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SMALLPOX ERADICATION

Report of a WHO Scientific Group

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WHO SCIENTIFIC GROUP ON SMALLPOX ERADICATION

Geneva, 17 to 24 October, 1967

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SMALLPOX ERADICATION

Report of a WHO Scientific Group

1. INTRODUCTION

The WHO Scientific Group on Smallpox Eradication met in Geneva from 17 to 24 October, 1967. Dr A. M.-M. Payne, Assistant Director-General, opened the meeting on behalf of the Director-General. He stated that, in accordance with a resolution adopted by the Nineteenth World Health Assembly, the Organization had embarked during 1967 on an intensified global programme of smallpox eradication which it was hoped could be completed during the next decade. An Expert Committee on Smallpox had already been convened by WHO in January, 1964 and its report had been published.[1] The task before the present Scientific Group was to review recent advances in knowledge regarding smallpox and smallpox eradication and to consider particularly the methodology and strategy for eradication in the light of the impetus provided the programme by the Assembly resolution. In addition, the Group was asked to give careful consideration to a technical and operational manual for smallpox eradication recently prepared by the Organization.[2]

Dr A. W. Downie was elected Chairman, Dr S. S. Marennikova Vice-Chairman, and Dr M. F. Polak Rapporteur.

2. DEFINITION AND CRITERIA OF SMALLPOX ERADICATION

For the present, eradication of smallpox can be defined as the elimination of clinical infection by variola virus. As there is no carrier state in smallpox and sub-clinical infections are rare, and as no animal reservoir of the disease is known, the absence of clinically apparent human cases may be assumed to signify the absence of naturally occurring smallpox. Proof that smallpox has been eradicated presupposes the presence of a case detection system sufficiently effective to reveal the presence of smallpox in an area before more than two or three generations of cases have occurred. When within an arbitrary period of two years, no endemic case of smallpox has been detected, and outbreaks following imported infection have been promptly controlled, it may be said that a country is " smallpox-free ".

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[1] *Wld Hlth. Org. techn. Rep. Ser.*, 1964, **283**.

[2] *WHO Handbook for Smallpox Eradication Programmes in Endemic Areas*, July 1967.

Because of the ease with which smallpox may be spread from one country to another, the word " eradicated " should be applied only when the disease is absent from an entire continent. At present, smallpox may be said to have been eradicated from Europe, North America and Australia, as well as from Oceania; it is still endemic in Africa, Asia and South America.

On the basis of epidemiological and technical considerations and the experience of many countries that have conducted successful programmes, it is believed that the global eradication of smallpox, as defined, is well within the bounds of possibility.

3. TRENDS IN SMALLPOX CONTROL AND DISEASE INCIDENCE

Whereas smallpox was once endemic throughout the world, its geographic limits have been increasingly constricted. In the first half of the present century, the countries of Europe and North America became smallpox-free through extensive, sometimes compulsory, vaccination and energetic containment measures. In the Americas, a regional eradication programme initiated in 1950 succeeded in virtually eliminating the disease from all countries with the exception of Brazil and Colombia. Also, during this period, several countries of North Africa, Asia and the Eastern Mediterranean area were freed of the disease by intensified vaccination programmes.

The continuing threat of the introduction of smallpox to all countries and the evident success of programmes even in countries with comparatively limited health services led the Eleventh World Health Assembly in 1958 to propose that smallpox eradication be undertaken on a global basis. All endemic countries were asked to initiate programmes; donations of vaccines and other material were solicited from the member governments. Following this resolution, a number of countries began systematic vaccination programmes directed towards smallpox eradication. Notable successes were achieved in Ecuador, Iran, Ivory Coast, Senegal, Sudan and Thailand. These countries all ceased to report endemic smallpox between 1962 and 1966. However, during major programmes in India and Pakistan, initial reductions in smallpox incidence have been followed in the last year or two by significant increases in the numbers of reported cases; smallpox was reintroduced into Peru in 1963 and again became endemic. A number of countries that had carried out systematic programmes of vaccination experienced repeated smallpox introductions from neighbouring endemic countries.

The experience of the first eight years of the global eradication programme clearly demonstrated the importance of developing and co-ordinating programmes on a regional and global basis. The need for technical

assistance in the planning and execution of programmes and in the establishment of surveillance and assessment procedures was apparent. Many programmes were hampered by inadequate transport and vaccination equipment; supplies of good-quality freeze-dried vaccine were insufficient and vaccine produced in a number of laboratories failed to meet the required standards (see section 8).

Having considered these problems and having carefully reassessed the prospects for smallpox eradication, the Nineteenth World Health Assembly in 1966 unanimously reaffirmed the intention of the Member States to pursue global eradication of smallpox, declared this programme to be a major objective of the Organization, appropriated funds specifically for this purpose, and called on member governments and other organizations to provide additional support. In response to this request, voluntary contributions to the Organization as well as bilateral and other support for the programme were increased significantly compared to previous years. Two-thirds of the countries in endemic areas planned to initiate or intensify eradication programmes by late 1967. Most of the remaining countries planned to begin programmes during 1968.

3.1 World morbidity and mortality

Although the reported numbers of smallpox cases represent only a portion of the total that occur, the data do serve to convey information about general trends in incidence of the disease in different parts of the world.

The annual total of reported cases by continent since the inception of the eradication effort in 1959 is shown in Table 1. No definite trend in the world incidence is evident. After an initial decline during 1960, the number

TABLE 1

ANNUAL NUMBER OF SMALLPOX CASES BY CONTINENT, 1959–1966 *

	1959	1960	1961	1962	1963	1964	1965	1966	1967 [a]
Africa	16 307	16 823	26 060	24 329	16 863	12 506	16 784	14 127	9 554
Asia	71 309	39 843	53 957	63 616	98 784	43 537	39 145	50 494	50 958
Europe	26	47	24	136	129	—	1	71	3
North America	—	—	—	1	—	—	—	—	—
South America	5 490	7 931	9 026	9 718	7 151	3 398	3 515	3 092	426
Oceania	—	1	—	—	—	—	—	—	—
Total	93 132	64 645	89 067	97 800	122 927	59 441	59 445	67 784	60 941

* Consolidated data compiled by WHO from various sources.

[a] Until July 15.

of reported cases rose progressively to 123 000 in 1963. An abrupt drop in reported cases occurred in 1964 but subsequently the incidence again increased. Information to date indicates that the total for 1967 will be considerably greater than that for 1966.

Asia usually accounts for two-thirds or more of all cases; of those reported from Asia in 1966, virtually all were in India, Indonesia and Pakistan (Table 2). Variations in the world incidence of smallpox depend largely on variations in incidence in these three countries; the latter variations, in turn, reflect the characteristic five- to seven-year cycles of smallpox incidence and the relative success of smallpox control in these countries. In Indonesia and Pakistan, reported cases have increased steadily since 1964; in India, a sharp increase in the number of cases was observed beginning late in 1966 and extending into 1967. Afghanistan and Nepal also report small numbers of smallpox cases each year, but reporting in these two countries tends to be incomplete. Burma recorded only a single case in 1966, during the third year of an intensive eradication programme.

In the Americas, most cases are reported by Brazil, with only scattered cases and outbreaks in adjacent countries. Although there has been a progressive but irregular decline in recorded cases from Brazil since 1962, it is impossible to draw conclusions regarding trends because of incomplete reporting.

In Africa, smallpox is endemic in most countries south of the Sahara. The total number of reported cases has varied little in recent years, sharp decreases in incidence in Zambia and the Ivory Coast, for example, being balanced by increases in Niger, Nigeria and Tanzania. In a number of African countries, the rates per 100 000 population in 1966 were higher than anywhere else in the world (Table 2 and map).

Reported case-fatality rates are higher in Asia than in Africa or America. Even when allowance is made for unreliable reporting and incomplete registration of deaths, the difference appears to be real. This may be explained by differences in the comparative prevalence of variola major and variola minor viruses and/or the existence of virus strains of intermediate virulence.

As the eradication programme proceeds, accurate reporting becomes ever more important in measuring progress towards eradication of the disease. As reporting improves during the early phases of the programme, reported figures may, for a period, suggest a higher incidence of smallpox despite definite progress toward eradication. This factor will need to be borne in mind.

3.2 Review of selected smallpox programmes

The Group reviewed reports of smallpox programmes in several countries in an attempt to determine significant factors that might have been

TABLE 2

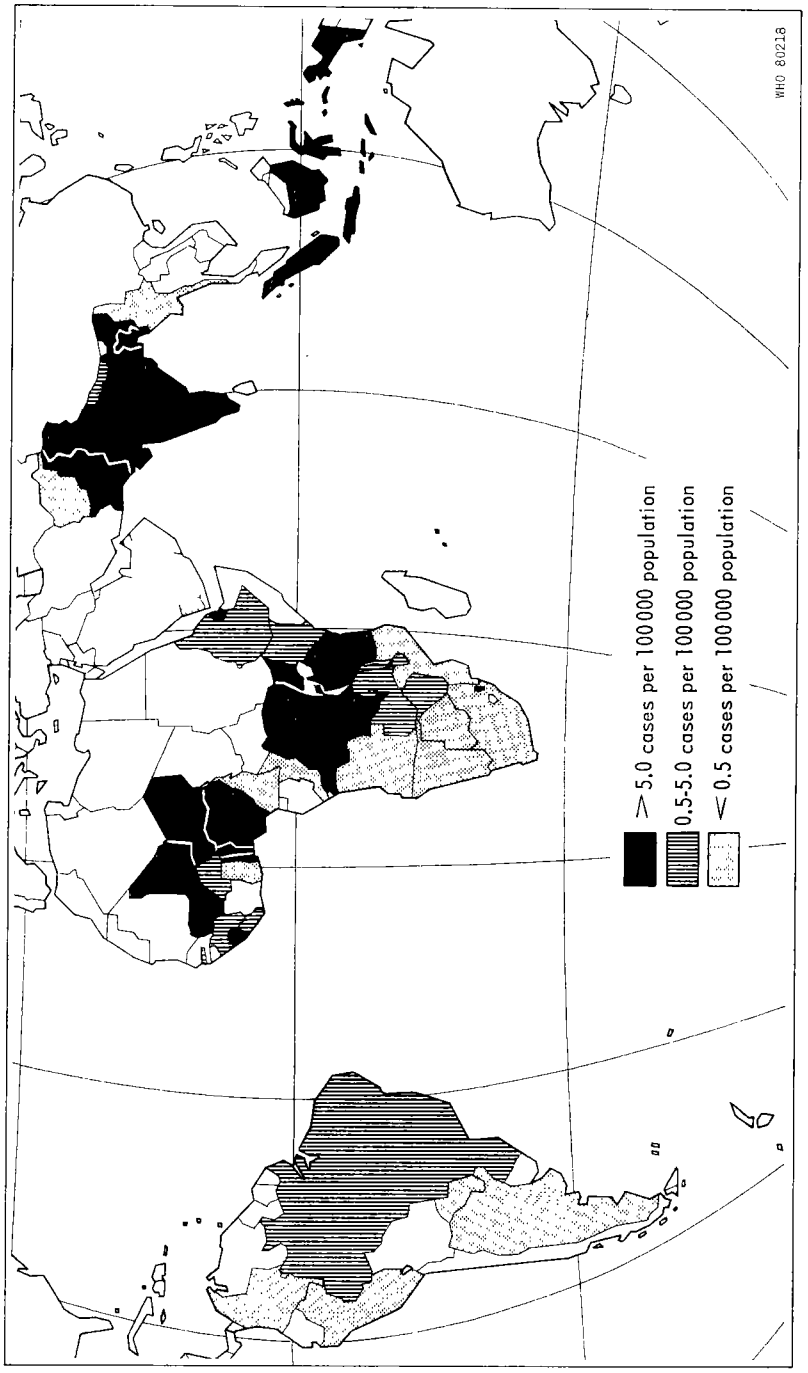
SMALLPOX INCIDENCE IN AFRICA, ASIA AND SOUTH AMERICA, 1966 *

Continent and country	Population (in thousands) [a]	No. of cases	Cases/100 000
AFRICA—WEST			
Cameroon	5 103	3	0.06
Congo (Republic of)	826	2	0.20
Dahomey	2 300	530	23.04
Gambia	324	3	0.92
Ghana	7 537	13	0.20
Guinea	3 420	56	1.63
Liberia	1 041	32	3.07
Mali	4 485	281	6.26
Niger	3 237	1 147	35.43
Nigeria	56 400	4 924	8.73
Sierra Leone	2 240	293	13.08
Togo	1 603	199	12.41
Upper Volta	4 750	76	1.60
AFRICA—EAST AND SOUTH			
Angola	5 084	3	0.06
Burundi	2 800	363	12.96
Congo (Democratic Republic of)	15 300	1 913	12.50
Ethiopia	22 200	228	1.03
French Terr. of the Afars and the Issas	81	52	64.20
Kenya	9 104	159	1.75
Malawi	3 900	88	2.26
Mozambique	6 872	19	0.28
Southern Rhodesia	4 140	33	0.80
Swaziland	288	29	10.07
Tanzania	9 990	3 207	32.10
Uganda	7 367	591	8.02
Zambia	3 600	63	1.75
ASIA			
Afghanistan	15 227	75	0.49
Burma	24 229	1	0.004
India	471 624	32 616	6.92
Indonesia	102 200	11 296	11.05
Nepal	9 920	385	3.88
West Pakistan	47 000	2 935	6.24
East Pakistan	54 000	3 181	5.89
Yemen	5 000	1	0.02
SOUTH AMERICA			
Argentina	22 022	21	0.09
Brazil	78 809	3 039	3.86
Colombia	17 482	8	0.05
Paraguay	1 968	5	0.25
Peru	11 298	19	0.17

* Consolidated data compiled by WHO from various sources.

[a] Mid-year estimate for 1964.

INCIDENCE OF SMALLPOX IN ENDEMIC COUNTRIES, 1966



responsible for their success or failure. Several countries have instituted smallpox control programmes with complete or partial success, followed by recrudescence of the disease.

A programme was initiated a century ago in Indonesia consisting of systematic primary vaccination of infants 3-6 months old four times a year in each area and revaccination of 10% of the general population annually. This was initially arm-to-arm vaccination, but later animal lymph was used. However, success was not attained until the 1920-1940 period, after several changes had been made in the programme. Supervision was made easier by the separate organization of primary vaccination and revaccination. Full-time regional public health physicians were appointed with whom the vaccinators worked out their vaccination programmes. In this way, the regional medical officers knew exactly where each vaccinator was working each day and could make unannounced inspections. Not only were vaccination sessions checked, but the district medical officers also assessed the work of each vaccinator by determining the percentage of children with scars of primary vaccination, and the success of revaccination by observing take rates. At this time also, a dried vaccine was developed for use in remote areas. By 1937, smallpox had ceased to be an endemic disease. However, during the Second World War, there was a complete breakdown in the programme of vaccination and revaccination. In the post-war period, smallpox was present in adjacent countries, and was reintroduced in 1947; in 1951, over 100 000 cases were reported. Endemic smallpox has persisted up till the present time.

Review of smallpox control and eradication programmes in East Pakistan, Argentina, Iran, Ghana and India disclosed several common features that appeared to be mainly responsible for their relative success or failure.

First and most important, failure appeared to be associated with inadequate supervision and assessment. Programmes that failed normally showed the following shortcomings: (*a*) supervisory personnel did not check at the family level to assure that broad coverage by vaccination of the population was being achieved, (*b*) supervisors were too burdened by other responsibilities to give more than nominal supervision, (*c*) inadequate provisions for travel and expenses, and (*d*) disinclination of supervisors to undergo the inconvenience of field work. As a consequence, in certain programmes the number of vaccinations reported sometimes exceeded the total number of persons in the population. Sometimes this was due to fraudulent reporting or to repeated revaccinations of readily accessible groups of the population, such as schoolchildren. Lack of assessment permitted the assumption that a large proportion of the population was being successfully vaccinated when follow-up would have shown that vaccination coverage was not good and that take rates were low because of faulty technique or impotent vaccine.

Secondly, concealment of cases and lack of prompt notification resulted in epidemic spread when otherwise the health department could easily have

contained the infection. In some areas, disease (and vital statistics) reporting was the responsibility of village policemen, ignorant of health matters. Laws requiring hospitalization of smallpox patients, while designed to contain the infection, resulted in concealment when the facilities provided were still those of the obsolete pest house.

Thirdly, failure to use the more stable freeze-dried vaccine, particulary in tropical and sub-tropical areas, frequently resulted in failure of an otherwise well-conducted programme.

Fourthly, in several instances, a well performed initial programme was not sustained. This permitted the accumulation of a susceptible population composed of children born after the initial vaccination programme and of immigrants. As smallpox persisted in other countries of the Region, there were repeated introductions resulting in localized outbreaks in border areas or urban centres where immigrants congregated. In the absence of an adequate surveillance-containment programme, smallpox was re-established in some countries as an endemic disease.

Finally, well organized campaigns have made it possible for some countries, such as Iran, to maintain a smallpox-free status. This was achieved by virtue of close supervision and continued assessment followed by assiduous case finding and containment.

4. CLINICAL SMALLPOX

4.1 Classification and frequency of the principal types of variola major

A detailed description of the various clinical types of smallpox is not attempted here, but to facilitate subsequent discussion, subdivision into five main types is considered. These types merge into each other but, broadly considered, the prognosis, diagnostic difficulties and possible infectiousness vary with the type of case. The old subdivision of clinical cases according to the density of the focal eruption has been shown by Dixon [1] and by Rao [2] to have less prognostic value than the nature and evolution of the eruption; consequently the broad classification adopted here is based on the latter features. The five types are (1) haemorrhagic, (2) flat, (3) ordinary, (4) modified, and (5) variola sine eruptione.

1. *Haemorrhagic.* In this type, the pre-eruptive illness, which may be prolonged, is marked by fever, intense headache and backache, restlessness, a dusky flush or sometimes pallor of the face, extreme prostration and generally a toxic appearance. There is little or no remission of fever throughout

[1] Dixon, C. W. (1962) *Smallpox*, London, Churchill.

[2] Rao, A. R., cited in Ramsay, A. M. & Emond, R. T. D. (1967) *Infectious diseases*, London, Heinemann.

the illness. In the more fulminating form, haemorrhagic manifestations appear on the second or third day as subconjunctival bleeding, bleeding from the mouth or gums, petechiae in the skin, epistaxis, haematuria and, in women, bleeding from the vagina. In this type of case, death often occurs suddenly between the fifth and seventh day of illness when only a few insignificant maculopapular lesions of the focal eruption are present. In patients who survive for 8-10 days, the haemorrhages appear in the early eruptive period, in and between the focal lesions, and the elements of the focal rash are flat and do not progress beyond the vesicular stage.

2. *Flat.* This type also exhibits a severe pre-eruptive illness with fever persisting through the eruptive phase. The focal lesions are slow to mature and the vesicles tend to be flat, so that they project little from the surrounding skin and are soft and velvety to the touch. Among patients who survive, the lesions resolve without pustulation. Cases with haemorrhages into the base of the lesions may not be readily distinguishable from late haemorrhagic cases. Whether the eruption be confluent, semi-confluent or discrete, the prognosis is bad.

3. *Ordinary.* This type comprises the majority of cases, both in the vaccinated and unvaccinated, and corresponds to the classical description of smallpox. The pre-eruptive illness of varying severity may last 2-4 days, but the temperature usually drops as the focal eruption develops. At this time, the patient feels much better, but fever may return with the development of the pustular stage, depending on the severity of the rash. The focal lesions appear as macules on the third or fourth day of illness; they rapidly become papular and fluid collects in them, usually within 24 to 48 hours. The vesicles may be umbilicated and the contents usually become turbid in a day or so. Drying up of the pustules and scabbing generally begin from the eighth to tenth day of the eruption. The lesions are usually sharply raised from the skin, the papules are hard, and vesicles and pustules tend to be tense and firm to the touch. The eruption shows a centrifugal distribution; lesions in any given area are at the same stage of development. In general, the severity of the clinical picture parallels the extent of the rash.

4. *Modified.* In this clinical type, which occurs mostly in vaccinated patients, the modification relates to the character and development of the focal eruption; crusting is complete within 10 days. The pre-eruptive illness may be severe and is not necessarily of short duration, but secondary fever during the evolution of the eruption is usually absent. The skin lesions tend to evolve more quickly, are more superficial, and may not show the uniformity characteristic of the typical smallpox eruption. The lesions are often few in number, but even when they are numerous they show some pleomorphism and evolve rapidly.

5. *Variola sine eruptione.* After a usual incubation period, this type produces a febrile illness such as occurs in the pre-eruptive phase of the

ordinary type of smallpox. However, no eruption follows. This type of illness is uncommon and is seen in well-vaccinated individuals; its nature can be confirmed only by antibody studies or rarely by virus isolation.

The relative frequency of these five clinical types as observed by Rao in a series of several thousand cases in Madras is shown in Table 3. These figures illustrate what has long been accepted, namely the milder nature of the disease in the vaccinated, but bring out one feature not generally known—the proportion of haemorrhagic cases is approximately the same among vaccinated persons as among those unvaccinated. The distribution of cases by age and type in the vaccinated and unvaccinated (Table 4) illustrates the loss of immunity with the passage of time after vaccination in infancy and the effect of vaccination in modifying the disease. Further analysis of the data shows that the haemorrhagic form was more common in adults than in children and especially common among pregnant women, irrespective of previous vaccination. The haemorrhagic type of infection is usually associated with a more intense and prolonged viraemia, but the factors in pregnancy that favour this type of infection are as yet unknown.

4.2 Variola minor (alastrim)

This form of smallpox generally shows a milder clinical picture than variola major. The febrile pre-eruptive illness has approximately the same duration but is usually less severe. Haemorrhagic cases are extremely rare (Marsden [1] recorded only three among thirteen thousand cases) and the case-fatality rate is usually less than 1.0%. The focal eruption has the same distribution and evolution as in variola major, although the lesions may be somewhat more superficial and the eruptive phase, from the first appearance of rash until scabbing, tends to be a day or two shorter. As in variola major, lesions are usually present in the mucosa of the mouth from the onset of the skin eruption. Even when the eruption is confluent on the face and accompanied by considerable oedema the patient does not have the toxic appearance seen in variola major with a similar type of rash. Nevertheless it is impossible, on clinical examination, to distinguish alastrim from the milder types of variola major; the identity of the infecting virus can be determined only by laboratory investigation.

4.3 Incubation period and period of infectiousness

The incubation period—that is, the time elapsing between infecting contact and onset of fever—is fairly constant at 11-14 days, although in a few well-documented cases, it has been as short as 8-9 days and occasionally as long as

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[1] Marsden, G. P. (1948) *Bull. Hyg. (Lond.)*, **23**, 735.

TABLE 3

FREQUENCY OF DIFFERENT TYPES OF SMALLPOX IN VACCINATED AND UNVACCINATED PATIENTS, MADRAS, 1961–1967 *

Clinical variety	*Vaccinated patients*			*Unvaccinated patients*		
	No. of cases	Frequency (%)	Case mortality (%)	No. of cases	Frequency (%)	Case mortality (%)
Haemorrhagic	112	3.3	93.7	84	2.4	96.4
Flat	46	1.4	72.5	230	6.5	96.5
Ordinary	2355	69.8	2.8	3126	88.9	30.2
Modified	862	25.5	0.0	78	2.2	0.0
Totals	3375	100.0	6.0	3518	100.0	35.5

* Data reported by A. R. Rao, as quoted in Ramsay, A. M. & Emond, R. T. D. (1967) *Infectious diseases*, Heinemann, London.

TABLE 4

FREQUENCY OF CLINICAL TYPES WITH REFERENCE TO AGE AND VACCINIAL STATUS, MADRAS 1961–1967

	Age	No. of cases	Distribution of cases by age (%)	Incidence of clinical types (%)			
				Haemorrhagic	Flat	Ordinary	Modified
Vaccinated	0– 4	94	2.8	1.1	5.3	57.4	36.2
	5–14	387	11.5	1.8	0.5	59.2	38.5
	15–44	2677	79.3	3.6	1.2	71.1	24.1
	45+	217	6.4	3.7	3.2	77.9	15.2
Unvaccinated	0– 4	2077	59.0	1.1	8.1	87.9	2.9
	5–14	851	24.2	0.9	3.9	94.3	0.9
	15–44	525	14.9	8.0	4.2	86.3	1.5
	45+	65	1.9	16.9	10.8	70.8	1.5

16-17 days. The available data indicate that the incubation period in alastrim is the same as in variola major.

It has been commonly assumed that smallpox patients are infective from the onset of fever, but eminent authorities such as Haygarth, Ricketts and Stallybrass did not consider patients infectious during the pre-eruptive illness. The period of maximum infectiousness of the smallpox patient, as determined by clinical and epidemiological investigation, appears to be from the third to the eighth day after onset of fever, that is during the week following development of rash. Virological examinations of patients and their environments have shown that the largest quantities of virus are recovered from the mouth, circumoral skin and bedclothes from the 6th to the 9th day of illness. Lesions in the mouth and upper respiratory tract, from which the infecting virus is mainly excreted, do not usually appear until the beginning of the eruptive phase. Virus is present in the skin lesions and crusts but this source appears to be less important in the transmission of infection.

4.4 Inoculation smallpox (variolation)

Inoculation smallpox (variolation), as described by writers in the eighteenth century, was generally milder than the usual sort, but acute fulminating cases were occasionally encountered. However, inoculation smallpox is as contagious as that naturally acquired, owing presumably to the enanthem that accompanies the generalized focal eruption on the skin, and therefore the practice of inoculation is to be condemned.

Smallpox is occasionally transmitted by accidental inoculation through the skin to doctors, nurses, or laboratory workers and the disease conforms in type to that described in the early years of inoculating the smallpox in the eighteenth century. The incubation period is usually shorter than that following infection by the usual route and a local lesion appears at the site of inoculation before constitutional symptoms appear about the seventh or eighth day.

5. PATTERNS OF TRANSMISSION

5.1 Reservoir of infection

Man has been considered to be the only natural host of variola virus, and no convincing evidence has yet been presented that any other species is or has been infected in nature. Reports of disease resembling smallpox occurring in monkeys or orang-utans and in reputed association with human disease have not been supported by conclusive virological evidence. Cynomolgous monkeys have been infected with aerosols of variola virus in the

laboratory, resulting in a mild, self-limited, generalized disease; the disease has also been transmitted to healthy cage-mates. It is pertinent, however, that areas where cynomologous monkeys abound have become smallpox-free, with no recrudescence of disease. There have been no reports of " smallpox in monkeys " in the highly endemic Indian subcontinent, where large numbers of monkeys have lived in the closest association with man.

A new poxvirus " monkeypox " was first recognized in 1958 among captive monkeys. This virus is distinguishable from other viruses of the group; vaccinia affords cross protection. Epidemic disease with monkey deaths has occurred in some monkey colonies, but no human infections have been reported amongst the numerous human contacts. This virus is considered to be comparable to other species-specific poxviruses, such as rabbit pox, sheep pox, etc., posing no apparent threat to man.

Thus, it is felt that the only natural source of variola virus is man and that there is no evidence of a hidden animal reservoir.

5.2 Transmission within family groups

As with most infectious diseases, the household is the fundamental epidemiological unit in smallpox; transmission is most frequent in the close association of the family group. Nevertheless, not all exposed, unvaccinated family members become infected when an infectious case occurs in the household. For example, during an epidemic in West Africa, 25 of 36 unvaccinated family contacts, and, in a study in India, only 37 of 103 unvaccinated family contacts, became infected.

The probability of transmission depends on the infectiousness of the case, the susceptibility of the contact, and the physical, social and environmental factors that may influence exposure.

Although, as noted, all cases of smallpox are potentially infectious from the development of the enanthem until the last scab separates from the skin, most family contact infections take place within the first week and few after the second week. Various studies suggest that transmission results predominantly from virus shed from the respiratory tract; this virus may be recovered from the skin, clothing and bedding of patients. Transmission may take place following resuspension of infectious material from these sources.

The infectiousness of the ill person is primarily related to the extent and severity of the enanthem in the mouth and throat and this, in turn, depends on the clinical character of the case. The likelihood of transmission depends upon the frequency and intimacy of contact between case and susceptible associate and this is largely related to whether the patient is ambulatory, confined to bed or removed to hospital. In a recent study in Madras, India, of transmission in 254 families, including 1249 contacts, 8.2% of whom were unvaccinated, the following observations were made :

(*a*) When the index patient was unvaccinated, nearly three times as many persons were infected as when the index patient had been vaccinated.

(*b*) Transmission was greatest from cases of intermediate severity (flat and ordinary), and markedly lower form the mildest cases (modified). In this series, no household transmission took place from haemorrhagic cases presumably because of their early removal to the hospital.

(*c*) Excluding haemorrhagic cases, transmission was greater from index patients who died than from those who survived.

Transmission is high from patients with flat and ordinary forms of smallpox because they have severe and extensive enanthems; patients with modified forms are less frequently sources of transmission, despite the fact that they are ambulatory, presumably because of the limited shedding of virus. Despite this, the mildest cases may be important in a smallpox eradication programme. Being fully ambulatory and not shunned by their associates, persons with such mild infections may be the unsuspected means of carrying infection from one locality to another.

The most important factor determining whether or not an exposed contact will become infected is his degree of immunity. Recovery from smallpox almost always protects the individual from infection; vaccination greatly reduces susceptibility, but the degree of protection depends upon the interval between last vaccination and exposure. However, the protection conferred by vaccination appears to be less in pregnant than in non-pregnant women. There is no evidence that nutrition, general health status, and intercurrent infection influence the likelihood of smallpox infection.

In the home, frequency of transmission is related to crowding and intimacy of contact. Transmission is more likely when case and contact share the same bed. The association of high smallpox attack rates with lower socio-economic status is a reflection of household crowding and a lower vaccination rate. The greater the number of unvaccinated persons in a family the greater is the risk of intra-household transmissions, even to the vaccinated.

In the Madras study, the age and sex of both index cases and contacts influenced the likelihood of transmission. The secondary attack rate was higher from index cases 5 to 14 years of age than from those younger or older, and it was higher from boys in this age group than from girls. Among unvaccinated contacts, the secondary attack rate was highest in those under 5 years of age.

The interplay of case, contact and environment may result in an irregular and sporadic pattern of transmission in households. Not all susceptibles may be infected and those infected may not be infected at the same time. As many as four or five generations of cases have been observed in a household. Thus, vaccination of family contacts is strongly indicated, even if the primary case has been discovered some weeks after onset of illness.

5.3 Transmission within populations

The factors involved in the transmission of smallpox within the community are, in general, the same as those involved in intrafamilial spread. Spread from community to community is usually caused by an infected individual who has travelled while incubating the disease. Mild illnesses that do not immobilize the patient may be important in the dissemination of infection. Movements of groups (nomads, refugees, seasonal migrants, pilgrims, etc.) afford greater opportunity for contacts between infectious cases and susceptible individuals, but the movements of individuals may be of greater importance in maintaining the endemic disease. Cities, and particulary their slum areas, offer many opportunities for personal contacts and tend to maintain a smouldering reservoir of smallpox. In many parts of the world, urban unskilled labour is provided by villagers who act as a permanent link between rural and urban centres. When ill, these people frequently return to their villages, either for want of care or from a desire to die at home. Urban centres often constitute the reservoir for dissemination of infection to the periphery, a process facilitated by the movement of people transporting produce to markets and bazaars. While the disease can persist for a time in peripheral foci, susceptibles tend to be exhausted after a limited period and infection stops.

Environmental factors and social practices have a very great influence on the spread of infection within the community. Congestion encourages or assures inter-family contact. Household-to-household spread within the village depends heavily on the mobility of children, who more often form the unvaccinated part of the population. Minority groups, especially if they hold anti-vaccination beliefs, provide even greater opportunities for contacts between infectious cases and susceptible persons. Local customs of caring for the sick influence patterns of exposure, and where relatives from neighbouring villages help in ministering to the ill or in burying the dead, intercommunity dissemination is promoted.

Hospitals and other medical establishments where patients congregate have frequently served as points of contact between susceptible persons and infectious smallpox cases. Transmission has most frequently occurred before smallpox was diagnosed and the patient isolated; this is particularly liable to occur with haemorrhagic cases. Infection has also occurred when isolation procedures have been inadequate.

Passive transfer of virus on the clothing of an immune person who has been in close contact with an infectious case, or on fomites, particulary the clothing and bedding of a smallpox patient, is well established as a means of spreading the disease. The laundry worker, in non-endemic as well as endemic areas, is at high risk of exposure.

5.4 Miscellaneous factors in transmission

5.4.1 *Contaminated houses*

The room or house occupied by a smallpox patient may be made safe by proper disinfection. If this is not done, the possibility of infection persists for some time. There are documented instances in which infection has occurred in nurses, cleaners, and others handling contaminated materials or occupying rooms vacated by patients several days previously. Virus has been recovered in the laboratory from pillow covers for up to six weeks after use by patients. It appears that a contaminated house may be a source of infection at least for some weeks. Variola virus present in scabs may remain viable for years, but virus in this situation appears to be less infectious.

5.4.2 *Airborne dissemination over distances*

It has been difficult to isolate virus from air samples collected in the vicinity of patients. Furthermore, experience has shown that when intermingling of patients is prevented smallpox rarely occurs among other patients housed in nearby hospital wards. The great dilution of virus in air and its rapid inactivation when exposed to sunlight makes airborne dissemination unlikely in transmission beyond the immediate environment of the patient.

5.4.3 *Other methods of transmission*

Transmission through ingestion of contaminated food has not been reported. Infection or mechanical contamination of blood-sucking arthropods has not been demonstrated. Contamination of flies attracted to the open lesions of smallpox patients has been shown to occur, but there is no evidence that this is important in transmitting disease. Finally, animals such as dogs, cats and birds may be contaminated on the fur or feathers or by eating scabs, but transmission resulting from such contamination has not been demonstrated.

6. IMMUNOLOGY

The value of vaccination as a prophylactic measure against smallpox depends on the antigenic similarity of vaccinia and variola viruses. Laboratory tests have failed to detect qualitative serological differences, and cross-protection tests in both man and animals show almost complete reciprocal immunity. This was firmly established soon after Jenner's introduction of vaccination when many observers regulary failed to infect with

smallpox matter those who had been successfully vaccinated. Similarly, those who have recovered from smallpox will for a few years fail to give a successful take on vaccination.

There is, however, no such thing as permanent immunity. Resistance to reinfection after a primary infection, whether by variola or vaccinia virus, slowly lessens with the passage of time and there is wide individual variation in the rate of this loss. Moreover, whether or not infection occurs in the person with waning immunity depends on the dose of the challenge virus, be it the concentration of virus in the vaccine used for revaccination or the intensity of exposure to a smallpox patient.

6.1 Antibody response

Antibodies may be measured by tests on serum for neutralizing, complement-fixing, precipitating or haemagglutinin-inhibiting activity. These tests have different significance as measures of immunity and in the diagnosis of suspected smallpox. The neutralization test measures the anti-infective power in a serum and therefore is probably the best serological indication of the immunity of the donor. Neutralizing activity persists in diminishing amount for many years. The other tests measure antibody to soluble viral antigen and haemagglutinin; these antibodies are not identical and their concentrations may differ widely. The complement-fixing, haemagglutinin-inhibiting and precipitating antibodies persist in significant amounts for a much shorter period than does neutralizing antibody. Consequently, detection of their presence in serum may be useful as a diagnostic test in suspected cases of smallpox.

6.1.1 *Antibody response after smallpox* [1]

Neutralizing antibody usually appears by the sixth day of illness—except in the very severe forms of the disease—reaches a high concentration in convalescence, and persists for many years. The antibody concentration is generally considerably greater in patients who have been previously vaccinated.

Complement-fixing antibody appears about the eighth day of illness and usually reaches a higher titre than is found after vaccination. Normally, this antibody does not persist at significant concentrations for more than a year.

Precipitating antibodies are detectable in the sera of convalescent smallpox patients by the precipitation-in-gel technique for at least 4-6 weeks. Such antibodies are rarely found in post-vaccination sera.

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[1] Downie, A. W. & McCarthy, K. (1958) *J. Hyg. (Lond.)*, **56**, 479.

Haemagglutinin-inhibiting antibodies usually appear about the fifth or sixth day of illness. Like complement-fixing antibodies, they reach a maximum in 2-4 weeks and diminish to low concentrations within a year.

6.1.2 *Antibody responses after primary vaccination* [1]

Neutralizing antibody is not usually detectable until after the tenth day. Concentrations attained are low in comparison with those following smallpox or revaccination, but neutralizing antibodies persist for many years. Multiple, as opposed to single, insertions have not been shown to influence the final antibody concentration but after generalized vaccinia the concentration may be high. Complement-fixing antibodies can be detected towards the end of the second week in only a proportion of cases, depending on the sensitivity of the test used. Precipitating antibodies are not demonstrable. Haemagglutinin-inhibiting antibodies appear from about the tenth day in most cases, reach a maximum after 3-4 weeks and fall to low levels within a year.

6.1.5 *Antibody responses after revaccination* [1]

A major reaction following revaccination results in a marked increase in neutralizing antibody detectable towards the end of the first week. Concentrations attained are on an average about ten times greater than after primary vaccination.

Complement-fixing antibody after successful revaccination may also appear rather more quickly than after primary vaccination, but despite a good neutralizing antibody response there may be no detectable complement-fixing antibody. Similarly, the haemagglutinin-inhibiting antibody response is variable and may be absent when there is a marked increase in neutralizing antibody.

About half the revaccinated persons with an equivocal reaction show a significant increase in antibody.

6.2 Duration of immunity

From the data available, it is impossible to state accurately the duration of immunity in any individual or population. Available information is derived from epidemiological evidence, such as the incidence of smallpox in endemic areas or during epidemics in those previously vaccinated or revaccinated as compared with that in the unvaccinated. The resistance to revaccination may also be a measure of immunity, but vaccination with a highly potent vaccine is probably a more severe challenge than exposure to smallpox infection within the family; for example, vaccination of those who have had smallpox only a few years before may result in a major reaction.

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[1] McCarthy, K., Downie, A. W. & Bradley, W. H. (1958) *J. Hyg. (Lond.)*, **56**, 466.

6.2.1 *Duration of immunity after smallpox*

Epidemiological observation indicates that after an attack of smallpox immunity to the disease is virtually life-long. However, second attacks, though rare, do occur, usually after an interval of many years.

6.2.2 *Duration of immunity after primary vaccination*

In persons vaccinated only in infancy, the incidence of smallpox increases with age as contrasted to the incidence in the unvaccinated, thus reflecting the waning of immunity. The data indicate protection of a high order for the first 4-5 years, slowly diminishing thereafter. The occurrence of a few cases within four years after vaccination is attributable to individual variation in the durability of protection. The relatively frequent occurrence of smallpox in endemic areas among vaccinated adults similarly indicates a loss of immunity to infection; but the milder nature of their disease as compared with disease in the unvaccinated shows that some residual immunity persists.

Similarly, the difficulty of producing a major reaction on revaccination diminishes with the passage of time, but even after 10 or 20 years the vaccine required to produce a high percentage of takes has to be at least 5-10 times more potent than that required for the same proportion of primary vaccination successes.[1] Vaccine that meets the minimum requirements published by WHO [2] (see section 8) will elicit a satisfactory proportion of revaccination responses in all populations.

6.2.3 *Duration of immunity after revaccination*

Not enough is known about the occurrence of smallpox in those who have been successfully revaccinated to permit a decision about the duration of immunity; but the greater concentration of antibody resulting from revaccination and the likelihood of its longer persistence suggest that immunity to infection is more durable than that following primary vaccination.

6.3 Significance of antibody levels

6.3.1 *Relationship of circulating antibody to protection against disease*

There are few data directly relevant to this question but on the basis of experience with other viral diseases high concentrations of neutralizing antibody may be expected to correlate with protection against infection by smallpox virus.

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[1] Espmark, J. A. (1965) *Acta path. microbiol. scand.*, **63**, 97.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1965, **323**.

6.3.2 *Relations of circulating antibody to vaccination response*

Revaccination within a few years of primary vaccination, when neutralizing antibody is still present, is often successful. This suggests that circulating antibody does not prevent cutaneous infection if a sufficiently potent vaccine is used. Failure to take on repeated revaccination with potent vaccine is usually related to high antibody concentration, assuming good technique.

7. STRATEGY OF SMALLPOX ERADICATION

The objective of the smallpox eradication programme is achieved by reducing the prevalence of smallpox to the point where transmission of the disease is terminated. Normally, as a first step, this requires systematic mass vaccination with potent freeze-dried vaccine to reduce the prevalence of disease. Simultaneously, however, a case-detection and reporting system should be established or improved to permit prompt application of containment measures, thereby interrupting further transmission. Both these aspects of the eradication programme must receive adequate attention but perhaps greater weight should be given to mass vaccination in highly endemic, poorly vaccinated areas, shifting the emphasis to case-detection and reporting as endemic disease declines and a more satisfactory state of herd immunity is achieved.

The detection of every case of smallpox that occurs is nearly impossible when the disease incidence in a country is high. As the incidence is reduced, however, the need to detect and trace the source of each infection becomes of paramount importance. This requires an alert and comprehensive reporting network as well as the epidemiological capacity to investigate all suspect cases and the clinical laboratory capacity to confirm or refute the diagnosis. The reporting of " no cases " must be as dependable a routine as that for reporting the occurrence of cases.

Theoretically, the interruption of smallpox transmission could be accomplished by the simultaneous immunization of the whole community. Since this is not feasible, mass vaccination is unlikely to result in eradication if it is the only method employed. Mass vaccination serves to reduce the volume of variola virus transmission but other techniques must be employed to eliminate the residuum. When the number of cases occurring in an area is reduced to relatively few a year, case detection and containment by vaccination of contacts, isolation of patients and disinfection will be effective in eliminating residual foci of infection or sporadic importation.

After a large proportion of the population has been covered by mass vaccination, and while surveillance and outbreak-containment teams are seeking out and eliminating the remaining pockets of infection, an effective immune barrier must be maintained. This is accomplished by systematic

primary vaccination of newborns and revaccination of those previously protected.

The programme of smallpox eradication can be considered as progressing through a series of phases, commencing with the institution of the systematic mass vaccination programme and continuing until continental eradication has been achieved. Three general phases can be broadly defined: attack phase (phase I), consolidation phase (phase II), and maintenance phase (phase III). These terms can be applied to regions of a country or to the country as a whole; in most programmes, progression from phase to phase may be expected to occur in some regions earlier than in others. Only when all regions have progressed to a higher phase can the country as a whole be considered to have entered that phase. The criteria that distinguish areas in the three phases are as follows :

Attack phase (phase I): Endemic areas with an incidence of smallpox of 5 or more cases per 100 000 population per year and with less than 80% of all segments of the population showing scars of primary vaccination.

Consolidation phase (phase II): Areas with an incidence of smallpox of less than 5 cases per 100 000 and in which over 80% of all segments of the population show scars of primary vaccination.

Maintenance phase (phase III): Areas free of endemic smallpox for more than 2 years but geographically situated in endemic continental areas (at present, Africa, Asia and South America).

The nature and intensity of the component activities of the eradication programme will vary from phase to phase. They are summarized in Table 5.

In summary, the strategy of a smallpox eradication programme consists of mass vaccination to reduce the volume of transmission, maintenance vaccination to consolidate and continue this accomplishment, surveillance to detect each sporadic case that occurs, and containment activities to prevent further spread. The surveillance network must be so organized that the reporting of zero cases truly reflects the complete eradication of the disease.

8. FREEZE-DRIED SMALLPOX VACCINE

Only freeze-dried vaccines are recommended for use in the endemic countries, because liquid vaccine undergoes rapid deterioration at ordinary air temperatures, particularly in tropical and subtropical countries. The requirements for smallpox vaccine, last reviewed by a WHO Expert Group in 1965,[1] relate to potency, stability to heat, and freedom from pathogenic bacteria.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1965, **323**.

TABLE 5

PHASES OF THE ERADICATION PROGRAMME

	ATTACK PHASE (PHASE 1)	CONSOLIDATION PHASE (PHASE 2)	MAINTENANCE PHASE (PHASE 3)
VACCINATION	Systematic mass vaccination	Continuing maintenance vaccination	Continuing maintenance vaccination
SURVEILLANCE	*Reporting*—prompt and regular reporting of smallpox by all existing health facilities. *Field investigation*—epidemiological investigation of major outbreaks throughout the country and of all cases in areas where systematic mass vaccination has been done.	*Reporting*—extension of case-detection system to assure that all suspected smallpox cases are reported. *Field investigation*—prompt epidemiological investigation of all cases to establish sources of infection and to exclude the possibility of unreported cases.	*Reporting*—continuation of case-detection system to ensure that all suspected smallpox cases are reported. *Field investigation*—investigation of each case as an emergency by an epidemiologist.
LABORATORY	Establishment of techniques and methods for the submission and examination of specimens for confirmation of diagnosis.	Study of specimens from all isolated cases and representative samples from each outbreak.	Study of specimens from every suspect case.
CONTAINMENT	Localized, intensive vaccination in communities where cases or outbreaks occur; isolation of cases, if feasible, and disinfection.	Vaccination and observation of case contacts; isolation of cases and appropriate disinfection; localized, intensive vaccination in the community.	Vaccination and observation of case contacts; isolation of cases and appropriate disinfection; localized, intensive vaccination in the community.

8.1 Stability

8.1.1 *Before reconstitution*

The vaccine is required to have a titre of more than 10^8 pock-forming units per ml on the chorioallantois; after incubation at 37°C for 4 weeks, it should retain 10% or more of its initial infectivity and have a titre of more than 10^8 pock-forming units per ml. Properly prepared dried smallpox vaccine should readily meet this requirement since good batches have been stored at 37°C for months with little loss in potency. Such dried vaccine stored at 10°C or lower retains its potency for several years. For this reason, vaccines in central depots and distributing centres should always be kept at 10°C or lower.

8.1.2 *After reconstitution*

Once dried vaccine has been reconstituted, it is no more stable than liquid vaccine, and therefore should be used only on the day that it is reconstituted. Reconstituted vaccine exposed to sunlight loses its potency in a few hours; in the field, therefore, the vaccine should be kept in the shade.

8.2 Potency

8.2.1 *Testing at time of production*

The virus content should be titrated by pock count on the chorioallantois of the chick embryo. A standard vaccine of known potency, comparable to the International Reference Preparation of Smallpox Vaccine,[1] should be titrated at the same time in chorioallantoic membranes of the same batch of embryos to ensure that the assay is sensitive enough. The potency of the vaccine is expressed as pock-forming units/ml.

The pock-counting method may be replaced by parallel line assays in cell cultures, when the potency of the vaccine is compared with that of the standard preparation. This type of assay gives more information than the pock-counting methods usually applied, but it is more complex to use and cannot be recommended, except to experienced workers who have carefully studied it.

8.2.2 *Testing during field use*

The final criterion for the potency of a vaccine in the field, if the technique of vaccination is satisfactory, will be determined by the take rate in those vaccinated. A suitable vaccine should give over 95% major reactions on primary vaccination and a figure approaching 90% on revaccination after 10 or more years. If the performance in the field is less than this, the method of storage of the vaccine, the skill of the vaccinator and the technique of vaccination should be checked. If these are satisfactory, the vaccine should be withdrawn and retested for potency in the laboratory.

8.2.3 *Relationship of titre to frequency of vaccine take*

Several well conducted studies have been made in recent years on this subject. Unfortunately, it is not possible to make a close comparison between the data from different studies, because of differences in the production strains of vaccine virus, differences in the vaccination techniques used and possibly in the skill of the vaccinators, and differences in the method of titrating potency. However, certain points have emerged. The concentra-

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1965, **323**.

tion of vaccine required to give more than 95% takes on primary vaccination of children and young adults is more than 10^7 pock-forming units per ml, but to achieve the same success rate in infants less than 10 weeks old, a concentration 5-10 times as great may be required. The potency of vaccine required to give a satisfactory take rate in revaccination is 5-10 times that needed for primary vaccination.

8.3 Requirements of vaccine for jet injection

Freeze-dried vaccine containing up to 5 non-pathogenic bacteria per ml of reconstituted vaccine has been used in recent extensive trials without untoward effect. Nevertheless, the aim should be to produce and use bacteriologically sterile vaccine. Vaccine to be used for jet injection must be as stable as conventional dried vaccine. The results of vaccination trials employing one particular make of jet injector suggest that a reconstituted vaccine diluted to a virus content of at least $10^{6.5}$ pock-forming units per ml gives satisfactory take rates when 0.1 ml is injected intradermally.[1] Some other jet injectors, however, have not given satisfactory take rates with this concentration of virus.

8.4 Comparative immunogenicity and reactogenicity of different vaccine strains

Of the various vaccinia virus strains that have been used in the preparation of smallpox vaccine, some produce more severe local and systemic reactions than others. Some strains also produce more necrotic skin lesions in laboratory animals and are neuropathogenic when administered by the intracerebral route.[2] The strains used in vaccine production should be those that have been found to give satisfactory immunity in man without producing severe local lesions or marked systemic disturbance. It is important that further studies be made to determine which strains best meet these conditions.

8.5 Comparative merits of vaccine production in chick embryo, in tissue culture, and in animal skin

Vaccines produced from virus grown in chick embryos and in tissue culture have the advantage that they may be readily produced free from bacterial contamination; they appear to be particulary suitable for administration by jet injection. However, it is more difficult to prepare a stable, dried vaccine from the chick embryo and from tissue culture. Moreover, after prolonged passage in chick embryos or tissue culture, the immunogenicity is impaired; consequently, it is recommended that the seed inoculum should not have had more than five successive passages in chick embryos

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[1] Millar, J. D. & Roberto, R. R. (1964) *Bol. Ofic. sanit. panamer.*, **57**, 537.

[2] Marennikova, S. S. & Tašpulatov, G. M. (1966) *Vop. Virus.*, **11**, 266.

or tissue culture. The requirement that chick embryos or chick tissues used in the preparation of smallpox vaccine must be free from avian leucosis viruses imposes limitations on the production of such vaccine.

9. VACCINATION AGAINST SMALLPOX

Vaccination consists of introducing into the basal layers of the epidermis sufficient amounts of vaccinia virus to infect susceptible cells and produce a local lesion. Vaccinia usually consists of localized skin involvement, with mild systemic symptoms. When the lesion heals, a scar is left. More virus is required to induce infection in the previously vaccinated individual than in unvaccinated persons, even if his immunity has waned. Vaccine administered subcutaneously has relatively little immunizing capacity.

9.1 Techniques of vaccination

The preferred site for vaccination is the outer aspect of the upper arm over the insertion of the deltoid muscle, or slightly behind the midline. This area is usually easily accessible, the lesion that develops is less likely to be traumatized than elsewhere and less likely to become macerated from body moisture. Local dress customs may require the use of other, less suitable sites. Unless the selected site is obviously dirty, no treatment of the skin is needed. Disinfectants inactivate vaccinia virus more effectively than they kill skin bacteria; cleansing may create slight abrasions which can then become infected with vaccinia virus to form " satellite pocks ". If the area is obviously dirty, it should be gently wiped with a cloth or cotton wool moistened with water, and permitted to dry.

Vaccine may be introduced by a variety of techniques, but only a few have proved satisfactory. In general, the multiple-pressure method and vaccination by jet injection give the highest percentage of successful vaccinations.

In the *multiple-pressure* technique, a small drop of vaccine is placed on the skin and a series of pressures is made within the smallest possible skin area (not more than $\frac{1}{4}$ inch or 6 mm in diameter) with the side of a sharp needle held tangentially to the skin. The pressures are made with the side of the needle, not the point; 30 strokes are completed in 5 to 6 seconds, with an up-and-down motion perpendicular to the skin, using sufficient pressures to induce a trace of blood to appear at the vaccination site.

The use of the newly developed bifurcated needle facilitates multiple-pressure application of vaccine. The bifurcated needle is dipped in the vaccine and then touched to the surface of the skin. Vaccination is performed

by 15 strokes through the droplet. This needle is economical of vaccine; 5-10 times as many vaccinations can be performed than by conventional techniques with a given amount of vaccine. However, sterilization methods must be such that the needles are dry when dipped into the vaccine to avoid its dilution. The efficacy of this needle for multiple-puncture rather than multiple-pressure vaccination is being studied.

The *scratch method* gives excellent results when properly performed by experienced workers. A single linear scratch not more than ¼ inch or 6 mm long is made through the vaccine with a suitable instrument. The scratch should be deep enough to cause a trace of blood to appear at the vaccination site within 30 seconds. The vaccine is rubbed into the scratch with the side of the needle.

Various instruments for vaccination have been devised over the years; they may produce excessive trauma and severe reactions, thus causing fear of vaccination and non-cooperation. As commonly used, they carry a risk of transmission of diseases, such as hepatitis.

Jet injectors are available which inject vaccine through a very small orifice into the superficial skin layers without hazard of transmitting infection from one individual to another. The injected dose is 0.1 ml but not all the fluid penetrates the skin; intradermal deposition should be confirmed in each case by sight or touch. Only those injectors that have proved effective under field conditions should be used.

No dressing should normally be applied at the time of vaccination; if the lesions should ooze later, a loose, non-occlusive dressing protects the clothing.

Although insertions at more than one site increase the likelihood of successful vaccinial infection, there may be an increase in the frequency of systemic reactions. Since severe reactions are deterrents to full co-operation, a single insertion is preferable, except in contacts of a smallpox patient.

9.2 Classification and interpretation of vaccination results

Successful *primary vaccination* evolves through the typical stages of vaccinia, with the development of a vesicle after 3-5 days. The fluid becomes pustular and the lesion achieves its greatest size after 8 or 9 days, going on to form a scab which separates at 14-21 days leaving a typical vaccination scar. On examination at one week, a vesicle or pustule is present.

Successful *revaccination* is one in which multiplication of vaccinia virus occurs and is manifest, on examination after one week (6-8 days), by a vesicular or pustular lesion, or an area of definite induration or congestion surrounding a central lesion which may be a scab or ulcer. This is termed a " major reaction "; all other findings are termed " equivocal reactions ". A " major reaction " is seen when there has been virus multiplication

although virus multiplication may also occur in some " equivocal reactions ". " Major reactions " include primary takes and reactions that were previously classified as " primary types of revaccination reaction " and " vaccinoid reactions ". An " equivocal reaction " may occur in the previously vaccinated individual whose high level of immunity inhibits virus multiplication; such a reaction may also follow insertion of inactivated vaccine or a potent vaccine applied with poor technique. The cutaneous response that may be observed in an " equivocal reaction " is a consequence of hypersensitivity to vaccinial protein. The hypersensitivity is recognizable by the appearance of papules (and not infrequently vesicles) with maximal skin involvement in the first 48 hours after vaccination. This allergic reaction may decline after 48-72 hours and may be completely gone by one week; or, if vesiculation has occurred, a scab may still be present at one week. After equivocal reactions, immunity cannot be assumed, although about half of such reactions are followed by antibody increases. If an equivocal reaction is observed, vaccination should be repeated.

9.3 Age at first vaccination in endemic areas

Infants can be successfully vaccinated during the neonatal period. Although the presence of passively transferred maternal antibodies makes infection more difficult,[1] it can be achieved if the vaccine meets the standards of potency referred to in section 8.2. Reactions in the child may be less marked than usual if the mother has been vaccinated, but the antibody response is comparable to that in older children. Although immunity may wane more rapidly following neonatal vaccination, the poor prognosis of the infant with smallpox warrants the earliest possible protection. Revaccination at one year of age provides an effective booster and should be done routinely.

9.4 Frequency of revaccination

The frequency of revaccination depends on the risk of exposure. In endemic areas, the general population should be revaccinated every 3-5 years. For those at risk of heavy exposure, such as certain hospital personnel (physicians, nurses and attendants in fever wards, laundry workers, etc.), adequate protection requires annual revaccination.

9.5 Complications of vaccination

Vaccination carries a finite risk to the patient, and the benefits must be weighed against this risk. While it protects against a potentially epidemic disease with a 40-50% mortality among the unvaccinated, vaccinia virus is

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[1] Espmark, J. A. & Rabo, E. (1965) *Acta paediat. scand.*, **54**, 149.

slightly but definitely pathogenic for man. It normally produces a localized necrotic lesion, regional lymphadenopathy, and systemic symptoms of malaise and fever. Very rarely, a more serious illness occurs which may be fatal.

Vaccinia virus can be transferred from the vaccination site by the fingers to mucous membranes or to abraded skin surfaces and initiate *autoinoculation* lesions. These may result in scarring, but except for the very rare instance where pre-existing excoriation permits infection of the cornea, this complication has no serious consequences. A transient viraemia may occur resulting in *generalized vaccinia*, with the appearance of vesicles 5-10 days after vaccination; prognosis is favourable. These cases are sometimes indistinguishable from a group of self-limited *exanthematous reactions* in which virus is not present; urticarial, morbilliform, and multiform eruptions are seen, often with vesiculation. Of greater concern is the danger of virus dissemination to the eczematous skin of either a vaccinated person or the contact of a vaccinated person, causing *eczema vaccinatum*. While many cases resolve satisfactorily, some, particulary those infected by contact, tend to have extensive lesions and require vaccinia immune globulin for recovery. *Progressive vaccinia* (or vaccinia necrosum) is very rare, occurring in immunologically defective individuals; the defect may be congenital, or it may be the consequence of tumours of the reticulo-endothelial system (leukaemia, multiple myeloma, etc.), therapy with immunosuppressive or corticosteroid drugs, or radiation therapy. *Post-vaccinial encephalitis* is a serious complication occurring 7-15 days after primary vaccination and occasionally revaccination. Although the majority of patients recover completely, a certain proportion die, and a few of those who recover have residual neurological sequelae.

Vaccination complications are usually seen only after primary vaccination but occasionally occur following revaccination after an interval of 10 or more years. In areas where smallpox has disappeared, it has been argued that this preventive measure produces greater morbidity and mortality than does the target disease. This takes no account of the fact that absence of smallpox depends on a high state of immunity among travellers, coupled with a basal immunity in the population. Although reactions are sometimes serious, significant reactions are relatively rare. For example the frequency of *eczema vaccinatum* in England and the USA has been estimated to be between four and eighty per million vaccinations. The incidence of postvaccinial encephalitis has been reported as 3 per million in the USA and 15 per million in England; it is more common after primary vaccination of older children and adults. Progressive vaccinia occurs in about 1 per million vaccinations. Only 20 cases of vaccinia of the foetus have been reported since 1932. Fatal complications of vaccination in the USA and England have occurred in approximately 1-10 per million primary vaccinations.

9.6 Contraindications to vaccination in endemic areas

In endemic areas, the risk of acquiring fatal smallpox far exceeds the danger of vaccination complications. In general, serious acute illness is the only recognized contraindication to vaccination. Vaccination of a critically ill individual may be contraindicated on general medical grounds; in addition, there is a further risk that death may be wrongly attributed to vaccination and may jeopardize the vaccination campaign. While the eczematous individual is at high risk from vaccination, the risk may well be greater from accidental contact inoculation, and thus eczema should not be considered a contraindication in endemic areas. Pregnancy is not a contraindication to vaccination in endemic areas.

9.7 Simultaneous administration of several vaccines

The concurrent administration of several immunizing agents, either mixed or separately, is sound public health practice, provided that there is no interference with the immunological responses, reactions are not intensified, and risks not increased. Smallpox vaccine has been given simultaneously, but at different sites, with diphtheria, pertussis, tetanus, typhoid, and inactivated poliomyelitis vaccines; the vaccines maintained their full efficacy and there was no intensification of reactions. Smallpox vaccine has been given to newborns at the same time as BCG, but in the other arm, with responses to each vaccine comparable to those after single vaccines only. Concurrent administration of smallpox and 17-D yellow fever vaccines does not result in any increase in adverse reactions; when given as separate injections, results have been comparable to those following administration of the agents singly, but when given combined, a reduction in the percentage developing yellow fever antibodies has been noted. When smallpox vaccine has been administered at the same time as measles vaccine, either mixed or separately, febrile reactions have been somewhat more marked, but serological responses have been unaltered. In the programme in West Africa, millions of smallpox and measles vaccinations have been performed concurrently without significant problems. Smallpox vaccination has also proved to be compatible with simultaneous oral poliomyelitis vaccination.

Thus, smallpox vaccine has been given concurrently with all the usual immunizing agents with no resulting difficulties. In fact, in one study, a mixture of smallpox, yellow fewer (17-D strain) and measles vaccines was administered without complications but, as noted above, with some reduction in serological response to yellow fever.

10. PLANNING AND EXECUTION OF MASS VACCINATION PROGRAMMES

10.1 Principles and objectives

The objective of a mass vaccination programme is the successful vaccination of the entire population of an area in the briefest possible time and in co-ordination with similar programmes in adjacent areas.

The minimum *overall coverage* is difficult to specify because requirements differ according to the density of population and the frequency of interpersonal contacts. In general, when 80% of each village, social, sex and age group are immunized, smallpox transmission should cease. In densely populated areas, however, higher proportions may be required. To achieve an 80% immune population necessarily requires that more than this proportion be vaccinated. If, for example, 90% successful takes result from vaccination, then 89% of the population would need to be vaccinated in order to achieve an 80% immune status ($90\% \times 89\% = 80\%$).

It must, however, be kept in mind that although 80% of the population in an area are initially immunized by a vaccination team, the influx of migrants from less well vaccinated rural areas, new births, and the natural waning of immunity will decrease the overall protection in the community. In areas where there are many migrants, frequent systematic vaccination programmes may be required to maintain satisfactory immunity.

In establishing the geographical sequence for the vaccination programme, account must be taken of the relative incidence of smallpox and of population density, past efficacy of vaccination programmes, and of geographical, administrative and political factors. As a general rule, the programme should be started in currently endemic areas in order to establish effective barriers to transmission as early as possible in the campaign and to eliminate rapidly sources of infection. Particular attention should be given to those at greatest risk of contracting the disease. In most areas, these are children of all ages, and the lower economic " floating " population. Special efforts should be made to ensure that everyone has had a primary vaccination.

The timing of the vaccination activities must be adapted to the habits and customs of the people and be sufficiently rapid to avoid extensive dilution of the target populations by people moving from one area to another. When possible, vaccination programmes should fit in with the seasonal timing and routes followed by migratory groups. Daily scheduling should be based on locally variable daily movements of the people for occupational and social reasons. The best coverage is obtained by concentration of the vaccination teams in such a way as to achieve saturation of a limited area, rather than their dispersal to many separate sites. A wave-like progression of vaccination achieves most complete coverage. For the

country as a whole, the aim should be to complete systematic vaccination within 3 years. Campaigns in adjacent areas, districts, provinces and countries must be co-ordinated, since extensive population movements often take place across political borders.

An integral part of the mass vaccination programme is the *concurrent* assessment of coverage and effectiveness of immunization. This permits early recognition and prompt correction of technical flaws, which, if undetected, would result in partial or total failure of the programme (see section 11 on assessment).

Smallpox outbreaks should not be permitted to disorganize routine vaccination schedules. They should instead be handled by specially designated containment or " fire-fighting " teams, which must respond promptly.

10.2 Type of programme

A mass vaccination programme may be so organized that the vaccinees come to the vaccinators (collecting point programme) or that the vaccinators go to the vaccinees (house-to-house programme). Each method has advantages and disadvantages; these must be weighed and a decision made to employ one or both methods depending on local factors.

The principal advantage of the collecting point method is its speed, conservation of manpower, and relative ease of supervision; its principal disadvantages are a somewhat poorer coverage of the population and the need for more detailed planning, advance notification, and a more complex organization.

Well-trained vaccinators employing the multiple-pressure or scratch technique can vaccinate one or two hundred people per hour. With the jet injector, the rate of vaccination is normally limited only by the rate at which vaccinees move past the vaccinating point. Since speed is the principal advantage of the collecting point method, record-keeping must be reduced to the absolute minimum (see section 11).

In the house-to-house method, vaccinators working singly or in pairs go from house to house vaccinating all residents in the community. Appropriate simple records are made. This method normally provides more complete coverage of the population and assessment is somewhat easier. However, the number of vaccinations performed per day by each vaccinator is sharply reduced. Supervision is difficult but can be effective if supervisors make frequent, unannounced spot checks. The greatest difficulty with house-to-house campaigns is that they frequently miss nomadic, migratory, and transient populations.

A highly effective approach may be to combine both methods to realise the advantages of each. One week after a collecting-point mass vaccination, house-to-house assessment is made to determine adequacy of cover-

age and effectiveness of vaccination, to vaccinate those missed in the initial campaign and to revaccinate those without a major reaction.

The local health services, ultimately responsible for control of all disease, must at all times play a key role in the eradication programme. In addition to continuing and gradually intensifying their normal vaccination programme, they should participate in planning and executing the local mass vaccination programme. They should have an important part in health education and publicity, and will, in many instances, provide a base of operations for mobile teams.

Where a malaria eradication programme is in progress, useful assistance may be obtained in the form of maps, census data, and experience in reaching the people. However, integration of malaria eradication and mass vaccination programmes has not yet proved practicable.

Other immunizing agents may be given or disease control activities may be undertaken by smallpox vaccination teams, provided that the teams are appropriately augmented and that the basic requirements of an effective smallpox vaccination campaign are not compromised.

10.3 General administrative elements

The smallpox eradication programme must have a full-time central directorate responsible for planning, training, support, supervision, coordination, analysis and evaluation. Its authority should be clearly defined and commensurate with its responsibilities. Lines of communication and command to subordinates and field units should be clearly specified and understood. Financing requires the establishment of realistic annual budgets.

The eradication programme should be supported by appropriate legislation, including, as a minimum, laws to compel vaccination, case reporting and isolation, and to prohibit variolation.

10.4 Programme Execution

The best plans, an ideal organization, and the availability of adequate supplies of potent vaccine are of no avail unless the programme is properly executed. This requires the recruitment of a qualified staff, proper training of each man in his responsibilities, and the development of an administrative system that assures a high performance of all personnel. These objectives require skill in personnel management. Each man, to contribute his best, must be sure he will be fairly treated and that he will receive a living wage, promptly paid; superior performance must be recognized and there must be opportunities for promotion. Workers must be reasonably secure in their job and provided with adequate accommodation and food while in the field.

Requirements must be constantly anticipated and arrangements assured for the continuing provision to the field workers of the necessary supplies and working equipment. Unforeseen eventualities, both in supply and field activities, will occur, calling for prompt and decisive action. Clear delegation of authority permits these crises to be handled immediately at the lowest level, so that the programme does not falter.

Success in smallpox eradication requires proper handling and application of vaccine, intensive coverage of all sections of the population, effective case finding, and prompt containment of outbreaks. Regular reports from the field furnish a measure of progress but provide, at best, a broad picture, and they can be woefully misleading. Ultimately, success depends on continuing close supervision to ensure conscientious performance by the field workers. Successful programmes have been characterized by having national health administrations whose members have personally and frequently observed all phases of field operation.

10.5 The use of vaccination certificates

Vaccination certificates may be useful in augmenting the overall vaccination state of the community if they are required for such purposes as travel, employment, school etc. Minimum identification should include the name, age and sex of the vaccinee. However, recording of the minimum necessary data requires considerably more time than the act of vaccination itself, and the cost of providing certificates may not be insignificant. For most purposes a recent vaccination scar is a far more satisfactory record.

11. ASSESSMENT

Continual assessment is required by supervisors at all levels to ensure that the planned vaccinations are being performed, that an adequate proportion of all sections of the population is being vaccinated, and that major reactions are being induced in at least 95% of primary vaccinees and in an adequate proportion of revaccinees.

A simple recording system is recommended for tabulation of numbers of vaccinations. The simplest form is a " tally sheet " on which each individual vaccinated is recorded by a mark by age group, vaccination status and sex. In the past, in some areas, efforts have been made to register by name in a permanent record, each individual vaccinated. Although such a system should provide a complete record of all persons with their vaccination status and should facilitate complete vaccination coverage, preparation of such records has proved costly in manpower and has rarely worked

efficiently. Alternatively, in house-to-house vaccination programmes, tally sheets might be completed for each household.

A simple count of vaccinations has proved helpful in charting the progress of a programme but it does not provide information about the adequacy of coverage in epidemiologically important groups. Since schoolchildren, for example, are most accessible, vaccinators are able to report large numbers of vaccinations by repeatedly vaccinating these children, whereas preschool children remain poorly vaccinated. Failure to observe vaccination responses may result in the widespread use of impotent vaccine or improper techniques leaving large numbers unprotected.

Although various approaches have been successfully employed in the assessment of vaccination coverage and vaccine takes, it is evident that the most successful have relied on the principle of continual systematic appraisal of the work of the vaccinators by independent assessors and by supervisory staff. When vaccination coverage is found to be inadequate or the number of takes insufficient, methods should be reviewed and appropriate action taken, including, if necessary, replacement of ineffective personnel.

After vaccinators have completed work in an area, assessors who are administratively independent of the team should examine all or a sample of the population to determine the proportion in different age groups and segments of the population who have been vaccinated and to determine the proportion of successful vaccinations. Examination of both primary vaccinees and revaccinees is desirable, but variations in classification of revaccination responses by different observers and variations in immunity in different areas make interpretation difficult. Examination of revaccination responses requires that the assessment team visit the area during the sixth to eighth day after vaccination, that is, at a time when such responses can be satisfactorily interpreted.

For operational purposes, responses of pre-school children to primary vaccinations are most useful and can be readily interpreted over 7 to 21 days. If cutaneous responses are observed in more than 95% of primary vaccinees, the vaccine and the technique can be assumed to be satisfactory. The assessor should again vaccinate all those without major reactions whom he observes.

Periodic assessment of the vaccination status of lower socio-economic areas, urban regions with considerable floating populations, as well as other areas in the country should be undertaken as a regular part of the programme. This may be done most simply by examination of different age groups and segments of the population for vaccination scars. Periodic assessment of this type by supervisory personnel at all levels of the programme can serve as a valuable stimulus to more effective work by vaccination teams.

Final assessment of the smallpox eradication programme as a whole must, of course, be based on the presence or absence of smallpox cases as reported by adequate surveillance.

12. SURVEILLANCE

A most important part of every country's eradication programme is surveillance. This includes schemes for the prompt detection and reporting of all suspected smallpox cases, the immediate investigation and confirmation of such cases, and the institution of appropriate containment measures. Concurrent analysis and interpretation of reported data and the dissemination of this information to responsible local, national and international authorities is essential.

All cases,whether thought to be variola major or variola minor (alastrim), should be considered as smallpox and reported as such. Every effort should be made to encourage the prompt reporting of all clinically suspect cases. Prompt reporting permits immediate measures to be taken for case confirmation and containment of infection. Should the case not prove to be smallpox, the erroneous provisional report may be easily revised, while failure to report cases until they are fully confirmed may permit extensive spread of infection before action is taken.

At the inception of each programme, posts should be designated or established to make regular reports on the occurrence of smallpox. In addition to the normal telegraphic or telephonic notification now generally practised, weekly or bi-weekly reports should be submitted *whether or not* cases have been observed. This provides assurance that reporting posts are functioning. The data about each case should be limited to essentials. Name, age, sex, locality, date of onset, death (if applicable) and vaccination status before exposure should be adequate. With this information, seasonal and geographical relationships can be defined; concentrations of cases within particular age groups may be detected; or cases among recent vaccinees noted. These analyses may serve to guide or redirect vaccination and containment activities. The collection, consolidation and interpretation of information concerning smallpox should be performed concurrently by regional and national smallpox eradication authorities.

A regular publication that charts the progress of the programme, notes the occurrence of smallpox, and provides technical and specialized information will assure more effective performance at peripheral posts. Prompt, helpful reaction at higher levels in response to reports of smallpox encourages prompt, regular reporting from intermediate and peripheral levels.

To supplement the official reporting scheme, based on hospitals, health centres, etc., information about smallpox cases should be sought from school teachers, malaria surveillance workers, political party leaders in villages, newspapers, etc. Although not a substitute for regular reporting by established health agencies, these sources may provide information, especially from remote areas, that permits more prompt identification of a problem and earlier effective containment.

As the number of smallpox cases in a country decreases, each case becomes highly significant, demanding clinical and laboratory verification and careful epidemiological tracing to determine the source of infection.

13. LABORATORY DIAGNOSIS [1]

In endemic areas and during epidemics, the diagnosis of the great majority of cases will be made on clinical grounds with due regard to the history of contact and previous vaccination. In a minority of cases, especially of the acute fulminating or modified type, the clinical picture may leave the issue in doubt and thus require supplementation by suitable laboratory investigation. Additionally, as eradication programmes progress and cases become infrequent the laboratory confirmation of each is necessary. The method of collecting and despatching specimens to the laboratory, and details of the simpler tests, are given in the Annex, where the tests available for detection of virus, of virus antigen, and of antibody in the patient's serum are discussed in relation to their value at various stages of illness.

Where laboratory facilities are limited, some of the tests described below may not be possible. The microscopic examination of stained smears, the precipitation-in-gel method for identification of antigen, and culture on the chorioallantois of the chick embryo are especially useful in diagnosis, and they require little experience and minimal equipment.

13.1 Detection of virus

Microscopic examination

(*a*) *Stained smears* [2]

Large numbers of elementary bodies are present in smears made from early maculopapular lesions and from scrapings from the base of vesicles. The elementary bodies can be demonstrated under the oil-immersion lens after staining the smears by Gutstein's methyl violet method or Gispen's silver impregnation technique. These staining methods are simple, rapid, and especially valuable in the early stages of illness, but experience in interpretation is necessary. Gispen's method is more useful than Gutstein's when the lesions are becoming pustular; scrapings from the base of vesicles and pustules rather than the fluid contents should be examined. A negative finding does not exclude the diagnosis of smallpox.

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[1] Kempe, C. H. & St Vincent, Leone (1966) *Variola and vaccinia*. In : Lennette, E. H. & Schmidt, N. J., ed., *Diagnostic Procedures for Viral and Rickettsial Diseases*, 3rd ed., New York, American Public Health Association, pp. 665-692.

[2] See Annex 2.

(*b*) *Electron microscopy* [1]

In the hands of those experienced in this technique, the examination of material from the patient's skin lesions at all stages of the disease will give positive identification of a pox virus within an hour or two. The negative staining method using phosphotungstic acid allows some of the surface structure of the particles to be seen and enables pox virus particles to be distinguished from those of the varicella-herpes group.

Isolation and identification of the infecting virus

Propagation of the virus on the chorioallantois of 11-13-day chick embryos (Annex 2) or in tissue culture is the most reliable of all laboratory methods and should be used for confirmation of other laboratory tests and to differentiate variola from vaccinia virus. The method should give positive results in 2-3 days from skin lesions at all stages of the disease and from the blood in haemorrhagic cases. The chick embryo technique is perhaps simpler, requires less complicated materials, and enables variola and vaccinia viruses to be readily distinguished. Varicella virus does not produce lesions on the chorioallantois. Herpes simplex typically produces smaller lesions than does variola virus and the inclusions demonstrable in the lesions are intranuclear and not cytoplasmic like those of the vaccinia-variola viruses. Variola virus grows in a variety of tissue cultures but most readily in cultures of human embryo and monkey kidney cells; if the virus content of the inoculum is large, it may be demonstrable in 12-48 hours by immunofluorescence or haemadsorption with suitable fowl red cells. Further confirmation of the identity of the virus may be made by precipitation-in-gel or complement-fixation tests.

13.2 Detection of virus antigen

The tests for antigen in the skin lesions of the patient may give positive results in 2-24 hours but, like microscopic examination, will not serve to differentiate variola from vaccinia virus nor the virus of variola major from the virus of variola minor.

Precipitation-in-gel test [2]

This will often enable a tentative diagnosis to be made in 1-4 hours provided that a sufficient amount of material is submitted for tests with an immune rabbit serum. A tiny drop of vesicle or pustule fluid or two or three crusts should suffice. The test is applicable at all stages of illness from the early vesicular to the end of scabbing.

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[1] Cruickshank, J. G., Bedson, H. S. & Watson, D. H. (1966) *Lancet*, **2**, 527.

[2] See Annex 2.

Complement-fixation test

As in the precipitation-in-gel tests, the material from skin lesions is tested with a high-titre antivaccinial serum prepared in the rabbit. This test is more sensitive than precipitation-in-gel, but requires more technical experience and results are available in 18-24 hours.

13.3 Tests for antibody in the patient's serum

Although isolation of the virus is always desirable, antibody tests may be the only practicable laboratory procedures in the retrospective diagnosis of patients whose scabs have separated. The patient's serum may be examined for antibody by neutralization, complement-fixation, precipitation, and haemagglutinin inhibition tests. The last three tests are less time-consuming and more suitable for diagnostic purposes than the neutralization technique. It is useful to have paired sera from each patient, one taken in the first few days of illness and a second after the first week, so that an increase in antibody may be determined. Frequently, however, only a single specimen of serum is available. If the patient has not been vaccinated in the previous year the presence of significant complement-fixing antibody, specific precipitation or high titres of haemagglutin-inhibiting antibody suggests that the case is one of smallpox.

13.4 Laboratory differentiation of vaccinia and variola viruses

In endemic areas or during outbreaks of smallpox in non-endemic areas, generalized vaccinia may occur in consequence of widespread vaccination. Microscopic examination, tests for virus antigen, and tests for antibody, in the patient's serum will often not distinguish the case of generalized vaccinia from that of smallpox. Differentiation of the viruses may be made by the size of the lesions on the chorioallantois after two or three days' incubation of inoculated embryos or, more rapidly, by growth of vaccinia virus in tissue culture. If doubt remains, it may be resolved by culture by either method, first at 37°C and then at 39-40°C. At the lower temperature, both viruses produce lesions, but at the higher one, only vaccinia virus will do so.

13.5 Laboratory differentiation of smallpox viruses

Epidemiological and clinical observations will usually identify an outbreak of smallpox as either variola major or variola minor (alastrim); laboratory methods will differentiate the causal viruses. The virus of variola major produces visible lesions within 3-4 days on the chorioallantois or in tissue culture at temperatures as high as 38.5°C, and an inoculum of 10^5 pock-forming units is lethal for the chick embryo at 35-37°C in four days. The virus of variola minor does not produce lesions at 38°C and is not lethal for the chick embryo with an inoculum of less than 10^7 pock-forming units.

Strains have been isolated in Tanzania that are intermediate in their laboratory characteristics. Though of low chick virulence, they produce some lesions at 38.3°C but many more at 36°C. The characteristics of the individual strains have remained constant. The intermediate case-fatality rates of up to 10% reported among unvaccinated cases from African countries may represent concurrent variola major and variola minor within the population, or, alternatively, may be due to the presence of intermediate strains. This possibility can be resolved only by additional study of the viruses isolated, together with adequate clinical and epidemiological data from various outbreaks.

14. CONTAINMENT MEASURES

The containment measures to be applied depend on the number of cases of smallpox in the area and the provision of facilities for isolation of cases, observation and isolation of contacts, etc. In areas of high incidence, emphasis will be placed on community vaccination programmes. When smallpox is occurring sporadically or in small outbreaks, it should be possible to isolate patients, to vaccinate contacts and keep them under observation for 16 days, to trace sources of infection, and, in due course, to proceed with terminal disinfection. As stressed above, prompt reporting of all suspected cases is essential for successful containment.

14.1 Isolation and quarantine

In endemic areas, hospital accommodation for the isolation of cases may be wanting. Provisional accommodation may have to be arranged in huts or houses, preferably distant from other habitations. Patients should be cared for by persons immune because of previous smallpox or recent vaccination. The care provided in hospitals or other premises should be of such quality that patients will readily submit to isolation. Visiting should be restricted to a minimum and limited to those who are immune. The patient should be isolated until all scabs have separated.

Contacts of the patient should be vaccinated promptly and observed at intervals for 16 days after last contact with the patient, so that any who become sick may be isolated before they become infectious to others. When the cases are few in number, home or hospital isolation of close contacts is desirable.

Where a high incidence of smallpox is limited to a relatively small area, as for example, a village, it might seem desirable to restrict movement in and out of the area of persons not recently vaccinated. This, however, is usually impracticable.

14.2 Contact and community vaccination

The appearance of smallpox in a community calls for an immediate energetic vaccination programme. All contacts of the patient should be sought out and vaccinated by two insertions. Vaccination within the first few days after exposure may confer protection. Although the first essential is vaccination of all close contacts, intensive vaccination in the immediate vicinity should be conducted, regardless of the fact that the community may have been recently vaccinated.

14.3 Disinfection

The object of disinfection is the destruction of virus contaminating the patient's clothing, bedclothes and the room. Even when the patient has not been sufficiently sick to be confined to bed or to his house during the infective period, the house should be disinfected.

The methods for disinfection will depend on the facilities available. Destruction by fire is the most effective and is particularly recommended for disposal of discharges from the nose and throat.

Where chemical disinfectants or special equipment are not available, simple procedures have been found effective for the disinfection of premises, bedding and other objects. Bedclothes and covers should be boiled. The room and other hard surfaces should be washed copiously with soap and water and left for 48 hours. Items so treated should, when possible, be exposed to direct sunlight for several hours.

It is clearly preferable to destroy the virus *in situ*. This can be done by soaking fabrics and items for 2-24 hours in solutions of disinfectants known to kill poxviruses. The disinfectant should be chosen among the following groups of substances: *chlorine preparations*, such as chlorinated lime in concentrations that leave 25 ppm or more of free chlorine after the chlorine demand has been satisfied (note: these are bleaching and oxidizing agents that may bleach or damage material); *coal tar derivatives* (phenolic compounds); currently marketed *quaternary ammonium compounds*, such as benzethonium, benzalkonium and cetylpyridinium chlorides at concentrations of 1% or greater; *formaldehyde* solution at 1% or greater concentration. For chemical disinfection of premises, floors and surfaces should be sprayed or mopped with the disinfectant solution and left for at least 4 hours before a final washing with water.

When fumigation is practicable, spaces can be safely disinfected by exposure to formaldehyde vapour for 6 hours. This can be accomplished by boiling commercial formalin in two volumes of water (500 ml of formalin plus 1 litre of water per 30 m^3), or by adding potassium permanganate to commercial formalin in large jars (170-200 g to 500 ml of formalin plus 1 litre of water per 30 m^3). Ethylene oxide has excellent disinfecting and

penetrating ability; however, fumigation with this gas requires highly specialized equipment.

14.4 Antiviral agents

In recent years, some promising results have been attained in the protection of family contacts by the use of antiviral drugs. It should, however, be emphasized that the use of such compounds is in no way a substitute for vaccination as a general prophylactic procedure. These drugs may be used in protection of previously unvaccinated family contacts and in the treatment of certain severe complications of vaccination, particularly vaccinia necrosum or progressive vaccinia. Vaccination of these family contacts should not be neglected. Only *N*-methylisatin-β-thiosemicarbazone (methisazone) has so far been found effective in chemoprophylaxis; it has the disadvantage that it may induce nausea and vomiting in a substantial proportion of those receiving it. Further work may produce preparations without undesirable side effects. Antiviral drugs have not yet been shown to have therapeutic value in smallpox.

Vaccinia immune gamma globulin is valuable in the prophylaxis of smallpox in previously unvaccinated family contacts and in the treatment of some vaccination complications. However, supplies of this product are not generally available in endemic areas.

15. MAINTENANCE VACCINATION

The success of the smallpox eradication programme depends upon a high level of herd immunity during the consolidation and maintenance phases after the more dramatic mass vaccination programme. Normally, the necessary vaccinations will be performed by the personnel of the general health services, although, in areas without health services, special mobile vaccination teams can effectively do this work.

Maintenance vaccination should begin immediately after the mass campaign in each programme area and should be continued at least until eradication on a continental scale has been achieved. Particularly important is vaccination of the newborn; of children, who most commonly introduce infection into the family group; and of others who were not vaccinated during the mass campaign. The last group most frequently comprises "migrants", persons living in slum areas and children not attending school. Systematic revaccination should be performed so that all the population is vaccinated every 3-5 years.

Maintenance vaccination can be accomplished, in part, through vaccination clinics at established centres, in schools, and among governmental and industrial groups. In addition, it is necessary to ensure adequate coverage of women, migrants, children not attending school, etc. This

will require the organization of a vaccination programme in or near the place of residence, either by house-to-house campaign or at collecting points.

In some areas, the use of multipurpose health workers is under consideration. This approach has theoretical advantages but the problems involved in the proper handling of reconstituted vaccine and the difficulty of assessing vaccination performance do not recommend it as a solution to the problem of maintenance vaccination. Best results are obtained when vaccinations are done by well-trained personnel for whom this is the principal responsibility.

The effectiveness of maintenance vaccination must be continually assessed to ensure that a high level of immunity does, in fact, exist in all sections of the population.

16. RECOMMENDATIONS

As previously noted, smallpox eradication is realizable only on a continental or global basis. Close co-ordination of programmes between countries is therefore of major importance. The World Health Organization can play a key role in this activity and it should endeavour to ensure that the programmes in proximate countries progress at a comparable pace. The countries themselves must, of course, assume the chief responsibility for this. Since, however, the smallpox eradication programme has been agreed upon by all countries and represents a major function of the Organization, it is to be hoped that all Member States will willingly share the responsibility.

Effective co-ordination requires that all countries regularly report the occurrence of smallpox and the progress of their eradication programmes. It is suggested that WHO should collect this information and make it widely available to all concerned with the eradication effort.

Laboratories for diagnosis should be developed in each of the larger countries and regional laboratories should be designated to serve groups of smaller countries. Regional reference laboratories are needed to assist in the development of national laboratories and training of their personnel, to supply standard reagents such as antisera and antigens, to examine representative specimens from these laboratories and to perform the more complicated tests. Until regional reference laboratories are established, essential reagents may be obtained from WHO.

Provision of enough freeze-dried vaccine of the required standard will probably be a problem for years to come. WHO should arrange for consultation and training for vaccine production laboratories in endemic areas, should provide for independent testing of vaccines, and should endeavour

to obtain from Member Governments enough vaccine to ensure that the programmes are not disrupted.

Provision should be made for WHO to provide, if required, assistance to non-endemic countries faced with the problem of imported smallpox.

Research is still required on several problems, including vaccine properties, vaccine production, vaccination methods, characteristics of variola and other poxviruses, epidemiological behaviour of smallpox in different ecological conditions, and duration of immunity following vaccination and revaccination.

This programme deserves the attention of all countries throughout the world. Non-endemic countries have as much to gain from its success as the less fortunate endemic ones. Those countries able to help should do so by direct donation to WHO and by bilateral aid.

Annex 1

COLLECTION AND DESPATCH OF SPECIMENS

Collection

Material from skin lesions. For microscopic demonstration and isolation of virus, material should be obtained by scraping macules, papules or the base of vesicles with a Hagedorn needle or small scalpel. The scraping should be smeared on clean slides. Five or six lesions should be sampled; gross admixture of blood is undesirable. The slides should be allowed to dry in air and should not be heated or exposed to fixatives or disinfectants. They should be separated from each other by means of rubber bands or small pieces of cardboard, wrapped in greaseproof paper, and placed in a container for despatch to the laboratory.

Vesicle fluid and pustular fluid. These are best collected in small glass capillary tubes which are put in screw-capped bottles or other suitable containers for despatch. If capillary tubes are not available, the material may be spread thickly on glass slides and allowed to dry in air. In the laboratory, the material on the slides may be washed off in a small volume of saline and used for the detection of antigen and/or for culture.

Blood. When required for antibody studies, the blood should be allowed to clot and the serum then removed.

Scabs. At least six scabs per patient should be collected and placed in a screw-capped bottle for despatch.

Despatch

All specimens mentioned above and the instruments used for their collection must be regarded as highly infective. It is therefore essential that all specimens and containers should be securely packed in metal, wooden, or strong cardboard containers before being sent to the laboratory.

Specimens should reach the laboratory as soon as possible. If they cannot be delivered by hand they should be sent by the most rapid means of transport available and the receiving laboratory should be informed by telephone of the estimated time of their arrival. Packages sent through the post should conform to national and international postal requirements.

With all specimens sent to the laboratory, details should be given of the patient's age, name, address, history of vaccination, date of onset of illness, and date of appearance of rash.

For postal shipment of specimens, special precautions must be taken. The following is quoted from Article 120 of the requirements established by the International Postal Convention (Vienna, 1964).

Perishable biological substances consisting of living pathogenic micro-organisms or of living pathogenic viruses shall be enclosed in a bottle or tube of glass or plastic materials with thick sides, well stoppered, or in a sealed vial. This container shall be impermeable and hermetically sealed. It shall be surrounded with a thick and absorbent material (medicated cotton wool, swan's down cloth or flannelette) wrapped round the container several times and bound both above and below it so as to form a sort of cocoon. The container so wrapped shall be placed in a solid, well-fastened, metal box. The absorbent material placed between the inner container and the metal box shall be of sufficient quantity to absorb, in case of a breakage, all the liquid contained, or capable of being formed, in the inner container. The metal box shall be made and fastened in such a way as to make any contamination of the outside of the box impossible. The metal box itself shall be wrapped in cotton or spongy material and enclosed in its turn in a protective box in such a way as to prevent any movement. This outer protective box shall be hollowed out from a block of solid wood, or shall be of metal, or may be of a material and construction of equivalent strength, and furnished with a well-fitting lid fastened so that it cannot open in course of transmission. Special provision, such as drying by freezing or packing in ice, shall be made to ensure the preservation of substances sensitive to high temperatures. Air transmission, which entails changes in atmospheric pressure, makes it necessary that the packing should be strong enough to withstand these variations in pressure. Moreover, the outer box (as well as the outer wrapping if there is any) shall be furnished on the side which bears the addresses of the officially recognized laboratories sending and receiving the item with a violet coloured label with the following indication and symbol:

(Dimensions 62 x 44 mm)

Annex 2

LABORATORY TESTS FOR DIAGNOSIS OF SMALLPOX

Examination of stained smears

Smears from skin lesions (see Annex 1) should be fixed and stained for virus particles by Gutstein's or Gispen's modification of Morosow's technique.[1] If such smears are properly prepared from early cases of smallpox, innumerable virus particles will often be found. When the lesions have become pustular, the results are less satisfactory. The only other lesions that may present a similar picture are those of vaccinia or cowpox. In smears from varicella or herpes simplex, elementary bodies are scanty, stain poorly and appear smaller. Experience of this method may be gained by the examination of smears from the base of an early vaccinial vesicle. A supply of such slides kept unstained should be used as a positive control in the tests on diagnostic specimens.

The simplest staining method, that of Gutstein, is described below.

Slides smeared with scrapings are fixed by flooding with methanol for 10-30 minutes; alcohol is added as needed to prevent drying. The methanol is washed off with distilled water. A freshly prepared mixture of equal parts of 1% aqueous solution of methyl violet and 2% aqueous solution of sodium bicarbonate is filtered on to the slide. The slide is heated gently until steam rises and this heating is repeated three or four times during a 5-minute period. The stain is then flushed off the slide with distilled or tap water and the slide is blotted dry on filter-paper.

The smear is examined under an oil-immersion lens. A tentative positive report is made only if innumerable elementary bodies are seen. They are uniform in size—about one-quarter that of staphylococci—and are uniformly deeply stained and confined to the area of the smear.

Virus particles are less numerous in vesicle fluid, and material from pustular lesions is unsatisfactory.

Detection of antigen

Precipitation-in-gel technique [2]

The precipitation technique provides a reliable diagnostic test if sufficient material is used. A small drop of vesicular or pustular fluid, or two

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[1] Gispen, R. (1952) *Antonie v. Leeuwenhoek*, **18**, 107.

[2] Nizamuddin, M. & Dumbell, K. R. (1961) *Lancet*, **1**, 68.

or three crusts should suffice. A highly potent antiviral immune serum (obtainable through WHO) is used, although smallpox convalescent serum will sometimes give satisfactory results.

The test is best carried out on microscope slides. A 1-mm layer of agar is prepared on a slide, using a 1-1.5% concentration of agar in isotonic phosphate-saline buffer at pH 7.3 containing 0.01% thiomersal. This agar layer is made by permitting molten agar to harden between two slides (one preferably of transparent plastic for easy removal) separated at the ends by pieces of 1-mm glass slides. Reservoirs or cups 4 mm in diameter, with centres 5-6 mm apart, are prepared by using a cork borer of requisite size or other suitable instrument.

Antigenic extracts are made from scabs by extraction with the phosphate-buffered saline used for preparing the agar. Crusts are crushed with a glass rod and allowed to stand for one hour at 37°C with a few drops of buffer to make approximately a 10% w/v suspension. Vesicle and pustule fluid should not be diluted more than 1 in 5. Even material submitted as a thick smear on a slide may not provide sufficient material to give a positive result. The immune serum should be placed in one cup and vesicular or pustular fluid or crust extracts should be placed in surrounding cups. A known positive extract of smallpox or vaccinial material must be included, and extracts should also be tested against a normal rabbit serum as an additional control. If available, a good convalescent herpes zoster serum may be included, as this will give a positive result with undiluted vesicle fluid in 24 hours if the case should be one of varicella. The slide is placed on moist cotton wool or filter paper in a closed container at room temperature. If the material is from a smallpox patient, precipitation lines should appear in the agar between the antigen and antiserum cups within 2 hours, linking with those in the positive control within 4-5 hours. If a weak serum is used, a final reading should be delayed until 24 hours.

(Most convalescent smallpox sera used undiluted will form lines of precipitate in gel when diffused against a vaccinia or variola antigen; this is not usually observed in post-vaccination sera. Vesicle fluid from a primary vaccination will serve as a vaccinial antigen.)

Virus isolation in chick embryos

Virus may be isolated by inoculating material from the lesions of the patient on the chorioallantois of chick embryos. Fertile hens' eggs should be incubated at 37-39°C for 11-13 days. The eggs are then examined by transillumination in a dark room and if the embryos are alive the outline of the air space, usually at the blunt end of the egg, and the spot over the vascular chorioallantois should be marked on the egg shell. The shell is punctured over the air space, on the side of the egg above the chorioallantois. With a needle or pen nib inserted into the opening so formed the

shell membrane is torn over the chorioallantois through a drop of sterile broth or saline, taking care to avoid tearing the underlying vascular chorioallantois. By using gentle suction with a rubber teat placed over the opening in the shell above the air space, the contents of the egg are displaced, thus producing a new air space with a base formed by the vascular chorioallantois. To the inoculum—suspension of scraping from macules, vesicle or pustule fluid, or a saline extract of crusts—penicillin and streptomycin are added to produce concentrations of 500 units/ml and 500 μg/ml, respectively. 0.1 ml of the fluid is inoculated with a syringe on to the chorioallantois of each of three or four eggs and the opening over the new air sac sealed with cellophane tape or a drop of melted paraffin wax. It is advisable to inoculate also 1/1000 dilution of the material so as to produce discrete lesions on the chorioallantois. The eggs are rotated gently to spread the inoculum. After 2 or 3 days' incubation at 36-37°C the egg shell is broken away with forceps over the new air space to expose the inoculated chorioallantois. The typical, relatively small (1-mm) rounded pocks produced by variola virus on the chorioallantois within 3 days permit a diagnosis to be reached by the experienced worker which can be confirmed by serological tests—for example, precipitation-in-gel of a concentrated extract of the lesions with a potent vaccinia immune serum.

This is the most reliable and sensitive of the simpler laboratory techniques for the diagnosis of smallpox and, where possible, should be used as a confirmatory test to supplement any other diagnostic method. It should give a positive result at any stage of the disease, from the appearance of the first macule to the disappearance of all crusts from the patient's skin. In patients with severe and fulminating infections, virus may be isolated from the blood in the first few days of illness; the buffy coat is more likely to give a positive result than whole blood.