EXPERT COMMITTEE ON
POLIOMYELITIS

First Report

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EXPERT COMMITTEE ON POLIOMYELITIS

First Session

Rome, 14-19 September 1953

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EXPERT COMMITTEE ON
POLIOMYELITIS

First Report *

The Third World Health Assembly stressed the desirability of international guidance on the measures to be taken to prevent the spread of poliomyelitis, and recommended the establishment of an expert committee to co-ordinate research with a view to obtaining the information necessary eventually to specify effective preventive measures.¹

The first session of the committee was held in Rome from 14 to 19 September 1953 following the Sixth International Congress for Microbiology.

The session was opened by Dr. W. M. Bonne, Director, Division of Communicable Disease Services, WHO. Professor J. R. Paul was elected Chairman, Professor S. Gard, Vice-Chairman, and Dr. A. J. Rhodes, Rapporteur.

1. INTRODUCTION

The transformation of the relatively uncommon “infantile paralysis” of the 19th century into “epidemic poliomyelitis” of almost worldwide distribution presents today one of the most formidable public-health problems. It is not only that the incidence of the paralytic disease is increasing and extending to areas previously believed to be free, because even now, in most areas, the incidence is considerably less than that of

* The Executive Board, at its thirteenth session, adopted the following resolution:
  The Executive Board.
  1. NOTES the first report of the Expert Committee on Poliomyelitis;
  2. THANKS the members of the committee for their work;
  3. REQUESTS the Director-General to report to the fourteenth session of the Board on the co-ordinating role of WHO in poliomyelitis research;
  4. REQUESTS the Director-General, when forwarding this report to governments, to draw attention to the importance of fellowships in virology which are available in the programme of the Organization; and
  5. AUTHORIZES publication of the report.
  (Resolution EB13.R6, Off. Rec. Wild Hlth Org. 52, 3)
¹ Resolution WHA3.21, Off. Rec. Wild Hlth Org. 28, 22
many other infectious diseases. It is rather the failure, so far, to control the disease that causes concern, and the relatively low incidence is counterbalanced by the permanence of the resulting disability, since treatment, prolonged and expensive as it is, is mainly palliative; a nerve cell once irreparably damaged cannot be replaced.

It is not surprising, therefore, that news of fresh discoveries in laboratory methods and in the epidemiology and control of poliomyelitis should arouse widespread interest. It was primarily to consider the place of these discoveries in future efforts to control the disease that the committee was convened. It was necessary, however, to review in some detail current knowledge and opinion on various aspects of poliomyelitis, so that theories which can no longer be considered valid could be discarded and replaced by interpretations in keeping with the observed facts. Only then can recent advances be incorporated in a logical plan for the future.

2. POLIOMYELITIS VIRUS

2.1 Definition of poliomyelitis virus

The term "poliomyelitis virus" has been used somewhat indiscriminately in the past, so that a statement as to what is considered under that term in this report appears to be necessary. At present it would be unwise to try to fit the virus of poliomyelitis into any comprehensive system of classification, because of the incomplete information available about the mechanism of multiplication, the nature of the cell-virus interaction, and the morphological, physical, and chemical properties. The variability of such characteristics as pathogenicity and host-range increases the difficulties of classification.

Systems have been proposed in which the poliomyelitis virus was included in a genus or a group together with a number of other viruses. In order to indicate presumed relationships, the viruses were either assigned binomial Latin names, or designated as "poliomyelitis", "para-polio-myelitis", and "pseudo-poliomyelitis" viruses.

Although viruses can be readily distinguished by differences in certain physical or chemical properties, or immunological characteristics, there are few known criteria by which true relationships between viruses can be established. It is the opinion of the committee that research aimed at a better understanding of the essential properties of viruses should be encouraged so that eventually a rational classification may be possible. At present there seems to be no justification for the use of nomenclatures which carry such far-reaching implications as do the above-mentioned
systems. In particular, terms like "para-poliomyelitis" or "pseudo-
poliomyelitis" are apt to create great confusion and should therefore be
avoided.

However, even if a comprehensive classification is not at present
practicable, some viruses have so many characteristics in common that
they seem to fall naturally into groups. Thus, it is now generally agreed
that the viruses of poliomyelitis in man and encephalomyelitis in mice
(Theiler's disease) form such a group. The reasons for grouping these
viruses together are the similarities in the natural history of the respective
diseases as well as in the morphological, physical and chemical properties
of the causative agents.²

2.2 Types of poliomyelitis virus

Three immunological types of human poliomyelitis virus have been
identified, the prototype strains of which are known as "Brunhilde"
(Type 1), "Lansing" (Type 2), and "Leon" (Type 3). Strains are iden-
tified as of a certain type by virus neutralization tests with specific antisera.
The types seem to be quite distinct, and overlapping of neutralization
between types is usually not observed. There may, however, exist common
or group complement-fixing antigens.

Observations have indicated that outbreaks of epidemic proportions
are attributable mainly to Type 1 strains. However, epidemics apparently
caused by Type 3 strains have recently been described. Type 2 strains
are known to be widely disseminated but, although sometimes found in
epidemics, they have usually been detected only in sporadic clinical cases.

Recently, several viruses have been isolated from cases of poliomyelitis
which differ immunologically from the established types of poliomyelitis
virus, and which have not been otherwise identified. It is not possible
at present to express an opinion as to the nature of these viruses. It is
desirable that such strains be subjected to comparative studies.

In the present report, only those viruses are considered and defined
as poliomyelitis viruses that fall into one of the recognized immunological
types, and otherwise conform with the descriptions presented in the following
section (see also section 6, page 29).

2.3 Some physical and chemical properties of poliomyelitis viruses

None of the characteristics to be described is strictly specific, and it is
at present not possible to identify a poliomyelitis virus by physical and

² A subcommittee under the Nomenclature Committee of the International Asso-
ciation of Microbiologists is, at present, concerned with the question of the application
of a provisional nomenclature to selected groups of animal viruses, including the
poliomyelitis group.
chemical examination alone. If, on the other hand, an unknown agent differs significantly from the typical pattern, its classification as a poliomyelitis virus should be only provisional.

2.3.1 Size and morphology

Information concerning these properties can be obtained by electron micrography, and by filtration end-point and sedimentation-diffusion rate determinations.

The particle size has been estimated to be 10-30 μ, the poliomyelitis virus thus being one of the smallest of the animal viruses. For practical purposes of identification, filtration end-point determination seems to be the most suitable method; virus will not pass through a filter with an average pore-diameter of less than 30 μ.

2.3.2 Resistance to physical and chemical agents

In contrast to most other viruses, poliomyelitis virus seems to be highly sensitive to complete desiccation, even in the presence of protein, glucose, or other preservatives, and even when dried from the frozen state.

Resistance to heat is influenced to some extent by the nature of the suspending medium. Thus, milk and cream exert a marked protective influence. However, even under conditions of optimal stability, temperatures above 60°C cause rapid inactivation. Application of heat is probably the most practicable means of disinfection of materials containing poliomyelitis virus. Ultra-violet irradiation cannot be relied upon to disinfect materials contaminated with poliomyelitis virus.

Resistance to chemical agents, on the other hand, is remarkable. The optimal pH is about 8, but rapid inactivation is not observed until below pH2 or above pH10. Of organic solvents, ether is without destructive effect; alcohol and acetone inactivate the virus slowly, but more rapidly at higher temperatures. Detergents have no effect; disinfectants such as phenol and formalin only inactivate slowly, and comparatively high concentrations are needed. However, sterilization of delicate instruments such as bronchoscopes, catheters, etc., in formaldehyde vapour at 50°C seems to be practicable. Oxidizing agents seem to be the most active chemical disinfectants; 0.05 parts per million (p.p.m.) of free chlorine is sufficient for complete inactivation in 15 minutes in the absence of extraneous organic matter; iodine acts even faster. It must be pointed out, however, that the critical concentration of all chemical agents is largely dependent upon the medium in which they have to exert their action. For this reason, no standard concentrations can be recommended. The treatment of stools with chloride of lime in a concentration sufficient to destroy typhoid bacilli does not destroy poliomyelitis virus. It may suffice to say that the presence of organic material, especially protein or degradation products
thereof, provides a considerable protection against inactivation, and, in
this connexion, it may be mentioned that infected stools kept at room
temperature may remain active for several months, provided that they
are not allowed to dry.

3. ESSENTIAL CLINICAL CHARACTERISTICS OF THE
ACUTE STAGES OF POLIOMYELITIS

3.1 Varieties of illness

The manifestations of infection by the virus of poliomyelitis are varied,
and range from an inapparent to a severe paralytic illness. In some countries,
the only form of illness regarded by physicians as indicative of infection
is the paralytic form of the disease. In others, poliomyelitis is regarded
as an acute febrile disease with evidence of meningeal irritation, which
often but not always proceeds to spinal or bulbar paralysis. It must be
strongly emphasized that paralysis is an infrequent complication of polio-
myelitis infection in man, and that most persons who become infected
either show no symptoms or else develop a transient abortive or “minor”
ilness. These asymptomatic infections and minor illnesses are of consi-
derable importance in the understanding of the epidemiology and immu-
nology of poliomyelitis. The main features of the various manifestations
of poliomyelitis infection are as follows:

(a) Inapparent infection. Associates of cases of poliomyelitis are
frequently infected without showing any clinical evidence of this process.
Such infections are known as inapparent, asymptomatic, or “silent”,
and can be recognized only in the laboratory by the recovery of virus or
the demonstration of an increase in serum antibody.

(b) Abortive poliomyelitis. Clinical manifestations of abortive infections
or minor illnesses include fever, headache, sore throat, listlessness, anorexia,
vomiting, constipation, and muscle and abdominal pain. These illnesses
usually last for only 24-48 hours, and are commoner in children than
adults. Abortive poliomyelitis cannot be diagnosed with certainty by
clinical methods, but a tentative diagnosis can be made in those known
to be close associates of a definite case of poliomyelitis.

(c) Non-paralytic poliomyelitis. This term refers to a more severe
illness in which definite manifestations of central-nervous-system involve-
ment are added to those of the minor illness. The clinical features include
fever, headache, vomiting, pains in the back, neck, trunk or limbs, paraes-
thesiae, and stiffness of the neck or back. There is usually an increase
of cells and protein in the cerebrospinal fluid. The features are those of
benign aseptic or “virus” meningitis, and are not specific for polio-
myelitis.
The onset of these manifestations marks the beginning of what is now usually called the "major" illness, in which virus is present in the central nervous system. The onset of the major illness is usually abrupt, but may be gradual over several days. In some cases, especially of children, illness may occur in two phases, the so-called "dromedary" form of poliomyelitis, in which a few days of well-being separates an initial minor illness from the second or major illness.

A non-paralytic illness may clear up completely within 4 to 7 days, or may become more severe and paralysis develop. In some instances, a mild degree of paralysis may occur which is detected only when the patient is re-examined two to four weeks later.

It is not possible to make an accurate diagnosis of non-paralytic poliomyelitis without resort to virological tests, which unfortunately are time-consuming, expensive, and available in only a few centres. A tentative diagnosis on clinical grounds may reasonably be made during the poliomyelitis season and especially if there is a history of association with a paralytic case.

(d) Spinal paralytic poliomyelitis. Paralysis usually develops after 1 to 4 days of a non-paralytic illness, as just described. This period is often referred to as the "pre-paralytic" phase. Less commonly, there is a very brief pre-paralytic phase, paralysis being almost the first manifestation of illness. Muscle pain may become more prominent with the onset of paralysis. Paralysis is of the flaccid or lower motor neurone type, and is characteristically asymmetrical in distribution, but may have any distribution. Even in a limb severely affected, it is usual to find that certain muscle groups are spared completely, or are less severely paralysed. The muscles commonly paralysed, in order of frequency, are those of the legs, arms, back, thorax (including the intercostals), and diaphragm. Paralysis of the muscles of respiration may therefore occur. It is unusual for the higher centres to be involved, and the sudden development of flaccid paralysis in a mentally alert patient should strongly suggest a diagnosis of poliomyelitis.

(e) Bulbar paralytic poliomyelitis. In this variety of poliomyelitis, there is paralysis of one or more muscle groups innervated by the cranial nerves, especially those of the soft palate and pharynx, and this gives rise to dysphagia, dyspnoea, nasal speech; paralysis of the muscles of the face, tongue, jaw, and eye may also occur. Paralysis of the circulatory and respiratory centres may develop with great rapidity and this is of serious prognosis. Bulbar poliomyelitis may represent the only paralytic manifestation of the infection, or may supervene as the final stage in the progression of a spinal paralysis. Practically all the acute deaths from poliomyelitis are attributable to bulbar involvement.
The diagnosis of bulbar paralysis is not infrequently missed, at any rate in the early stages, with consequent delay of appropriate treatment. Bulbar poliomyelitis should always be considered in an acutely ill patient showing paralysis of the cranial nerves, particularly if poliomyelitis is known to be prevalent contemporaneously.

(f) Encephalitic manifestations. Encephalitic manifestations, such as coma and tremors, are sometimes observed and may be common; paralysis may be associated with such manifestations.

3.2 Changes in the cerebrospinal fluid

Examination of the cerebrospinal fluid usually affords valuable confirmation of the clinical diagnosis, but negative findings may occur. In the non-paralytic illness, as well as the paralytic illness, there is an increase in the cell count, which may range from 10 cells to over 500 per mm³. In the early stages, up to 85% of these cells may be neutrophils. With the progress of the illness, the cell count falls, and lymphocytes predominate. The protein content is often raised in the early stages of the illness, and may continue to rise (to 500 mg% or higher) for two to three weeks even though the cells may have disappeared by that time.

3.3 Differential diagnosis

The non-paralytic illness may be diagnosed tentatively on the results of clinical examination, suitable tests on the cerebrospinal fluid (see above), and if there is a history of association with a paralytic case of poliomyelitis. It must be realized, however, that many other agents cause an aseptic meningitis that cannot be differentiated from non-paralytic poliomyelitis, except by elaborate laboratory tests. The commonest viruses causing aseptic meningitis simulating poliomyelitis are the following: mumps, lymphocytic choriomeningitis, herpes simplex, herpes zoster, and epidemic encephalitis. Coxsackie viruses have been incriminated by various workers. A considerable number of cases of aseptic meningitis caused by Leptospira have been reported. In addition, in the initial stages of an illness, it may be difficult to distinguish between tuberculous or pyogenic meningitis and aseptic meningitis caused by viruses.

As a general rule, a diagnosis of spinal paralytic poliomyelitis may be made without much difficulty on clinical grounds. The features of mental clarity, a history of a pre-paralytic phase, pains in the back and limbs, an asymmetrical flaccid lower motor neurone paralysis, and changes in the cerebrospinal fluid are highly suggestive of poliomyelitis.

Some difficulty may be experienced in making a diagnosis of bulbar poliomyelitis, and this condition must always be considered in the presence
of paralysis of the cranial nerves, especially when there is involvement of the muscles of deglutition and respiration.

The syndrome known variously as acute infectious polyradiculitis, acute radiculoneuritis, infectious neuronitis, Landry's paralysis, or the Guillain-Barré syndrome, may be confused with an extensive paralysis due to poliomyelitis. Although there are various forms of the syndrome, in a typical case there is a symmetrical flaccid paralysis of all four limbs, the distal portions being mainly affected. In the initial stages of the Guillain-Barré syndrome, there is often a normal cell count in the cerebrospinal fluid with a high protein content, the so-called "albuminocytologic" dissociation. There is usually a relatively rapid and complete recovery even from quadriplegia.

During recent years, a few cases of clinically typical Guillain-Barré syndrome have been found to excrete poliomyelitis virus, but there is no evidence that the majority of cases of this syndrome are infected with the virus of poliomyelitis.

4. EPIDEMIOLOGY

4.1 Host factors predisposing to or precipitating paralysis

The possibility that factors other than the biological characteristics of the strain of virus causing the infection may influence the development of paralysis has been recognized for many years.

Various factors inherent in the host have been held to predispose to the development of paralysis. For example, it has been suggested that genetic constitution may play a part. It is reasonably well established that poliomyelitis during pregnancy may run a severe course. The latter may be an example of the effect of endocrine factors in increasing susceptibility, just as cortisone increases the susceptibility of experimental animals (see page 32).

Various observers have claimed that bacterial or virus infections of the throat or alimentary tract may predispose to paralytic poliomyelitis.

Some experience suggests that injuries, which may range from a trauma of moderate severity to a fracture, may be followed by the appearance of paralysis. An important example of the effect of trauma is the excessive incidence of bulbar paralysis in persons subjected to tonsillectomy and adenoidectomy within the previous month. Some observers have also incriminated dental extractions.

There appears to be little doubt that over-exertion about the time of onset of the major illness is particularly liable to be followed by severe paralysis.
Of particular interest in this connexion is the demonstration of an association between intramuscular injections and the subsequent appearance, within a month, of paralysis in the limb injected. There is definite evidence that intramuscular injection of adsorbed combined diphtheria-pertussis vaccine or of arsenicals may have this effect. It has been suggested that injections may predispose to or precipitate paralysis in persons who otherwise might have developed an inapparent, abortive, or non-paralytic attack. An alternative explanation is that the injection leads to the localization in the injected limb of a paralysis which would have occurred in any case. However, experience in an outbreak in Tahiti in 1951 indicated that the intramuscular injection of organic arsenicals and heavy metals did, in fact, increase the incidence of paralytic poliomyelitis.

4.2 Portals of entry and exit; distribution of poliomyelitis virus in the body

Because of the fact that monkeys can be infected by the olfactory route, it was earlier inferred that infection was spread mainly by inhalation of respiratory droplets into the nose. More recently, however, it has been shown that virus is not commonly detectable in nasal secretions of patients, and that involvement of the olfactory bulbs is an infrequent occurrence. Evidence has, at the same time, accumulated to establish that the portal of entry is the mouth, and that the primary site of infection is in the pharynx and the rest of the alimentary tract.

The virus is most readily found in the oropharynx for 3-5 days before and for 3-7, rarely 11, days after the onset of illness; it is also found in the throats of persons who exhibit no clinical evidence of disease. Apart from this limited period, the virus has only rarely been detected in the throat, although further studies using tissue-culture techniques for virus isolation are desirable to confirm these findings.

Virus has been recovered from the stool as long as 3 weeks before onset of symptoms. During the first 10-14 days after onset, practically every patient excretes virus in the stool. By 3 weeks after onset, approximately half the patients no longer excrete virus; by 5-6 weeks, only 25% of patients still excrete virus in the stool, and in a small percentage excretion may continue for 12 weeks. In inapparent infections, faecal excretion of virus occurs over much the same period. Chronic excreters of virus have not been found, but this problem has not been adequately investigated. Examination of post-mortem material has shown that the virus is present in the walls of the pharynx and intestines, and not merely in the intestinal contents. Moreover, alimentary infection (without fever or physical signs) has been

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established by feeding virus to human subjects, and antibodies have developed. These observations indicate that the virus is actively multiplying in the alimentary tissues, and that the presence of virus in the pharynx and intestinal contents does not represent merely passive carriage.

In a small proportion of infected persons, involvement of the central nervous system follows, resulting in the clinical pictures of non-paralytic or paralytic poliomyelitis. It is not completely clear how the virus spreads from the alimentary tract to the central nervous system. Until recently, it was generally held that virus spread only along nerve fibres. Observations on the presence of virus in the blood-stream (viraemia) have gone far towards offering an alternative explanation. Thus, it has been shown that, following the feeding of virus to monkeys and chimpanzees, there is frequently a short period of viraemia before the onset of illness. This provides a means by which the virus can reach the central nervous system. Observations on viraemia in primates are supported by the demonstration, in a small number of persons, that viraemia also occurs in man during the incubation period. Most of the isolations of virus from the blood of patients have been made in persons who did not develop paralysis. Further observations on the incidence and significance of viraemia in the human disease are clearly necessary.

The evidence that the mesenteric lymph glands show inflammatory changes, and that virus has been recovered from those nodes, suggests that virus may spread from the alimentary tract by the lymphatic system.

The orthodox concept of spread of virus along nerve fibres was based largely on studies in primates, and there was little direct confirmation from a study of the disease in man. Nevertheless, it would appear that in bulb poliomyelitis following tonsillectomy the virus travels to the medulla along the cranial nerves supplying the pharynx.

In some instances the possibility of entry through abrasions of the skin or wounds, or by injections through contaminated skin, must be considered.

4.3 Immunity in man

The best index of immunity to poliomyelitis is at present obtained by a study of the distribution of the disease in various age-groups. In those areas in which infection is highly prevalent, the occurrence of cases is usually limited to the lowest age-groups—a fact which suggests that the older members of the population have acquired an effective resistance. In

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4 The word “immunity” is often used to refer only to resistance of various degrees to infection. It should, however, be borne in mind that, in poliomyelitis, a person who is not immune to alimentary infection might nevertheless be resistant to the development of serious lesions in the central nervous system, and hence resistant to paralytic poliomyelitis, a difference obviously of fundamental importance to the individual.
temperate climates, and in those portions of populations in which hygienic conditions are advanced, immunity is apparently acquired later, less solidly, and less uniformly. As a result, clinical poliomyelitis is increasingly common in persons of older age in these areas. These conclusions are supported by studies which measure the age of appearance and the frequency of antibody in such populations.

Serological data indicate that immunity to all three types of virus is generally acquired by natural exposure. Pools of adult sera or of gamma-globulin have been commonly found to contain comparable levels of antibody to each of the types, even though the frequency of clinical disease caused by the three types may differ considerably. In urban populations, acquisition of antibody to the different types appears to take place at approximately the same age, but serological tests in rural and isolated communities indicate that the times at which the respective viruses appear in the community are not necessarily identical; they may appear in successive waves. These time relationships vary widely in different parts of the world.

It is clear from experimental data that immunity to one type of virus does not furnish protection against the other two, although in human populations, as suggested above, antibody to Type 2 virus may indicate the probability that previous experience with the other types has occurred.

The recovery of poliomyelitis virus from the stools of adults with either apparent or inapparent infection strongly indicates that a single previous experience does not prevent the subsequent establishment of an alimentary infection. In a few limited experiments it has been possible to re-establish alimentary infection with the same strain in a proportion of human subjects. Second paralytic attacks are uncommon; the probability of their occurrence, even in the absence of immunity, can be estimated statistically to be very low. However, such attacks do occur, but there is insufficient information to determine whether they are caused by the same or by different strains of virus. It is hoped that further work will clarify this important problem.

There is divergence of opinion as to whether durable immunity is acquired from a single infection, or whether it is dependent upon repeated experience. It appears, nevertheless, that where environmental pollution is great and the probability of successive infections is high, a durable immunity is rapidly acquired. In contrast, where the chance of infection is small and the probability of reinfection reduced, immunity is less uniformly observed in all age-groups.

The presence of antibody in the blood is primarily a record of earlier antigenic experience. The fact that the level of antibodies after infection tends to decline with the passage of time suggests that the high levels of later life are developed by reinforcement from multiple infections.
There are as yet insufficient measurements to establish a correlation between levels of antibody in the blood and the degree of resistance to poliomyelitis virus. It may be difficult to obtain this information, because the variation in response to infection is great among non-immunes, the differences being not strictly related to immunological resistance. Nevertheless, experimental demonstration that animals can be protected against the paralytic disease by administration of sufficient antibody, as can man to some extent, clearly establishes an important role for circulating antibody in immunity. That it is not necessarily a complete preventive is seen in chimpanzees, for passively administered antibody does not always prevent the establishment of alimentary infection. Similarly, present data indicate that circulating antibody does not necessarily prevent alimentary infection in man. Nevertheless, it seems probable that, following extensive experience with natural infection, a stage can be reached in which infection of any degree is resisted; this may be the situation in populations with continued heavy exposure to virus.

Up to the present, the major correlations between immunity and epidemiology have been made by study of the results of measurement of serum antibody to Type 2 (Lansing) virus. In keeping with evidence that exposure to the three types of virus occurs within the same general time period, it has been interesting to observe that antibody to Type 2 virus tends to reflect in a population the presence of antibody and resistance to other types. Moreover, in infected familial aggregations, it has been found that the virus is less likely to become established in persons with antibodies to Type 2 virus in moderate amounts, and presumably therefore to other types as well, than in other members of the family. This field of knowledge still requires much investigation, and with the advent of more practicable laboratory procedures advances can be anticipated.

4.4 Methods of spread

All the evidence points to the fact that poliomyelitis virus is a highly infectious agent which is spread by means of the transfer of pharyngeal and intestinal excretions of infected human individuals. Poliomyelitis can be acquired by association with infected persons of such an intimate nature that direct transfer of virus to the alimentary tract of the susceptible is made possible. Under such circumstances, the importance of virus in pharyngeal excretions is particularly evident. Similarly, contamination of hands and utensils by pharyngeal virus may be involved.

In an infected family, it is usual to find that most members become infected at the same time, probably from a common exposure, although multiple paralytic cases occur in less than 10% of infected families. Such families form foci with a high density of infection. In contrast there is a
much lower incidence of infection among extra-household associates, and
a still lower incidence among non-contacts in the same neighbourhood.
Intimate association appears to be an important factor. Indeed, it has
been suggested that even during an epidemic, in areas with good sanitation,
infection is not widely or evenly diffused through the community, but tends
to be concentrated in the households and institutions where cases have been
recognized. Laboratory studies indicate that for every person with symp-
toms, there may be 10 to 100 infected individuals with no obvious illness.
Epidemiological evidence suggests that under some circumstances the
proportion may be even higher. Studies indicate that infection tends to
follow lines of movement of human beings particularly from infected
households and institutions. In what direction and how widely the infection
spreads is determined by the state of immunity of the exposed persons and
the nature of their association with infected individuals.

All means which permit of the transfer of infected pharyngeal excretions
are naturally suspect, but there is a great need for systematic and quanti-
tative studies for clarification of this subject.

Faeces provide a rich and persistent source of virus. As much as one
million infectious doses for monkeys can be detected in a gram. It seems
probable that poliomyelitis virus can be as readily distributed by faecal
contamination as is bacillary dysentery. Since opportunities for the direct
transfer of faecal contamination to the mouth are very frequent, it is readily
understood how infection with poliomyelitis virus can be easily acquired.

Sewage contains large amounts of virus when infection occurs in the
community served. Polluted water used for bathing and washing is a
constant risk. In a few outbreaks there have been reasons for incriminating
the water-supply, but otherwise there is essentially no evidence to suggest
that it has played any part. Milk or foodstuffs which are contaminated
with virus constitute potential vehicles of transfer but here again actual
demonstrations that the disease is spread in this way have been few. Never-
theless, the fact that infection is acquired early in life and is so uniform in
areas and communities with poor sanitation and much faecal pollution of the
environment supports the likelihood that these materials are an important
and a major source of distribution of virus. Conversely, in areas of good
sanitation, exposure and infection are reduced so that the dissemination of
virus is more sharply limited.

The role of flies in the dissemination of poliomyelitis is not entirely clear,
but they are certainly not essential. That they can become readily conta-
minated with virus from infected faeces has been adequately demonstrated.
Evidence indicates that in many areas, especially the more temperate zones,
the housefly is not commonly involved, but rather the sarcophagids and
blowflies. On the other hand, where fresh excreta are a significant source
of moisture, flies of the genus Musca will also feed. Virus has been isolated
from flies in cities with good sanitation where the possibilities of contamination with faeces in open privies were not obvious. The potential importance of flies is evident under conditions in which they can ingest virus and contaminate food which may be eaten by a susceptible subject. However, attempts to control the disease by suppressing the fly population with DDT sprays during or preceding an epidemic have not been successful.

Virus has also been recovered from cockroaches, but up to the present biting insects have not been implicated.

As already mentioned, the possibility may be considered that infected excretions introduced into wounds or abrasions, or by injections made through contaminated skin, can be a mode of infection.

Although poliomyelitis virus can be transmitted by many methods, it seems likely that different methods operate in various circumstances. Further work is needed to establish the relative importance of many of the suggested methods of spread.

4.5 Geographical distribution

Poliomyelitis is a worldwide infection. Paralytic cases have been reported from all continents and most islands. Epidemic outbreaks were not reported until about a century ago. Probably the first to be described (by Sir Charles Bell) occurred in 1834 in the Island of St. Helena. Subsequently widespread epidemics occurred in Scandinavia and the USA, and in other countries with high standards of living.

Before the second World War, poliomyelitis was thought to be a rare disease in tropical countries, but it is now known that the incidence of paralytic cases in these areas does not reflect the incidence of infection (see section 4.6, page 17).

Recently serious epidemics have occurred in many countries and islands, in many of them for the first time. Following outbreaks in the Eastern Mediterranean in 1941, Malta was affected in 1942 and 1943, South Africa in 1945, Mauritius and St. Helena in 1945, Northern Rhodesia in 1946, France in 1943, 1945, and 1946, Italy in 1946, Britain, Germany, and Austria in 1947, and the Nicobar Islands in 1947. During this time several severe epidemics occurred in the countries of the Western hemisphere.

In North America, the relative importance of poliomyelitis among the infectious diseases has increased progressively since the beginning of this century. Almost every year it occurs in epidemic proportions in some region of the USA and in 1952 the figures were the highest yet reported. In Latin America a notable increase in the number and extent of epidemics has been reported within the last decade; for example: Argentina, 1950, 1951, 1952; Chile, 1949, 1952; Costa Rica, 1944; Cuba, 1946, 1952; Mexico, 1948, 1951; Puerto Rico, 1946; Uruguay, 1947. In Europe there
has also been an increase in prevalence. Since the first extensive outbreak in Britain in 1947, widespread epidemics have occurred in 1949, 1950, and 1952. Other European countries, notably Norway in 1951, have had similar experiences. There has been no indication that the tendency for the disease to occur in epidemic form in Scandinavia is lessening. In 1952, Denmark, Germany, and Belgium suffered serious epidemics. Sweden and Iceland were severely affected in 1949.

The situation in the Eastern Mediterranean and in North Africa is of particular interest. Except for the lowest age-groups, the indigenous population is largely spared from paralytic attacks, but the disease continues to be quite a serious hazard for recently arrived adult immigrants, as well as children, from Europe and North America. Israel has experienced serious epidemics for the past three years.

In southern Africa local epidemics have occurred in several territories in which the main brunt of the paralytic form has characteristically also been on the recently arrived immigrants from Europe and the Union of South Africa. Such outbreaks have occurred in Northern and Southern Rhodesia, Angola, and Mozambique. A similar situation has been reported from various parts of Asia, for example, Bombay, Singapore, Bangkok, Japan, Korea, and the Philippine Islands.

Thus, while the incidence of paralytic poliomyelitis varies significantly in different parts of the world, in countries with mixed populations there is also a significant difference in the incidence in the different races. It has been suggested that there is an inherent racial or genetic factor to account for this difference, or that the state of nutrition, as determined by the staple diet, may exert an influence on the incidence of paralytic poliomyelitis; most primitive people subsist on a monotonous cereal diet whereas the majority of more advanced people have a varied mixed diet. However, as explained below, the most likely explanation is that in primitive surroundings people are more often exposed to endemic strains of virus, and so acquire an immunity not shared to the same degree by people living in more advanced communities.

4.6 Influence of climate and environment

As pointed out in the preceding section, the geographical distribution of poliomyelitis is worldwide, and there is at present no reason to suspect that there are inhabited regions where the disease will not appear. Climate and season nevertheless exert a profound effect on epidemiological behaviour, for within both northern and southern temperate climates the disease is more prevalent, in both its epidemic and non-epidemic forms, in summer than in winter. By contrast, in tropical areas, the cases occur more uniformly throughout the year. There are two possible explanations:
for this: either something happens during the summer which enormously facilitates the dissemination of the virus; or something happens which enormously reduces the resistance of the human host. Certain meteorological conditions apart from temperature, such as rainfall and humidity, have attracted the attention of investigators repeatedly, but no consistent correlation with the prevalence of poliomyelitis has been noted.

Another effect not so clearly related to climate, but which has been noted more frequently in tropical climates, often where crowded living conditions exist within cities or villages, is that the cases within such areas are almost entirely restricted to infants, i.e., the old-fashioned "infantile paralysis". An explanation offered for this is that within such areas poliomyelitis virus is plentiful, and so widely distributed throughout the environment that practically all indigenous children acquire infection before the age of five years. There is increasing evidence to support the view that the infantile poliomyelitis infection acquired between the ages of one and five years tends, by and large, to be milder and is in fact far less likely to be recognized than if acquired by a child below the age of one year or over five years. (The presence of transmitted maternal antibody modifies this situation in the early months of life.) For this reason, sporadic "infantile poliomyelitis" often lies as a hidden disease within a community, and its prevalence does not appear in statistics of notified cases. Thus, there are many countries, particularly in tropical and subtropical regions, in which the potential dangers of poliomyelitis to visitors and immigrants from other countries have not been recognized. To support this view there are frequent examples within the past decade in which military populations from temperate climates, when stationed in such an area, developed far higher case-rates for poliomyelitis than existed in the corresponding military populations in their homeland. This phenomenon has been recognized in the Eastern Mediterranean, in India, and in the Far East—notably in the Philippine Islands—but it also occurs elsewhere. It is a common situation in the tropics. The extent to which sanitary conditions influence this situation is not known, but it is suspected that they play an important role, along with other factors.

It is also clear that living conditions, or social or socio-economic conditions, particularly crowding, can affect the age distribution of poliomyelitis, as is the case with other diseases spread by human association, such as measles. The difference in the age distribution of urban as compared to rural cases of poliomyelitis within the same general area is one example of this, the urban cases being found among younger children. Similarly, within a given city it has been shown that children brought up under crowded and less favourable circumstances acquire poliomyelitis at an earlier age than do children brought up under more favourable conditions. These effects of environment have also been corroborated by studies on the ages
at which neutralizing and complement-fixing antibodies to Type 2 (Lansing) poliomyelitis virus are acquired. Such antibodies appear at an earlier age among those children living under poorer socio-economic circumstances than among children who are more favourably situated. The implication is that poor and crowded living conditions facilitate the spread of poliomyelitis virus. However, persons brought up under more favourable conditions may not derive any permanent advantage as regards poliomyelitis, for they may acquire the infection in later life, and it is questionable if this is desirable in view of the increased severity of the disease in the higher age-groups.

In general, it would seem that the poorer the standard of living and sanitation of a people, the more extensively is poliomyelitis virus disseminated among them and the lower is the apparent incidence of paralytic poliomyelitis. But primitive living conditions, per se, within the tropics or elsewhere are no guarantee of this relative freedom from paralytic poliomyelitis. This was abundantly clear from the experience in St. Helena in 1945 and the Nicobar Islands in 1947 where, although living conditions were relatively primitive, the outbreaks were among the most severe on record. In these epidemics, all age-groups up to the age of 20 were almost equally and severely affected. Similar severe epidemics have been reported from isolated communities in the Arctic, where all age-groups, including children, their parents, and even grandparents, have been involved. Such isolated communities may have been out of contact with poliomyelitis virus for long periods, and so even the adults have no immunity when a virulent invasive strain is introduced and severe epidemics are liable to occur.

Nevertheless, it is of considerable significance that widespread epidemics of poliomyelitis first occurred in Scandinavia, the northern USA, Australia, and New Zealand, countries with high standards of living and hygiene. As other countries have attained similar standards, so they in turn have begun to experience epidemics. As still other countries attain such standards, it may be anticipated that they too will have epidemics of poliomyelitis.

4.7 Environmental distribution of virus

In planning measures for the control of a disease it is essential to know the distribution of the infection in the environment of the affected community.

The virus of poliomyelitis has been isolated from the throats and from the faeces of cases of poliomyelitis and their associates (see section 4.2, page 11); their immediate environment must therefore be regarded as highly contaminated. The fate of the large amounts of virus excreted in the faeces of both overt and inapparent cases is therefore an important problem. In
cities and towns with water-borne sewage it has been shown that the virus is present in large amounts in the raw sewage. It also survives several stages of the process of sewage purification; thus it has been detected in the raw moist sludge and in the settled sewage, and, in lesser amounts, is sometimes found in the effluent from sprinkler filter beds, but it has not been detected in the effluent from the final sand filter beds or in dried sludge.

In many purification plants the sewage does not undergo such complete treatment and it has been demonstrated that the effluent may contain poliomyelitis virus. In other tests virus has been detected in the effluent from septic tanks and in the effluent from an improperly supervised composting works. From this it may be concluded that the effluents from sewage purification plants and from septic tanks may contaminate streams and rivers and if used for irrigation may contaminate vegetables and fruit. Thus, it has been suspected that polluted water and contaminated fruit or foodstuffs may be responsible for spreading infection, but up to the present there is insufficient information concerning their role in disseminating the disease (see section 4.4, page 14).

4.8 Survival of virus in inter-epidemic periods

No factual information is available on how virus survives in inter-epidemic periods in countries subject to periodic epidemics. It has been shown on several occasions that cases of infection may continue to occur through the winter, thus bridging the period between one epidemic season and the next. It is not known whether it is the usual method of survival of the virus in these communities, nor whether epidemics in such countries result from activation and spread of virus already present, or from the introduction of a strain of virus from without. In support of the latter theory it has been suggested that the virus strains causing the epidemics following the second World War in various countries were introduced by service personnel returning home from the Middle East and other highly endemic regions. Further study on this problem is necessary, and will depend on the development of more refined techniques for the differentiation of individual strains.

Further study is also necessary to determine the distribution of virus in tropical and subtropical regions where poliomyelitis is often highly endemic, and where large epidemics of the paralytic form of the disease affecting the indigenous population are less likely to occur. There is no doubt that the virus is widespread in these tropical communities, but how it is maintained throughout the year remains to be discovered. Although cases tend to occur all the year round, studies by actual isolation of the virus suggest that, even in these regions of high endemicity, infection
tends to occur in waves at particular times of the year which, in the absence of paralytic cases, remain unrecognized.

4.9 Extra-human reservoirs

Mention has already been made of the presence of poliomyelitis virus in sewage which is therefore a type of extra-human reservoir.

The possible occurrence of poliomyelitis virus in hosts other than man is also a consideration and, although still theoretical, it has been frequently investigated. Claims have repeatedly been made that human outbreaks may be accompanied or preceded by paralytic disease, sometimes in epidemic form, in birds, such as fowls, ducks, turkeys, budgerigars, and canaries, or in mammals, such as mice, dogs, cats, pigs, and cattle. However, all attempts to isolate poliomyelitis virus from droppings, faeces, or spinal-cord suspensions of such animals have failed so far. In a number of instances, Newcastle or distemper virus has been isolated, while in others no clue as to the etiology of the disease was found. Efforts to cause disease or subclinical infection by inoculation of poliomyelitis virus in animals of the above-mentioned species have not succeeded (with the exception of certain rodent species which are used for experimental work). On the other hand, it has been shown that a varying proportion of sera from domesticated, but not from wild, animals neutralize considerable amounts of poliomyelitis virus. Further work is indicated to evaluate the significance of these findings. Actually, the only spontaneous poliomyelitis infection described in animals is that of a few chimpanzees presumably infected from human sources.

Virus has been recovered from flies and cockroaches in nature, but it has not been shown that the virus multiplies in these insects; it is apparently transmitted mechanically (see section 4.4, page 14). The presence of virus in blood-sucking insects caught under natural conditions has not been demonstrated.

In order to explain the abundance of virus in sewage, attempts have been made to demonstrate virus multiplication in certain water protozoa, with negative results. Virus has not been demonstrated in plants grown on a sewage farm nor in tomatoes grown in hydroponic solution contaminated with virus.

5. CONTROL MEASURES

5.1 Introduction

From the time when poliomyelitis was first recognized to be an infectious disease until about 1930, various types of control measures were applied in an endeavour to check its spread. However, none of the methods used appeared to be successful, and so in recent years the common attitude
of health authorities has been that the general control measures usually applied to other infectious diseases are of little avail in poliomyelitis. This idea has received support from the pronouncements of certain authorities that, at the time of an epidemic, there are many hundreds of persons with inapparent infection for every case of paralysis. Although this may be true in very extensive and severe epidemics, virological studies of certain communities have indicated that the virus has been found mainly in the intimate associates of the paralytic case. For this reason, it appears possible that some reduction in the number of paralytic cases may be achieved by quarantine measures centred particularly around the first paralytic cases occurring in a community.

Institution of control measures will probably have an even greater chance of reducing the number of paralytic cases in isolated rural or island communities. Here not only may measures be exerted against an individual infected household, but it should also be possible to prevent the entry into an apparently healthy community of individuals from infected localities.

Many attempts have been made in the past to use convalescent serum as a prophylactic or therapeutic measure in poliomyelitis, without any conclusive evidence of its efficacy having been obtained. Improved methods of the fractionation of plasma have been introduced and it is apparent that antibodies against poliomyelitis, as well as against many other infections, are concentrated in the gamma-globulin fraction of pooled adult human plasma. Experiments in monkeys and chimpanzees and to a lesser extent in man have shown that if this gamma-globulin is given before exposure paralysis may be prevented. Thus, this material, which is now in short supply, may be a useful measure of control under special circumstances (see section 5.4.1, page 27).

A more promising method of control is the possible use of prophylactic vaccines (see section 5.4.2, page 29) which are not yet available. However, the results of numerous experiments in primates and a growing experience from the experimental use of vaccine in man indicates the probability that a poliomyelitis vaccine may become available to the health officer in the not too distant future.

The various methods of control of the disease will be discussed under separate headings.

5.2 Measures to reduce the spread of infection

5.2.1 Notification of cases

All available aids, both clinical and laboratory, should be used in an attempt to make a definite diagnosis. Cases considered to be poliomyelitis should be notified either as non-paralytic or paralytic. Although the
diagnosis of non-paralytic poliomyelitis is less reliable than that of paralytic poliomyelitis, the figures so obtained, along with those of mortality rates, permit of some estimate of the severity of an epidemic, of a comparison to be made with data from other epidemics, and of an evaluation of the validity of the reporting. A patient is considered clinically to have poliomyelitis for purposes of notification if the symptoms and signs correspond with the following descriptions (see also section 3, page 7):

(a) *Non-paralytic poliomyelitis*

An illness characterized by fever, headache, vomiting, sore throat, listlessness, stiffness of neck and back; pains in the back, neck, trunk, or limbs, and hyperaesthesias; cerebrospinal fluid changes are usually found. The diagnosis is often strongly supported by epidemiological evidence; for example, known contact with a paralytic case or residence in an epidemic area.

(b) *Spinal paralytic poliomyelitis*

Signs and symptoms of non-paralytic poliomyelitis with the addition of partial or complete paralysis of one or more muscle groups, detected on two examinations at least 24 hours apart.

(c) *Bulbar paralytic poliomyelitis*

Signs and symptoms of non-paralytic poliomyelitis with involvement of the cranial nerves and/or medullary centres.

5.2.2 *Isolation of the patient*

It is established practice in some countries for patients to be isolated for 1-3 weeks from the onset of the major illness if paralytic, or from the onset of symptoms in non-paralytic cases. Periods of isolation longer than three weeks may be considered advisable under special circumstances, since excretion of virus in faeces may continue for several weeks (see section 4.2, page 11).

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8 The standard public-health procedures of isolation and quarantine have little or no effect on the spread of such diseases as measles or influenza but have proven exceedingly valuable in combating enteric infections like typhoid fever. In some countries, similar procedures have been applied to poliomyelitis for almost 30 years, but without apparent success. Since poliomyelitis is essentially an alimentary infection, this might seem confusing. It must be remembered, however, that whereas typhoid carriers and excreters can be traced by the aid of comparatively simple laboratory methods, it was until recently a technical impossibility as far as poliomyelitis was concerned. This was all the more serious because of the much larger proportion of otherwise unrecognizable disseminators of the virus. With the introduction of the tissue-culture technique, virus laboratories for the first time are technically in a position to offer health authorities at least some support in attempts at controlling the spread of infection, although the present number and capacity of laboratories able to carry out this work is entirely inadequate to permit of a systematic application of methods similar to those used in the control of typhoid fever.
When conditions permit, isolation of the patient in his home should be considered. If the patient is removed from his home, it should be to a hospital or unit for infectious diseases, a special hospital for poliomyelitis patients, or an isolation unit (one or more rooms) in a general hospital.

Suspected cases who are removed to hospital should preferably be isolated from known cases of poliomyelitis until the diagnosis is confirmed.

At some future date, it may be possible to determine the periods of isolation for individual patients by using tissue cultures as a means of detecting the presence of virus in the faeces.

*Concurrent disinfection.* Throat discharges and faeces are infectious and should be disposed of as quickly and safely as possible. Soiled articles should be promptly disinfected by heat. Patients should have individual bed-pans unless immediate cleansing and sterilization by heat is possible.

All those attending the patient should be instructed that the disease is highly infectious and that they must practise maximum hygienic precautions (e.g., those precautions which would normally be adopted in attending a case of typhoid fever). Hand washing before and after handling the patient is essential. Nurses need not be isolated but, where it is practicable, should not attend other patients while caring for acute poliomyelitis patients.

*Terminal disinfection.* A hospital isolation unit (or room), after being used for poliomyelitis patients, and before being opened to receive other cases, should be washed thoroughly with soap and water.

Patients should not be moved to an orthopaedic ward or hospital until the locally approved period of isolation is complete. Poliomyelitis convalescents may still be excreting virus in the stool, and therefore should not associate for 6-8 weeks from the onset of the disease with other orthopaedic patients or others in swimming-baths for rehabilitation or pleasure. If possible, poliomyelitis patients should have completely separate rehabilitation units.

### 5.2.3 Measures regarding contacts

*The family.* Family and intimate associates, especially children, should be considered as probably infected. Children with familial or intimate exposure should be confined to their homes for 21 days, avoiding over-exertion. Adults \(^6\) need not be confined, but should refrain from over-exertion and should observe maximum hygienic precautions; they should

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\(^6\) Infection of one or both parents, as a result of handling a young infant with abortive or inapparent poliomyelitis in the infectious stage, is becoming more common in countries where the age at which poliomyelitis is contracted continues to advance.
refrain from association with children other than their own, and should avoid intimate contact with adults. They should not handle foodstuffs served outside the family. Any associates who do not feel well should go to bed and a physician should be consulted.

Day-nurseries and nursery schools. Numerous investigations have demonstrated a very high infection rate in infants associated with paralytic cases in such institutions. If a case occurs, nursery schools should be closed and the staff, all the children, and their siblings should be treated in the same manner as family associates. The parents of such children should observe maximum hygienic precautions and refrain from over-exertion.

Residential nurseries, schools, and children's camps. If a case occurs in such a community, the other residents should be kept under observation for at least 21 days and instructed to avoid over-exertion; no new children or adults should be introduced. It should be remembered that if the residents are dispersed to their own homes they may seed the virus in a number of presumably unaffected communities.

5.2.4 Measures regarding the community

The public should be instructed in the probable modes of spread of the disease, and advised to take the following precautions in epidemic periods:

1. Wash hands frequently, especially after defaecation and before eating.

2. Protect food from flies, and thoroughly wash uncooked food, such as fruit and vegetables.

3. Avoid intimate associations (shaking hands, common eating utensils, communal towels, etc.) with members of a family in which a case of poliomyelitis has occurred within three weeks.

4. Treat all febrile illnesses with caution; bed rest or at least the avoidance of over-exertion for a period of a week is advisable.

5. Avoid over-exertion, particularly if not feeling perfectly well.

6. Unnecessary travel into or out of communities where the disease is prevalent should be discouraged.

7. In the presence of a severe local epidemic it would be wise to delay opening schools after the summer holidays, but normally schools need not be closed nor public gatherings forbidden. Swimming-pools with adequately chlorinated water need not be closed, but should not be overcrowded. Unchlorinated pools should be closed.

If, in the future, isolation and quarantine applied on a national level should prove to be of real value as methods of prevention of epidemics,
some countries, particularly those where the general level of immunity
in the population is low, might be inclined to apply quarantine on an
international level as well. The committee feels that, at present, restrictions
of international travel would not be justified and recommends that the
developments in this field be closely followed by the proper authorities.

5.3 Measures to reduce the incidence of paralysis

It has already been mentioned in section 4.1 (see page 10) that a number
of factors may predispose to or precipitate paralysis. Some reduction in
the incidence of paralysis may be expected to result from attention to the
following principles:

1. Elective operations for the removal of tonsils and adenoids should
not be carried out during epidemic prevalence of poliomyelitis.

2. The activity of persons suffering from an illness in which there is
reason to suspect poliomyelitis should be restricted for a week, preferably
by rest in bed.

3. Persons in intimate association with a case of poliomyelitis should
take the minimum amount of exercise during the period in which symptoms
might be expected to develop, that is, between 5 and 21 days after exposure.
Fatigue from any cause, including travel, should be avoided.

4. With regard to immunizations and injections, of which mention has
been made in section 5.1, the following extract from the report of the
WHO Conference of Heads of Laboratories Producing Diphtheria and
Pertussis Vaccines is endorsed by the committee:

"The conference feels that the effectiveness of diphtheria- and whooping-cough-
immunization campaigns should be disturbed as little as possible by the fear of subsequent
poliomyelitis. Immunization against diphtheria and whooping cough should normally
be continued during the poliomyelitis season; but if the disease should assume serious
epidemic proportions in any given area, all immunization should be temporarily suspended
in that locality. If, in the opinion of the local health authority, the epidemic is of minor
severity, then immunization with diphtheria and whooping-cough vaccines may be
continued, but the use of adsorbed combined vaccine should be discouraged." 7

5. It seems advisable to suspend during epidemics of poliomyelitis the
large-scale use of intramuscular injections of an irritant character, for
example, organic arsenicals and heavy metals.

6. In view of the possibility that the skin may be contaminated with
poliomyelitis virus, before administering an injection, cleansing with tinc-
ture of iodine is recommended, and separate heat-sterilized syringes and
needles should be used for each patient. 8

8 Alternatively, if individual syringes cannot be used, devices designed to prevent
contamination of the nozzle of the syringe may be considered, e.g., that devised by
Professor R. Gispen (see Lancet, 1952, 2, 171).
5.4 Measures for the production of specific immunity

5.4.1 Passive immunization

Gamma-globulin prepared in the USA and other countries from the pooled blood of large numbers of adult donors contains antibody to the three types of poliomyelitis virus. Gamma-globulin has been shown to be effective in the prophylaxis of poliomyelitis in primates infected with virus by feeding or other peripheral routes (see also section 4.3, page 12), and it would appear that a low level of circulating antibody may be sufficient to confer protection. It has been shown that when gamma-globulin is injected intramuscularly in doses of approximately 0.1 ml per lb (0.22 ml per kg) of body-weight into persons whose serum does not contain Type 2 poliomyelitis antibody, small amounts of such antibody become demonstrable in the serum. Studies on the persistence of passively administered poliomyelitis antibody in the serum of primates suggest that the antibody level declines in a linear fashion, the “half-life” being about 7-10 days. Other observations suggest that in man the half-life may be somewhat longer. These results indicate that there is a regular, and rather rapid, decline in serum antibody level following passive immunization. (This is in contrast to the behaviour of antibody stimulated by active immunization.)

Against the background of these experimental observations, and having regard to the protective effect of gamma-globulin in measles and infectious hepatitis, Hammon and his collaborators in the USA carried out in 1951 and 1952 a large-scale trial of gamma-globulin as a prophylactic during times of epidemic prevalence. Injections were given intramuscularly in children aged 1-11 years, and the average dose was 0.14 ml per lb (0.31 ml per kg) of body-weight. Control groups received intramuscular injections of gelatin. Gamma-globulin appeared to afford protection against the paralytic disease over a period of 5 or 6 weeks, whereas no protection was demonstrated during the week immediately following the inoculation. However, it has been claimed that children who received gamma-globulin during the week prior to onset of illness developed a modified disease, and that paralysis was mitigated.

A serious disadvantage in the practical application of gamma-globulin as a prophylactic in poliomyelitis is that, even when employed under the most favourable conditions, hundreds or thousands of persons will be unnecessarily inoculated for each one who actually derives protection. It is clear that the ultimate answer to the control of poliomyelitis by immunization lies in active and not in passive immunization. In this regard, one conclusion that can be drawn from the experiments with gamma-globulin in man is that low levels of circulating antibody appear to afford a degree of protection against paralysis.
The committee fully realizes that in many countries no gamma-globulin is yet available, whereas in others it is in short supply. Therefore, although a number of individual cases may be prevented as a result of mass inoculation of large groups of children and adults, this method of use of gamma-globulin does not seem at present to be practicable for the control of poliomyelitis epidemics. It may, nevertheless, be employed in certain other circumstances. For example, it may be administered to household contacts of clinically diagnosed cases, and to pregnant women.

Although it is not common to have multiple cases of paralysis in a family, the incidence is said to be from 5 to 20 times the incidence of poliomyelitis in the general population. The available data on the time of onset suggests that only about 10% of the additional cases occur long enough after the initial case to permit gamma-globulin to exert its maximal protective effect. However, if the administration of gamma-globulin shortly before the onset of illness does in fact modify the disease, as has been claimed, then some modification may be expected in approximately another 30% of the multiple cases.

The committee considers that the administration of gamma-globulin to household associates represents a reasonable distribution of the available supplies. The administration of gamma-globulin to intimate associates who are not actually members of the infected household is also reasonable. The committee expresses the hope that accurate information on the results of the administration of gamma-globulin will be collected so that a proper evaluation can be made.

It would also seem a reasonable practice to give gamma-globulin to patients in a hospital ward or to children in a nursery school following the recognition of a case of poliomyelitis. Gamma-globulin is also indicated in the protection of individuals coming from an uninfected area into an infected home or institution. Conversely, it can be given to otherwise unexposed groups into which an infected person is knowingly introduced. The new-born infants of mothers developing poliomyelitis present another instance where gamma-globulin may be given. Tonsillectomy is generally avoided during a poliomyelitis epidemic, but if the best interests of the patient are to be served by performing the operation, then a protective dose of gamma-globulin should be given.

In view of the shortage of this valuable substance in almost all countries, the committee considers that the administration of gamma-globulin should be carefully planned, and in general restricted to the circumstances outlined above. Gamma-globulin has no therapeutic value and should not be given after the onset of symptoms.

In conclusion, the committee points out that the use of gamma-globulin as a prophylactic in poliomyelitis is still in its infancy, and only after more
experience will it be possible to estimate its value and the best methods of using the available supplies.

5.4.2 Active immunization

Whereas passive immunization can afford only temporary protection and thus has limited indications, it is the opinion of the committee that considerable expectations are to be placed on active immunization against poliomyelitis, especially in areas in which naturally acquired immunity tends to develop at an older age, and is less uniformly distributed throughout the population.

Results have been reported recently, showing that different methods of immunization against poliomyelitis are being investigated in a number of laboratories. The general trend of research is directed towards the preparation of two different types of vaccines. One contains inactivated material obtained from each of the three antigenic types of viruses, and is injected parenterally with or without adjuvants. In the other line of approach, attempts are being made to attenuate strains of virus of the three antigenic types, in the hope that it may be possible to administer a living but avirulent virus by the oral route, thus duplicating the mechanism of natural infection.

Experiments in animals, and a limited but significant number of tests in human volunteers, have shown that vaccines of such types consistently induce the formation of specific antibodies and, at least in the monkey, result in subsequent resistance to infection with a challenge virus.

Nevertheless, the committee points out that, however promising these results may be, vaccination procedures against poliomyelitis are still in an experimental stage, and that "poliomyelitis vaccines" of unquestionable value are not yet available for general use. It is highly desirable, therefore, that further studies of methods of immunization be carefully pursued, and carried out under proper scientific supervision. Mass immunization should not be generally adopted until such time as sufficient data are available on the innocuity and efficacy of the vaccines, and information on the level and duration of induced immunity should be sought.

6. EXPERIMENTAL POLIOMYELITIS

6.1 Host-range and methods of infection

The virus of poliomyelitis has a limited host-range. Although a great number of species of domestic and wild animals and birds have been tested for susceptibility, it has been found that, on primary isolation, it regularly produces paralytic disease only in primates. The primates that
have been shown to be susceptible and used in experimental studies include various species of macacus, cebus, and cercopithecus monkeys, several species of baboon, and the chimpanzee.

6.1.1 Monkey

Different strains of virus vary considerably in their virulence and some are relatively non-pathogenic for monkeys; in particular, a number of Type 3 (Leon) viruses have been found by some workers to be relatively avirulent in these animals. However, monkeys may be readily infected with most strains isolated either by monkey inoculation or by tissue culture.

In experimental studies, monkeys have been infected by various routes of inoculation, including the intracerebral, intraneural, intramuscular, and intraperitoneal routes. Intranasal instillation has also been widely used.

Most species of monkeys are less susceptible to infection via the gastrointestinal tract. However, the cynomolgus monkey may be infected by the oral route by swabbing the virus on the pharynx or by the ingestion of contaminated food.

Some strains of virus readily infect primates when given into or under the skin whereas others do not.

Viremia occurs during the incubation period of a significant number of experimentally infected monkeys, especially cynomolgus monkeys. This finding has called for a review of the previously generally accepted idea that in poliomyelitis the invasion of the central nervous system takes place along the neural pathways. There is little doubt that, under experimental conditions, migration of the virus along nervous tissue does occur. In cynomolgus monkeys infected by the oral route, a number of experiments have suggested that the virus spreads from the oropharynx along the Vth, IXth, and Xth cranial nerves, or along the sympathetic nerves from the intestine. Virus has been recovered from the corresponding nerve ganglia which show characteristic pathological changes. It has also been observed that recent tonsillectomy predisposes cynomolgus monkeys to paralytic poliomyelitis after oral infection.

The occurrence of viremia after experimental feeding in chimpanzees further emphasizes the need for study of this important aspect of experimental poliomyelitis.

The incubation period varies from 5 to 35 days, but is most commonly 7-21 days. At the onset of illness, the animal develops fever, its coat becomes slightly ruffled, and it sits quietly in its cage. Later, tremors are observed and paralysis develops which progresses until the animal dies. Some monkeys have an inapparent infection, some a non-paralytic attack, and others recover from the acute stage with residual flaccid paralyses of varying degrees.
At post mortem, apart from enlargement of the lymph glands there may be no macroscopic evidence of disease except in the central nervous system. The blood vessels of the meninges and brain are congested. On histological examination, the distribution of lesions is similar to that found in fatal human cases. A description of the histological appearances will be found in Annex 1 (see section 4.2, page 55).

6.1.2 Chimpanzee

The disease caused by poliomyelitis virus in chimpanzees most closely simulates that naturally occurring in man. These animals may readily be infected by feeding the virus as well as by parenteral inoculation.

After feeding, the great majority of chimpanzees have an inapparent infection only; about 20% or less develop a paralytic attack. In both the paralytic and the inapparent form, the virus may be detected in the blood of a significant number of animals for a few days and in the faeces for about three weeks.

Chimpanzees which have recovered from an alimentary infection nearly always resist a second alimentary infection by feeding with the same strain but are still susceptible to infection with virus of another type. It is not yet clear whether this resistance applies uniformly to all strains of the same type, and further study of the relationship is desirable, for it may be possible to differentiate strains of virus within the three recognized types by this procedure.

6.1.3 Rodents

Some strains of Type 2 (Lansing) virus have been adapted to white Swiss mice and other rodents, including the cotton-rat and the Syrian hamster. Once established, these strains can be passaged indefinitely in these rodents. With prolonged passage, their virulence for rodents increases, while they become less virulent for monkeys.

Leon, the prototype strain of Type 3, and Mahoney, a strain of Type 1, viruses have been adapted to white Swiss mice and Syrian hamsters by intraspinal inoculation, but passage of these viruses in rodents by other routes of inoculation has not been achieved.

Various important observations have been made on factors determining the resistance of mice to the Type 2 virus. Infection with most strains of this type is usually only possible by the intracerebral route. The incubation period varies greatly in the same batch of mice—from 2 to 30 days. The infected animals then either die acutely or develop flaccid paralysis followed by death.

The inoculation of a variety of substances intramuscularly, including vaccines, horse serum, and snake venom in sublethal doses, prior to the
inoculation of virus produces a significant shortening of the average incubation period. On the other hand, the inoculation of adult human serum exerts a protective effect (see Annex 2, page 57).

A diet deficient in certain essentials such as thiamine induces a state of resistance to the virus.

After administration of cortisone there is a marked increase in susceptibility to the infection and mice become susceptible to peripheral inoculation of the virus.

Interference between Coxsackie B virus and Lansing virus infections in mice has also been reported from one laboratory.

6.1.4 The developing chick embryo

Recently it has been reported that the MEFI strain of Type 2 poliomyelitis virus has been propagated in the developing chick embryo by inoculation both into the yolk sac and into the allantoic cavity.

6.2 Immunity in experimental poliomyelitis

6.2.1 Convalescent immunity

Monkeys which have suffered a paralytic infection are highly resistant to a second inoculation of the same strain but may readily be infected with strains of other types. Our knowledge of the three immunological types of poliomyelitis virus has come largely from studies on monkeys based on this fact.

6.2.2 Induced immunity

6.2.2.1 Active

Immunity to each of the three known types of poliomyelitis has been developed in chimpanzees and monkeys by repeated inoculation of active or formalin-inactivated suspensions of virus. Immunity has been suggested by the development of serum antibodies, and has been proved by the demonstration of resistance to challenge with active virus of the same type. This work has raised the hope that a similar immunity may be produced in human beings by the administration of formalin-killed vaccines. It may be anticipated that monkeys will be essential for testing the safety and potency of vaccines against poliomyelitis developed for human use. Mice can also be actively immunized against strains of poliomyelitis virus to which they are otherwise susceptible.

6.2.2.2 Passive

As already mentioned (see section 5.4.1, page 27), the experimental disease in monkeys, chimpanzees, and mice can be suppressed by the injection
of human gamma-globulin prior to the administration of the challenge virus. The place of gamma-globulin in the control of poliomyelitis is considered in section 5.4.

6.2.3 Protection (neutralization) tests

The presence of serum antibody against all three types of virus in the blood of human beings and of animals may be demonstrated by serum protection or neutralization tests carried out in monkeys. However, because of the difficulty and expense of obtaining large numbers, few large-scale studies have been made in these animals. Such studies may now readily be made in tissue cultures.

The Lansing strain of Type 2 virus has been used extensively in mouse-protection tests. Serum surveys of populations in different parts of the world using this test have contributed largely to our knowledge of the epidemiology and extent of poliomyelitis virus infection. (See Annex 2, page 56.)

6.3 Tissue specificity

The classical description of the clinical picture of acute anterior poliomyelitis implied that the virus was strictly neurotropic and had a specific affinity for the anterior horn cells of the spinal cord; in fact, only the presence of changes in these cells was accepted as indisputable evidence of transmission of the disease to primates. It was considered that the site of multiplication was essentially limited to the spinal cord.

It is now apparent that the virus is not so strictly neurotropic as was thought, and has an affinity for other tissues. In certain primates, when the virus is fed, or inoculated parenterally, infection may take place with or without signs of involvement of the central nervous system. Virus is excreted in the faeces for such a long time and in such quantities that one must accept either that multiplication of the virus occurs at some point between mouth and anus, or that the virus is being actively excreted into the gut from some other site of multiplication. Virus has been recovered in appreciable amounts from the wall of the intestine in such animals; exactly which layer or system is involved is, however, not known. Poliomyelitis virus has also been recovered from mesenteric lymph-nodes following feeding, and local lymph-nodes after intracutaneous or subcutaneous inoculation, but it has not been proven that multiplication of virus occurs in these tissues. The virus has also been recovered from the heart and spleen but, as virus has been shown to be present in the bloodstream, it is difficult to determine by a single examination whether the virus has actually multiplied in these organs. There have also been suggestions that local multiplication of virus has occurred at the site of intra-
muscular injection of certain strains of virus, but there are insufficient data on which to decide whether this occurs in all species of primates and whether it is a common property of many strains of virus.

One of the most striking facts in this field of investigation is that the kidney and testicle of the monkey, which appear to support little or no multiplication of the virus in vivo, support growth of virus in tissue cultures even when the organs are obtained from immune animals.

In adult mice, multiplication of virus appears to be confined to nervous tissue. However, at least one strain of Type 2 (MEFI) has infected suckling mice by the intra-abdominal route. The injection of cortisone before peripheral inoculation of this virus in adult mice or hamsters is followed by apparent growth of the virus in the peri-adrenal fat, and in the limb and paravertebral muscles; more or less coincidently viraemia occurs, and then multiplication takes place in the spinal cord. In contrast to these findings in vivo, even the Lansing type of virus has not been cultivated in tissue cultures of embryonic or adult tissues of the mouse.

6.4 Animal infections with the poliomyelitis group of viruses as patterns for the study of poliomyelitis in man

By systematically planned and controlled animal experiments, information can be obtained on some aspects of the infection in man that cannot otherwise be readily studied. It must be borne in mind, however, that the results of model experiments are not necessarily directly applicable to the human disease. The history of poliomyelitis research provides an example of this kind. The early findings that rhesus monkeys regularly contracted the disease after intranasal inoculation, whereas feeding of the virus only exceptionally produced infection, were presumed to apply equally to man. As a result, it was held that poliomyelitis was spread exclusively by droplet infection through the olfactory route, a theory that was not seriously challenged until 25 years later. Undoubtedly, attention to this theory did much to retard progress in the field of the epidemiology of poliomyelitis. Nevertheless, animal experiments cautiously interpreted often provide valuable information. In the preceding section important studies in chimpanzees which have shed light on the natural disease of man have been outlined.

Furthermore, there is a naturally occurring infection of mice—mouse encephalomyelitis (Thelier's disease)—which shows many analogies with human poliomyelitis in its natural history and epizootiological pattern. Thelier's virus is widely spread and is perpetuated in all stocks of mice so far examined. Production of a virus-free mouse colony has therefore been a difficulty, which has been solved by rearing new-born mice on white rats as foster-mothers. The infection offers an opportunity for the
study of a virus, apparently closely related to the poliomyelitis virus, in its natural host.

7. LABORATORY TECHNIQUES FOR THE ISOLATION AND IDENTIFICATION OF POLIOMYELITIS VIRUSES AND FOR THE STUDY OF ANTIBODIES

7.1 Animals

Poliomyelitis virus may be readily isolated at autopsy from patients who have died in the first or second week of the acute disease. The tissues and material from which it is most readily obtained include those of the central nervous system, notably the pons, medulla, and the cervical and lumbar regions of the spinal cord. Occasionally, it can be found in other tissues, such as lymph glands and the intestinal wall, but a more reliable extraneural source is the intestinal contents.

In clinical and subclinical cases of poliomyelitis, the virus can be found in the oropharyngeal secretions during the early days (about one week) of illness, and also during the days immediately prior to onset. This also is true of the faeces, where the virus remains for much longer periods (see section 4.2, page 11).

Two methods of isolation and identification of the virus are now in use, namely, the inoculation of monkeys and tissue cultures.

Techniques for the isolation and identification of poliomyelitis virus by inoculation of monkeys, including the collection of appropriate material, its storage, and preparation for shipment to other laboratories, are given in Annex 1 (see page 47).

The technique of carrying out neutralization tests in mice with Type 2 (Lansing) virus is given in Annex 2 (see page 56). The use of monkeys for carrying out neutralization tests with Types 1 and 3, or for typing of strains, is expensive, requiring many animals. In view of the availability of tissue-culture methods, it is recommended that these newer (tissue-culture) methods be employed in future studies. However, type-specific antisera required for these tests are generally prepared in monkeys.

7.2 Tissue cultures

The discovery by Enders, Robbins & Weller that poliomyelitis viruses will proliferate in vitro in tissue cultures of human tissue has revolutionized the methods used in the poliomyelitis laboratory. During the past few years, very rapid advances have been made in this field, and experience has shown the methods of tissue culture to be more rapid, more convenient, and fully
as accurate as methods involving the inoculation of mice or monkeys. Tissue-culture techniques have been developed for the isolation, subcultivation, typing, and quantity production of poliomyelitis viruses; estimations of poliomyelitis antibody in serum can likewise be performed in tissue cultures.

At the present time many different technical methods are in use. Examination of published data indicates that essentially similar results can be obtained by techniques in which a variety of tissues and nutrient media are employed. The committee does not consider that it would be desirable, in the present stage of rapid progress and changing techniques, to recommend a standard procedure. Indeed, the choice of method may be largely dictated by the local availability of materials. In some countries, for example, there are real difficulties in obtaining human embryonic tissue, whereas in others monkey tissue is not readily obtainable. Many of the ingredients of the nutrient media commonly employed by workers in North America cannot easily be obtained elsewhere.

The results with the tissue-culture technique that have been obtained by independent poliomyelitis laboratories in various parts of the world indicate that these techniques can be successfully adopted by workers experienced in the methods of virology, and in particular in the study of poliomyelitis virus. It is, however, clearly desirable that more laboratories should undertake this type of work, especially in countries where no facilities at present exist. The committee suggests that arrangements should be made for selected workers desirous of learning tissue-culture techniques to study in a laboratory already experienced in this work. It is believed that such a period of study and training is essential for the successful use of these methods. In the meantime, arrangements might be made whereby selected laboratories would undertake the examination of a limited number of specimens from countries where there are as yet no adequate facilities. Such an arrangement would provide information without too much delay as to the antigenic types of virus causing poliomyelitis in many parts of the world. Consideration might be given to the support of established laboratories to act as reference centres, with the major function of examining and comparing strains of human poliomyelitis virus, and those strains which resemble human poliomyelitis virus in some respects but which apparently differ antigenically from the recognized types. The preparation of standard strains of virus and type-specific antisera for laboratory purposes might also be undertaken.

For the reasons given above, only a brief review of existing methods is given here.  

* Selected references on tissue-culture techniques will be found in Annex 3, page 66.
Poliomyelitis virus may be cultivated by two main methods, in flasks and in roller tubes. The roller-tube technique is the more popular but flask ("suspended cell") cultures are still used for certain purposes.

7.2.1 Materials

The following tissues have been successfully employed: human embryonic skin muscle, intestine, kidney, and lung; post-natal human kidney, testis, uterus, prepuce, tonsil, and subcutaneous tissue; monkey testis, kidney, and lung. In addition, a strain of epithelial cell (HeLa) derived from a human carcinoma has been used. It is difficult to recommend one of the above sources in preference to another, and the final choice must depend on local availability. However, most work has been carried out with human embryonic tissue, human uterus and tonsil, and monkey kidney or testis; all these tissues are known to give satisfactory results.

Several media have been recommended by different workers. Most of the media contain a balanced salt solution with the addition of animal protein-containing materials such as horse serum and beef- or chick-embryo extract; ox-serum ultrafiltrate may be added; bovine amniotic fluid, with its very low protein content, can be used with advantage and does not require the addition of balanced salt solution. These media are excellent for most applications of the tissue-culture technique. Another valuable medium is lactalbumin hydrolysate. A completely synthetic nutrient, which does not contain animal protein, has been employed for the development of an experimental poliomyelitis vaccine from tissue cultures. It is customary to add antibiotics to all media, and this enormously facilitates the maintenance of bacterial sterility. Phenol red is commonly used as an indicator.

7.2.2 Preparation of cultures

Flask cultures are prepared by adding minced tissue to nutrient medium in conical flasks. No active cell growth takes place under these conditions, but if the nutrient fluid is replaced twice weekly, cell metabolism continues for several weeks. These cultures have been used for the isolation of viruses from human specimens, especially from stools.

Roller-tube cultures have now largely replaced flask cultures, mainly because the effects of virus action can be seen by direct microscopical examination of the tube. Fragments of tissue are embedded in a clot of chick plasma, and nutrient fluid is added. The tubes are placed horizontally in a slowly rotating drum at 37°C. After 3-7 days, depending on the tissue and the medium, an abundant growth of cells develops around each fragment. The material which presumably contains virus is then added. After a further period of incubation, the fragments are examined
microscopically. In the presence of poliomyelitis virus, a most striking change occurs in the cells. They become granular, necrotic, and rounded, and finally disintegrate. These changes have been referred to as the "cytopathogenic" changes. The time required for the cytopathogenic changes to become visible is variable. Some strains produce the effect in 24 hours, others not for 7 days or longer. In general, the change develops more quickly in outgrowths of epithelium, as from monkey kidney, than in fibroblasts, as from human tissue or monkey testis. Several workers report that good results can be obtained in similar cultures that are not rotated, but merely kept stationary in the horizontal position.

7.2.3 Isolation of poliomyelitis virus in tissue culture

The tissue-culture technique has been found to be very suitable for the isolation of poliomyelitis viruses from pathological specimens. Suspensions of central nervous system or throat washings are inoculated without further treatment. Stools are prepared as an aqueous suspension and usually clarified by centrifugation. Some workers prefer to concentrate the virus by ultracentrifugation. The material to be tested is inoculated in roller-tube cultures which, after incubation, are examined for the cytopathogenic changes. Subcultures are then made, and eventually the agent is identified by a virus neutralization test with type-specific antisera. Some difficulty may be experienced with stool preparations which may digest the culture or cause a non-specific degeneration of the cells. For this reason, some workers prefer to inoculate stool preparations in flask cultures, testing for the presence of virus by making subcultures in roller tubes.

7.2.4 Quantity production of poliomyelitis viruses

Considerable quantities of virus-infected fluids can be obtained from tissue cultures prepared in larger containers. Roller cultures can be prepared in large bottles; as a modification of the flask technique, cultures can be set up in large bottles which are suitably rocked. By various methods that have been described in the literature, quantities of 500 ml or more of infected fluid can be obtained from each container.

7.2.5 Titration of virus in tissue culture

Virus-containing preparations can be readily and accurately titrated in roller-tube tissue cultures, by inoculating serial dilutions in groups of five or more cultures and calculating the 50% end-point of infectivity.

7.2.6 Titration of antibody in tissue culture

One of the most valuable applications of the tissue-culture technique depends on the fact that specific antibody inhibits the cytopathogenic
action of the virus. Accordingly, poliomyelitis antibody can be titrated by inoculating mixtures of known virus and serial dilutions of serum into roller-tube cultures. The 50% neutralizing end-point of the serum can then be calculated. Such tests are of value in testing paired sera for antibody, in serum surveys, and in the assay of gamma-globulin or animal immune serum.

7.2.7 Typing of poliomyelitis viruses in tissue culture

Until the introduction of tissue-culture techniques, strains of poliomyelitis virus could be typed only by expensive and time-consuming methods in monkeys. Strains can now be typed inexpensively, rapidly, and probably more accurately in tissue culture. Various modifications of method are in use, but they all depend on the fact that poliomyelitis antibody specifically inhibits the multiplication of poliomyelitis virus in vitro. Series of cultures are prepared, to which are added mixtures of the virus to be typed, and standard antisera to the three types of poliomyelitis virus. Usually the results of such typing tests are clear-cut, the strain being inhibited by one of the type-specific sera only.

Apart from the fact that at least one type of Coxsackie virus grows and produces cytopathogenic changes in tissue culture, recent work has revealed the existence of agents, presumably virus in nature, which also produce well-marked cytopathogenic changes in tissue culture and are not inhibited by poliomyelitis antisera. These strains have been recovered most commonly from the stools of cases of aseptic meningitis. Some of these agents are pathogenic for suckling mice, but most are not, nor do they infect monkeys. Further studies of these agents should be undertaken.

7.3 Complement fixation

The complement-fixation test has proved of great value in the diagnosis of many virus diseases. Only in recent years, however, has its use in poliomyelitis become possible. The failure of earlier attempts to demonstrate complement fixation must probably be attributed to the comparatively low virus content of the materials then available for the preparation of antigens.

Two types of antigen are now being tested. One is prepared from the brains of mice infected with a specially adapted strain of virus belonging to Type 2 (Lansing). The other antigen is obtained from tissue cultures of each of the three types. The results so far obtained indicate that the complement-fixation reaction is not always as strictly type specific as is the neutralization test and that a positive reaction is of shorter duration, so that it may be used as an indication of recent infection. Results are
undoubtedly encouraging, but further studies are needed before judgement
can be passed on the value of the method as a routine diagnostic laboratory
test.

8. SUMMARY AND CONCLUSIONS

Poliomyelitis is a problem of ever-increasing importance in all parts
of the world, and it is the primary object of this report to present an account
of present concepts of the essential nature of this disease, with a review
of recent advances that have been made in many aspects, especially in
methods of diagnosis and possibilities of prevention.

The manifestations of infection by the virus of poliomyelitis range
from an inapparent infection to a severe paralytic illness. In some countries,
the only form of illness regarded as indicative of infection is an acute
febrile disease usually followed by paralysis. It must, however, be empha-
sized that paralysis is an infrequent complication of poliomyelitis infection
in man, and that most persons who become infected either show no symp-
toms, or else develop a milder illness, which may or may not show evidence
of involvement of the central nervous system.

There is no particular difficulty in making a diagnosis of the paralytic
form of poliomyelitis on clinical grounds. There is, however, considerable
difficulty in making a diagnosis of the non-paralytic form of the disease.
Many other agents cause an aseptic meningitis that cannot be differentiated
from non-paralytic poliomyelitis except by elaborate and time-consuming
laboratory tests.

An interesting consideration in the clinical field is that factors other
than the virus itself may predispose to or precipitate the development of
paralysis. It has been suspected for some years that genetic and hormonal
factors may predispose to paralysis, and it is well accepted that specific
trauma, especially tonsillectomy, and over-exertion may precipitate para-
lysis. Of particular interest is the demonstration that intramuscular injec-
tions of certain substances may produce a similar effect. Specific recom-
mandations on this point regarding the procedures to be followed during
times of epidemic prevalence have been given.

A complete understanding of the epidemiology of poliomyelitis involves
knowledge of the portals of entry and distribution of virus in the body.
It was earlier believed that infection was spread mainly by respiratory
droplets and that virus entered by the olfactory route. However, more-
recent evidence suggests that the portal of entry is usually the mouth and
that the primary site of infection is in the pharynx and the remainder of
the alimentary tract. Virus probably multiplies actively in the tissues of
the alimentary tract. It is not completely clear how the virus spreads from
the primary site of multiplication to the central nervous system. Until recently, it was thought that virus passed along the nerve fibres. However, the more recent finding of virus in the blood-stream has suggested an alternative explanation. It appears possible from experiments with monkeys and chimpanzees that virus may reach the central nervous system by the blood. Only a few tests have so far been made on cases of human poliomyelitis and, although viraemia has been found during the incubation period, further observations on its incidence and significance are necessary.

During the incubation period the virus appears at more or less the same time in the throat, in the blood-stream, and in the intestinal tract. Virus can subsequently be demonstrated in the throat for about 10 days and in the faeces for as long as 12 weeks in some cases. It will be evident that the previous concept of poliomyelitis virus as mainly neurotropic is no longer tenable. The virus evidently has an affinity for the alimentary tract and perhaps other tissues.

The accumulated laboratory and epidemiological evidence indicates that poliomyelitis is a highly infectious disease spread by intimate association with infected persons. It is probable that the virus is actually transferred directly or indirectly by means of pharyngeal excretions and faecal matter. The previous belief that poliomyelitis was spread by respiratory droplets is no longer widely held, and the new concept is one of transmission mainly by faecal contamination as occurs in intestinal infections such as bacillary dysentery. The fact that virus has been found in sewage, and that infection is acquired early in life in communities with poor sanitation, suggests that environmental contamination by virus is actually a means of distribution. Flies have been incriminated as potential sources of infection, and while this may be true in areas where they have free access to faecal matter there is no evidence that they play an essential role. In particular, flies do not appear to become actively infected with the virus and therefore do not serve as reservoirs of infection but merely as mechanical carriers.

The best index of immunity to poliomyelitis may be obtained from a study of distribution of the disease in various age-groups, particularly if correlated with results of serum antibody determinations. Experience indicates that most primary infections are acquired in childhood. In those areas with poor living conditions where the virus is highly prevalent, infection is acquired at an earlier age than in populations where hygienic conditions are more satisfactory.

It is becoming increasingly evident that the presence of Lansing-type antibody in the general population, as determined by serum antibody surveys, runs parallel with that of antibodies for the other two types, so that determination of Type 2 antibody has been used as an indication of experience with the other types.
There is a difference of opinion as to whether solid and durable immunity is acquired from a single infection or whether it is dependent upon repeated exposures. Evidence suggests that a more durable immunity is produced by repeated exposures as occurs in areas of high environmental pollution.

Experience from experiments with primates suggests that the level of serum antibody plays a role in determining resistance to infection. There are insufficient observations serving to establish a similar correlation between antibody and resistance in man. While circulating antibody does not necessarily prevent alimentary infection in man, it may well serve to interfere with spread of virus to the central nervous system, and therefore with the production of paralysis.

Poliomyelitis is a worldwide infection and paralytic cases have been reported from all continents and many islands. It is of particular interest that paralytic poliomyelitis, which before the second World War was thought to be rare in the tropics, is being increasingly recognized in such areas. In view of the fact that the clinical disease is apt to be much less severe in children under five years than in older children or adults, poliomyelitis often lies hidden in tropical communities, and the true incidence is not recorded in official returns.

Climate and season both exert a profound effect on the epidemiological behaviour of the disease, for in the temperate regions of both hemispheres poliomyelitis is more prevalent in summer and autumn than in winter. In contrast, in tropical areas cases occur more uniformly throughout the year. The reason for this phenomenon is not yet known.

As has already been pointed out, poor sanitary conditions greatly facilitate the spread of virus. It is also clear that socio-economic conditions, particularly crowding, can affect the age distribution of poliomyelitis, as is the case in other diseases spread by human association. In urban as compared to rural areas, the disease attacks younger children. The implication is that crowded and poor living conditions facilitate the spread of poliomyelitis, but persons brought up in more favourable surroundings may not necessarily derive any permanent advantage, as poliomyelitis infection may merely be postponed to later life, when the disease tends to be more severe.

The standard methods of control as applied by health officers with many infectious diseases have been found to be of little avail in poliomyelitis. Part of the reason for the apparent failure of isolation and quarantine measures to check the spread of infection may lie in the fact that, for every case of paralysis, there may be large numbers of persons with mild or inapparent infections. However, virus studies have shown that, during epidemic periods, virus is found mainly in the intimate associates of the case. It appears possible, therefore, that some reduction in the number of cases of poliomyelitis might be achieved by vigorous quarantine and
hygiene measures directed at the first recognized cases. Such measures will probably have a greater chance of proving effective in isolated communities.

It is probable also that a reduction in the incidence of paralytic cases in times of epidemic prevalence can be achieved if efforts are made to avoid conditions known to predispose to or precipitate paralysis, such as over-exertion, tonsillectomy, and the administration of certain intramuscular injections. In addition, all febrile illnesses occurring at times of epidemic poliomyelitis should be treated with caution, and as far as possible such persons should rest for a few days.

Because of the prolonged excretion of virus in the stools, it is probably advisable that patients should be isolated, if necessary at home, but it is difficult to make a firm recommendation as to the length of time because it is not known when the individual case becomes free from infection. There would appear to be much to commend the practice in some countries of isolating cases and quarantining contacts for three weeks. Under conditions where virus may spread readily, such as nursery schools and residential nurseries, it may be desirable to exclude convalescent poliomyelitis cases for several weeks.

At the present time, more hope for the control of poliomyelitis is being placed in methods of immunization. Experiments in primates have shown that paralysis can be prevented passively by the inoculation of poliomyelitis antibody and, in particular, of gamma-globulin. It appears from these experiments that low levels of circulating antibody serve to protect against infection following oral administration. Gamma-globulin has been widely used in the USA in an attempt to control the incidence of paralysis. Some limited success has been achieved. Despite drawbacks arising from the low morbidity of poliomyelitis, the use of gamma-globulin is recommended in certain instances which are detailed.

At the moment, research is being undertaken on a more promising method of control of poliomyelitis by active immunization or vaccination. On the one hand, attempts are being made to discover avirulent strains or to attenuate virulent strains of poliomyelitis virus, so that, when they become devoid of virulence, they may be safely administered by mouth in the hope that natural infection and its resulting immunity will thereby be simulated. At the moment, relatively little progress has been made in applying experimental results to man. The other trend of research concerns the development of a vaccine containing chemically inactivated virus prepared from tissue cultures. Work has not yet passed the experimental stage and there is, as yet, no direct evidence that such preparations can induce resistance in man, although it is known that antibody develops following inoculation.
Throughout this report frequent reference has been made to tissue-culture techniques. It can be said without exaggeration that the introduction of the tissue-culture method of cultivating poliomyelitis virus has revolutionized the study of the disease. It has become abundantly clear that poliomyelitis virus can be studied more readily, and more accurately, by this newer technique than by the older methods in which monkeys were required. It is expected that the increasing use of tissue-culture techniques in laboratories in many parts of the world will, before long, lead to increased recognition of the fact that poliomyelitis is of universal prevalence. Not only is the tissue-culture technique of value in the isolation and antigenic typing of poliomyelitis viruses, but it can be used in facilitating the study of the basic immunological behaviour of the disease by means of the examination of specific serum antibody levels.

Another application concerns the development of an experimental vaccine from tissue cultures, and much hope is expressed that such a product may prove safe and effective. Although great gains in knowledge have already been made, further progress is limited by the scarcity of workers trained in the techniques of tissue culture. The committee recommends that consideration be given to the support of selected laboratories in parts of the world where poliomyelitis has been little studied. Such assistance should include the training of virologists.

Laboratory diagnosis of poliomyelitis has been rendered very much easier by tissue-culture methods, and the isolation of virus can be readily accomplished. It should not be forgotten, however, that, in parts of the world where monkeys can be easily obtained, isolation by the well-tried method of monkey inoculation will serve essentially the same purpose.

There is still great need for a rapid serological test that might assist in the laboratory diagnosis in the first few days of illness. Progress with the development of a complement-fixation test is encouraging, but the practical stage has not yet been reached.

It is the hope of the committee that, with the increasing knowledge of poliomyelitis, and with recent technical improvements, it may prove possible before too long to introduce some measure of effective control. At the same time, the committee wishes to stress that much has still to be learned about the disease and that, for a time, particularly in some countries, there may be a continued increase in prevalence.

9. RECOMMENDATIONS FOR FURTHER RESEARCH AND FUTURE DEVELOPMENTS

It will be evident from this report that there are still many unanswered problems in human poliomyelitis. Solutions to some of these are urgently needed for the intelligent application of the methods of control now under
intensive development. More fundamental research into the many aspects of viruses and their interaction with the host-cell is not less important, since it may eventually lead to even more valuable prophylactic or therapeutic measures.

The committee considers it desirable to call special attention to the need for further research on the following problems of immediate or potential practical importance, especially those concerned with the control of the infection.

1. The practicability and effectiveness of isolation and quarantine as control measures when applied to the first families affected with poliomyelitis in a community.\(^{10}\) (This important problem presents an admirable opportunity for research by health officers in co-operation with epidemiologists and virologists.)

2. \((a)\) The comparative importance of pharyngeal and faecal excretions in the transmission of the disease.
   \((b)\) The frequency and importance of other modes of indirect transmission.
   \((c)\) The possibility of the existence of chronic faecal excreters ("carriers") of virus.
   \((d)\) The manner of propagation of infection in inter-epidemic periods.

3. Continued epidemiological surveys are recommended to determine:
   \((a)\) The incidence of the various types of poliomyelitis virus in different regions.
   \((b)\) The age incidence of infection by the different types of virus in different communities, as determined by local virus isolation and antibody surveys.

These problems could well be studied by virus laboratories not equipped for the more difficult research techniques.

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\(^{10}\) The value of such measures would be greatly increased if their application were supported by laboratory examinations for the presence of virus which are now technically possible as a result of the development of tissue-culture techniques. In particular, the inter-epidemic season in temperate climates seems to offer opportunities for health officers and laboratories to undertake joint efforts to attempt to trace the infection in the winter, and in the hope of preventing the propagation of an epidemic from one season to the next. In the winter, the number of silent infections is probably exceedingly small, and the spread of virus slow, reducing the demands on the laboratory very considerably. At the present time, however, few virus laboratories are able to undertake even this restricted volume of work. More and more laboratories should enter upon this field, and it should eventually be possible to provide such facilities as are necessary by the development of more-adequate public-health laboratory services so as not to interfere with the essential programmes of research laboratories. If this is done, it may in the future become possible to detect and control disseminators of poliomyelitis virus as readily as is now possible with typhoid carriers.
4. The possibility of strain differences within types.

5. (a) The relative duration and intensity of infection in children and adults.
(b) The relation of serum antibody levels to the intensity of infection and to the degree of resistance to infection and clinical disease.
(c) The significance of repeated infections in the establishment of immunity.

6. The incidence and significance of viraemia.

7. (a) Studies on the optimum methods of use of prophylactic gamma-globulin.
(b) Biological standardization of gamma-globulin.

8. Studies on potency and innocuity tests for poliomyelitis vaccines. It need hardly be mentioned that the development of safe and effective poliomyelitis vaccines should be pressed forward with all possible speed.


10. Standardization of neutralization tests and the development of the complement-fixation or other test for routine diagnosis early in the acute stage of the disease.

It is clear that many of these studies cannot be carried out without extension of the available laboratory facilities. Many countries have no laboratories capable of undertaking this work. As an interim measure, the committee recommends that laboratories, chosen from among the research laboratories of scientific institutes experienced in the necessary techniques, should be designated as regional laboratories for the purpose of examining limited numbers of specimens from countries where there are as yet no facilities. The principal task of the regional laboratories would be the identification, typing, and further study of poliomyelitis virus and of unidentified viruses isolated from stools in all parts of the world. In addition, the preparation, storage, and distribution of strains of poliomyelitis virus, as well as type-specific antisera for diagnostic and typing purposes, should be undertaken to assist other laboratories working in this field.

Consideration should be given to the provision of fellowships for the training of virologists, with special emphasis on tissue-culture techniques.

Finally, the committee wishes to stress that the complete control of an infectious disease by vaccination alone has rarely, if ever, been achieved. The part played by hygienic measures in the control of most infectious diseases must not be forgotten, although their place in the control of poliomyelitis is not yet clear.
Annex 1

ISOLATION AND IDENTIFICATION OF POLIOMYELITIS VIRUS IN THE LABORATORY

Foreword

The directions given in sections A-E apply to material intended for the inoculation either of animals or of tissue cultures. Section F is concerned mainly with the techniques of animal inoculation. Recommended techniques for tissue-culture work cannot be laid down at the present stage of their development. Annex 3 (see page 66) therefore gives a list of references in which further information on current practices may be found.

A. Precautions

Isolation of poliomyelitis virus from human and other sources usually requires a team of two or more workers and their work is not without danger, since the percentage of laboratory workers who have accidentally acquired poliomyelitis in the laboratory is appreciable. For obvious reasons, therefore, sterile precautions must be taken in handling infective materials. Gowns should be worn at all times, and for certain procedures gloves, cellophane masks, and eye-shields are necessary as well. Persistent vigilance is required on the part of the director of the team engaged in this work to ensure that the individual members consistently take the necessary precautions.

B. Sources of Material

Poliomyelitis virus can be isolated by monkey inoculation and by tissue-culture inoculation, from a number of materials derived from man, and from extra-human sources such as sewage and flies. However, even using the most careful technique, negative results are a common experience. If human material is being tested, the chances of successful isolation are greater if the material is obtained early in the disease, within the first 7-8 days, dating the onset of the disease from the first onset of fever—even though the symptoms be slight—(i.e., the "minor illness" if such occurs) and not from the onset of paralytic symptoms, which may actually occur late in the acute infection. Sometimes when it is important to obtain a strain of poliomyelitis virus quickly from a given outbreak, it is useful to
visit the homes of hospitalized patients, and to determine whether any other members of the family are ill with symptoms suggesting an earlier stage of the disease. If so, they may furnish more-valuable specimens than does the patient in the hospital.

1. **Human autopsy material** from which the virus has been frequently isolated includes:

   (a) pons, spinal cord, and medulla, provided death has occurred 7-10 days from the onset, and

   (b) intestinal contents and intestinal wall.

   In removing central-nervous-system tissue at autopsy, an assistant should be ready with sterile gloves and several sets of sterile instruments, or at least with the means for reboiling the same instruments frequently.¹ Favoured sites from which virus may be isolated are the medulla, and the cervical and lumbar sections of the spinal cord; pieces about 2 cc in size should be taken from these areas and placed in a sterile Petri dish. The cauda equina is not recommended as a source of virus. At the same time, other appropriate sections of the cord should be placed in fixing solution for subsequent histological examination.

2. **Clinical cases and carriers as a source of material**

   Poliomyelitis virus has been isolated frequently from:

   (a) faeces;

   (b) Rectal swabs (some faecal material should be obtained on the swab, if possible);

   (c) pharyngeal washings (15-30 ml);

   (d) throat swabs (2 from each patient).

   A reliable source of virus is human faecal material, since poliomyelitis virus is found for a longer time in the intestinal tract than elsewhere within the body, and has been isolated from faeces during the incubation period, during the acute disease, and in convalescence. Nevertheless, the optimal time for collecting specimens in all cases, paralytic, non-paralytic, and abortive, is early in the course of the infection (see section 4.2, page 11). Virus may also be recovered from asymptomatic carriers who are often present in association with known cases of poliomyelitis.

   The virus is not found in spinal fluid, but recently it has been detected in the blood during the incubation period and during the minor illness. However, blood is not a favoured source of virus for isolation.

¹ The main reason for maintaining a sterile technique is that the chances of bacterial contamination are minimized, which is important when central-nervous-system tissue is to be used for intracerebral monkey inoculation or for tissue-culture inoculation.
C. Collection of Material from Patients and Carriers

1. Faeces

A 15-25 g specimen is desirable. Small wide-mouthed jars are useful as containers. The specimen should preferably be kept frozen, or at least kept cold, until tested.

2. Rectal swabs

If a stool specimen cannot be obtained, a rectal swab is often a useful substitute. A moist sterile swab is inserted well into the rectum and manipulated until faecal material is found to be adhering to it. The swab is then placed in a test-tube containing 1 ml of sterile water or broth. It is important that the specimen be tested promptly or else kept frozen.

3. Pharyngeal washings

Various types of irrigating fluid, such as sterile distilled water or broth, may be used to obtain pharyngeal washings from patients or suspected carriers of the virus. The irrigating fluid is introduced into the patient's mouth, either from a drinking glass or through a large glass syringe without a needle attached to it. The patient is then encouraged to gargle the material which is collected in a glass or a small sterile basin. The procedure is carried on over a period of at least three minutes, using the same fluid repeatedly. Not more than 30 ml of irrigating fluid should be used.

4. Throat swabs

Material is obtained by rubbing the oropharynx vigorously with two sterile cotton swabs, which are immediately transferred to a test-tube containing 1-2 ml of sterile water or broth. The specimens should be tested promptly or kept frozen.

D. Storage of Material

Material awaiting testing or shipment may be held for short periods at refrigerator temperature (0°-4°C). For longer storage it should preferably be frozen, or it may be kept in 50% glycerol.

Freezing

Ordinary glass test-tubes containing more than 1 ml of fluid are liable to crack when frozen; therefore, if fluid material is to be frozen it should be
placed in special containers, i.e., either nitro-cellulose tubes or thick-walled glass containers. Freezing may be accomplished by placing the tubes in the freezing compartment of an electrically driven refrigerator, or in a specially constructed insulated box containing solid carbon dioxide (dry ice) which may maintain a temperature of from \(-20^\circ\) to \(-70^\circ\)C. For the preservation of poliomyelitis virus, unlike certain other viruses, temperatures below \(-20^\circ\)C are not essential.

Glycerol

Only the purest brands of glycerol should be used for the preservation of poliomyelitis virus. The glycerol should be mixed with an equal volume of physiological saline before use.

Some points with regard to the use of glycerol are:

1. do not put more than 4 or 5 small pieces of tissue in 50 ml of glycerol-saline, and
2. do not allow the tissue to remain untested any longer than is necessary.

However, as 50% glycerol has a slow bactericidal action, it may be useful to allow bacteriologically contaminated specimens to remain in it for a few days before testing them for virus. Although poliomyelitis virus has been known to survive in 50% glycerol for many years, it has also died out in this medium after a few months or even weeks.

Lyophilization

As a method of preserving poliomyelitis, lyophilization has been accomplished with various diluents such as 10% monkey serum, and mucin. However, results are irregular and lyophilization is not recommended for this purpose.

E. Shipping of Specimens

If frozen material is to be shipped short distances, it should be sent in a proper container, such as an insulated box containing dry ice, or a well-packed thermos flask containing dry ice. Special care in packing thermos bottles is essential or breakage may easily occur.

The necessity of keeping all specimens cool probably varies with the circumstances.\(^2\)

\(^2\) The survival time of poliomyelitis virus in human stools at room temperature is variable, but the virus remains viable in this medium for several weeks at refrigerator temperature.
A more practical method for shipping involves the use of 50% glycerol. Autopsy specimens, stool specimens (small in amount), and the sediment from rectal swabs, pharyngeal washings, and throat swabs may all be sent at room temperature in 50% glycerol. For this purpose it is convenient to use small wide-mouthed bottles with tightly stoppered or capped orifices, and, for safety, the top of each bottle should be wrapped with several layers of water-proof tape. Before preparing such material for inoculation the fragments of tissue or particulate matter should be washed several times in saline solution to remove some of the glycerol.

F. Monkey Inoculation

The following species of monkeys have been most often used in poliomyelitis work:

*Macaca mulatta*: the rhesus monkey, usually from India.

*Macaca cynomolgus and/or irus or mordax*: the cynomolgus (or Java) monkey from the East Indies, Philippine Islands, or Malaya.

*Cercopithecus aethiops sabaews and griseoviridis*: the green African and grievet monkeys from West and East Africa.

*Cercopithecus aethiops centraulis*: the vervet monkey from West, Central, and South Africa.

*Cebus capucina*: the ringtail or capuchin monkey from South America.

Of this series the rhesus monkey has been most widely used.

One, two, or three monkeys may be used for testing each specimen. It is considered conservative practice to use one animal.

The choice of the route of inoculation is based on the following principles. The intracerebral route is the most delicate, but if the inoculum contains an excess of bacteria, the inoculated monkey may succumb with a brain abscess before it acquires experimental poliomyelitis. The intranasal route is quite reliable and not dangerous to the animal, but is cumbersome and time-consuming. The intra-abdominal route is less reliable, but is simple and safe and is often used as an adjunct to the other two.

1. Preparation of inocula from nervous tissue

Weighed fragments of medulla or spinal cord (1.0-1.5 g are usually sufficient) are ground in a sterile mortar containing sterile sand or an abrasive, with enough sterile water, not more than 1 or 2 ml at first, to
produce a fairly thick paste. Grinding is usually continued for at least five minutes. Sufficient cold, sterile, distilled water is then added to make a 10% suspension, and antibiotics are added to yield a final concentration of 500 units of penicillin and 500 micrograms of streptomycin per ml. The suspension is then transferred to a cold centrifuge tube where it is spun at low speed (2,000 revolutions per minute (r.p.m.)) for five minutes. The supernatant fluid will be opalescent, but should contain no particles large enough to plug the lumen of a small needle. It is advisable to prepare from 10 ml to 15 ml of suspension so that some of the material may be kept frozen for possible future use.

1.1 Intracerebral inoculation

The monkey to be inoculated should be properly marked beforehand (by tattooing if feasible). It is anaesthetized, commonly with ether; the hair is clipped away from the forehead and top of the head, and the area is then shaved. The site of inoculation is the central area over the frontal lobe on the right or left side. The skin over the area is rubbed well with iodine and alcohol. Trehpinning of the skull can be accomplished by using a sharp instrument (half of a pair of scissors is satisfactory) which will bore a hole 1-2 mm in diameter, through which 1 ml of the suspension to be tested can be injected intracerebrally. It is common practice to inject 1 ml or less, to a depth of about 1 cm. Some workers advise dividing the inoculum into two parts of 0.5 ml each; each part is then inoculated deep into the hypothalamus, 0.5 ml on the left and 0.5 ml on the right.

If it is particularly important to demonstrate virus, the animal may be re-inoculated intracerebrally with the same material at intervals of one, two, or three weeks. Furthermore, the same monkey can be inoculated by other routes: (1) intranasally; 2 ml of the original suspension should be instilled into the etherized animal’s nares, and the process repeated on the subsequent days (see below); and (2) intra-abdominally; 5-15 ml can also be given intra-abdominally.

1.2 Intranasal inoculation

In carrying out intranasal instillation, it is useful but not essential to anaesthetize the monkey lightly with ether. 2 ml of the inoculum are allowed to drop directly into each nostril either from a pipette or from a syringe fitted with a blunt needle. During this process an assistant should hold the monkey underneath a fixed glass plate in order to limit splattering of the infectious material. Both operators should wear gowns and gloves, and if no glass plate is available they should wear goggles and cellophane masks. Instillation of material should preferably be repeated daily over a period of six days.
2. Preparation of inoculum from faecal material

Relatively large (25-50 g) specimens are sometimes desirable (but not essential). In preparing the material for monkey inoculation, various procedures may be used; none of them is easy, and a laboratory which is beginning to work in this field may expect to encounter difficulties which can be overcome through a process of trial and error.

It may be desirable to divide the original specimen of faeces (or fluid from the rectal swab) into two equal portions, one being kept frozen or in the refrigerator for future use should the test be unsatisfactory, or should there be other reasons to retest the specimen.

The intracerebral route of inoculation of faecal material (alone or in combination with other routes) has been more successful in some hands than in others. The chief difficulty is the readiness with which some faecal suspensions give rise to brain abscesses in the inoculated monkeys.

A satisfactory method is to prepare a 10% suspension from the stool specimen in cold, sterile, distilled water in a tightly-stoppered 250-ml flask containing glass beads; after frequent shaking, the specimen is allowed to settle in the cold. The supernatant fluid is then poured off into another flask, and it is again well shaken and allowed to settle. From the supernate of the second flask, the material is divided into two parts, I and II, generally amounting to between 20 and 25 ml each.

Part I (20 ml), without further treatment, is kept at refrigerator temperature for intranasal use (see above).

Part II (25 ml) is immediately centrifuged at relatively low speed (15 minutes, 2,000 r.p.m.), and to the supernate, 15% ether is added as a bactericidal agent together with penicillin and streptomycin solution to make a final dilution of 500 units and 500 micrograms per ml, respectively. The etherized suspension is kept in a stoppered container in the refrigerator.

A sample of Part II (5-10 ml) is placed in a small centrifuge tube within the centrifuge cup in the refrigerator to chill the specimen. In order to remove bacteria, it is again centrifuged at 4,500 r.p.m., or at higher speeds if such are available, for half an hour to one hour. 3 ml are removed from the supernate and 0.1 ml of this is cultured on a blood-agar plate. This specimen, Part III, is set aside in the refrigerator for intracerebral inoculation.

If, after 24 hours, the growth of bacteria is minimal or absent, the fluid from Part III below the layer of ether is removed, and an amount not exceeding 1 ml is inoculated intracerebrally.

* If the material is to be inoculated into tissue culture ether need not be added.
The same monkey may be inoculated intra-abdominally with the residuum of Part II, using an inoculum of not more than 10-12 ml. The concentration of ether (15%) and antibiotics used in Part II usually, but not always, destroys or diminishes the number of bacteria in the suspension sufficiently to permit of intra-abdominal injection of 10-12 ml without fear of inducing fatal peritonitis.

If only the washings from a rectal swab are available, the volume of material will be small, but may be diluted to a volume of 3-5 ml; 15% ether and antibiotics as above are added, and the suspension is treated as are Parts II and III and used for intracerebral inoculation.

3. **Pharyngeal washings and throat swabs**

The washings are transferred to a sterile flask containing glass beads; the flask is tightly stoppered and shaken for 10 minutes. The suspension is subjected to light centrifugation (2,000 r.p.m. for 10 minutes), 10% ether and antibiotics are added, and it is allowed to stand in the refrigerator overnight. On the following day, the etherized material is inoculated intracerebrally in 1-ml amounts. Intracerebral inoculation may be supplemented by intra-abdominal injection of 10 ml of the etherized suspension.

**Other methods.** More-delicate methods of handling these suspensions exist, which include in particular the use of the ultracentrifuge in preparing material for intracerebral inoculation. This instrument is a valuable but not an essential part of the usual poliomyelitis laboratory’s equipment.

4. **Observation of inoculated monkeys**

Monkeys inoculated with material suspected of containing poliomyelitis virus should be observed for at least four weeks. It is good practice to examine and exercise these animals daily during this period. By this method early signs of experimental poliomyelitis, such as tremor, ataxia, and weakness of the limbs, can be detected, and the animal can promptly be sacrificed at an appropriate time if a strain of virus is desired for passage or storage. A further reason for daily examinations is that, soon after an intracerebral inoculation, the animal may develop a spastic paralysis, often taking the form of hemiplegia, resulting from an upper motor neuron lesion caused by local trauma or necrosis of the brain. It is important not to confuse this with the flaccid paralysis resulting from poliomyelitis.

Temperature readings should be taken daily, preferably at the same time each day, using individual rectal thermometers which are sterilized in a strong disinfectant solution between use. The usual rectal temperature of rhesus monkeys varies from 102.2°F (39°C) to 103.5°F (39.7°C), but temperatures up to 104°F (40°C) are not particularly abnormal. The onset
of the experimental disease (induced with human strains of virus), may follow an incubation period of from 4 to 25 days. This is usually, but by no means always, heralded by a rise of temperature to between 104°F (40°C) and 106°F (41.1°C). Fever is maintained from one to six days, during which time other signs may appear quickly or slowly. These consist of ruffled fur, nervousness, tremors (often first noticeable as a fine tremor of the ears), ataxia, and finally weakness, to be followed by definite paralysis most easily detectable in the extremities, although it may involve the face, neck, or back. With the development of considerable paralysis there is usually an abrupt fall in temperature. Even in the absence of signs of infection it may be wise to sacrifice the animal as a routine at the end of the 30-day period of observation. It is not good practice to use the same animal again for poliomyelitis investigation.

4.1 Autopsy of monkeys

An animal can be sacrificed by the injection of ether into the heart. Before incising the skin, the fur of the back and head of the animal should be swabbed with lysol solution. The brain and cord are removed first, using two or three changes of sterile instruments. It is best to remove the cord with the dura intact, and then to open the dura with sterile scissors and forceps. Several sections are taken from the cervical, dorsal, and lumbar regions of the cord, and from the medulla. One of each is placed in fixing solution for histological examination, and several of each are kept for passage or storage, either frozen or in 50% glycerol.

4.2 Criteria for a positive result

The most important evidence of the experimental disease in the monkey is found on histological examination of sections taken from the spinal cord. The lesions should be unequivocal before a positive diagnosis of the isolation of poliomyelitis virus is accepted. Such lesions are generally manifest in the grey matter of the spinal cord involving in particular the ganglion cells in the anterior horns. The lesions pass through several stages, but when fully developed they are characterized by destruction of neurons and neuronophagia, and, prominently, by perivascular and interstitial round-cell infiltration. In the brain, lesions are not likely to be extensive and may be scattered, often in the base of the brain and not uncommonly in the motor cortex. The cerebral lesions are not regarded as convincing evidence of poliomyelitis unless other lesions are also found in the medulla and spinal cord. By adopting this standard, experimental poliomyelitis is less likely to be confused with other types of encephalomyelitis, or with the areas of inflammation surrounding a brain abscess or brain injury. In many instances it may be wise to pass the strain in
tissue culture and to identify it by neutralization tests using the three types of poliomyelitis antisera; or to pass the strain of virus to another monkey for confirmatory evidence. Under certain circumstances, it may also be wise to inoculate the strain intracerebrally in adult and suckling mice, rabbits, and guinea-pigs. If these animals develop encephalomyelitis it is probable that either the virus is not poliomyelitis virus, or if mice alone are infected, it may be a Type 2 (Lansing) strain of poliomyelitis virus which can infect rodents. These possibilities can be checked by further tests, preferably including attempts to type the virus.

A negative result should be recorded if the animal fails to show typical lesions in the spinal cord. In general, the failure of the inoculated animal to show the appropriate signs of infection during the period of observation is a fair indication of a negative result, but non-paralytic or apparently silent infections may occur in a small percentage of inoculated monkeys.

An incomplete or unsatisfactory result is recorded if the inoculated monkey dies from some cause other than poliomyelitis before the 30-day period of observation is complete.

Annex 2

NEUTRALIZATION TESTS AS PERFORMED IN MICE *

The neutralization test as carried out with poliomyelitis virus in mice is being supplanted in many laboratories by the use of tissue cultures for this purpose, but these directions have been prepared for those to whom tissue-culture facilities are not available. The use of monkeys in the performance of neutralization tests with poliomyelitis virus is no longer recommended.

In general the principles of the test, regardless of what virus is used or of whether it is performed in mice or tissue culture, are the same.

* The directions for performing these tests have been taken in large measure from the following sources:
  
  

  The committee is indebted to Dr. J. L. Melnick for assistance in preparing this annex.
General principles of neutralization tests

The principle of the test is that specific protective, or virus neutralizing antibodies, can be measured by adding serum containing them to virus, and then injecting the mixture into a susceptible experimental animal, or preferably into a group of such animals (eggs or tissue cultures). The failure of the animals to develop the disease (or "lesions") which is acquired by the control animals receiving virus alone, or virus plus a serum free of the antibody in question, is proof of the presence of neutralizing antibodies. The level of such antibodies can be determined by using a constant amount of virus and serial dilutions of serum, or by employing a constant amount of serum and serial dilutions of virus. For clinical diagnosis, one must be able to show a significant rise in antibody titre in serial blood samples obtained during the course of the infection. A positive test in a single sample of serum is not of diagnostic value in so far as acute, recent infections are concerned, for neutralizing antibodies can persist for years and their mere presence may indicate a past infection in a given individual. In view of the latter fact, neutralization tests are useful in the field of serological epidemiology when one is interested in knowing the viral agents with which a given population has had previous experience. Therefore, for epidemiological work, a more simple "screening" test is advised than that used for clinical diagnosis. The technique advised for the latter test appears at the end of this annex (see page 64).

Although simple in principle, neutralization tests by their very nature are expensive in time and in materials, and the results may be difficult to interpret, chiefly because of the variability in the titration end-point and the possible non-specificity of the neutralization. For each viral agent, the technique of performing the test for neutralizing antibodies must be standardized. Among variables which concern the test as performed with animals are: the selection of the experimental animals which in poliomyelitis might include mice (adult and suckling), hamsters, or cotton-rats; route of inoculation of the virus-serum mixture; age of the test-animals; stability of the test virus and reproducibility of the end-point; relative heat stability of the specific antibody and of possible interfering substances in serum; use of one concentration of virus and varying dilutions of serum, or vice versa (and the relationship between varying concentrations of each); temperature at which the neutralizing mixture of virus and serum is held, and the time of incubation of the mixture.

Collection of blood specimens for antibody tests

There are a number of reasons which may prompt one to determine whether a given sample of serum does or does not contain neutralizing antibodies against poliomyelitis, and, if so, in what amount. Thus, the test is used both for epidemiological and clinical diagnosis. For the former,
single blood samples from the same individual sometimes suffice. For the latter, two or more serial samples of serum are desirable if antibodies are to be adequately tested and evaluated. These samples should be collected as follows: (i) as soon as possible after the onset of illness; (ii) three weeks after onset; (iii) later, if necessary.

In poliomyelitis, neutralizing (and complement-fixing) antibodies are usually already present at the time the patient is admitted to hospital, i.e., usually one or two days after the onset of the major illness. The relationship of the antibodies to the infection is indicated by their quantitative increase during the next few weeks.

Individual blood specimens should be drawn with sterile precautions as for a blood culture. It is desirable to obtain 15-25 ml of fasting blood for each determination. The serum should be removed about two hours after clotting or, if it is not possible to do this at that time, the blood should be placed in a refrigerator as soon after collection as possible, and the serum removed within the next 24 hours. Sterile glass-ware should be used throughout. Serum should be centrifuged, if necessary, to remove traces of erythrocytes, and should be kept in the refrigerator. If there is to be a delay of more than a few days before the tests can be performed, the sera should be frozen. A commercial low-temperature refrigerator set at $-20^\circ$C is adequate, or a solid carbon-dioxide refrigeration box may be used. The latter is particularly convenient for use in the field and for transportation of frozen specimens. In the event that a refrigerator which will maintain serum below freezing temperatures is not available, it is advisable first to inactivate the serum at $56^\circ$C for 30 minutes, and then store the material.

**Directions for the performance of a quantitative neutralization test**

In carrying out the following technique, which has been in use for several years for the clinical diagnosis of certain viral encephalitides and for poliomyelitis, the following points deserve emphasis, although the same basic principles apply to virtually all virus neutralization tests:

(a) *Source of virus.* Although it is useful to have virus suspensions of known titre available in the frozen state, it is nevertheless necessary to titrate the virus each time a test is performed.

(b) *Diluent.* Since it has been found that certain preparations of broth may inactivate some viruses in the higher dilutions, it is desirable in such cases to use 10% normal rabbit serum in physiological saline as the diluent, and for the control titration.

(c) *Test-animal.* A stock of mice of known uniform susceptibility should be selected and mice three to four weeks of age should be used routinely.
(d) **Calculation of results.** The 50% end-points (LD$_{50}$) are calculated according to the method of Reed & Muench,\(^1\) the titre being expressed as the logarithm of the concentration of the injected tissue. From the standpoint of definition, the 50% end-point is that dose at which half the injected animals become infected or die. ID$_{50}$ indicates the dose that will infect 50% of the inoculated animals; LD$_{50}$ indicates the dose that will kill 50% of the inoculated animals.

(e) **Neutralization index.** This can be expressed as the ratio of the control LD$_{50}$ titre over the LD$_{50}$ titre of the serum-virus mixture. (Log of LD$_{50}$ titre of control minus log of LD$_{50}$ titre of serum-virus equals X. Antilog of X = neutralization index.) Some workers prefer to give the results logarithmically, as the difference between the log of the control titration and the log of the titration of the virus-serum mixture. This difference is the logarithm of the neutralization index.

- Neutralization index 1-9 = negative
- Neutralization index 10-49 = equivocal
- Neutralization index 50 or more = positive

When an equivocal result is obtained the serum should be retested. When in the second test the neutralization index is 1-9, the serum should be considered negative; an index of 10-49 still leaves the serum in the equivocal category. An index of 50 or more in the second test will put the serum in the positive category.

**Details of the neutralization test for neurotropic viruses**

(a) **Virus.** Ten or more brains removed from mice showing signs of experimental infection of the nervous system (ataxia, convulsions, paralysis, prostration) are ground with sterile sand or other abrasive and 10 ml of inactivated (56°C for 30 minutes) undiluted rabbit serum are added for each gram of brain tissue. The suspension may receive additional homogenization in a motor-driven blender; this should be done with great care to avoid contamination of the air with fine droplets.\(^2\) After centrifugation at 2,000 r.p.m. for 10 minutes, the supernatant fluid is drawn off and regarded as a 1 in 10 dilution of virus. Part of it is titrated immediately (i.e., its infective or lethal dose is established), and the rest is distributed in ampoules of 1-ml amounts. The sealed ampoules are quickly frozen.

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2. As already mentioned in Annex 1, the handling of these particular agents is dangerous and one must proceed with utmost care if laboratory infections are to be avoided. Because of the fine mist produced, blending of virulent material is a particular hazard, as is also the handling of lyophilized active virus.
and stored in a solid CO₂ refrigerator or electrically driven low-temperature refrigerator. Depending upon needs, one or more ampoules are thawed and used in each test, the unused portion of the virus being discarded.

(b) Preparation of virus dilutions, and control and serum-virus mixtures. Starting with the 1:10 dilution from the frozen ampoule, dilutions of 1:50, 1:500, 1:5,000, etc., are prepared using 10% rabbit serum in saline as the diluent and a separate pipette for each dilution. Then 0.2 ml of the selected dilutions are added to labelled tubes containing 0.2 ml of the undiluted unknown sera being tested. As controls, a serum known to contain antibodies and one free of antibodies are included in each test.

(c) Selection of critical dilutions. If the preliminary titration has indicated that the LD₉₀ titre of the virus to be used is in a range as high as 10⁻² dilution (although with poliomyelitis virus much lower titres are the rule), then the control dilutions with the normal serum will be 10⁻⁶, 10⁻⁴, 10⁻³, and 10⁻², and the dilutions with the unknown serum 10⁻³, 10⁻⁴, 10⁻⁵, and 10⁻⁶.

These dilutions are selected in order to be within the range of 100% mortality at one end, and 100% survival at the other, in the control titration, bearing in mind the variations in titre that may be expected with virus suspensions preserved in the frozen state. When the test is to be used for diagnosis with a virus that has been found to maintain its titre well, one may dispense with the lowest dilution in the serum mixture (i.e., the 10⁻³) and use 3 instead of 4 serum-virus dilutions.

A virus suspension which yields titres less than those customarily given by the virus, as well as suspensions which, during storage, have fallen off in titre by 1.0 or more log dilutions, should be discarded.

The virus suspension which is added to the serum must be twice as concentrated, for it will be diluted twofold by the serum. Thus if, for example, virus-infected central-nervous-system tissue in a concentration of 10⁻¹.₃ is required,

\[
20\% \text{ suspension} = 10^{-0.7} \text{ concentration of mouse brain} \\
\text{Desired concentration} = 10^{-1.2} \\
\text{Dilution necessary} = 10^{0.5} \\
\text{Antilog of 0.5} = 3.2
\]

Therefore, the virus suspension to be used in the test is prepared by adding 2.2 ml of distilled water to each ml of 20% suspension.

With each series of neutralization tests, control titrations of virus are done in the presence of saline. If the control titration is unsatisfactory, only certain sera need be retested. For example, if the control titration should show that only 10 ID₉₀ were added to the sera under test, then those sera negative in the test need not be run again, for they will not neutralize.
larger doses of virus. On the other hand, if the control titration should show that 1,000 ID₅₀ were used in the test, the positive sera need not be run again, for they will neutralize smaller doses of virus. In deciding whether or not a test should be repeated, a variation of ±0.4 log is permissible, i.e., control titrations ranging from 10⁻³ to 10⁻² are allowable for a virus which has given an average titre of 10⁻² and if the test in question was based on this latter value. Thus, although the calculated dose is 100 ID₅₀, tests in which the control titrations show that 40-250 ID₅₀ were used need not be repeated.

To 0.2 ml of serum in a 10 mm × 75 mm test-tube, there is added 0.2 ml of the virus suspension. The mixture is allowed to stand at room temperature (25°C ± 5°C) for one hour. For each serum to be tested, eight mice are inoculated intracerebrally with 0.03 ml of serum-virus mixture.

(d) Incubation, numbers of mice, and period of observation. The controls and serum-virus mixtures are incubated in a water-bath at 37°C for two hours and then placed in an ice-bath until inoculated. The highest dilutions are inoculated first (i.e., 10⁻¹, 10⁻², etc.) and the control mixtures should be the last. Groups of 5-8 mice of proved uniform susceptibility are to be inoculated with each dilution. The intracerebral route of inoculation is used, employing 0.25-ml tuberculin syringes to permit of accurate delivery of a standard 0.03-ml dose. A single syringe may be used for injecting each serum provided that the highest dilution of virus is inoculated first, followed in turn by the successively lower dilutions of virus. The syringe must be emptied after each dilution and the inside rinsed thoroughly by filling and discharging the next lower dilution.

Mice are observed daily for signs of central-nervous-system disease to establish the specificity of the deaths. Deaths are recorded for at least 21 days. All deaths within 24 hours after inoculation are regarded as due to traumatic or non-specific causes.

(e) Determination of the neutralization index

**EXAMPLE**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Virus dilution</th>
<th>LD₅₀ titre negative logarithm</th>
<th>Log of neutralization index</th>
<th>Neutralization index (antilog)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>10⁻⁵</td>
<td>10⁻⁶</td>
<td>10⁻⁷</td>
</tr>
<tr>
<td>Negative control</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Positive control</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Acute serum</td>
<td>5/5</td>
<td>5/5</td>
<td>3/5</td>
<td>3/5</td>
</tr>
<tr>
<td>Convalescent serum</td>
<td>4/5</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Fraction 3/5 indicates that five mice were inoculated and three died as a result of the virus infection.
POIOMYELITIS

Procedure used in calculating LD₅₀ titre by the Reed-Muench formula.³

NEGATIVE CONTROL MIXTURE

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mortality ratio</th>
<th>Died</th>
<th>Survived</th>
<th>Accumulation totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁴</td>
<td>5/5</td>
<td>4</td>
<td>6</td>
<td>6/7</td>
</tr>
<tr>
<td>10⁻³</td>
<td>4/5</td>
<td>2</td>
<td>3</td>
<td>2/6</td>
</tr>
<tr>
<td>10⁻²</td>
<td>0/5</td>
<td>0</td>
<td>0</td>
<td>0/9</td>
</tr>
</tbody>
</table>

Therefore, LD₅₀ titre, log of dilution = 8.0

Arrows indicate direction of addition for accumulation totals.

CONVALESCENT SERUM

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mortality ratio</th>
<th>Died</th>
<th>Survived</th>
<th>Accumulation totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁴</td>
<td>4/5</td>
<td>5</td>
<td>1</td>
<td>6/7</td>
</tr>
<tr>
<td>10⁻³</td>
<td>2/5</td>
<td>0</td>
<td>3</td>
<td>2/6</td>
</tr>
<tr>
<td>10⁻²</td>
<td>0/5</td>
<td>0</td>
<td>0</td>
<td>0/9</td>
</tr>
</tbody>
</table>

Proportionate distance between the two log dilutions (in the example between 10⁻⁴ and 10⁻³ where the 50% end-point lies =

\[
\frac{\text{% mortality above } 50\%}{\text{% mortality below } 50\%} = \frac{86 - 50}{53} = 0.68 \text{ or } 0.7
\]

Log LD₅₀ titre = log of dilution above 50% + factor (proportionate distance × log of serial dilution used in titration), or in this case = 4.0 + 0.7 = 4.7, and the LD₅₀ = 10⁻⁴.³

Procedure used to calculate LD₅₀ titre according to Kaerber.⁴ This method, like that of Reed & Muench, applies strictly only to complete

³ The Reed-Muench formula may be used even in the absence of a zero mortality end-point or of two dilutions below the 50% or more mortality range, with the clear understanding that the results are not absolutely accurate. This is the best available procedure for a standard method of expressing the results under circumstances which make it impractical to increase the number of dilutions to cover the strict requirements of the Reed-Muench formula.

⁴ Kaerber, G. (1931) Arch. exp. Path. Pharmak. 162, 480
titration series, i.e., a series covering the whole reaction curve from 100% to 0% mortality. Even if such conditions are not fulfilled, it gives results as accurate as those of Reed & Muench but is much simpler to calculate, and for that reason is included here. The Kaerber formula for calculating the LD₉₀ for virus titrations carried out in serial tenfold dilutions is as follows:

\[
\text{Log LD}_{90} = 0.5 + \log \left( \frac{\text{log of highest concentration of virus used}}{\text{sum of percentage of dead animals}} \right) \frac{100}{100}
\]

For the examples shown above the logarithms of the LD₉₀ are:

**Control mixture**

\[
0.5 - 6.0 - \frac{100 + 87 + 50}{100} = 0.5 - 6.0 - 2.4 = -7.9
\]

**Convalescent serum**

\[
0.5 - 4.0 - \frac{86 + 33}{100} = 0.5 - 4.0 - 1.2 = -4.7
\]

*Calculation of neutralization index for convalescent serum.* Log of neutralization index = log of LD₉₀ titre of negative control minus log of LD₉₀ titre of convalescent serum. The antilog of this difference is the neutralization index.

- Negative control titre, log of dilution = 8.0
- Convalescent serum titre, log of dilution = 4.7
- Log of neutralization index = 3.3
- Neutralization index = antilog of 3.3 = 2,000

*(f) Interpretation.* In the above example, the acute-phase serum had a neutralization index of less than 10, and the convalescent serum an index of 2,000, indicating a marked rise in neutralizing titre during convalescence. For diagnostic significance, the increase in neutralization index during convalescence must be at least 100. As neutralizing antibodies for many viruses are of long duration it is essential to demonstrate a rise in titre in paired sera, in order to establish a causal relationship between the virus and a given illness. Such a positive test in a single specimen may be the result of an earlier and perhaps subclinical infection and is useful for epidemiological and other purposes but not for the clinical diagnosis of the acute disease.
Qualitative Lansing poliomyelitis neutralization test for survey purposes

An example of a simple method which has been used by several workers for detecting antibodies to Lansing (Type 2) poliomyelitis virus may be cited. This test is run to see whether the individual from whom the serum has been taken has, or has not, been previously exposed to and infected by an agent which gives rise to Lansing antibodies. It is in essence a screening-test which is adequate for certain epidemiological purposes.\(^6\)

1. *Virus.* The stock virus pool, obtained from the brains and spinal cords of infected mice, should have an \(LD_{90}\) titre of not less than \(10^{-1.5}\) by the intracerebral route in mice (at least three weeks old) from the stock to be used in carrying out the neutralization tests. A control titration is carried out with each neutralization test.

The pool of infected mouse brains (and spinal cords) in the form of a 20% suspension in distilled water, should be distributed in ampoules or in tightly stoppered tubes, frozen, and kept at \(-20^\circ\mathrm{C}\) or below. Samples are thawed as needed and centrifuged at low speeds, and the supernatant fluid is diluted to the proper amount. (See below.)

2. *Sera.* For adequate survey purposes samples of blood should be collected from normal individuals covering as wide an age-group as is feasible. If the series is to span all age-groups, there should be adequate representation of the lowest age-groups if possible, i.e., one month to five years, and the whole series should include upwards of 200 samples.

Sera should be handled aseptically\(^6\) and ideally should be stored frozen from the time of collection, and thawed just before use. (Sera may be heated at \(56^\circ\mathrm{C}\) for 30 minutes before storage, the antibody being stable under these conditions, in order to obviate the possibility that thermolabile non-specific factors might give false-positive tests for antibody.)

3. *Procedure.* From the accumulated values of four titrations of the virus pool, using eight mice per dilution, the extent to which the 20% suspension must be diluted is calculated so that the final virus-serum mixture contains 100 \(ID_{90}\)\(^7\) per 0.03 ml, the actual volume inoculated into the brain. For example, if the virus pool has an average titre of \(10^{-2.5}\) based on the wet weight of the mouse brains used to prepare the suspension, then the concentration of infected mouse brains desired in the final mixture is \(10^{-1.8}\).

---


\(^6\) In some instances the use of a preservative may be necessary but it is not recommended.

\(^7\) \(ID_{90}\) rather than \(LD_{90}\) is used if some indication of infection other than death is taken, in this instance, paralysis.
Mice are examined once (or twice) daily for three weeks, the paralysed and dead animals being recorded. In a small percentage of the tests, one or two of the eight mice may die during the first 24 hours and such deaths are not tabulated. Under such circumstances the results are based on six or seven rather than on eight mice.

(4) Critical level of antibody. The criteria for the "presence" of neutralizing antibody depends upon the number of surviving mice one day following the injection and the number surviving 21 days later, and these criteria are listed below:

<table>
<thead>
<tr>
<th>Number of mice surviving one day after inoculation</th>
<th>Number of mice succumbing during observation period of 3 weeks</th>
<th>Positive test: antibody present</th>
<th>Negative test: antibody absent</th>
<th>Questionable test *</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0,1,2</td>
<td>6,7,8</td>
<td>3,4,5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0,1,2</td>
<td>5,6,7</td>
<td>3,4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0,1</td>
<td>5,6</td>
<td>2,3,4</td>
<td></td>
</tr>
</tbody>
</table>

* Should be repeated if possible.

(5) Quantitative serum titres. If these are desired, the serum should be diluted to appropriate levels, such as 1:5, 1:25, 1:125, 1:625.

To 0.2 ml of each serum mixture, there is added 0.2 ml of the test virus. After incubation as above, eight mice are inoculated with each serum-virus mixture. 50% serum end-points are calculated according to Reed & Muench.
Annex 3

SELECTED REFERENCES ON TISSUE-CULTURE TECHNIQUES


13. Ledinko, N., Riordan, J. T. & Melnick, J. L. (1952) Multiplication of poliomyelitis viruses in tissue cultures of monkey testes. I. Growth curves of Type 1 (Brunhilde) and Type 2 (Lansing) strains and description of a quantitative neutralization test. *Amer. J. Hyg.* 55, 323-338


47. Youngner, J. S., Ward, E. N. & Salk, J. E. (1952) Studies on poliomyelitis viruses in cultures of monkey testicular tissue. II. Differences among strains in tissue culture infectivity with preliminary data on the quantitative estimation of virus and antibody. *Amer. J. Hyg.*, **55**, 301-322
| Number | Price  \
<table>
<thead>
<tr>
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<tr>
<td>Adoption, Joint UN/WHO Meeting of Experts on the Mental-Health Aspects of Final report</td>
<td>70</td>
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<td>Alcohol, Expert Committee on First report</td>
<td>84</td>
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<td>Alcoholism Subcommittee See under Mental Health Antibiotics, Expert Committee on Report on the first session</td>
<td>26</td>
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<td>Bilharziasis, Expert Committee on First report</td>
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<td>Bilharziasis in Africa, Joint OIHP/WHO Study-Group on Report on the first session</td>
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<td>Biological Standardization, Expert Committee on Report on the third session</td>
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<td>36</td>
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<td>Brucellosis, Joint FAO/WHO Expert Committee on Report on the first session</td>
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<td>Communicable Diseases of Childhood, Active immunization against common Report of a group of consultants</td>
<td>6</td>
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<td>Diphtheria and Pertussis Vaccination Report of a conference of heads of laboratories producing diphtheria and pertussis vaccines</td>
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<td>25</td>
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<tr>
<td>Report</td>
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<td>Hepatitis, Expert Committee on</td>
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<td>First report</td>
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<td>First report</td>
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<td>73</td>
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<td>Mentally Subnormal Child, Report of a Joint Expert Committee convened by WHO with the participation of United Nations, ILO, and UNESCO</td>
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<td>Physically Handicapped Child, Joint Expert Committee on the (WHO, United Nations, ILO, and UNESCO)</td>
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