The Diagnosis of Poliomyelitis

BY

JAMES GEAR, M.B., CH.B., D.P.H., D.T.M. & H., DIP.BACT.

Director of Research, Poliomyelitis Research Foundation, South African Institute for Medical Research, Johannesburg.

Classical poliomyelitis is a biphasic illness with two bouts of fever separated by an interval of one or more days of normal temperature (Chart 1). The primary wave of fever or minor illness is associated with sore throat, headache and bodily pains, and often with upper respiratory symptoms, and sometimes with nausea, vomiting and abdominal symptoms. The secondary wave of fever or major illness is ushered in with severe headache, vomiting, stiff neck and back and pains in the limbs, and in a variable proportion of cases these symptoms are followed by the occurrence of flaccid paralysis of groups of voluntary muscles.

Manifestations of Infection by Poliovirus

This classical picture of poliomyelitis, fortunately, is one of the rarer manifestations of infection by poliovirus, which may take one or other of the following forms:

1. *Inapparent Infection.*—This is the commonest form, but of course can only be recognised in the laboratory by the recovery of poliovirus or by the demonstration of an increase in the serum antibody against poliovirus.

2. *Abortive Poliomyelitis.*—In abortive poliomyelitis the disease does not progress further than the minor illness, and the patients have fever, headache, sore throat, listlessness, anorexia, vomiting, constipation and muscle and abdominal pain. The illness usually lasts only 24 to 48 hours. This form of infection is more commonly seen in children than in adults. It cannot be diagnosed with certainty on the clinical findings alone, but should be suspected in times of epidemic prevalence, especially in those known to have been in close contact with a patient with poliomyelitis or with the siblings of such a case.

3. *Non-Paralytic Poliomyelitis.*—In non-paralytic poliomyelitis manifestations of involvement of the central nervous system occur after those of the minor illness, and the patient then has fever, headache, vomiting, pains in the muscles and stiffness of the neck and back. These features are those of benign aseptic or virus meningitis and are not specific for poliomyelitis. Thus an accurate diagnosis of non-paralytic poliomyelitis cannot be made without the help of the laboratory. A tentative diagnosis can be made during the poliomyelitis season, especially if there is a history of association with a paralytic case or with the siblings of such a case.

4. *Paralytic Poliomyelitis.*—In this form paralysis usually develops after one to four days of non-paralytic illness, and its occurrence may be heralded by an increase in muscle pain or fibrillary tremors of the muscles. The paralysis is of the lower neurone type. The sudden development of flaccid paralysis in a mentally alert patient strongly suggests poliomyelitis.

In the spinal form the muscles affected are those innervated by the spinal nerves. The lower limbs are more often involved than the upper limbs, but the paralysis may have any distribution and is characteristically assymmetrical.

In bulbar paralytic poliomyelitis there is paralysis of one or more groups of muscles supplied by the cranial nerves, especially those of the soft palate and pharynx, giving rise to dysphagia, dyspnoea and nasal speech. Facial paralysis or paralysis of the muscles of tongue, jaw and eye may occur. The circulatory and respiratory centres may be involved. Nearly all deaths from poliomyelitis result from bulbar involvement.

In the encephalitic form, tremors and coma may occur. This form is probably the rarest manifestation of infection with the virus of poliomyelitis.

These forms may occur separately or in various degrees of combination in a single patient. Thus in a case beginning as a spinal paralysis the pathological process may spread to involve the brain stem, the basal ganglia and even the motor area of the cerebral hemispheres.

Clinical Differential Diagnosis

In the diagnosis of poliomyelitis difficulties arise in each of the stages of the illness.

Causes of Illness Resembling the Minor Illness.—The minor illness of poliomyelitis cannot on clinical findings be differentiated from a large number of other conditions with fever, headache, sore throat, listlessness and bodily pains associated with upper respiratory and gastro-intestinal disturbance, but may be suspected in a case with these signs and symptoms when poliomyelitis is epidemic. The suspicion that such an illness may be due to poliovirus can only be confirmed

by the isolation of the virus. Now that this can be readily done in the laboratory, much greater accuracy of diagnosis is possible even at this stage of the infection.

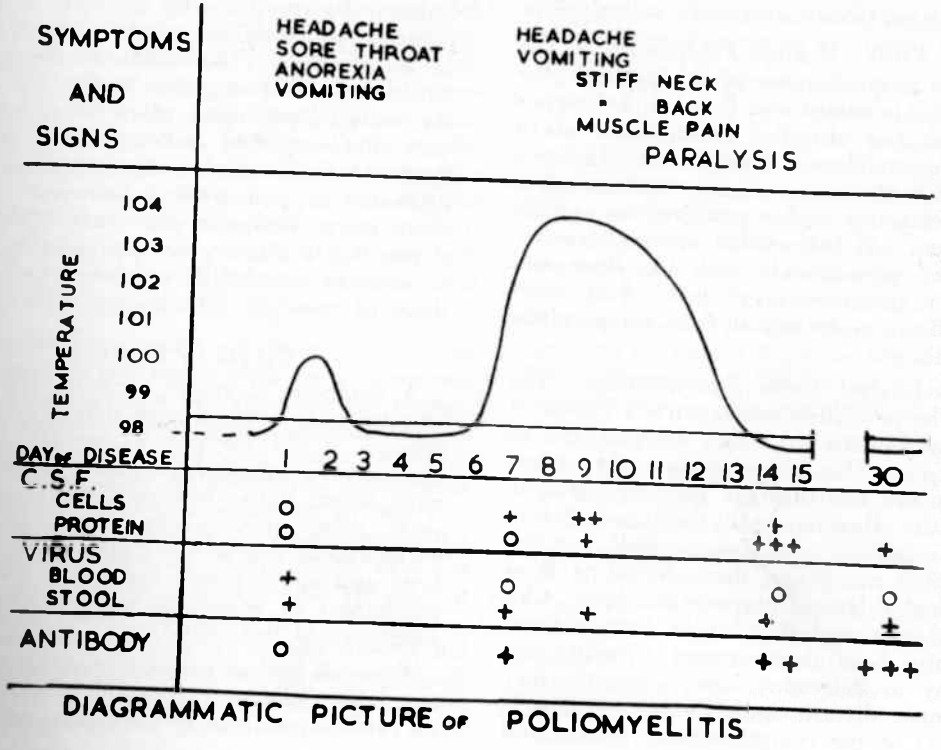
Causes of Meningo-Encephalitis.—In those cases developing the major illness with involvement of the central nervous system, other conditions associated with meningo-encephalitis have to be considered. Meningo-encephalitis may be caused by bacterial, fungal, protozoal and leptospiral agents, and in the differential diagnosis of non-paralytic poliomyelitis these have to be excluded. This is often possible on the clinical findings, but sometimes cannot be done without the aid of the laboratory. There are several virus infections causing aseptic meningitis which often cannot be differentiated clinically from non-paralytic poliomyelitis. To assess the importance of these other virus infections a study was made of all cases of meningo-encephalitis admitted to the Johannesburg Fever Hospital as cases of incipient poliomyelitis. A comprehensive series of tests to detect the possible causes of meningo-encephalitis was systematically carried out. This study revealed that in this region Coxsackie group A and B viruses and mumps virus were the most important causes of virus meningo-encephalitis simulating that occurring in cases of non-paralytic poliomyelitis.

Coxsackie Virus Meningo-Encephalitis.—The commonest identified cause was Coxsackie group B virus. This virus is also the cause of pleurodynia or Bornholm disease, which was prevalent at the time of the occurrence of these cases of meningo-encephalitis. In some of these cases the involvement of the central nervous system was preceded by symptoms of Bornholm disease. In others the preceding illness was less definite in character. Coxsackie group B type 4 virus was also identified as the cause of an outbreak of meningo-encephalitis which occurred in Southern Rhodesia, also at a time when Bornholm disease was prevalent.

Coxsackie group A virus may also cause meningo-encephalitis, the signs and symptoms of which may suggest non-paralytic poliomyelitis. These may be preceded by an attack of herpangina or by a febrile illness with indefinite muscular pains.

ECHO virus meningoencephalitis: The ECHO group of viruses was brought to light by the use

Chart 1



of the tissue culture technique in the study of cases diagnosed as poliomyelitis. In these studies a number of non-polio viruses producing destruction of the tissue culture cells were isolated. Some proved to be Coxsackie viruses, most of them of the Coxsackie B group, and a few of the A group, in particular the A9 type. There were a large number of what are now known as the ECHO group of viruses which were distinct from the Coxsackie and poliovirus groups. The word ECHO is derived from the descriptive term applied to these viruses: (E)ntero-(c)ytopathogenic (h)uman (o)rphan viruses. The word orphan was coined because these viruses at the time of their isolation had no disease which they could claim as their parents. Now it is clear that some of the 17 different types which have been identified so far are important causes of disease of man.

Among the ECHO viruses, serotypes 4, 6 and 9 have been associated with regional outbreaks of aseptic meningitis and, in addition, serotypes 2, 3, 7, 14 and 16 have been recovered from patients ill with aseptic meningitis.

It is clear then that this newly discovered group of viruses includes important pathogens of man and some of the commonest causes of the aseptic meningitis syndrome, which has to be differentiated from non-paralytic poliomyelitis.

Mumps Virus Meningo-Encephalitis. — The second most frequent cause of meningo-encephalitis during this period was found to be mumps virus, which was identified as the cause of 14 cases by the complement fixation test. In seven of these cases there was an associated parotitis. In the other seven neither parotitis nor orchitis was apparent. It follows that mumps virus infection may present only with the signs and symptoms of meningo-encephalitis. Such cases may be difficult to distinguish from non-paralytic poliomyelitis.

Arthropod-Borne Virus Encephalitides.—The arthropod-borne viruses are important causes of meningo-encephalitis in North America and in the Far East. The clinical picture they cause often simulates non-paralytic or even paralytic poliomyelitis. How important they are in Southern Africa has not yet been assessed, but the presence of a number of them, including West Nile, Bwamba fever, Bunyamwera fever, Chikungunya fever and Rift Valley fever viruses, has recently been demonstrated. Studies are under way to determine how frequently they cause human disease and how often there is involvement of the central nervous system and how often these conditions are mistaken for the illness of poliomyelitis. This study has been undertaken by a team headed by Dr. K. C. Smithburn and Dr. R. H. Kokernot and sponsored jointly by the Rockefeller Foundation, the South African Institute for Medical Research and the Council for Scientific and Industrial Research. The determination of the distribution, incidence and clinical manifestations of these virus infections should help in the clarification of the picture of meningo-encephalitis as a whole in Southern Africa.

It is of interest that herpes virus was not found to be a frequent cause of meningo-encephalitis. Only three cases were identified—one by the isolation of the virus from the brain of a fatal case and two by the demonstration in the complement fixation test of the development of antibodies to a significant titre in their convalescent serum.

It also is of interest and perhaps somewhat surprising that no cases due to the virus of lymphocytic choriomeningitis were identified. It is even more noteworthy that of 75 cases admitted with the diagnosis of poliomyelitis and tested for its presence, poliovirus was detected in only three of them.

At the same time attempts were made to isolate poliovirus from the faeces of 27 cases of paralytic poliomyelitis and these were successful in 23 of them. The technique therefore was sensitive and the negative findings in most of the non-paralytic cases of meningo-encephalitis were thus supported as being valid. However, it should be noted that during this period the incidence of poliomyelitis was relatively low. More recent studies in the years 1955-56 have shown that a much greater proportion of cases of meningo-encephalitis are due to poliovirus in times of epidemic prevalence.

CAUSES OF PARALYSIS

A diagnosis of spinal paralytic poliomyelitis may be made without much difficulty on clinical grounds. The features of mental clarity, a history of a preparalytic phase, followed by a more severe illness with painful stiffness of the neck, pains in the back and limbs and an asymmetrical flaccid lower motor neurone paralysis and an increased cell count with a preponderance of neutrophil leucocytes are highly suggestive of this condition.

Cases of bulbar paralysis present more difficulty, but poliomyelitis should be considered in all cases of paralysis of the cranial nerves.

Post-Diphtheritic Paralysis.—Palatal paralysis occurring in some cases of bulbar poliomyelitis may be confused with the palatal paralysis occurring as a complication of diphtheria. However, the history of the preceding illness and a survey of all the clinical findings usually differentiate the two conditions.

Rabies.—Bulbar poliomyelitis may be mistaken for rabies and, conversely, cases of rabies may be suspected of having bulbar poliomyelitis. The history of a bite by a possibly rabid animal may give a clue to the correct diagnosis, but this is sometimes not forthcoming. The evolution of the disease to its fatal termination may not be of diagnostic value. The true cause may only be identified by the isolation and characterisation of the virus in the laboratory.

Coxsackie Virus Infections.—In Coxsackie group B virus meningo-encephalitis the patient may develop facial nerve paralysis. This, unlike most cases of poliomyelitis, is usually transient and does not often last longer than 48 hours.

Guillain-Barre Syndrome.—Greatest difficulty in the differential diagnosis is caused by the Guillain-Barre syndrome or radiculoneuronitis. This condition is not uncommon. Its aetiology remains obscure, but it may develop as a post-infective condition and it has been suggested that it is analogous to post-infective encephalitis. Other cases are apparently related to drug treatment and others again may be due to virus infections. Of value in differentiating it from poliomyelitis are the findings that in the Guillain-Barre syndrome the weakness or paralysis is symmetrical, there is often paraesthesia or other sensory symptoms, and in the cerebrospinal fluid a normal or slightly raised cell count is associated with a raised protein, often of over 100 mg. per cent.

Chemical Intoxication.—Some chemicals may cause neuritis and motor weakness or paralysis. The heavy metals are well known in this respect. Acute arsenical, phosphorus and triorthocresyl phosphate neuritis may also result in motor paralysis simulating somewhat the paralysis of poliomyelitis. Cases of poisoning by the new insecticides, especially the cholinesterase inhibitors, may manifest with marked motor weakness which may raise a suspicion that the patient has poliomyelitis. In these cases of chemical intoxication the patients are usually afebrile and the distal groups of muscles are most affected, giving rise to foot drop and wrist drop, and the cerebrospinal fluid is usually normal.

Metabolic Disturbances.—Porphyria: Some cases of porphyria develop lower motor neurone paralysis which may progress in landriform fashion and end fatally. The condition may closely simulate paralytic poliomyelitis, but usually these patients do not have fever and the cerebrospinal fluid is normal. The detection of porphyrins in the urine gives the clue to the correct diagnosis.

Local Conditions.—Some local conditions, including trauma affecting the spinal column, the spinal cord, the nerve roots or the peripheral nerves, occasionally cause difficulty. Cases of osteomyelitis, septic arthritis and rheumatic fever may be wrongly diagnosed as paralytic poliomyelitis. A careful clinical assessment of such cases should avoid these errors.

Brief mention will now be made of a number of other conditions which occasionally may cause confusion in the differential diagnosis of poliomyelitis.

Leptospiral Infections.—Although not yet identified as a cause of meningo-encephalitis in Southern Africa, leptospiral infections, particularly those due to *Leptospira canicola* and *L. pomona*, have been shown to be important causes of this condition in North America and Europe. Studies to determine their incidence and importance in this region are under way.

Malaria.—Cerebral malaria may also be confused with virus meningo-encephalitis and with the encephalitic form of poliomyelitis. The difficulty of course only arises in countries where malaria occurs or in individuals who have come from or have passed through such areas. It is important to remember this possibility in patients who have recently arrived in an aircraft which has landed at an airport in a malarious region, especially if the aircraft has been delayed at such an airport for some hours. The correct early diagnosis of these cases may mean a difference between life and death of the patient.

Helminthic Infections.—Rarely some helminthic infections may cause motor paralysis and at first suggest the possibility of poliomyelitis. Amongst these is bilharzial myelitis, which may be wrongly diagnosed as poliomyelitis. However, the onset is usually gradual, the course chronic, and at the time of the involvement of the spinal cord the patient is afebrile. The finding of ova in the urine or faeces of the eosinophilia and of a positive result in the bilharzial complement fixation test will suggest the correct diagnosis.

Hydatid cysts and the cysticercosis may also, but very rarely, involve the spinal cord and may give rise to symptoms suggestive of poliomyelitis. However, these too are chronic conditions with an afebrile course and thus may be differentiated from acute anterior poliomyelitis.

Post-Infective and Post-Vaccinal encephalomyelitis.—Encephalomyelitis may occur as a complication of most of the acute infectious fevers, most frequently after measles and chickenpox. In most cases the signs and symptoms first become manifest about two weeks after the onset of the primary illness. In cases complicating measles the condition often begins on about the fifth day of illness. Pathologically these cases are characterised by foci of perivascular demyelinisation, associated with an inflammatory cell infiltrate in the brain and spinal cord. The neurones are not much affected in the process and are relatively normal in appearance.

Clinically these patients often exhibit clouding of consciousness and even stupor which may progress to coma and death. Paralysis, when it occurs, most often affects the muscles supplied by the cranial nerves. This picture contrasts with the findings in most cases of poliomyelitis in which the patients are mentally clear, but the encephalitic and bulbar forms of poliomyelitis may present a somewhat similar picture and be difficult to differentiate. The history of a preceding acute exanthematous or other acute illness helps in distinguishing the two conditions.

Neuroparalytic Accidents Complicating Rabies Vaccination.—Encephalomyelitis may also occur as a complication of the prophylactic treatment of persons bitten by animals suspected of rabies. These vaccines are prepared from central nervous system tissue and may give rise to autoallergic reactions. The myelitic, especially the dorso-lumbar form, may mimic the paralysis of poliomyelitis. However, there are often sensory changes and the clear history of antirabies treatment will distinguish the condition.

Encephalomyelitis may also result from a hypersensitivity reaction to drug treatment, especially with the "sulpha" and arsenical drugs. This condition may present as a transverse myelitis, which may simulate paralytic poliomyelitis, but in addition to the motor weakness or paralysis, there are usually marked sensory changes.

Patients suffering from periarteritis nodosa not infrequently may develop peripheral neuritis with marked motor weakness, and this may simulate poliomyelitis. The other stigmata of this condition will be apparent, or will become apparent, and so the true cause of the condition be revealed.

Tick-bite paralysis has been described in South Africa. The condition is rare, but when it occurs it may be mistaken for paralytic poliomyelitis. The finding of the attached tick and the improvement in the condition soon after its removal, and the absence of signs of meningeal involvement, will give the clue to the cause of the patient's illness.

Malignant Disease.—Tumours of the spinal cord and those involving the spinal column may also occasionally create problems in the differential diagnosis, especially relatively rapidly growing tumours of young infants. The further progress of these cases of course clearly separates them from cases of poliomyelitis.

Hysteria and malingering are rare in young children, but these conditions not infrequently have to be considered in cases simulating paralytic poliomyelitis in adolescents and adults. A careful survey of all the evidence usually suffices to distinguish them from cases of true paralysis.

Iceland Fever.—In 1950 Sigurdsson and others published an account of an outbreak of an illness simulating poliomyelitis that had occurred in Iceland in the winter of 1948-49. This epidemic involved particularly the inhabitants of a town, Akureyri, in which 6.5 per cent. of the inhabitants were affected. The age group 15-19 years, including especially the scholars at the high school, were most severely involved. This disease appeared to be infectious, for it spread widely through the town and beyond to the countryside and neighbouring towns along the routes of travel. Judging by the time of onset after their exposure in individuals who had had only one such exposure, the incubation period was from five to nine days. The illness was characterised sometimes by a sudden, more usually by a rather gradual, onset, by sore throat and sometimes vomiting and upper respiratory infection, and by pain in the neck and back and pains in the limbs, and at this time by a feeling of numbness and tingling in the limbs, and often a feeling of heaviness which progressed to weakness sometimes so marked as to simulate paralysis. An important finding was muscle tenderness on deep pressure. Disturbances of sensation were not infrequently observed. There appeared also to be some mental disturbance and complaints of nervous instability, sleeplessness, loss of memory and inability to concentrate.

After some improvement, relapses were common. Six months later many of the patients still complained of weakness, headache and inability to concentrate. Six years later many of the patients still complained of nervousness, tender muscles, pains and tiredness. Signs of paresis have tended to improve, but in some patients the improvement has been slow.

Faeces were tested for poliovirus and for Coxsackie virus, but no virus was found.

Since 1950 several other outbreaks of a similar condition have been described: one in New York, one in Adelaide and three in England—one in Coventry, one in Middlesex Hospital and one in the Royal Free Hospital. In the outbreaks in England nurses living in nurses' homes were involved.

In 1955 a similar outbreak affected a number of nurses on the staff of the Addington Hospital in Durban. These cases were characterised at the onset by a low grade fever and by painful neck muscles, and by a feeling of heaviness and weakness of one or more limbs, which often became so marked as to simulate paralysis; the deep tendon reflexes were often exaggerated and sometimes gave a tetanoid or clonic response. In many patients there were patchy areas of anaesthesia. The course of the condition was variable. Some patients recovered quickly; others after early improvement had relapses, and even a year later had not improved sufficiently to resume work. In their chronic course and tendency to relapse, these cases also recall the outbreak in Iceland.

Detailed laboratory studies were carried out in this outbreak. In attempts to isolate virus, suspensions prepared from throat swabs, faeces, blood and cerebrospinal fluid were inoculated into various animals, including monkeys, rabbits, guinea-pigs, adult and baby mice, and tissue cultures of monkey kidney cells. No virus was isolated. Examination of the urine also failed to reveal any chemical intoxicant. Evidence of intoxication by the insecticides was looked for in particular.

The aetiology of this condition thus remains unknown. It is of interest and perhaps significant that each of the described outbreaks has followed in the wake of a poliomyelitis epidemic, and in each instance the early cases were diagnosed as having poliomyelitis. It has been suggested that the condition is possibly a manifestation of poliomyelitis in an immune individual. Psychological and mental aspects of the cases have also been emphasised by most investigators and are especially noteworthy in the chronic cases. However, it appears unlikely that an illness having such a similar picture and occurring in such widely separated parts of the world would be due entirely to psychological factors, although this possibility has not been entirely excluded.

From the foregoing account it is apparent that some of the many conditions which have to be considered in the differential diagnosis of poliomyelitis can be excluded on the history and clinical findings. In others this is not possible and the final diagnosis depends on the results of laboratory tests. To include all the causes of illness simulating non-paralytic or paralytic poliomyelitis a comprehensive series of tests is needed. These will be briefly described and their application and interpretation briefly discussed.

LABORATORY DIAGNOSIS

A clinical diagnosis of poliomyelitis may be supported by the isolation and identification of poliovirus in the throat secretions or in the faeces of the patient. This procedure can now be applied in all cases of poliomyelitis in which there is doubt about the diagnosis, and by appropriate tests the true cause of those cases which prove not to be due to poliovirus may also be identified. To achieve this the specimens which should be collected and sent to the laboratory are set out in Table I with the laboratory tests of value in the differential diagnosis of poliomyelitis.

The blood count in cases of poliomyelitis may show a normal number of white cells; more usually there is a slight or moderate leucocytosis due to an increase in the number of neutrophil leucocytes. Cases of meningo-encephalitis with high leucocyte counts of over 20,000, with an increase in the percentage of the neutrophil leucocytes to about 90 per cent., are more likely to be caused by pyogenic bacteria such as meningococci or pneumococci than by poliovirus or by the other viruses.

The sedimentation rate in most cases of poliomyelitis shows only a slight or moderate increase. A marked increase suggests some other cause than poliovirus.

Examination of blood smears will exclude or confirm malaria and relapsing fever, the cerebral forms of which may sometimes be mistaken for poliomyelitis, and also the rare cases of trypanosomiasis in which the signs may suggest polio-

TABLE I

The Type of Specimens to be Collected and the Laboratory Tests of Value in the Differential Diagnosis of Poliomyelitis.

Time of Collection.	*Specimen.*	*Tests.*	*To Confirm or Exclude.*
Acute phase.	1. Blood.		
	(i) Oxalate tube.	Full Blood count.	
		Sedimentation rate.	
	(ii) Slides.	Differential count.	
		Parasites.	Malaria.
			Relapsing fever.
			Trypanosomiasis.
	(iii) Plain tubes with no preservative.	(a) Agglutination tests. Widal.	Enteric fever.
		Weil Felix.	Typhus.
		Brucella.	Brucellosis.
		Paul Bunnell.	Glandular fever.
		(b) Complement fixation tests.	Rickettsial and virus diseases.
		(c) Neutralisation tests in mice and tissue culture.	Virus Diseases.
		(d) Inoculation of egg culture. Baby and adult mice. Tissue culture tubes.	Virus Diseases.
	2. Cerebrospinal fluid.	(a) Direct smear.	Bacterial meningitis.
		(b) Cell count.	Meningoencephalitis.
		(c) Chemical analysis.	Inflammatory changes.
			Guillain Barre syndrome.
		(d) Inoculation of mice. Tissue culture tubes.	Coxsackie and other virus.
			Meningoencephalitis.
	3. Faeces.	Inoculation of baby mice. Tissue culture tubes.	Virus isolation esp. poliovirus.
			Coxsackie virus. Echo virus.
	4. Urine.	Chemical analysis.	Toxic elements.
			Porphyria.
Convalescent phase.	1. Blood.	Repeat tests in parallel with acute phase serum for antibodies in agglutination, complement fixation, neutralisation tests.	Virus and rickettsial infections. Occasionally bacterial, fungal and helminthic infections.

myelitis. Missing the correct diagnosis in cases of malaria or relapsing fever may have fatal consequences and it is important that blood smears should be taken from all cases, in which the possibility exists, to exclude them.

Influenzal, tuberculous and salmonella meningitis will be revealed in the finding of the bacilli in smears made from the cerebrospinal fluid and in a positive culture. *Torula histolytica* will also be revealed in this way.

Q fever is not infrequently mistaken for poliomyelitis in its early stages because of the intense headache often associated with a stiff neck. The true diagnosis becomes apparent by the further course of the illness and by the development of complement-fixing antibodies

against *Rickettsia burneti* demonstrable in the convalescent phase serum compared with their absence in the acute phase serum.

ISOLATION OF VIRUS

Poliovirus may be isolated from the blood if this is collected early in the course of the illness. It has rarely been found at other times. The period in which it occurs in the blood is of short duration and occurs at a time when poliomyelitis is often not yet suspected. The isolation of virus from the blood is thus seldom relied on for confirmation of the diagnosis of poliomyelitis.

The virus is also rarely found in the cerebrospinal fluid, and its detection there is thus not attempted as a routine method of laboratory diagnosis. It is readily detected in the throat during the period extending from one week before to one week after the onset of paralysis. It may be found on occasion prior to and later than this period. However, it is present in the faeces for a longer time, often for several weeks, and in greater amount than in the throat secretions. Thus reliance for laboratory confirmation of a clinical diagnosis of poliomyelitis is usually placed on attempts to isolate poliovirus from the faeces. For this test it is necessary to send a specimen of faeces, with no preservative added, in a sterile specimen bottle, preferably under refrigeration. If more than 24 hours elapse it should be sent under refrigeration or, if this is not practicable, the specimen should be preserved by mixing with 50 per cent. glycerine in physiological saline to form about 10 to 20 per cent. emulsion.

On receipt at the laboratory a 10 per cent. suspension of the faeces is prepared and to this 100 units of penicillin and 100 ug. of streptomycin are added for their bacteriocidal and bacteriostatic effect. This suspension is then inoculated into tissue culture tubes. The tissue usually used is monkey kidney tissue, but a variety of monkey and human tissues including human amnion have been used successfully for this purpose.

The presence of poliovirus is suspected when a characteristic destruction of the tissue cells is observed, often within 24-48 hours, usually within one week of inoculation. The viruses so isolated may be passaged in series in other tissue culture tubes, and finally identified by noting that its tissue destructive or cytopathogenic effect is neutralised by the corresponding poliovirus type specific antiserum.

For the isolation of Coxsackie virus the present practice is to inoculate a litter of one-day-old baby mice with the same suspension prepared from a throat swab or from the faeces. Each mouse is inoculated with 0.03 c.c. subcutaneously. The litter is then observed for 14 days for signs of weakness and paralysis. Any mouse showing these signs, or even a suspicion of them, is sacrificed and its organs are removed for histological section. A suspension prepared from its carcase is then inoculated into a further litter of one-day-old mice.

It may be necessary to passage the virus several times before the typical picture develops.

Histological sections reveal characteristic lesions on which the diagnosis may be made. In mice dying of Coxsackie A infections there is a diffuse extensive myositis affecting the voluntary muscles, but the other organs are not affected. In mice dying of Coxsackie B infections there is necrosis and acute inflammation and sometimes early calcification of the interscapular fat pad. In addition, foci of acute necrosis and inflammation may be found in the voluntary muscles, pancreas, liver and in the brain. In the brain there may be considerable destruction of neurones associated occasionally with areas of softening and with an infiltration of inflammatory cells and perivascular cuffing.

The final identification of a Coxsackie virus is made by noting that it is neutralised in a baby mouse protective test by the corresponding specific antiserum.

Attempts at isolation of poliovirus from cases of meningo-encephalitis have resulted in the isolation of a number of viruses, which subsequent investigation has shown are neither poliovirus, Coxsackie virus nor adenovirus. These newly discovered viruses have been labelled "Echo" (enteric cytopathogenic human orphan) viruses, and at least 14 different antigenic types have been identified. Their importance in causing human disease is presently under investigation. There is no doubt that some at least of these viruses may cause meningoencephalitis, the clinical picture of which may closely simulate non-paralytic poliomyelitis.

The ECHO viruses may be isolated from throat swabs or from the faeces of infected patients by the inoculation of human or monkey kidney tissue. Their identification is finally made by noting that their cytopathogenic effect on these cell cultures is not neutralised by poliovirus antisera, but is neutralised by the corresponding ECHO type antiserum.

Adenovirus, of which several different antigenic types have been identified, is a recently

discovered cause of acute respiratory disease, including some cases of atypical pneumonia. Some of these cases may be complicated by meningo-encephalitis and therefore require consideration of the diagnosis of poliomyelitis. These viruses so far have proved non-pathogenic to the commonly used experimental animals. They may be isolated from throat swabs and the faeces of infected patients by the inoculation of tissue culture tubes and their identity established in tissue culture neutralisation tests.

With increasing use of the tissue culture technique it may be anticipated that still other groups of viruses will be unearthed. In particular it may be anticipated that the number of cases of meningo-encephalitis in which the cause is not identified will steadily diminish.

TESTS FOR ANTIBODY

Tissue culture neutralisation tests may be applied to the detection of antibody against poliovirus. In this test the serum of the individual to be tested is mixed in three separate tubes with a suspension of each of the three types of poliovirus. The virus suspension is diluted to contain about 100 tissue culture doses, so that the serum-virus mixture contains about 50 TC_{50} doses of virus. After incubation at 37° C. for two hours, these mixtures are each inoculated into two tissue culture tubes.

Known negative and positive control sera for each of the three types of poliovirus are included in the test. Readings are taken on or about the fourth and seventh day after inoculation.

If the serum contains the corresponding antibody the tissue destructive or cytopathogenic effect of the virus is inhibited. Thus it is possible to determine which, if any, of the three types of poliovirus antibody is present. However, it has been found that at the time of admission to hospital most patients have already developed antibody in high titre against their infecting virus, and a comparison between the serum of the acute phase and convalescent phase of the illness does not reveal a difference of diagnostic value, nor whether the antibody detected is a recent or remote acquisition. It is therefore often not possible on the results of the antibody tests alone to confirm a diagnosis of poliomyelitis, unless the immunity status of the patient before the onset of the major illness is known. This, of course, is exceptional.

The same limitation has been found to apply to the results of complement fixation tests for poliomyelitis. However, the titre of antibody demonstrable by complement fixation tests tends to fall much more rapidly than neutralising antibody. The detection of high titre antibody in the complement fixation tests therefore implies recent or fairly recent infection with poliovirus. In general the antibody tests are not as valuable in the diagnosis of poliomyelitis as is the case in so many other virus diseases. The usual and most reliable procedure for confirmation of the diagnosis of poliomyelitis as of the Coxsackie virus infections is the isolation and specific and type identification of the virus. However, a comparison between the antibody content of the acute phase serum and the convalescent phase serum is of diagnostic value in a number of other virus diseases which may be complicated by meningo-encephalitis. Included amongst these are mumps, lymphocytic choriomeningitis, lymphogranuloma inguinale, and a number of the arthropod-borne virus infections. In the case of mumps virus infection, which is one of the commonest causes of meningo-encephalitis, the complement fixation test is usually relied on for confirmation of the diagnosis. In the others mentioned, either the complement fixation or neutralisation tests may give the required information.

The tests for antibody by complement fixation tests are also of value in detecting non-viral causes of meningo-encephalitis, which may be mistaken for poliomyelitis. Notable examples of these are the leptospiral infections and toxoplasmosis.

The application of this comprehensive series of laboratory tests, of course, is only necessary in some cases. However, by the proper use of laboratory facilities the diagnosis of many cases can be established with certainty, and with this certainty come the proper treatment and preventive measures. The number of cases of unknown aetiology should also be steadily diminished until this admission of failure should be the exception rather than the rule, as it is at present.