such powders would be still less satisfactory.

*Sodium fluoride*

Sodium fluoride (NaF), known as an insecticide for more than fifty years, was recommended in 1940 by Roubaud\(^\text{182}\) for flea control in houses. Roubaud found that, while the action of this compound on adult fleas was too slow to make it useful for disinfesting animals such as dogs, it exerted a larvicidal action, killing for instance in a concentration of 10% *Ctenocephalides canis* larvae in 18-24 hours. An *X. cheopis* population kept in a glass jar was found to be annihilated a few days after small doses of NaF had been applied. Premises infested with dog- or cat-fleas could be freed from these parasites by spreading sodium fluoride either in pure form or in a concentration of 50% on the floors at the rate of 2 g per square metre. The price of this chemical was low and its toxicity for man and domestic animals was 30 times less than that of arsenites. In Roubaud's opinion sodium fluoride was apt to be useful for combating loose *X. cheopis* living in grain debris.

It might be interesting to establish whether or not NaF could be used together with pyrethrum.

*Comparative tests*

Smith,\(^\text{204}\) comparing the action exerted within 24 hours by 619 insecticides on *Ctenocephalides felis* and *X. cheopis* with that of DDT, found that at a concentration of 0.5%, 29 of these compounds proved more effective than DDT, 24 were within the range of effectiveness of DDT, and 566 were less effective than DDT. He stated in particular that:

"The outstanding toxicants were heptachlor, dieldrin (compound 497), aldrin (compound 118) and benzene hexachloride (95 per cent gamma isomer). Chlordane and parathion were more effective than DDT at concentrations of 0.5 and 0.05% and toxaphene and pyrethrum extract plus piperonyl butoxide at 0.5% only. Methoxychlor and other analogs were about equal to DDT ".

Interesting as these findings are, they are of only limited practical value because (a) the residual action of the compounds found to compare favourably with DDT or to be as effective as the latter insecticide was not determined, and (b) the DDT concentrations used in these comparative tests (0.5%, 0.05%, and 0.005%) were far below those applied in actual practice.
Fumigants

The comparative value of various fumigants for the destruction of rodent-fleas is well illustrated by the observations of Stewart & Mackie,\textsuperscript{209} whose principal findings are embodied in table XXIII.

Though, as shown in the table, hydrocyanic acid gas gave by far the best results, Stewart & Mackie\textsuperscript{209} stressed that in actual practice the experiences made with the application of calcium cyanide were not fully satisfactory in the case of the wild-rodent fleas or in that of the rat-fleas. The two workers advocated, therefore, the use of methyl bromide which, if applied to each opening of ground-squirrel burrow-systems in quantities of 10 ml, was found to be fully effective against the fleas as well as against the rodents. There can be no doubt, however, that nowadays it would be both more expedient and far more safe to use DDT instead of methyl bromide for the purposes of flea control.

However, fumigation procedures and, still more, application of calcium cyanide continue to be of importance for the disinfestation of grain consignments under the conditions usually prevailing in plague-affected localities—the more so as it was established through the observations of Pandit et al.\textsuperscript{157} and of George & Webster\textsuperscript{62} that exposure to the sun or to hot air, as formerly often done, was unreliable for the disinfestation of grain bags.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
Fumigant$^a$ & Temperature & Volume of gas$^b$ & Time of exposure (hours) \\
& ($^\circ$F) & ($^\circ$C) & (ml) & \\
\hline
Carbon disulfide & 71 & 21.6 & 5 & 1 \\
Chloropicrin & 77 & 25.0 & 5 & 1 \\
Ethylene oxide & 76-50 & 23.3-26.6 & 2-10 & 0.5-4 \\
25% ethylene oxide — 75% ethylene dichloride & 73-82 & 23.3-27.8 & 5-10 & 1-2 \\
HCN & 71-87 & 21.6-30.4 & 0.1-0.5 & 0.186-0.5 \\
Methyl bromide & 72-76 & 22.2-23.9 & 0.5-10 & 0.186-1 \\
Propylene oxide & 75-85 & 25.5-29.5 & 6-10 & 1-2 \\
Sulfur dioxide & 76 & 24.5 & 10 & 1 \\
\hline
\end{tabular}
\caption{Relative Efficacy of Various Fumigants Against Adult Fleas (Diamanus Montanus and Hoplopsyllus Anomalus)\textsuperscript{*}}
\end{table}

\textsuperscript{*} Only 100%-kill recorded.
\textsuperscript{a} Some other fumigants, including carbon monoxide and pure ethylene dichloride, were found to exert no lethal action on fleas.
\textsuperscript{b} The tests were made in a glass fumigation chamber with a capacity of 126 ml.

The following experiment made by George & Webster is particularly instructive:

"On one occasion in Cumbum village a bandicoot was found dead of plague in a grain store. The fleas on the carcass, ten in number, were removed. About 700 bags of grain
were removed and placed singly on a cement platform exposed to bright sunlight throughout a whole day in November, 1932. The maximum shade temperature that day was 82°F (28°C). At night twelve de-flead guinea-pigs were allowed to wander loose among the bags. Next day eleven of these [animals] were caught and searched for fleas. Thirty-six *cheopis* and *astia* fleas were recovered."

Application of hot air, used on dull days to disinfest grain in bags, was equally unsatisfactory because, as stated by George & Webster,62 it was not possible in this way to maintain a temperature of 120°F (49°C) for 45 minutes as required for the destruction of fleas.

Further observations in the Cumbum Valley, Madras State, showed, however, that satisfactory results could be obtained by applying calcium cyanide (a) to grain bags put in fumigation chambers, or (b) to the bags loaded on bullock carts, which were covered for this purpose with oiled tarpaulins, in sheds erected on the roadside.60, 61, 93 It was found that the following HCN concentrations and minimum periods of exposure were required to de-flea the various commodities usually encountered in these wayside plague-control stations:

<table>
<thead>
<tr>
<th>Articles</th>
<th>Quantities of HCN * required per 1,000 cubic feet ** in airtight chambers ***</th>
<th>under oiled tarpaulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal effects</td>
<td>1 oz for ½ to 1 hour</td>
<td>1 oz for 1 hour</td>
</tr>
<tr>
<td>Rice, paddy, lentils, coriander, etc.</td>
<td>3 oz for 3 hours</td>
<td>4 oz for 3 hours</td>
</tr>
<tr>
<td>Bran, coffee-husks, sesame seeds, etc.</td>
<td>4 oz for 8 hours</td>
<td>5 oz for 8 hours</td>
</tr>
<tr>
<td>Cotton and flour</td>
<td>12 oz for 5 hours</td>
<td>16 oz for 8 hours</td>
</tr>
</tbody>
</table>

* Obtained through application of approximately 4 ounces of calcium cyanide dust.

** 1 ounce per 1,000 cubic feet = 1 g/m³

*** Capacity of about 3,000 cubic feet. Enclosed on three sides and on top by solid masonry work. Entrance tightly closed with the aid of an oiled tarpaulin. Calcium cyanide was pumped in through lead pipes in walls.

Remarks: (a) To test the efficacy of these procedures, observations were made in 1935 with live fleas in muslin bags left at various levels inside the grain bags and outside them.

(b) Penetration of the gas was facilitated if the bags were not too tightly packed.

The use of methyl bromide for the purpose of freeing grain cargoes from plague-infected fleas was recommended by Stewart & Mackie.209 It must be noted, however, that recently van Tiel 215 considered further tests necessary to exclude the possibility of an absorption of methyl bromide into grain. He considered it preferable, therefore, to fumigate grain with methallyl chloride (β-methallyl chloride or 3-chloro-2-methylpropene) which is safer to handle in so far as it has a repulsive odour. However, this compound is inflammable and at certain concentrations explosive when admixed with air. Moreover, though found to be effective against bed-bugs and lice, its action on fleas has apparently not been explored.

Bouhelier & Foury 18 recommended trichloroethylene (CHCl₃:CCl₂) for grain disinfestation. Experimentally, this volatile, non-inflammable fluid, which is used as an anaesthetic in clinical medicine, killed *Ct. canis* in 19 ½ hours if used at the rate of 100 ml per cubic metre or 1 fluid ounce per 10 cubic feet at temperatures ranging from 20°-25°C (68°-77°F).
It would not seem that application of the above fumigants is more advantageous than that of calcium cyanide for the purpose of freeing grain supplies from fleas under field conditions. Hence, until further research leads to the introduction of improved procedures, calcium cyanide application may be considered as the method of choice in dealing with actually or potentially flea-infested grain consignments under the conditions usually prevailing in and near rural plague areas.

**DIRECT CONTROL OF BUBONIC (ZOOTIC) PLAGUE**

Having dealt in the preceding pages with the methods of indirectly controlling plague through steps taken against the reservoir rodents and the insect vectors of the infection, it is now necessary to review the measures by which man, the victim of the infection, may be safeguarded. For obvious reasons separate consideration must be given in this respect to the bubonic (zootic) and to the pneumonic (demic) type of the disease.

**Hospitalization of Patients**

It may be maintained that from the viewpoint of plague prevention it is immaterial whether or not patients suffering from bubonic plague not complicated by lung manifestations are isolated. Such sufferers are not directly contagious and, as proved by the usual absence of instances of secondary infection in their households, the danger of an indirect conveyance of *P. pestis* from them to those near them through insect vectors is, as a rule, inconsiderable. Even if the sufferers left in their houses should subsequently develop secondary lung involvement, the danger of a spread of pneumonic plague can now be effectively averted through abortive treatment of their contacts.

If, however, one disregards the somewhat arbitrary distinction between preventive and curative medicine, which are united in the endeavour of benefiting mankind, hospitalization of bubonic (zootic) plague patients is of great importance in view of the far superior facilities available in the wards, not only for efficiently treating but for adequately nursing the sufferers.

Hence, while preferably avoiding forceful means, plague workers should use every possible inducement to arrange that the patients are quickly hospitalized. However, in places remote from hospitals or possessing no adequate means of communication one ought to weigh the advantages of hospitalization against the risks to which a long and/or inadequately conducted transport exposes the patients. One should also keep in mind that among groups of people unfamiliar with, or prejudiced against,
modern medicine, insistence on hospitalization, while beneficial to the affected individuals, may greatly enhance the difficulties of obtaining the goodwill and co-operation of the people. It is important to note, in this connexion, that some workers, such as Mathur & Goyal,131 Shamanna & Hedge,193 Shamanna & Kalappa,194 and Simeons & Chhatre202 in India, and also the present writer in China, were able to obtain satisfactory results when treating bubonic plague patients in their homes.

If no permanent plague hospitals or wards are available, care must be taken to house the patients in buildings which are, or can be rendered, free from rats. A special ward, preferably in a separate building or in a shed or tent erected for this purpose, should be provided for, where the newly arrived patients are undressed, bathed or washed, if necessary freed from vermin, and dressed in hospital garments.

The clothes in which the patients come must be disinfested in steam sterilizers or delousers, or—if such permanent facilities are unavailable—with the aid of calcium cyanide or a fumigant in an airtight chamber or container. Application of DDT dust followed by storage of the clothes would also be satisfactory.

The staff handling the newly admitted patients or their clothes should be provided with flea-proof garments. Such garments should also be worn by the staff members attending the patients in the wards in cases where no hospital garments can be issued. If so, it might be advisable to apply DDT dust to the persons and clothes of the patients, as practised for the purposes of louse-borne typhus control.

The medical officers and nurses attending plague patients in the wards should preferably wear masks even if the sufferers show no manifest signs of lung involvement.

Management of Contacts

Though it was often considered necessary to isolate the contacts of bubonic plague patients for six days or even longer in quarantine camps, it may be asserted that, in view of what has been stated in the foregoing section, there was never any real need to insist upon this most unpopular practice as long as the patients had been hospitalized or, if left in their houses, remained free from lung complications. Nowadays, when DDT is available to render the affected houses safe, the method of confining the contacts of patients suffering from bubonic plague without lung involvement in camps is altogether obsolete.

Evacuation

Voluntary removal of the people from places where plague had appeared or had become rampant, is no doubt the oldest method used to prevent the
spread of this disease. For instance, it is definitely known that this procedure had been adopted for many centuries in northern India, where the people left their homes as soon as the rats began to die (Patel 160).

However, while one must admit the soundness of the principle of removing the people from places where hordes of plague-infected fleas lurk, several reasons militate against its implementation. Short of the barbaric practice sometimes adopted formerly of burning down plague-infected houses or even settlements, enforced evacuation is the most unpopular of all anti-plague measures. To make adequate arrangements for housing and feeding the evacuated is difficult. Moreover, unless proper methods of disinfestation are applied, the evacuated may bring along infected fleas in their effects or even on their persons, so that the spread of plague among them is not cut short.

While, for these reasons, the advisability of a mass evacuation of plague-infected settlements or precincts was questionable in the past, adoption of this method has now become superfluous, because, instead of removing the people from the flea-vectors, the latter may be effectively dealt with in loco.

At the same time, however, consideration ought to be given to the advisability of temporarily removing the inhabitants of individual houses or groups of houses which, on account of a particularly heavy rat-infestation, have become hot-beds of the infection, so as to get elbow-room and sufficient time to deal adequately with the rodent-harbourages.

**Mass Vaccination**

It is not surprising to find that in the field as well as in the laboratory the results obtained with plague vaccination by different workers and consequently the opinions they formed regarding the value of this prophylactic method, varied most markedly. The potency of the vaccines they used was bound to be markedly different. The same holds true of the dosage used—a factor of particularly great importance in the case of the killed vaccines which, instead of being given in two doses at an interval of 5–7 days, as is considered essential in principle, were actually most often administered once only. More important still, the statistical evidence presented in regard to the efficacy of plague vaccination, though ample, is often not satisfactory, mainly because the groups of the vaccinated and unvaccinated were not under the same risk of infection (see Otten 153).

Nevertheless, though it is not possible to accept the exaggerated claims made by some advocates of plague vaccination, there is no reason to revise the opinion reached after a careful consideration of the evidence by competent reviewers, such as the Indian Plague Commission (quoted by Simpson 203), Dieudonné & Otto,44 and recently Meyer,137 that the method is of definite value in the prevention of plague. It is important to note that Meyer was
able to base this conclusion not only upon reliable statistics, such as those of Otten (1936, 1940, 1941), Girard & Robic (1936, 1938), Girard (1946), and Patel & Rebello (1948), but also upon observations on the appearance of immune bodies in volunteers to whom plague vaccines had been administered (Meyer & Foster; Meyer 1948, 1953).

Being due to a process of active immunization, the protection conferred through plague vaccination reaches an effective degree only after a period usually assumed to last 5-7 days after administration of the vaccine. It is inevitable, therefore, that persons who incubate the infection at the time of vaccination, or who become infected soon afterwards, develop plague even though they have been vaccinated. It was undoubtedly due to such occurrences, which are bound to be particularly frequent if plague is rampant, that soon after Haffkine started to administer his vaccine, claims were made as to the existence of a "negative phase" following vaccination, during which the vaccinated were supposed to be more susceptible to the infection than the unvaccinated. As noted by Stevenson & Kapadia, in a discourse delivered in 1899 before the Royal Society, London, Haffkine definitely denied the existence of such a negative phase.

Calmette & Salimbeni, on the contrary, noted during the 1899 outbreak at Oporto that "animals during the period of immunization with heated cultures were extremely sensitive to very small doses of the virus, doses which were rarely mortal to non-vaccinated animals" (summary by Calmette, quoted by Stevenson & Kapadia). Since, however, Calmette & Salimbeni experimented with a few animals only and used a vaccine which, because it had been heat-killed at a temperature of 70°C (158°F), could have possessed but little immunizing value, not much credence can be given to their findings.

Nevertheless, during the years following the introduction of Haffkine's vaccine there was so much fear of a negative phase in Bombay that persons living in plague houses were given only half the vaccine doses used for people who apparently had been under no risk of infection. This practice was continued until 1905 when Liston, repeating Calmette & Salimbeni's experiments with a small number of guinea-pigs, found no evidence of the existence of a negative phase. Liston's findings were confirmed through ampler tests carried out by Stevenson & Kapadia which showed:

"(1) that there is no 'negative phase' or period of increased susceptibility of rats to plague after the administration of anti-plague vaccine even within one and a half hours after the operation;
(2) that the production of immunity among rats commences within a few hours of inoculation and increases in amount till the 2nd or 3rd day after anti-plague vaccination."

More important still, observations confirming the experimental findings of Stevenson & Kapadia have been made in the course of vaccination campaigns. Bannerman (quoted by Dieudonné & Otto) maintained in this connexion, with much reason, that if the susceptibility to plague were
increased immediately after vaccination, as was claimed by Calmette & Salimbeni, there ought to be a higher plague mortality among the recently vaccinated than among the unvaccinated. Actually, however, the mortality among 358 persons developing plague within 10 days after vaccination, including 43 who were already ill when vaccinated or fell ill on the same day, was 48% as against a death-rate of 73.7% in a control group of 5,079 unvaccinated. In a smaller group observed at Dharwar, only 36.5% of 74 persons developing plague within 10 days after vaccination succumbed as against 90.8% deaths in the control group.

Patel & Rebello, recently recording the results of a mass-inoculation campaign with Haffkine Institute vaccine, also found that "there was no adverse negative effect observed among those inoculated after exposure to infection and hence it would seem unwise to refuse inoculation to contacts from fear of negative phase effect ".

Summarizing the experiences made in China with agar-grown killed vaccines, Pollitzer likewise stated:

"We never found any evidence of a negative phase—on the contrary, when administering for curative purposes during the 1928 South Manchurian outbreak single vaccine doses to recently admitted bubonic plague patients, we saw that the life of these sufferers was often prolonged."

That administration of live avirulent vaccines does not produce a negative phase is well shown by the observations of Grasset who found only 15 cases of plague among 24,000 immunized persons within 10 days after vaccination.

Hence, though statements to the contrary have been made by a few recent observers, either on experimental grounds (Pons & Advier) or on account of field observations (see Simeons & Chhatre for example), it may be asserted that plague vaccination is not followed by a negative phase during which the vaccinated are temporarily more susceptible to the infection. Since, however, administration of plague vaccines does not prevent development of the disease in persons who were incubating the disease or who became infected during the days immediately following vaccination, it is obviously preferable to use this preventive method during the interepidemic period and not when plague, particularly human plague, has already become manifest.

As has been discussed in chapter 3, no doubt can exist that under adequate conditions equally good laboratory results can be obtained with killed and live avirulent vaccines respectively.

It is gratifying to note that an analogous conclusion has been reached in regard to human plague vaccination by Meyer who stated that:

"Any plague vaccine, whether killed or living, provided it either inherently contains 2 to 3 mg of Fraction I or is capable of producing this amount when inoculated into the
body of man, will favourably alter the susceptibility of 50 per cent of the inoculated. This basic immunity may even improve after the first month and may persist for at least 6 months”.

Meyer emphasized, however, the great importance of revaccination, concluding that:

“Annual revaccination progressively improves the immune status of the population groups and this creates the impressive results reported from countries in which this principle has been adopted as an aid in the control of plague in endemic regions”.

In view of the statements made above, the choice of the vaccines to be used for the purpose of plague prevention depends upon practical considerations rather than upon fundamental differences between the products. The casein hydrolysate vaccine now manufactured in the Haffkine Institute is certainly an excellent, exactly standardized product but shares with all killed vaccines the drawback that, in order to be fully effective, it must be administered in two doses given at an interval of 5–7 days—a procedure which as a rule is rather difficult to adopt. Live vaccines are fully effective in single doses but thus far have to be used soon after manufacture, a procedure not easy to adopt when it is necessary to carry out campaigns in remote areas. However, it deserves great attention that in contrast to previous experiences (see chapter 3) recent preliminary tests with a lyophilized EV vaccine prepared by Girard have given promising results in vitro and experimentally. Still, even if such lyophilized products should be found suitable for human vaccination, it might remain difficult to use them in localities distant from adequately equipped laboratories.

The dosages for the administration of the casein hydrolysate vaccine now produced in the Haffkine Institute, Bombay, are as follows (Greval):

<table>
<thead>
<tr>
<th>Age and sex</th>
<th>First dose (ml)</th>
<th>Second dose (in 5-7 days) (ml)</th>
<th>Annual revaccination (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Women</td>
<td>0.75</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Children, 1-4 years</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>5-10 years</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>11-15 years</td>
<td>0.4</td>
<td>0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Note: It should be kept in mind that these dosages, particularly those for women, have been worked out according to Indian standards.

Agar-grown killed vaccines are usually administered in an initial dose of 0.5 ml, followed 5–7 days later by administration of 1 ml. The latter dose (1 ml) is also adopted for the purposes of revaccination. Lesser doses must be given to adults of low body-weight and to children in proportions analogous to those indicated in the case of casein hydrolysate vaccine.

Live avirulent vaccines are administered once in an adult dose of 1 ml (Girard & Robic; Grasset). The proportionally lower doses recom-
mended by Grasset\textsuperscript{70} for children were 0.5 ml for the age-group of 5 to 12 years and 0.25 ml for those under 5 years.

If due regard is given to the principles of asepsis, the local and general reactions produced by the now available plague vaccines are as a rule mild or at most moderate. The conclusion arrived at in this connexion by the WHO Expert Committee on Plague,\textsuperscript{237} at its second session, was that:

"With living vaccines local reactions are essential, while with killed vaccines satisfactory protection can be obtained with preparations producing neither local nor general reactions."

**Serum Prophylaxis**

As summarized by Dieudonné \& Otto,\textsuperscript{44} some early workers reported favourable results obtained through mass prophylaxis with plague immune serum. However, Dieudonné \& Otto pointed out that the validity of some of these observations was doubtful, and stressed, moreover, that the method was disadvantageous in view of the short duration of the protection afforded and the high price of the serum, which would render it impossible to repeat the injections even if there were no danger of anaphylaxis.\textsuperscript{6} Dieudonné \& Otto maintained, however, that it would be advantageous to combine the prophylactic administration of plague immune serum with that of plague vaccine so as to obtain a rapidly effective as well as a longer lasting protection. They noted that the use of this combined method had given favourable results during an outbreak in Kobe, Japan.

Though good success with this combined method was also recorded by some recent workers, the serum doses they used were often so small (5 ml or even less) that it is difficult to consider them as effective. No doubt, more reliable results could be obtained with higher doses but the cost of using these during large-scale campaigns would be prohibitive. Moreover, as shown by an observation of Mackay-Dick,\textsuperscript{124} even plague-serum doses of 5 ml may produce severe urticarial reactions.

It would appear, therefore, that the method of combined plague serum and vaccine prophylaxis has little, if anything, to recommend it. As already stated, vaccination campaigns should be conducted before plague becomes manifest so as to avoid the appearance of the disease in persons already incubating the infection when they are vaccinated. Should it be necessary to vaccinate during a plague epidemic, application of DDT would be far more effective in preventing infection of the not yet immunized than combined serum administration. One may claim, therefore, that nowadays this combined method of prophylaxis is of historical rather than of actual interest.

\textsuperscript{6} That a second administration of prophylactic serum doses after an injudiciously long interval is rather dangerous, is proved by an observation made by Lloyd\textsuperscript{21} during the 1905 outbreak at Guayaquil, Ecuador. He stated that then "a number of private practitioners tried to immunize their clients against plague by injection of two small doses (usually 5 cm\(^3\)) of anti-plague serum, at an interval of 15-20 days. A great number of severe anaphylactic reactions resulted. Several of the patients were confined to their beds for days or even weeks, but I have not learnt that any death from anaphylaxis resulted."
Comparative Value of the Measures of Control

The comparative value of the various measures against bubonic (zootic) plague enumerated below has been, and still is, the subject of considerable debate.

Measures to be used permanently
Rodent control; intelligence work; public-health propaganda and education.

Measures to be used periodically or temporarily
Application of insecticides; rodent destruction; vaccination; quarantine measures.

General agreement exists that, in order to deal effectively with bubonic plague, a proper system of detecting manifestations of the infection, particularly of incipient epizootics, is indispensable. The great value of obtaining the goodwill and co-operation of the people through propaganda and health education is also generally realized. The necessity for measures to prevent the spread of plague at distance is likewise universally recognized.

However, even though the causative role played by the rodents is fully realized, the advisability, or even the necessity, of making direct campaigns against these animals the cornerstone of plague-preventive work is not rarely doubted. In the past, it was at one time believed that vaccination alone would suppress plague (Haffkine, quoted by Dieudonné & Otto 44). While the fallacy of this hope was soon realized, a number of workers were of the opinion that anti-rat campaigns were of no use for the control of plague because no really effective methods were available to combat these animals. Now that this impasse has been overcome, it is sometimes stated that in view of the success obtainable in plague control with DDT alone other measures, including rat control, are superfluous.

In evaluating this claim one must admit that:

(a) as long as the rodent-fleas have not become resistant to DDT, single applications of this insecticide, if adequately made, form a practically infallible means of preventing or suppressing plague manifestations in man;

(b) DDT campaigns, if repeated at proper intervals, continue to keep the rodent-flea incidence at low levels and this low flea incidence is apt to persist for considerably long periods even after the periodic use of the insecticide has been stopped.

However, whether this prolonged reduction of the flea rate will lead to a disappearance of rodent plague, as is sometimes claimed, remains to be seen. An observation made by Hill et al.85 in murine-typhus control work deserves great attention in this respect. As found by these workers, repeated DDT dusting led to suppressions of human typhus incidence, prevalence of complement-fixing antibodies, and rat-flea abundance, which persisted for over two years following the last dusting campaigns in September 1947. They added, however, that:
"A moderate rise in prevalence of murine typhus complement-fixing antibodies in the domestic rat reservoirs in the two treated counties in 1949 accompanied by a moderate increase in abundance of rat fleas suggests the necessity for further surveillance."

These observations tend to confirm the opinion held by the majority of plague workers and fully shared by the present writer that DDT application, though of great value in plague-control work, does not obviate the necessity of rodent-control work.

As stressed before, exclusion methods should be used as far as possible for the latter purpose in preference to eradicative work. The WHO Expert Committee on Plague (1952), 237 while admitting that it was impossible to recommend one rigid scheme of plague control, endorsed this view by stating that "under all circumstances the ideal method to fight plague was to cut short the contact between rodent and man through house improvement."

A further much debated question is whether the availability of DDT renders plague vaccination superfluous. There is no doubt that DDT application is the method of choice during plague outbreaks when it is preferable not to resort to vaccination so as to avoid the development of the disease in persons who are already infected when they receive the vaccine or who contract the infection soon afterwards because they have not yet become immunized.

At the same time, however, it would be unwise to discard the use of vaccination applied during the interepidemic periods and followed by yearly revaccination during those periods for the following reasons:

(a) As stated in chapter 8, vaccination considerably improves the chances of curing plague patients—a factor of particular importance in localities where no or limited possibilities for the use of antibiotics are available.

(b) In vast and sparsely populated rural plague areas, where it may be difficult to visit every house for the purpose of DDT administration, it may prove easier to vaccinate the people assembled for this purpose at suitable points or to vaccinate those assembled for other purposes, e.g., at markets or fairs.

Vaccination might also serve as a sheet-anchor in plague-infected localities where the fleas have become DDT-resistant.

**CONTROL OF PNEUMONIC (DEMIC) PLAGUE**

In contrast to the control of the insect-borne zootic form, the prevention of pneumonic plague, because it depends almost exclusively upon the protection of man from man, is quite easy to accomplish. The principal methods to be adopted when dealing with patients suffering from primary
pneumonic plague as well as with those developing secondary lung involve­ment in the course of a primarily bubonic attack will now be discussed.

Case Detection

The rapid suppression of pneumonic plague manifestations depends primarily upon a system of complete, as well as rapid, case detection. Every effort must be made, therefore, to detect the presence of the disease in either the secondary or the primary form at the first possible moment so as not to delay the measures necessary for dealing adequately with the situation. A prompt diagnosis of primary pneumonic plague is all the more important because, as emphasized in chapter 8, the patients are non-infective during the first 20-24 hours of illness.

The detection of cases during a pneumonic plague outbreak is greatly facilitated if the help of the people can be obtained in this respect. Hence, instead of trying to hide the occurrence of the disease, all available means of propaganda should be used to inform the people of its presence, to acquaint them with its symptoms and signs, and to urge them to report all suspicious cases at once, so that the sufferers may be properly cared for and their contacts may be protected.

Since, however, it is impossible fully to rely upon the help of the people in this respect, a system of house-to-house inspection must be adopted in communities, precincts, or smaller foci where pneumonic plague shows a tendency to spread. Preferably, the houses should be visited twice daily, as early as feasible in the morning and as late as possible in the afternoon or evening. All inmates of the houses should be seen, and their pulses, as well as their temperatures if possible, should be taken. Any person showing an increased temperature and/or an unusually rapid or otherwise abnormal pulse, or who appears otherwise to be ill, should be considered as plague-suspect, unless he obviously suffers from another disease, and should be kept under intensive observation or sent at once to the suspect-ward of the hospital.

Isolation and Treatment of Patients

Prompt hospitalization of pneumonic plague patients must be insisted upon, both in order to exclude the possibility of a spread of the infection to their families or visitors and to give them optimal facilities for treatment. In places remote from hospitals it may be inevitable to make exceptions to this rule, but even though it may be possible safely to isolate the patients in their houses or in outhouses or other buildings, the chances of effectively treating them at home are poor.

Both in order to initiate treatment at the earliest possible moment and to reduce chances for a spread of the infection from the patients during their
transport to the hospital, it is desirable to administer to them a dose of a suitable antibiotic before they leave their homes. Seal recommended the use of 1 g of streptomycin for this purpose.

Besides wards for the accommodation of the patients in the manifest stage of pneumonic plague, admission wards as well as sufficiently spacious wards for suspects must be provided for.

As in the case of bubonic plague hospitals, the admission ward should be installed in a separate building, or in a shed or tent erected for this purpose. The incoming patients must be undressed, cleaned, if necessary also disinfected, provided with hospital garments, and then sent to their proper wards. Contact between suspects and those suffering from manifest pneumonic plague must be strictly avoided.

It would be ideal to accommodate the suspects in individual rooms or cubicles, but this is as a rule impossible. However, a fair amount of protection can easily be afforded to the suspects by placing screens between the individual beds, which should not be placed close to one another.

Management of Contacts

It is no doubt desirable to accommodate the contacts of pneumonic patients for a period of 6-10 days in quarantine camps instead of leaving them at home—both in order to ensure that abortive treatment is effectively carried out and to detect signs of incipient illness at once. However, since removal of the contacts to a camp may be so much resented by the people as to lead to the hiding of cases, the plague staff should not go too far in insisting upon this measure, provided that it is possible to make adequate arrangements for observation and abortive treatment of the contacts in the houses. In particular, it may be advisable to leave one or two inmates in farmhouses to care for the livestock.

Abortive treatment should be started even before the contacts leave their houses. In the camp they must be seen at least twice daily, when their temperatures and pulses must be taken. Those appearing to be even slightly ill must be separated from the rest, closely observed, and sent to the suspect wards unless they rapidly improve or obviously suffer from another disease. Development of manifest pneumonic plague in a camp is a sign of bad management.

Protection of Staff

As shown by the deplorably large number of pneumonic plague infections among isolation-hospital workers or other staff members, proper precautions must be taken by all persons attending sufferers from this form of the disease. The most important of these precautions is the wearing of a proper mask.
As described by Chun,²⁹ the mask used in the Manchurian outbreaks "consists of two layers of gauze enclosing a flat oblong piece of absorbent cotton. It can be easily made by cutting the usual surgical gauze (9 inches wide [23 cm]) . . . into strips, each measuring 3 feet [1 m] in length. Each strip is then folded lengthwise so as to contain in the middle a piece of cotton wool measuring 4 inches by 6 inches [10 cm by 15 cm] in area and ½ inch [1.3 cm] in thickness. At either end of the gauze two cuts, each measuring 15 inches [38 cm], are made, thus turning the pad into a two-tail gauze bandage, with the central piece of wool over the nose and mouth. The upper tails of each side should be passed round the head above the ear and tied together behind. The lower tails should in a similar manner be passed under the ear and tied...

In individuals with prominent noses a lacuna is left on each side when the mask is adjusted. In order to obliterate the leak, the insertion of small cotton plugs within the upper margin of the mask to fill up the empty spaces on either side of the nose is to be recommended ³⁰.

As a further precaution, the hospital staff wore a hood made of cloth with a layer of silk, 6 inches (15 cm) wide, sewn in front of the nose and mouth area. This hood, which had apertures for the eyes, was tucked inside the overall at the neck of the wearer. Goggles were worn, but alternatively another kind of hood provided with a frontal mica window was worn over the gauze-cotton mask.

In addition to the masks, and, if necessary, hoods and goggles, the workers were protected by hospital gowns closing at the back, and one-piece linen trousers and stockings, as well as by rubber or high leather boots. Rubber gloves were worn when there was danger of coming in contact with sputum. The working apparel, particularly the masks, had to be worn not only in the wards but whenever there was a possibility of coming in touch with plague patients with lung involvement.

**Disinfection of Contaminated Objects**

While it seems unnecessary to carry out wholesale disinfection of the rooms or houses from which pneumonic plague patients have been removed, careful sterilization of all objects contaminated with their sputum is essential.

Removable articles of little value, which have become soiled with sputum, are best burnt. Garments and bedding, which have become contaminated, ought to be sterilized by steam or boiled. Water at boiling temperature should be poured over sputum deposited on bedsteads, floors, or walls. Deposits on level surfaces should then be covered with lime powder. Contaminated parts of the walls, or preferably the whole walls, should be freely treated with whitewash.

The patients should be given solid and large sputum cups or spittoons, which should be frequently sterilized by boiling them together with their contents.

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³⁰ It is interesting to note that, according to Rooks et al.,¹⁸¹ repeated laundering of the gauze material used for hospital masks greatly increased the bacterial filtering efficiency of the latter.
The urine and the stools of the patients should be mixed with generous amounts of milk of lime before they are disposed of. Boiling water ought then to be used to clean the containers, unless they can be sterilized by steam.

The methods of dealing with the dead bodies of pneumonic plague victims will be discussed later (see page 610).

Value of Vaccination

Killed vaccines

The problem of to what extent administration of killed vaccines was of value for the prevention of pneumonic plague was thoroughly discussed during the International Plague Conference held in 1911 at Mukden, Manchuria. Summarizing these discussions, Stanley stated that:

"From the evidence available it was not possible to draw definite conclusions as to the value of protective inoculation against plague pneumonia; but the general opinion was that the infection was too massive when the lungs were involved for the ordinary methods in use to be of much value."

As far as the present writer is aware, no further facts have been recorded to establish that administration of killed vaccines is of direct benefit in pneumonic plague epidemics. The method was not used during the 1917-18 Shansi outbreak, Young postulating that vaccination would have given a false sense of security which might have led to a neglect of essential precautions, especially the wearing of masks.

During the 1920-1 pneumonic epidemic in and near Manchuria, vaccination was used only in the Russian Coast Province, where the disease caused but little havoc. How far it was effective, it is impossible to say because (a) probably most of the 6,000 vaccinated Russians, who remained free from plague, were under no real risk of infection, and (b) nothing is known regarding the fate of the 3,000 vaccinated Chinese.

It is significant that Wu, discussing the management of pneumonic plague epidemics in general, made no reference to the use of vaccines.

Live vaccines

In the opinion of Grasset, the efficacy of live plague vaccine in the prevention of pneumonic plague was "shown by the small number of plague cases among immunized persons even among very close contacts of pneumonic cases." It is important to note, however, that most of the latter contacts received prophylactic serum doses as well as vaccine. Thus an old woman, who had remained healthy even though she had attended four pneumonic plague victims in her household, had been given in addition to live vaccine not less than 100 ml of plague immune serum prophylactically.
Compiling the statistics on a large-scale trial of his Tjiwidej vaccine, Otten omitted to consider the 84 pneumonic plague patients, 36 of whom had been vaccinated, "on the presumption based upon data available from literature and personal research, that this vaccination could not be expected to guarantee any protection against aerogenic infection, at least when subcutaneously administered at the relatively small dose adhered to till now ".

Girard, discussing the potential value of EV vaccine in the prevention of pneumonic plague, made the following statement:

"Our answer to this often asked question is that guinea-pigs are effectively protected against the bronchopneumonia which is invariably caused in the controls through intratracheal plague infection. And, despite the actual failure of this as well as of other modes of vaccination, it is undeniable that protection against bubonic plague leads ipso facto to a reduction of the pulmonary form which originates from the former type."

With this conclusion one must thoroughly agree. Though evidently of no direct value in warding off pneumonic plague infection in man, plague vaccination is of indirect value by reducing the frequency and/or severity of bubonic manifestations.

**DISPOSAL OF THE DEAD**

In the past, it was often considered necessary to cremate the dead bodies of plague victims, but except that it formed an easy means of disposing of numerous corpses, there was never any real need to resort to this practice which was often utterly repulsive to the people. Now, when one may expect that, owing to the availability of effective preventive and curative methods, there will no longer be any mass mortality from plague, it is certainly legitimate to bury the dead bodies instead of cremating them, unless cremation is compatible with the religious tenets or customs of the populations concerned.

As far as possible, the dead bodies of plague victims ought not to be handled and encoffined by their relatives or friends but by burial squads. The members of these should be provided with masks, protective garments, and boots, as well as with thick rubber gloves or stout canvas mittens.

It was formerly often recommended that the shrouds or clothes of the dead be moistened with strong antiseptics or that the dead bodies be covered with lime. Since, however, such practices, because apt to counteract putrefaction, may prolong persistence of the causative organisms in the dead bodies, they are not to be recommended.

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8 "A cette question maintes fois posée, nous répondrons que le cobaye est effectivement protégé contre la bronchopneumonie que détermine invariablement chez les témoins l’inoculation de virus dans la trachée. Et ce qui est indiscutable, malgré les échecs à l’actif de cette vaccination comme de toute autre, c'est que la protection contre la peste bubonique entraîne ipso facto la réduction des formes pulmonaires qui en dérivent."
It may be well, however, to place a layer of lime or another absorbent material into the coffins before the dead bodies are put in. Burial in graves sufficiently deep to prevent access of rodents or other rapacious animals is essential.

The garments and other belongings of bubonic plague victims dying in their houses must be de-flea’d unless ample previous use of DDT has been made. In the case of pneumonic plague victims, due attention must be paid to the sterilization of all sputum-soiled articles.

Funeral ceremonies in the houses of plague victims, which may involve assembly of many people, should be discountenanced as far as possible.

**CONTROL OF THE SPREAD OF PLAGUE AT DISTANCE**

As will be gathered from the statements made in earlier parts of this monograph, a spread of plague at distance may be effected:

1. by the arrival in a hitherto unaffected area of a plague patient who left an active focus when already ill or when incubating the disease;

2. by the transport of infected fleas on the persons or in the baggage of travellers;

3. by the carriage of infected rodents and/or fleas in transport vehicles or in goods carried by these.

As noted before, the appearance of pneumonic plague in a hitherto unaffected locality was quite frequently due to the arrival of a bubonic plague patient who had developed lung complications en route. Patients who fall ill with primary pneumonic plague while travelling and those who become ill after reaching their destination are equally apt to spread the infection in this form. It is, on the contrary, an unlikely contingency that rodent infection will be produced in a hitherto plague-free locality by the arrival of a bubonic patient who brought no infected fleas with him. Importation of such fleas by human agency, i.e., on the persons or in the baggage of travellers, who may or may not have contracted plague themselves, may take place and may lead to the infection of persons attacked by these fleas and/or to a transition of *P. pestis* to the rodents. On the whole, however, this mode of spread is not frequent, particularly not as far as long-distance spread is concerned.

Carriage of infected rodents and/or fleas in transport vehicles or in export goods is of ominous importance for the spread of plague.

In order to prevent the transport of bubonic plague infection by human agency, it is sufficient as a rule: (a) to ascertain the good state of health of the intending travellers through their medical inspection immediately before departure; (b) to de-flea their baggage with the aid of DDT, calcium cyanide,
or by other means; and (c) in the case of serious and/or not well-controlled outbreaks, to apply DDT to these persons as is done for the purpose of controlling louse-borne typhus. The administration of plague vaccine to intending travellers, though not required by the international quarantine regulations, ought to be given serious consideration as far as local traffic within or near plague-affected areas is concerned. However, in order to be effective, vaccination of the intending travellers must be completed at least a week before their departure.

While it is not difficult to adopt these procedures in regard to travellers intending to proceed by regular routes on larger ships or on trains or buses, it is often most difficult to implement these methods in the case of local traffic by primitive means of communication. However, the installation of temporary quarantine stations at strategic points round the affected localities is apt to remedy this situation to a considerable extent.

In the case of pneumonic plague outbreaks it is as a rule quite sufficient to examine the intending travellers immediately before departure, ascertaining at the same time that they have not been in contact with patients and have not lived in or near foci of the infection. Persons who have had recent contact with pneumonic plague patients, and preferably also those suspected of such contact, ought to be kept under quarantine for 6-10 days before they are permitted to leave.

It is quite easy to prevent the carriage of infected rodents and/or fleas in transport vehicles or in goods as long as one has to deal with the larger seagoing ships. As provided for in the international sanitary regulations, sea-going ships must be fumigated periodically unless, because they are kept free from infestation through rat-proofing procedures or regular anti-rodent measures, they have been granted exemption certificates. Suspect goods to be carried by them may be fumigated before loading or may be disinfested after unloading on lighters or on shore.

There ought to be no great difficulty in adopting similar procedures even on small vessels plying on inland waterways, particularly those within or near plague foci—the less so because 1080 and calcium cyanide may be used in place of elaborate fumigation methods.

DDT may be used to keep railway cars, buses, and trucks free from fleas, while calcium cyanide is satisfactory for dealing with the goods carried by railways and trucks. Again, however, it is most difficult to control adequately the local traffic of goods apt to harbour infected fleas or even rodents, particularly small grain consignments transported by primitive means of communication, such as carts or pack animals, or carried by porters. Temporary quarantine stations may prove useful in such cases, but it is far more important to prevent goods destined for export from becoming infested by providing rat-proof storage facilities for them. As has been noted, in emergencies such goods may be kept on elevated platforms, the supports of which are provided with rat-guards.
There can be no doubt, however, that the most effective method of preventing the spread of plague at distance is to deal rapidly and drastically with the local manifestations of the infection. Fully adequate methods are available for this purpose so that the task now confronting the plague workers is to apply these procedures intensively and universally.

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CONTROL AND PREVENTION

ANNEXES
LIST OF RESERVOIRS AND VECTORS OF PLAGUE

Lists enumerating (a) the animals in respect of which positive proof of the infection has been obtained, and (b) the suspected animals are given in tables I and II in order to show the occurrence of natural plague in rodent species or subspecies other than the commensal rats and mice, and in Lagomorpha.

Particular attention has been paid to make table I not only as accurate as possible, by closely following the standard nomenclature of Ellerman and of Ellerman & Morrison-Scott, but as complete as possible. For its compilation valuable information has been derived from unpublished working documents generously made available by Macchiavello and Davis.

These two documents as well as published material of Wu and of Chabaud were also used in the compilation of table V.

<table>
<thead>
<tr>
<th>TABLE I. WILD RODENTS AND LAGOMORPHA PROVED NATURALLY INFECTED OR STRONGLY INCriminated THROUGH POSITIVE FINDINGS IN THEIR ECTOPARASITES</th>
</tr>
</thead>
</table>

<table>
<thead>
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<th>family and subfamily</th>
<th>species</th>
<th>Locality</th>
<th>Bibliographical reference</th>
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<tbody>
<tr>
<td>BATHYERGIDAE</td>
<td>Cryptomys sp.</td>
<td>Angola</td>
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<tr>
<td>CAVIIDAE</td>
<td>Cavia porcellus</td>
<td>Brazil</td>
<td>95</td>
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<td></td>
<td>Cavia aperea</td>
<td>Ecuador</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Cavia pamparum</td>
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<td>160</td>
</tr>
<tr>
<td></td>
<td>Cavia tschudi alachalpae</td>
<td>Peru</td>
<td>99</td>
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<tr>
<td></td>
<td>Cavia australis australis</td>
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<tr>
<td></td>
<td>Cavia australis juannia</td>
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<td>Galea musteloides leucophrya</td>
<td>Argentina</td>
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<td>Galea musteloides littoralis</td>
<td>Argentina</td>
<td>9</td>
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<tr>
<td></td>
<td>Galea spizii &quot;Prea&quot;</td>
<td>Brazil</td>
<td>96</td>
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<tr>
<td></td>
<td>Kerodon rupestris</td>
<td>Brazil</td>
<td>96</td>
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</tbody>
</table>

*The common guinea-pig, Cavia porcellus (Cavia cobaya auctt.), though repeatedly found plague-infected (Wu Lien-teh and others), has not been included in this annex since it is a domestic rather than a wild rodent. Referring to the findings recorded in Peru, Macchiavello stated that "plague infection in Cavia, has been mentioned by several authors but without accurate taxonomic classification of specimens".*
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<th>Bibliographical reference</th>
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<td><strong>CHINCHILLIDAE</strong></td>
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<tr>
<td></td>
<td><strong>Lagostomus maximus immollis</strong>&lt;br&gt;&quot;Vizcacha&quot; or Peruvian hare</td>
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<td>94</td>
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<tr>
<td>Dipodinae</td>
<td><strong>Allactaga elater</strong>&lt;br&gt;Small five-toed jerboa</td>
<td>South-east Russia</td>
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<td></td>
<td><strong>Allactaga elater indica</strong></td>
<td>Iranian Kurdistan</td>
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<tr>
<td></td>
<td><strong>Allactaga sibirica subsp.</strong>&lt;br&gt;(A. saltens auctt.)</td>
<td>South-east Russia</td>
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<tr>
<td></td>
<td><strong>Allactaga sibirica sibirica</strong>&lt;br&gt;Mongolian five-toed jerboa</td>
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<tr>
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<td><strong>Dipus sagitta</strong>&lt;br&gt;Northern three-toed jerboa</td>
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<td><strong>ECHIMYIDAE</strong></td>
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<td>Echimyinae</td>
<td><strong>Cercomys cunicularius laurentius</strong></td>
<td>Brazil</td>
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<td><strong>GEOMYIDAE</strong></td>
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<td></td>
<td><strong>Thomomys bollae</strong>&lt;br&gt;Western pocket-gopher</td>
<td>California, USA : Colorado, USA (?)</td>
<td>133, 149</td>
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<tr>
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<td><strong>Thomomys fossor</strong>&lt;br&gt;(T. talpoides fossor auctt.)&lt;br&gt;Mountain pocket-gopher</td>
<td>Fleas only : Colorado</td>
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<td><strong>HETEROMYIDAE</strong></td>
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<td>Dipodomyinae</td>
<td><strong>Dipodomys sp.</strong></td>
<td>Texas, USA</td>
<td>114</td>
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<td></td>
<td><strong>Dipodomys ordi ordi</strong>&lt;br&gt;Ord's kangaroo rat</td>
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</tr>
<tr>
<td></td>
<td><strong>Perognathus sp.</strong>&lt;br&gt;Pocket-mouse</td>
<td>Fleas only : Washington, USA</td>
<td>138</td>
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<tr>
<td>Heteromyinae</td>
<td><strong>Heteromys anomalus anomalus</strong></td>
<td>Venezuela</td>
<td>68</td>
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<tr>
<td><strong>MURIDAE</strong></td>
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<tr>
<td>Cricetinae</td>
<td><strong>Akodon dolores</strong></td>
<td>Argentina</td>
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<td><strong>Akodon mollis mollis</strong></td>
<td>Peru-Ecuador border region</td>
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<tr>
<td></td>
<td><strong>Akodon mollis orophilus</strong>&lt;br&gt;Mountain field-mouse</td>
<td>Huancabamba, Peru</td>
<td>98, 99</td>
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<tr>
<td></td>
<td><strong>Cricetus cricetus</strong>&lt;br&gt;Common hamster</td>
<td>South-east Russia</td>
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<td><strong>Eligmodontia hirtipes jucunda</strong></td>
<td>Argentina</td>
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<td><strong>Eligmodontia moreni</strong></td>
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<td><strong>Graomys griseoflavus centralis</strong></td>
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<td><strong>Graomys griseoflavus griseoflavus</strong></td>
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<td><strong>Hesperomys fecundus</strong></td>
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<td><strong>Hesperomys murillus cordovensis</strong></td>
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### TABLE 1 (continued)

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<th>Bibliographical reference</th>
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<td><strong>MURIDAE</strong>&lt;br&gt;Cricetinae (continued)</td>
<td>Holochilus balteatum</td>
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<td>Holochilus salterus</td>
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<td>Mystromys albicaudatus</td>
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<tr>
<td></td>
<td>Neotoma albicrus albicrus</td>
<td>Arizona, USA; New Mexico, USA</td>
<td>134, 140, 145</td>
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<td></td>
<td>Neotoma cinerea occidentalis</td>
<td>California, USA</td>
<td>113</td>
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<td>Neotoma desertorum</td>
<td>Nevada, USA; Utah, USA</td>
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<td>Neotoma fuscipes</td>
<td>California, USA</td>
<td>104</td>
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<td></td>
<td>Neotoma fuscipes mohavensis</td>
<td>Nevada, USA</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>Neotoma intermedia intermedia (N. lepida intermedia auct.) Intermediate (Rhoads') wood-rat</td>
<td>California, USA</td>
<td>113</td>
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<tr>
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<td>Neotoma lepida lepida</td>
<td>Utah, USA (?)</td>
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<td></td>
<td>Neotoma mieropus</td>
<td>Texas, USA</td>
<td>114 b</td>
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<td>Onychomys sp.</td>
<td>Fleeas only: Texas, USA</td>
<td>114</td>
</tr>
<tr>
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<td>Onychomys leucogaster</td>
<td>Fleeas only: New Mexico and other Western areas of the USA; Texas</td>
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<tr>
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<td>Onychomys torridus</td>
<td>Fleeas only: New Mexico, USA</td>
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<td>Oryzomys undinus</td>
<td>Peru</td>
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<td>Oryzomys arenicola</td>
<td>Peru</td>
<td>99</td>
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<tr>
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<td>Oryzomys flavescens subsp.</td>
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<td></td>
<td></td>
<td>Bolivia</td>
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<tr>
<td></td>
<td>Oryzomys laticeps intermedius</td>
<td>Brazil</td>
<td>98 c</td>
</tr>
<tr>
<td></td>
<td>Oryzomys laticeps nilidus</td>
<td>Fleeas only: Ecuador</td>
<td>99</td>
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<td></td>
<td>Oryzomys palustris (Hesperomys palustris auct.)</td>
<td>New Orleans, La., USA</td>
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<td></td>
<td>Oryzomys phaeopus obesus</td>
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<tr>
<td></td>
<td>Oryzomys soldamani soldamani (O. longicaudatus soldamani auct.)</td>
<td>Huancasamba, Peru</td>
<td>96, 99</td>
</tr>
</tbody>
</table>

b Spontaneous plague in Neotoma sp. was recorded in Oklahoma (Hampton 4) as well as in other western parts of the USA.

c Macchiavello 44 also mentioned infection in "Oligoryzomys sp." and "Oryzomys sp. (?) sylvaticus".
### TABLE 1 (continued)

<table>
<thead>
<tr>
<th>family and subfamily</th>
<th>species</th>
<th>Locality</th>
<th>Bibliographical reference</th>
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<tr>
<td><strong>MURIDAE</strong></td>
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<td></td>
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</tr>
<tr>
<td>Cricetinae (continued)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oryzomys xanthaeolus xanthaeolus</strong></td>
<td>Ecuador; Peru</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td><strong>Oxymycterus sp. (?? paramensis)</strong></td>
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<td>99</td>
<td></td>
</tr>
<tr>
<td><strong>Peromyscus boylii</strong></td>
<td>Fleas only: Arizona, USA</td>
<td>145</td>
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</tr>
<tr>
<td><strong>Peromyscus leucopus</strong></td>
<td>Fleas only: New Mexico, USA</td>
<td>145</td>
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<tr>
<td><strong>Peromyscus maniculatus</strong></td>
<td>Fleas only: California, USA; Washington, USA</td>
<td>139, 152</td>
<td></td>
</tr>
<tr>
<td><strong>Peromyscus truei gilberti</strong></td>
<td>California, USA</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td><strong>Peromyscus truei truei</strong></td>
<td>California, USA; New Mexico, USA</td>
<td>111, 18</td>
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<td><strong>Phyllostis amicus maritimus</strong></td>
<td>Fleas only: Peru</td>
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<td><strong>Phyllostis darwinii vaccaurum</strong></td>
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<td><strong>Phyllostis fruticicolus</strong></td>
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<tr>
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<td>139, 137, 147</td>
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<td><strong>Rhipidomys sp. (?? leucodactylus)</strong></td>
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<td>99</td>
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<td><strong>Rhipidomys hirsutus</strong></td>
<td>Peru</td>
<td>98, 99</td>
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<td>Vermont, USA</td>
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<td><strong>Sigmodon peruanus</strong></td>
<td>Fleas and lice only: New Mexico, USA</td>
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<td><strong>Dendromys haymani</strong></td>
<td>Belgian Congo</td>
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<td><strong>Dendromys insignis kivu</strong></td>
<td>Belgian Congo</td>
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<td><strong>Malacothrix typicus</strong></td>
<td>South Africa</td>
<td>191</td>
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<td><strong>Desmodillus auricularis</strong></td>
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<td><strong>Gerbillus paebo</strong></td>
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<td><strong>Meriones meridianus</strong></td>
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<td>186, 44</td>
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</tr>
<tr>
<td><strong>(Pallasiomys merid. auctt.)</strong></td>
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</tr>
<tr>
<td><strong>Midday gerbil</strong></td>
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<td></td>
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<tr>
<td><strong>Meriones persicus persicus</strong></td>
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<td>2</td>
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<tr>
<td><strong>Persian jird</strong></td>
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*An unidentified subspecies of Oryzomys xanthaeolus was also found infected.*

*The presence of spontaneous plague in "Peromyscus sp." or their fleas was also repeatedly confirmed (Eskey & Haas and others).*
### ANNEX 1

#### TABLE I (continued)

<table>
<thead>
<tr>
<th>RODENTIA</th>
<th>species</th>
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<td>Meriones tamariscinus</td>
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<td>Rhombomys opimus</td>
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<td>Tatera brantsi</td>
<td>South Africa</td>
<td>118, 53</td>
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<td>Tatera indica</td>
<td>India, Iranian Kurdistan</td>
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<td>Tatera nigrilena beniensis</td>
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<td>Tatera schinzi</td>
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<td>Ellobius tulescens</td>
<td>Iranian Kurdistan</td>
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<td>Ellobius talpinus</td>
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<td>Lagurus curtatus</td>
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<td>Lagurus lagurus</td>
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<td>Arvicanthis abyssinicus rosen</td>
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*Spontaneous plague in "Microtus sp." was also recorded on several occasions.* 137, 139
TABLE 1 (continued)

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<td>Mus triton fors</td>
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<td>(Leggada triton fors auctt.)</td>
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<td>Oenomys hypoxanthus hypoxanthus</td>
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<tr>
<td></td>
<td>Rusty-nosed rat</td>
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Davis stated that "D.i. nudipes [Dasymys incomtus nudipes] is found in the enzootic plague area in Barotseland, N. Rhodesia, where it is subject to secondary infection from gerbils and Mastomys".
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<th>RODENTIA</th>
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<td>Pelomys campoae <em>h</em></td>
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<td>Rattus natatoris</td>
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<td>Rhabdomys pumilio</td>
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<td>Otomys angoniensis</td>
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<td>MURIDAE</td>
<td>Otomys roridus</td>
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<td>MURIDAE</td>
<td>Otomys tropicalis elgonis</td>
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<td>MURIDAE</td>
<td>Otomys unisulcatu5.</td>
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<td>Karoo rat</td>
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<td>MURIDAE</td>
<td>Parotomys brentsi luteolus</td>
<td>Eastern Karoo rat or Brants’ Otomys</td>
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<td>PEDETIDAE</td>
<td>Pedetes cafer</td>
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<td>SCIURIDAE</td>
<td>Citellus armatus</td>
<td>USA (western States)</td>
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<td>Citellus brevicei brevicei</td>
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<td>SCIURIDAE</td>
<td>Citellus brevicei douglasii</td>
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<td>SCIURIDAE</td>
<td>Citellus brevicei fisheri</td>
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<td>SCIURIDAE</td>
<td>Citellus brevicei nudipes</td>
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<tr>
<td>SCIURIDAE</td>
<td>Citellus beldingi beldingi</td>
<td>California, USA</td>
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<tr>
<td>SCIURIDAE</td>
<td>Citellus beldingi oregoneus</td>
<td>Oregon, USA</td>
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</table>

h Garnham * claimed that this rodent was afterwards identified as being Lermiscomys griselda.

i Davis * noted that “in Barotseland P. f. frater (Pelomys fallax frater) is associated with Otomys and Dasymys and has a similar flea fauna. It may act as a transient reservoir with these species”.

In the list of plague-affected wild rodents inserted in the 1936 Manual separate mention was made of three subspecies of Otomys (Myotomys auctt.). To make the present list tally with that of Davis, * Otomys unisulcaitus Cuvier and Geoffroy alone has been embodied.
PLAGUE

<table>
<thead>
<tr>
<th>family and subfamily</th>
<th>species</th>
<th>Locality</th>
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<tr>
<td><strong>SCIURIDAE</strong> (continued)</td>
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<td><strong>Citellus columbianus columbianus</strong> Columbian ground-squirrel</td>
<td>Washington, USA</td>
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<td></td>
<td><strong>Citellus columbianus ruficaudus</strong> Blue Mountain ground-squirrel</td>
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<td><strong>Citellus fulvus</strong> Large-toothed suslik</td>
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<td><strong>Citellus idahoensis</strong> Idaho ground-squirrel</td>
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<td><strong>Citellus lateralis chrysodeirus</strong> Golden-mantled ground-squirrel</td>
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<td><strong>Citellus lateralis lateralis</strong> Fleas only: Wyoming, USA</td>
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<td><strong>Citellus leucurus leucurus</strong> Antelope ground-squirrel</td>
<td>Fleas only: Arizona, USA</td>
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<td><strong>Citellus mexicanus</strong> Fleas only: New Mexico, USA</td>
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<td><strong>Citellus pygmaeus</strong> Little suslik</td>
<td>South-east Russia</td>
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<td><strong>Citellus richardsoni elegans</strong> Wyoming ground-squirrel</td>
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<td><strong>Citellus richardsoni nevadensis</strong> Nevada ground-squirrel</td>
<td>Also Colorado, Idaho, Montana, and Utah</td>
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<td><strong>Citellus richardsoni richardsoni</strong> Richardson's ground-squirrel</td>
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<td><strong>Citellus spilosoma major</strong> Fleas only: New Mexico, USA</td>
<td>Alberta, Canada</td>
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<td><strong>Citellus townsendi mollis</strong> Piute ground-squirrel</td>
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<td><strong>Citellus tridecemlineatus</strong> 13-striped ground-squirrel</td>
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<td><strong>Citellus variegatus grammurus</strong> Say's rock-squirrel</td>
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<td><strong>Citellus variegatus utah</strong> Utah rock-squirrel</td>
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* Plague was also confirmed in fleas from "Callospermophilus sp." in California, and in fleas and ticks from "Citellus lateralis" in Colorado. 
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<td>Washington ground-squirrel</td>
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<td><em>Cynomys</em> sp.</td>
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<td><em>Cynomys gunnisoni gunnisoni</em></td>
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<td>Zuni prairie-dog</td>
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<td><em>Cynomys leucurus</em></td>
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<td><em>Cynomys ludovicianus</em></td>
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### TABLE I (concluded)

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<td>Tamiasciurus douglasii albominutus Sierra Nevada chickaree</td>
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<td>Xerus erythropus Central African side-striped squirrel</td>
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<td>Xerus inauris inauris (Geosciurus capensis auctt.) Bristly ground-squirrel</td>
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<td>Lepus californicus Black-tailed jack-rabbit</td>
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<td></td>
<td>Sylvilagus audoboni</td>
<td>Ecuador</td>
<td>99</td>
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<tr>
<td></td>
<td>Sylvilagus bachmani California-brush rabbit</td>
<td>Peru</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Sylvilagus brasiliensis</td>
<td>Huancabamba, Peru</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Sylvilagus brasiliensis gibsoni</td>
<td>New Mexico, USA</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>Sylvilagus nuttalli nuttalli Washington cotton-tail rabbit</td>
<td>Fleas only (?) California, USA</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brazil</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Argentina</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td></td>
<td>California, USA</td>
<td>109</td>
</tr>
</tbody>
</table>

1 Davis stated in regard to these two hares that "it is doubtful whether the records of first infections can be relied on to be zoologically exact. *L. capensis* and *L. saxatilis* occur together over most of South Africa and extend northwards throughout the continent".

2 Instances of secondary plague manifestations among domesticated rabbits, due to the presence of the infection among rats, have been recorded by several workers, e.g. recently by Buck et al., who also referred to previous observations made in this respect in Madagascar.
### Table II. Wild Rodents and Lagomorpha Presumed To Be Naturally Infected

<table>
<thead>
<tr>
<th>Family and Subfamily</th>
<th>Species</th>
<th>Locality</th>
<th>Reference</th>
<th>Reason for Non-Inclusion in Table 1a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RODENTIA</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Caviidae Caviinae</td>
<td>Gatea sp.</td>
<td>Bolivia</td>
<td>99</td>
<td>B</td>
</tr>
<tr>
<td>Dipodidae Dipodinae</td>
<td>Jerboa</td>
<td>South-east Russia</td>
<td>116</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>(? Jaculus sp.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jerboa</td>
<td>Somalia</td>
<td>100</td>
<td>B b</td>
</tr>
<tr>
<td>Echimyidae Echimyinae</td>
<td>Citronyx sp.</td>
<td>Argentina</td>
<td>194, 4</td>
<td>A, B</td>
</tr>
<tr>
<td></td>
<td>(? Cl. mendocinus)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;Tucu-tucu&quot;</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Muridae Gerbillinae</td>
<td>Gerbillus sp.</td>
<td>Tunisia</td>
<td>42</td>
<td>A, B</td>
</tr>
<tr>
<td></td>
<td>Talera valida liodon</td>
<td>Barotseland</td>
<td>23</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Arvicolal sp.</td>
<td>Kenya</td>
<td>790</td>
<td>A, B</td>
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<td></td>
<td>Brachytyromus albicauda</td>
<td>Madagascar</td>
<td>96, 165</td>
<td>A</td>
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<tr>
<td></td>
<td>Microtus sp.</td>
<td>Angola</td>
<td>106</td>
<td>A, B</td>
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<tr>
<td>Murinae</td>
<td>Aethomys chrysophilus</td>
<td>South Africa</td>
<td>23</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Bandicota bengalensis varius (Gunomys varius auctt.)</td>
<td>Calcutta</td>
<td>55</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Saccostomus campestris</td>
<td>Nyassaland</td>
<td>55</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Capa pouchad-rat</td>
<td>South Africa</td>
<td>23</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Thallomys nigricauda</td>
<td>South Africa</td>
<td>23</td>
<td>A</td>
</tr>
<tr>
<td>Sciuridae</td>
<td>Citellus sp.</td>
<td>South</td>
<td>1</td>
<td>A, B</td>
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<tr>
<td></td>
<td>(? G. mongolicus umbratus auctt.)</td>
<td>Manchuria</td>
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<td>Marmota sp.</td>
<td>Tibet</td>
<td>168</td>
<td>A, B</td>
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<tr>
<td></td>
<td>(? M. himalayana robusta)</td>
<td>Kansu</td>
<td>123</td>
<td>A, B</td>
</tr>
<tr>
<td>Unclassified</td>
<td>Brush (coconut-tree) rat</td>
<td>New Caledonia</td>
<td>122</td>
<td>A, B</td>
</tr>
<tr>
<td></td>
<td>Field-mouse (mu'ot)</td>
<td>Algeria</td>
<td>127, 126</td>
<td>A, B</td>
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<tr>
<td></td>
<td>Field-rat</td>
<td>Kherson</td>
<td>43</td>
<td>B</td>
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<tr>
<td></td>
<td></td>
<td>Rhodesia</td>
<td>79</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tunisia</td>
<td>42</td>
<td>A, B</td>
</tr>
<tr>
<td>Lagomorpha Leporidae</td>
<td>Lepus sp.</td>
<td>Brazil</td>
<td>55</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>French West Africa</td>
<td>88</td>
<td>A, B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Russian Turkestan</td>
<td>75, 77, 131</td>
<td>A, B</td>
</tr>
</tbody>
</table>

*a* A = incomplete evidence of infection  
*B* = species undetermined or doubtful  
*b* The presence of plague in other desert rodents was also suspected.
<table>
<thead>
<tr>
<th>FAMILY AND SUBFAMILY</th>
<th>SPECIES</th>
<th>LOCALITY</th>
<th>BIBLIOGRAPHICAL REFERENCE</th>
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<tbody>
<tr>
<td><strong>BATHYERGIDAE</strong></td>
<td>Bathylagus suillus</td>
<td>South Africa</td>
<td>23</td>
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<tr>
<td></td>
<td>Cryptomys hottentotus</td>
<td>South Africa</td>
<td>23, 125</td>
</tr>
<tr>
<td><strong>DASYPROCTIDAE</strong></td>
<td>Dasypodidae aguti</td>
<td>Brazil</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>Golden aguti (&quot; Cottia &quot;)</td>
<td>Brazil</td>
<td>166</td>
</tr>
<tr>
<td><strong>DIPODIDAE</strong></td>
<td>Dasyproctidae</td>
<td>Brazil</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>Dasyproctinae aguti</td>
<td>Brazil</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>Golden aguti (&quot; Cottia &quot;)</td>
<td>Brazil</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Hairy-rumped aguti</td>
<td>Brazil</td>
<td>166</td>
</tr>
<tr>
<td><strong>ECHIMYIDAE</strong></td>
<td>Echimyidae</td>
<td>Brazil</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Echimys inermis &quot;Punaro&quot;</td>
<td>Brazil</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Echimys lamarum Rata de espinho</td>
<td>Brazil</td>
<td>166</td>
</tr>
<tr>
<td><strong>MURIDAE</strong></td>
<td>Muridae</td>
<td>China</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Cricetinae</td>
<td>Transbaikalia</td>
<td>69, 176</td>
</tr>
<tr>
<td></td>
<td>Cricetus barabensis barabensis (Cr. furunculus aucttt.)</td>
<td>Iranian Kurdistan</td>
<td>2</td>
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<tr>
<td></td>
<td>Mesocricetus auratus auratus</td>
<td>USA</td>
<td>105</td>
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<tr>
<td></td>
<td>Eversmann's hamster</td>
<td>Iranian Kurdistan</td>
<td>2</td>
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<tr>
<td></td>
<td>Sigmodon chonensis</td>
<td>Ecuador</td>
<td>99</td>
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<tr>
<td></td>
<td>Gerbillus campestris subsp.</td>
<td>Tunis</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>Gerbillus campestris dodsoni</td>
<td>Tunis</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>Gerbillus pyramidum hirtipes</td>
<td>Tunis</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>Meriones crassus charon</td>
<td>Iranian Kurdistan</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Meriones shawi grandis</td>
<td>Morocco</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Meriones shawi shawi</td>
<td>Tunis</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>Psammomys obesus roudairei Fat sand-rat</td>
<td>Tunis</td>
<td>197</td>
</tr>
</tbody>
</table>

*Baltazard et al. worked with *Mesocricetus auratus brandti* and *Mesocricetus auratus raddei* which they found as little susceptible to plague as the subspecies (?) *Mesocricetus auratus auratus* used by McMahon."
### RODENTIA

<table>
<thead>
<tr>
<th>family and subfamily</th>
<th>species</th>
<th>Locality</th>
<th>Bibliographical reference</th>
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<tbody>
<tr>
<td><strong>MURIDAE</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gerbillinae (continued)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gerbillinae</td>
<td>Tatera afra</td>
<td>South Africa</td>
<td>23</td>
</tr>
<tr>
<td>Gerbillinae</td>
<td>Tatera vicina</td>
<td>Kenya</td>
<td>157</td>
</tr>
<tr>
<td>Gerbillinae</td>
<td>Arvicolal amphibius</td>
<td>South-east Russia</td>
<td>44</td>
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<tr>
<td>Gerbillinae</td>
<td>Apodemus agrarius</td>
<td>Formosa</td>
<td>84</td>
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<tr>
<td>Gerbillinae</td>
<td>Apodemus sylvaticus</td>
<td>Europe</td>
<td>122</td>
</tr>
<tr>
<td>Gerbillinae</td>
<td>Gallus ellioti</td>
<td>India</td>
<td>40</td>
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<td>Gerbillinae</td>
<td>Lemniscomys barbarus</td>
<td>Tunis</td>
<td>197</td>
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<tr>
<td>Gerbillinae</td>
<td>Miceromys minutus</td>
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<td>44</td>
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<tr>
<td>Gerbillinae</td>
<td>Miceromys irani</td>
<td>Iranian Kurdistan</td>
<td>2</td>
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<tr>
<td>Gerbillinae</td>
<td>Thallomys namaquensis</td>
<td>South Africa</td>
<td>23</td>
</tr>
<tr>
<td>Gerbillinae</td>
<td>Tachyoryctes daemon</td>
<td>Central Africa</td>
<td>35</td>
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<tr>
<td>Tachyoryctinae</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>MUSCARDINIDAE</strong></td>
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</tr>
<tr>
<td>Graphiurinae</td>
<td>Graphiurus sp.</td>
<td>French West Africa</td>
<td>91</td>
</tr>
<tr>
<td>Graphiurinae</td>
<td>Dryomys nitidula</td>
<td>South-east Russia</td>
<td>44</td>
</tr>
<tr>
<td><strong>SCIURIDAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sciurinae</td>
<td>Allantoxerus getulus</td>
<td>Morocco</td>
<td>12</td>
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<tr>
<td>Sciurinae</td>
<td>Citellus dauricus mongolicus</td>
<td>Manchuria</td>
<td>207</td>
</tr>
<tr>
<td>Sciurinae</td>
<td>Citellus dauricus ramosus</td>
<td>Manchuria</td>
<td>121</td>
</tr>
<tr>
<td>Sciurinae</td>
<td>Citellus undulatus [Citellus eversmanni auctt.; Long-tailed Sizeran suslik]</td>
<td>Transbaikalia</td>
<td>70</td>
</tr>
<tr>
<td>Sciurinae</td>
<td>Citellus suslicus guttatus</td>
<td>South-east Russia</td>
<td>163, 164</td>
</tr>
<tr>
<td>Sciurinae</td>
<td>Marmota marmota</td>
<td>Europe</td>
<td>203</td>
</tr>
<tr>
<td>Sciurinae</td>
<td>Sciurus stramineus stramineus</td>
<td>Ecuador</td>
<td>99</td>
</tr>
</tbody>
</table>

---

*P. pestis* was isolated from *X. brasiliensis* found in a deserted nest of *Thallomys namaquensis*.\(^1\)

---

\(^1\) P. pestis was isolated from *X. brasiliensis* found in a deserted nest of *Thallomys namaquensis*.\(^1\)
<table>
<thead>
<tr>
<th>FAMILY AND SUBFAMILY</th>
<th>SPECIES</th>
<th>LOCALITY</th>
<th>BIBLIOGRAPHICAL REFERENCE</th>
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</thead>
<tbody>
<tr>
<td>LEPORIDAE</td>
<td><em>Sylvilagus calidus</em> <em>Tapeti</em></td>
<td>Ecuador</td>
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<tr>
<td>OCHOTONIDAE</td>
<td><em>Ochotona dauricus</em></td>
<td>Transbaikalia</td>
<td>99, 176</td>
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</table>

<table>
<thead>
<tr>
<th>ORDER</th>
<th>SPECIES</th>
<th>LOCALITY</th>
<th>REMARKS</th>
<th>BIBLIOGRAPHICAL REFERENCE</th>
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<tbody>
<tr>
<td>ARTIODACTYLA</td>
<td><em>Camelus sp.</em> Camel</td>
<td>South-east Russia</td>
<td>See chapter 6</td>
<td></td>
</tr>
<tr>
<td>CARNIVORA</td>
<td><em>Canis aureus</em> Asiatic jackal</td>
<td>India</td>
<td>Suspected by the Plague Research Commission</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td><em>Canis familiaris</em> Domestic dog</td>
<td>French West Africa, Manchuria Morocco</td>
<td>Suspected</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td><em>Cynictis penicillata</em> Yellow mongoose</td>
<td>South Africa</td>
<td>See chapter 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Felis catus</em> Domestic cat Fox</td>
<td>Brazil</td>
<td>Suspected</td>
<td>19</td>
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<tr>
<td></td>
<td><em>Herpestes sp.</em> Mongoose</td>
<td>India, Hawaii</td>
<td>Found infected, One specimen found infected</td>
<td>27, 47</td>
</tr>
<tr>
<td></td>
<td><em>Mustela sp.</em> Weasel</td>
<td>Argentina, USA: western States</td>
<td>94, 198</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Mustela altaica</em> Alpine weasel</td>
<td>Iranian Kurdistan</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Mustela putorius</em> European polecat</td>
<td>Near Odessa Russia</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Mustela putorius eversmanni</em></td>
<td>South-east Russia</td>
<td>66</td>
<td></td>
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</tbody>
</table>

* No mention has been made of the kangaroo because the solitary specimen found plague-infected by Thompson had been involved in an outbreak among animals kept in the Sydney Zoological Gardens.
<table>
<thead>
<tr>
<th>Order</th>
<th>Species</th>
<th>Locality</th>
<th>Remarks</th>
<th>Bibliographical reference</th>
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<tr>
<td><strong>CARNIVORA</strong></td>
<td><strong>Mustela putorius furo</strong></td>
<td>South Africa</td>
<td>Plague was confirmed in ferrets used to catch rats at Port Elizabeth</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td><strong>Mustela sibirica tarsi</strong></td>
<td>Japan</td>
<td>One specimen found infected</td>
<td>63a</td>
</tr>
<tr>
<td></td>
<td><strong>Mustela vison</strong></td>
<td>Canada</td>
<td>Incriminated</td>
<td>41</td>
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<td><strong>Suricata suricatta</strong></td>
<td>South Africa</td>
<td>Suspected</td>
<td>125</td>
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<tr>
<td></td>
<td><strong>Taxidea taxus neglecta</strong></td>
<td>Oregon, USA</td>
<td>Found infested with plague-infected ticks</td>
<td>133</td>
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<tr>
<td><strong>INSECTIVORA</strong></td>
<td><strong>Crocidura olivieri</strong></td>
<td>Senegal</td>
<td></td>
<td>3 :</td>
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<tr>
<td></td>
<td>(Cr. stampflii auctt.), Egyptian giant-shrew</td>
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</tr>
<tr>
<td></td>
<td><strong>Erinaceus sp.</strong></td>
<td>Senegal</td>
<td>Found infected</td>
<td>74</td>
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<tr>
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<td><strong>Suncus murinus</strong></td>
<td>India</td>
<td></td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>(Crocidura caerules auctt.), House-shrew a</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><strong>Sylvisorex gemmeus irene</strong></td>
<td>Belgian Congo</td>
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<tr>
<td><strong>MARSUPIALIA</strong></td>
<td><strong>Monodelphis domestica</strong></td>
<td>Brazil</td>
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<td></td>
<td>(Perameus domesticus Orissai)</td>
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<tr>
<td></td>
<td><strong>Didelphis marsupialis</strong></td>
<td>Brazil</td>
<td>Found infested with infected fleas</td>
<td>99</td>
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<td><strong>PERISSODACTYLA</strong></td>
<td><strong>Equus sp. Donkey</strong></td>
<td>Manchuria</td>
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<td><strong>Presbytis entellus</strong></td>
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<td></td>
<td>50, 51</td>
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<tr>
<td><strong>PRIMATES</strong></td>
<td><strong>Macaca radiata</strong></td>
<td>India</td>
<td></td>
<td>50, 51</td>
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</tbody>
</table>

\( a \) According to Bangxang, shrews also played a role in the plague outbreaks of Thailand.  
\( b \) Strong & Teague, who found donkeys insusceptible to plague infection by inhalation doubted the validity of Fujinami's findings.  
\( c \) According to Sáenz Vera, monkeys were possibly also involved in Ecuador.
### TABLE V. WILD-RODENT FLEAS FOUND NATURALLY INFECTED OR PROVED TO BE SUSCEPTIBLE TO EXPERIMENTAL INFECTION

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Usual hosts</th>
<th>Findings</th>
<th>Bibliographical reference</th>
</tr>
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<tbody>
<tr>
<td><em>Amphipsylla rossica</em></td>
<td>South-east Russia</td>
<td><em>Lagurus</em></td>
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<td>67</td>
</tr>
<tr>
<td>Anomiopsyllus sp.</td>
<td>New Mexico, USA</td>
<td><em>Neotoma</em></td>
<td>S</td>
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<td><em>Lagurus</em></td>
<td>S&lt;sub&gt;P&lt;/sub&gt;</td>
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<td><em>Atyphloceras multiidentatus</em></td>
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<td>S&lt;sub&gt;P&lt;/sub&gt;</td>
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<td><em>Chiastopsylla rossi</em></td>
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<td><em>Graomys</em></td>
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<td>Ctenophthalmus breviatus</td>
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<tr>
<td>Ctenophthalmus cabirus</td>
<td>East Africa, Belgian Congo</td>
<td><em>Arvicanthis</em> and other rodents</td>
<td>E, T</td>
<td>179</td>
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<td>Ctenophthalmus orientalis</td>
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<td>Ctenophthalmus phyrins</td>
<td>Belgian Congo</td>
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<td>South-east Russia</td>
<td><em>Citellus</em></td>
<td>S</td>
<td>189a</td>
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<tr>
<td>Ctenophthalmus wagneri</td>
<td>South-east Russia</td>
<td><em>Arvicola</em></td>
<td>E</td>
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<tr>
<td>Delostichus (Parapsyllus) talis</td>
<td>Argentina</td>
<td><em>Cavia</em></td>
<td>S, T, X</td>
<td>4-7, 178</td>
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<tr>
<td><em>Diamanus montanus</em></td>
<td>USA, western States</td>
<td><em>Citellus</em></td>
<td>S, T, X</td>
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*E* = experimentally infected  
*S* = found naturally infected  
*T* = transmitted plague in the laboratory  
*X* = known to bite man  
*S<sub>P</sub>* = found together with wild-rodent fleas belonging to other species in pools which proved plague positive.
<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Usual hosts</th>
<th>Findings *</th>
<th>Bibliographical reference</th>
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<td><em>Dinopsyllus ellobius ellobius</em></td>
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<td><em>Rhabdomys, Tatera, and other rodents</em></td>
<td>S, X</td>
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<td><em>Dinopsyllus ellobius lypusus</em></td>
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<td><em>Arvicornis and other wild rodents</em></td>
<td>S, T, X</td>
<td>25, 54, 156, 157</td>
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<td><em>Foxella ignota</em></td>
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<td><em>Thomomys</em></td>
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<td><em>Heclopsylla eskeyi</em></td>
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<td><em>Cavia and rats</em></td>
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<td><em>Hystrichopsylla dippiei</em></td>
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<td><em>Citellus and other wild rodents</em></td>
<td>E, T</td>
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<td><em>Listropsylla sp.</em></td>
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<td><em>Microtus Peromyscus</em></td>
<td>E, T</td>
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<td><em>Malarneus telchinum</em></td>
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<td><em>Microtus and other wild rodents</em></td>
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<td><em>Megabothris abantis</em></td>
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<td><em>Megarhigiogesus ditaus</em> (M. longispinus auctt.)</td>
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<td><em>Neotoma Tamias</em></td>
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<td><em>Meringis shannoni</em></td>
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<td><em>Onychomys</em></td>
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<td><em>Monopsyllus wagneri</em></td>
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<td><em>Lagurus, Peromyscus, and other wild rodents</em></td>
<td>S&lt;sup&gt;D&lt;/sup&gt;</td>
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<td><em>Neopsylla inopina</em></td>
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<td><em>Citellus</em></td>
<td>E</td>
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<td><em>Neothyphlocerus rosenbergi</em></td>
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<td>Species</td>
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<td>Findings*</td>
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<td><em>Nosopsyllus consimilis</em></td>
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<td><em>Nosopsyllus laeviceps</em></td>
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<td><em>Nosopsyllus nigiriensis</em></td>
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<td><em>Bandicota</em></td>
<td>E (suspected as vector)</td>
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<td><em>Opisocroslis bruneri</em></td>
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<td><em>Opisocroslis hirsutus</em></td>
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<td><em>Marmota</em></td>
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<td>Venezuela</td>
<td><em>Heteromys</em></td>
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<td><em>Sigmodon, rats</em></td>
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<td><em>Sigmodon, R. norvegicus</em></td>
<td>E, T</td>
<td>57</td>
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<td><em>Polygenis lilargus</em></td>
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<td><em>Sigmodon</em></td>
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<td><em>Polygenis platensis</em></td>
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<td>cisandinus</td>
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<tr>
<td><em>Thrassis acamantis</em></td>
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<td><em>Marmota</em></td>
<td>E, T</td>
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<tr>
<td>acamantis</td>
<td>Canada</td>
<td><em>Marmota</em></td>
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<th>Species</th>
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<td><em>Thrassis acamantis</em> boccetti</td>
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<td><em>Thrassis bacchi</em> bacchi (= <em>Thr. gladiolis</em>)</td>
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<td><em>Thrassis johsoni</em></td>
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<td><em>Lagurus</em> Peromyscus</td>
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<td><em>Thrassis fetus</em></td>
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<td><em>Xenopsylla sp.</em> (conformis group)</td>
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<td><em>Meriones</em></td>
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<td><em>Xenopsylla sp.</em> (<em>X. mycterii</em> auctt.)</td>
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<td><em>Otomyxys</em> and other wild rodents</td>
<td>Probably spontaneously infected</td>
<td>23</td>
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<td><em>Xenopsylla hirsuta</em></td>
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<td><em>Xenopsylla philoxera</em> (<em>X. eridos</em> auctt.)</td>
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<td><em>Lophuronyx</em> and other wild rodents</td>
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<td><em>Xiphiopsylla lippa</em></td>
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<td><em>Lophuronyx</em> and other wild rodents</td>
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IDENTIFICATION OF FLEAS

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British Museum (Natural History),
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Fleas are small (1-8 mm long), wingless insects with a holometabolous metamorphosis (egg—larva—pupa—adult). In the adult stage only, and generally temporarily, they parasitize (i.e., suck blood from) mammals and birds. Their bodies are strongly laterally compressed, usually heavily sclerotized, hairy and shiny, light-rufous to almost black. The larvae are apodous, elongate, strongly haired, and eyeless, but have biting mouthparts; they are not parasitic, but feed on organic matter which they mainly find in their usual abode, the nest of the host. The free pupa is enveloped by a cocoon.

At present, the number of described species and subspecies of fleas is approximately 1,400, but only a minority of these figure largely in the literature on plague.

According to the latest classification, the order of fleas, Siphonaptera, is divided into two superfamilies: Pulicoidea and Ceratophylloidea. The former superfamily, though the smaller, contains most of the better-known fleas, e.g., the genera *Pulex*, *Xenopsylla*, *Echidnophaga*, and *Ctenocephalides*, while the latter superfamily includes *Leptopsylla*, *Nosopsyllus*, and *Monopsyllus*. The two superfamilies together are divided into 17 families.

The object of this note is: (a) to show the way of preparing fleas for study and identification; and (b) to point out pictorially the more important and conspicuous taxonomic characters of fleas so as to make intelligible the illustrated key (see page 650) for the prima-facie diagnosis of those fleas which are predominant in medical literature.

Preparation of Fleas for Study and Identification

The specimens, immediately after their capture, are preserved in small tubes with 70%-80% alcohol or methylated spirit in which they may be stored indefinitely. If storage is to be for a long period, a drop of glycerin

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a For details of this classification, and a key to all families, see Vol. I of the *Catalogue of the Rothschild collection of fleas*, by G. H. E. Hopkins & M. Rothschild, published by the British Museum (Natural History), London.

b When there is any doubt as to the correctness of a determination, specimens should be sent for verification to a specialist; they would be welcomed by The Keeper, Department of Entomology, British Museum (Natural History), Cromwell Road, London, S.W.7.
may be added. Never use a formaldehyde solution as a preservative fluid. Adequate labelling of the tube is most important.

Fleas can only be studied and identified satisfactorily if they are mounted properly. The following method is still the best known. Pass the specimens (which should be kept in the same tube while changing the fluids; pin the label on the cork) successively through the following liquids:

(a) Water; one hour.

(b) 20% solution of potassium hydroxide at room temperature; one or more (generally two) days, till the specimens are yellowish or light rufous and somewhat transparent.

(c) Water; a few minutes.

(d) 5% aqueous solution of glacial acetic acid; half an hour.

(e) Water (renew once); one hour.

If permanent mounts are not desired, the specimens can, at this stage, be studied on a slide in water (under a coverslip) and afterwards returned to a tube containing alcohol. For making permanent mounts, the next steps are the following:

(f) Put the specimens on a clean slide, a short distance from each other (taking care to keep the batches apart). Remove with blotting-paper any superfluous water round the specimens but avoid drying up. Arrange— with the aid of two, very fine, mounted needles, under a dissecting microscope (or a powerful lens) —the legs in proper position, i.e., downwards and free from each other, as in fig. 1 (see page 655). Put on a coverslip and let absolute alcohol run from a small pipette underneath the slip (95% alcohol or dehydrated methylated spirit may be used). Since the alcohol evaporates quickly from underneath the coverslip, some more alcohol should be added regularly. Very large and thick fleas should be covered with half a slide, since the pressure of a coverslip would not be sufficient; leave for half an hour under coverslip.

(g) Take the specimens off the slide and put them in absolute alcohol; one hour or longer.

(h) Oil of cloves (oleum caryophyllorum); at least for some hours, preferably for a day or longer; at first the specimens will float on the oil, but eventually they will sink (if not, they should be pushed into the oil with a fine needle).

(i) Xylol (renew once); about 10 minutes.

(j) Mount the specimens in Canada balsam, dissolved in xylol. Every flea should be put on its right-hand side on the slide and it is advisable not to place more than one specimen under one coverslip.

If one prefers to use euparal as a mounting-medium (because of its colourlessness and more favourable refractive index), the procedure is the same up to (g) inclusive; one then proceeds as follows:

(h) Eucalyptol; one day

(i) A mixture of 1 part of eucalyptol and 1 part of euparal; one day

(j) Mount the specimens in euparal and use this medium also for ringing if so desired.
(k) Dry the slides on a hot-plate or in an oven at 80°-90°C for about half an hour.

(l) After some days, the slides may be ringed, if circular coverslips have been used, with Canada balsam; dry again at 80°-90°C, but only for about 10 minutes.

(m) Label the slides well. The data to be mentioned are: name of the host (preferably its scientific name), locality, altitude, date, and name of the collector; after identification, the name of the flea should be added.

One should always handle fleas with care and, for instance, lift them out of fluids with a lanceolate mounted needle or a similar instrument, so as not to damage specimens through loss of setae.

Illustration of Taxonomic Characters of Fleas

The more important taxonomic characters of fleas have been illustrated in fig. 1-40, in order to facilitate interpretation of the key to the identification of some common fleas (see below). Nearly all the characters mentioned in this key are shown in the figures; the drawings were not all made to the same scale. A list of abbreviations of technical terms used in the key and in figures 1, 2, 5, 6, 11, 15, 25, and 26 is given below.

<table>
<thead>
<tr>
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<th>Explanation</th>
<th>Abbreviation</th>
<th>Explanation</th>
</tr>
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<tr>
<td>a.</td>
<td>abdomen</td>
<td>l.</td>
<td>lacinia</td>
</tr>
<tr>
<td>a.ap.</td>
<td>aedeagal apodeme (also called penisplate)</td>
<td>l.p.</td>
<td>labial palp</td>
</tr>
<tr>
<td>a.s.</td>
<td>antesensial seta</td>
<td>m.</td>
<td>manubrium</td>
</tr>
<tr>
<td>a.st.</td>
<td>anal stylist of female</td>
<td>m.p.</td>
<td>maxillary palp</td>
</tr>
<tr>
<td>ac.s.</td>
<td>acetabular seta</td>
<td>mse.</td>
<td>mesepimeron</td>
</tr>
<tr>
<td>an.</td>
<td>antenna</td>
<td>mses.</td>
<td>mesepisternum</td>
</tr>
<tr>
<td>ap.t IX</td>
<td>apodeme of tergum IX of male</td>
<td>msn.</td>
<td>mesonotum</td>
</tr>
<tr>
<td>b.c.</td>
<td>bursa copulatrix</td>
<td>mss.</td>
<td>mesosternum</td>
</tr>
<tr>
<td>c.</td>
<td>coxa</td>
<td>mte.</td>
<td>metepimeron</td>
</tr>
<tr>
<td>cl.</td>
<td>claw of tarsus</td>
<td>mtes.</td>
<td>metepisternum</td>
</tr>
<tr>
<td>co.</td>
<td>corpus of clasper</td>
<td>mtn.</td>
<td>metanotum</td>
</tr>
<tr>
<td>ct.</td>
<td>ctenidium (a comb of spines)</td>
<td>mts.</td>
<td>metasternum</td>
</tr>
<tr>
<td>ct.a.st IX</td>
<td>distal arm of sternum IX of male</td>
<td>o.</td>
<td>occiput</td>
</tr>
<tr>
<td>d.a.st.IX</td>
<td>ductus bursae copulatrixis</td>
<td>o.s.</td>
<td>ocular seta</td>
</tr>
<tr>
<td>d.b.c.</td>
<td>ductus obturatorius (blind duct)</td>
<td>P</td>
<td>process of the corpus of clasper</td>
</tr>
<tr>
<td>d.o.</td>
<td>ductus seminalis (duct of spermatheca)</td>
<td>p.</td>
<td>pronotum</td>
</tr>
<tr>
<td>d.s.</td>
<td></td>
<td>p.a.st.IX</td>
<td>proximal arm of sternum IX of male</td>
</tr>
<tr>
<td>e.</td>
<td>eye</td>
<td>p.c.t.</td>
<td>pronotal ctenidium</td>
</tr>
<tr>
<td>ep.</td>
<td>epipharynx (an unpaired structure)</td>
<td>pl.r.</td>
<td>pleural rod of mesopleurum</td>
</tr>
<tr>
<td>F</td>
<td>movable process of clasper</td>
<td>r.sp.</td>
<td>reservoir of spermatheca</td>
</tr>
<tr>
<td>f.</td>
<td>femur</td>
<td>s.</td>
<td>sensillum</td>
</tr>
<tr>
<td>fr.</td>
<td>frons</td>
<td>s.f.</td>
<td>spiracular fossa</td>
</tr>
<tr>
<td>g.ct.</td>
<td>genal ctenidium</td>
<td>sp.</td>
<td>spermatheca (also called receptaculum seminis)</td>
</tr>
<tr>
<td>h.</td>
<td>head</td>
<td>st.</td>
<td>sternum (sterna II-X)</td>
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ANNEX
Abbreviation

sti.
t.
t.sp.

Explanation

stipes (also called maxilla or
maxillary lobe)
tergum (terga I-X)
tail of spermatheca (also called
appendix)

2

651

Abbreviation

ta.
th.
ti.
tr.

Explanation

tarsus
thorax
tibia
trochanter

Key to Identification of Some Common Fleas

1.

Without ctenidia . . . . . . . . . . . . . . . .
At least pronotum \Vith a ctenidium . . . . . . .

2
6

2.

Mesosternosome without an internal pleural rod (fig. 8)
Mesosternosome with an internal pleural rod (fig. 1 and 15, pl.r.).

3
4

3.

Frons angulate (fig. 3); genal lobe directed backwards; laciniae very
broad and coarsely serrate; occiput with two setae and usually with
a well-developed lobe in the female only: thorax dorsally narrower
than tergum I (fig. 3); fifth tarsal segment with three pairs of stout,
equally spaced, lateral setae and a smaller fourth pair, and with two
subapical plantar setae (fig.4); claws of tarsus without a large basal
projection (fig. 4); 6 processes F 1 and F 2 not quite reaching to the
middle of process P, the latter having a long and downward-pointing
apical seta (fig. 5); ~ sternum VII and spermatheca (rather variable
in shape) as shown in fig. 6. All zoogeographical regions, most common
in the Ethiopian region ; absent from the cooler areas including, for
example, nearly the whole of Europe, and also absent from the greater
part of the neotropical region ; on poultry and other birds, and on a
large variety of small mammals. A stick-tight flea, i.e., the female
buries its piercing mouthparts in the skin of the host and is not easily
removed.
Echidnophaga gallinacea (Westwood), 1875
Frons smoothly rounded (fig. 7) ; ocular seta placed below the conspicuous eye (fig. 7 ; cf. fig. 13, 14); one small spinelet at the genal
margin (seldom two, sometimes absent); occiput with only one strong
seta; 6 with a broad process P, covering the processes F 1 and F 2
(fig. 9) ; spermatheca with a globular reservoir and a curved tail
(fig. 10) ; outline of sternum VII as in fig. l 0, but usually hardly visible
in mounted specimens. Cosmopolitan; on man and other mammals,
particularly pig and badger.
Pulex irritans Linne, 1758 d

4.

6 antesensilial seta on a marginal cone (fig. 11); process P with seven
or eight strong setae, one of which is stouter than the others and

d The genus Pu/ex contains only two other species : porcinus Jordan & Rothschild. known from the
southern part of the ~earctic region, and sinoculus Traub, from Guatemala.


precedes an elbowed seta (fig. 11); process F longer than process P and slightly bent upwards towards its apex (fig. 11); sternum IX straight, with only a few small setae along its ventral margin (fig. 11); aedeagal apodeme rather narrow, its ventral margin not undulate (fig. 20). ♀ antesensilial seta not marginal (fig. 12); sternum VII as in fig. 12; reservoir of spermatheca for the greater part almost globular and much wider than the base of its tail (fig. 12). Originally an Ethiopian species, but has now been found in many other parts of the world; mainly on rats.

*Xenopsylla brasiliensis* (Baker), 1904

Antesensilial seta of both sexes not marginal (fig. 16 and 17); reservoir of spermatheca either not globular or much smaller than the base of its tail (fig. 17 and 19) . . . . . . . . . . . . . . . . . . . 5

5.  ♂ process P rather broad, with a straight or slightly concave apical margin and a number of fairly slender setae (fig. 16); process F with its tip a little curved downwards, never upwards (fig. 16); sternum IX straight and widened towards the apex which is not much or hardly at all turned upwards (fig. 16); aedeagal apodeme with a concave dorsal margin and broadest pre-apically, its ventral margin partly sinuate (fig. 21).
♀ sternum VII as in fig. 17; reservoir of spermatheca somewhat longer than broad, not broader than the base of its tail, while the lower margin of head and tail are about level (fig. 17). Cosmopolitan; principally on rats.

*Xenopsylla cheopis* (Rothschild), 1903

♂ process P not much widened apically, with six to nine slender setae along its dorso-apical margin (fig. 18); process F longer than process P (fig. 18); sternum IX with a strongly sclerotized ventro-marginal strip, bearing at its apical half a number of minute setae (fig. 18); aedeagal apodeme with a rounded apex, much wider pre-apically than basally (fig. 22).
♀ sternum VII as in fig. 19; reservoir of spermatheca smaller than the basal part of its tail, which is strongly ventricose, its outline being almost spherical (fig. 19). Oriental region, introduced on East African coast; on gerbils, also on rats.

*Xenopsylla astia* Rothschild, 1911

6. With a ctenidium on pronotum only (fig. 23) . . . . . . . . . . . 7
With a ctenidium on head and one on pronotum (fig. 1, 32-34) . . . . . . . . . . . 8

7. No setae in front of the row of three below the eye, but one or a few small ones above this row (fig. 23, 27); first hind tarsal segment, besides the lateral setae, with a row of setae on the outer surface; none of the
apical setae of the second hind tarsal segment reaches the apical margin of the third segment.

♂ sternum VIII vestigial (fig. 25); tergum VIII strongly rounded from behind the last dorsal marginal seta (fig. 24); manubrium shorter than (or at most as long as) the clasper, the latter measured from the apex of the angle formed by the apodeme of tergum IX and the manubrium (this angle is very obtuse) to the pair of acetabular setae (fig. 25); clasper and sternum IX as in fig. 25.

♀ bursa copulatrix long and spirally shaped (fig. 26, b.c.); tail of spermatheca not narrowed towards its apex (fig. 26); posterior margin of sternum VII slanting, not incurved (fig. 26). Cosmopolitan; on a large variety of rodents, chiefly on rats.

_Nosopsyllus fasciatus_ (Bosc), 1800

Head very much like that of _N. fasciatus_ (cf. fig. 23 and 27); no setae of hind tarsal segments I and II extending beyond the segment following.

♂ sternum VIII long and narrow, usually with a very narrow, membranous, pre-apical process (fig. 28); anterior margin of tergum IX forming with the manubrium an angle much smaller than 90°, this angle being rounded off (fig. 28); process P conical, bearing one long and two short setae (fig. 28); process F elongate; proximal arm of sternum IX strongly bent (fig. 28).

♀ posterior margin of sternum VII rounded (fig. 29); head of spermatheca, cylindrical, about three times as long as the tail (fig. 29). Temperate eastern Asia; on rats.

_Monopsyllus anisus_ (Rothschild), 1907

8. Genal ctenidium vertical, consisting of four spines (fig. 32); two of the setae near the frontal angle are spiniform (fig. 32); clasper as in fig. 30, sternum VII and spermatheca as in fig. 31. The tibiae bear at their dorso-posterior margin a row of spiniform setae which form a so-called false comb. Cosmopolitan; mainly on the house mouse, though in certain areas common on rats.

_Leptopsylla segnis_ (Schönherr), 1811

Genal ctenidium horizontal, generally consisting of eight to nine spines (fig. 33 and 34)

9. Head strongly rounded anteriorly in both sexes, first spine of genal ctenidium about half as long as the second (fig. 33); hind tibia with the setae A and B, as shown in fig. 35; ♀ manubrium with a dilated apex (fig. 37); ♀ apical part of the tail of spermatheca long (fig. 39, cf. fig. 40). Cosmopolitan, though rare in the Oriental and Ethiopian regions; mainly on Canidae.

_Ctenocephalides canis_ (Curtis), 1826
Head not strongly convex anteriorly,\textsuperscript{e} hence more elongate than that of \textit{Ct. canis} (fig. 1, 2 and 34); first spine of genal ctenidium about as long as the second; hind tibia only with seta A, seta B absent, though rarely represented by a minute seta (fig. 36); \textgreek{g} manubrium only a little dilated (fig. 38); \textgreek{q} apical part of the tail of spermatheca short (fig. 40, cf. fig. 39). Cosmopolitan; on a large variety of (preferably fairly large) mammals.

\textit{Ctenocephalides felis felis} (Bouché), 1835

\textsuperscript{e} In the subspecies \textit{felis strongylus} (from Africa) and \textit{felis orientis} (occurring from Ceylon to the Admiralty Islands, but not in Australia) the head is short and not unlike that of \textit{Ct. canis}; in \textit{felis damarensis} (south-west Africa) it is more like that of the nominotypical subspecies.
FIG. 1-2. FEMALE CTENOCEPHALIDES FELIS FELIS (BOUCHÉ) *

1. General appearance; 2. Head
* For explanation of the lettering see page 650.
FIG. 3-4. ECHIDNOPHAGA GALLINACEA (WESTWOOD)

3. Head, thorax, and tergum I, ♀; 4. Last hind tarsal segment, ♀
FIG. 5. ABDOMEN OF MALE ECHIDNOPHAGA GALLINACEA (WESTWOOD) *

* For explanation of lettering see page 650.
FIG. 6. TERMINAL SEGMENTS AND GENITALIA OF FEMALE ECHIDNOPHAGA GALLINACEA (WESTWOOD) *

* For explanation of lettering see page 650.
FIG. 7. HEAD, PROTHORAX, AND FORE COXA OF MALE PULEX IRRITANS LINNÉ
8. Mesothorax, ♀; 9. Modified abdominal segment IX, ♂ (i.e., clasper (= tergum IX) and sternum IX)
FIG. 10. TERMINAL SEGMENTS AND GENITALIA OF FEMALE
PULEX IRRITANS LINNÉ
FIG. 11. MODIFIED ABDOMINAL SEGMENTS OF MALE XENOPSyllA BRASILIENSIS (BAKER) *

* For explanation of lettering see page 650.
ANNEX 2

FIG. 12. TERMINAL SEGMENTS AND GENITALIA OF FEMALE XENOPSyllA BRASILIENSIS (BAKER)
FIG. 13. HEAD, PROTHORAX, AND FORE COXA OF MALE XENOPSyllA CHEOPIS (ROTHSCHILD)
FIG. 14-15. XENOPSyllA CHEOPIS (ROTHSCHILD) *

14. Head, ♀; 15. Mesothorax, metathorax, and tergum I, ♂

* For explanation of lettering see page 650.
FIG. 16. MODIFIED ABDOMINAL SEGMENTS OF MALE XENOPSyllA CHEOPIS (ROTHSCHILD)
FIG. 17. TERMINAL SEGMENTS AND GENITALIA OF FEMALE XENOPSyllA CHEOpIS (ROTHSCHILD)
FIG. 18. MODIFIED ABDOMINAL SEGMENTS OF MALE
XENOPSyllA ASTIA ROTHsCHILD
FIG. 19. TERMINAL SEGMENTS AND GENITALIA OF FEMALE XENOPSyllA ASTIA ROTHSCILD
FIG. 20-22. ADEEAGAL APODEME

20. Xenopsylla brasiliensis; 21. Xenopsylla cheopis; 22. Xenopsylla astia
FIG. 23. HEAD, PROTHORAX, AND FORE COXA OF MALE
NOSOPSYLLUS FASCIATUS (BOSC)
Fig. 24-25. *Nosopsyllus fasciatus* (Bosc), Male

24. Tergum VIII; 25. Modified abdominal segment IX and sternum VIII

*For explanation of lettering see page 650.*
FIG. 26-27. **Nosopsyllus fasciatus (Bosc), female**

26. Terminal segments and genitalia  
27. Head

* For explanation of lettering see page 659.
FIG. 28. MODIFIED ABDOMINAL SEGMENT IX AND STERNUM VIII OF MALE MONOPSYLLUS ANISUS (ROTHSCHILD)
FIG. 29. TERMINAL SEGMENTS AND GENITALIA OF FEMALE
MONOPSYLLUS ANISUS (ROTHSCHILD)
FIG. 30. TERMINAL SEGMENTS AND GENITALIA OF MALE
LEPTOSYLLA SEGNIS (SCHÖNHERR)
FIG. 31. TERMINAL SEGMENTS AND GENITALIA OF FEMALE LEPTOPSYLLA SEGNI (SCHÖNHERR)
FIG. 32. LEPTOPYLLA SEGNIS (SCHÖNHERR)
FIG. 33. CTENOCEPHALIDES CANIS (CURTIS)

32. Head and prothorax, ♂;
33. Head and pronotum, ♀
FIG. 34. *CTENOCEPHALIDÉS FELIS FELIS* (BOUCHÉ)

FIG. 35. *CTENOCEPHALIDÉS CANIS* (CURTIS)

FIG. 36. *CTENOCEPHALIDÉS FELIS FELIS* (BOUCHÉ)

34. Head and pronotum, ♀
35. Hind tibia, ♀
36. Hind tibia, ♀
FIG. 37-38. MODIFIED ABDOMINAL SEGMENT IX OF MALE

37. Ctenocephalides canis (Curtis); 38. Ctenocephalides felis felis (Bouché)
FIG. 39. TERMINAL SEGMENTS AND GENITALIA OF FEMALE CTENOCcephALIDES CANIS (CURTIS)
FIG. 40. TERMINAL SEGMENTS AND GENITALIA OF FEMALE CTENOCEPHALIDES FELIS FELIS (BOUCHÉ)
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