LYMPHOCYTIC CHORIOMENINGITIS WITH REPORT OF A CASE.

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The past twenty years have witnessed a great increase in our knowledge of the viruses capable of attacking the central nervous system of man. Encephalitis lethargica, first recognized as a clinical and pathological entity in Vienna by von Economo (1917), was responsible for a series of epidemics between 1920 and 1926, but has now almost entirely disappeared. Its present diminished prevalence suggests either a fall in the virulence of the causative agent, possibly a virus related to that of herpes simplex, or else a higher degree of immunity among the inhabitants of the countries affected. A number of other neurotropic virus infections have since been recognized. They include St. Louis encephalitis and equine encephalomyelitis in the United States of America; type B encephalitis in Japan; spring-summer encephalitis in the U.S.S.R.; swineherds’ disease (maladie des porchers) in Eastern France and Switzerland; X disease in Australia; louping ill, an infection of sheep in the border counties of England and Scotland and in laboratory workers, and B virus infection in individuals bitten by rhesus monkeys. The most widely distributed of these new virus diseases, however, is that known as lymphocytic choriomeningitis.

HISTORICAL.

During the transmission to rhesus monkeys of infectious material originally obtained from an individual who had died at St. Louis during the 1933 epidemic of encephalitis, Armstrong and Lillie (1934) obtained a previously unidentified virus which in rhesus monkeys caused round-celled infiltration of the meninges and choroid plexus. The disease was, therefore, designated lymphocytic choriomeningitis, the virus being quite distinct from that of St. Louis encephalitis. Later, Armstrong and Wooley (1935) obtained two other strains of the same virus, one from a woman who had died from an undiagnosed nervous affection, the other from a monkey which had been inoculated with the virus of poliomyelitis. These three strains were immunologically identical and gave rise to similar pathological changes,
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not only in monkeys but in mice, which also showed infiltration of the meninges and choroid plexus with lymphocytes. Almost at the same time Rivers and Scott (1935) obtained the virus of lymphocytic choriomeningitis from the cerebrospinal fluid of two laboratory workers. Shortly afterwards the virus was isolated from cases in England (Findlay et al., 1936) and later in France.

**Clinical Symptoms.**

For some time, cases of meningitis of unknown aetiology had been described under a number of names such as serous meningitis, acute aseptic meningitis, or acute benign lymphocytic meningitis. In 1924, Wallgren suggested that these were all names for one and the same disease. With the isolation of the virus of lymphocytic choriomeningitis it was believed that the causal agent of acute lymphocytic meningitis had been discovered. Further observations, however, have shown that not all cases of lymphocytic meningitis are caused by the virus of lymphocytic choriomeningitis, and that the clinical signs may vary from those of a mild febrile disease without nervous involvement to symptoms pointing to interference with the function of both cranial and spinal nerves. Thus Findlay, Alcock and Stern (1936) isolated the virus from the cerebrospinal fluids of two patients who showed considerable residual paralysis, one still having weakness in the legs more than a year after the onset of his illness. Hammis (1938) noted psychotic changes with delusions and hallucinations, Skogland and Baker (1939) headache, generalized weakness, nervousness, tinnitus, epigastric distress and disturbance of balance, and MacCallum and Findlay (1939) symptoms suggestive of poliomyelitis. Another patient developed obliterative arachnoiditis (Barker and Ford, 1937), while Viets and Warren (1937) and Machella, Weinerger and Lippincott (1939) reported fatal cases. On the other hand, Lépine, Mollaret and Kreis (1937), as the result of injecting the virus into a number of patients with cerebrospinal syphilis, found that only half developed signs of meningitis, the others suffering from a mild influenzal-like infection with moderate prostration. The general rule was for a febrile to be followed by an apyrexial period; only in those cases in which nervous symptoms developed was there a second period of fever. The virus could be detected in the spinal fluid of those cases that developed meningitis several days before the rise in the number of lymphocytes, but had disappeared some time before the cell count became normal. Virus was present in the blood from the onset of fever and disappeared usually at the end of the second or third week. It has not been detected in the saliva but is found in the urine of man (Lépine and Sautter, 1938).

In the blood, Mollaret, Lépine and Kreis (1939) noted during the first febrile and apyrexial phase a leucopenia followed by a lymphocytosis and mononucleosis, often associated with a relative eosinophilia. These changes disappeared with the onset of meningitis and renewed fever.
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Case History.

The following case presents certain interesting features:—

Clinical history and transmission to guinea-pig in France.

Pte. H. R. had completed a course of treatment with sulphapyridine for urethritis as an in-patient in a ward where intercurrent respiratory infections were occurring, and was about to be discharged when he suddenly became ill and showed signs of meningitis. He was transferred to a casualty clearing station where he complained of headache and vomited repeatedly. There was a temperature of 102° F. with a pulse of 80, the neck was found to be stiff, and Kernig's sign was positive. No other abnormal neurological signs were found. There was a very scanty urethral discharge which showed neither leucocytes nor gonococci. Lumbar puncture produced a faintly turbid fluid containing 500 leucocytes per c.mm., of which 50 per cent were lymphocytes and 50 per cent polymorphonuclear cells. Cultures were sterile and no organisms were seen in films of the centrifuged deposit. The blood showed a leucocyte count of 10,000 per c.mm. with a normal differential distribution. Treatment was carried out with sulphanilamide as the patient had been receiving sulphapyridine up to three days before admission. Clinical improvement was slow and there was a diphasic fever for three days, followed by a gradual settling of the temperature by the seventh day. Vomiting and headache continued for some days and convalescence was not established until the tenth day, when blood was removed and serum separated. A second lumbar puncture on the second day after admission gave similar findings to the first and the cerebrospinal fluid showed 700 cells with 60 per cent polymorphonuclear cells on this occasion.

0·2 c.c. of the first specimen of cerebrospinal fluid was inoculated intracerebrally by one of us (S.-H.) into a young guinea-pig kindly supplied by the Director of a Pasteur Institute. The animal showed a rise of temperature to 103·8° F. on the sixth day and on the eighth day had lost weight and was sick with rough fur, laboured breathing, and a temperature of 104·3° F. It was killed on the ninth day and at autopsy scattered purplish-brown patches of consolidation were found in both lungs which closely resembled the areas of atelectasis seen in the lungs of mice infected experimentally with influenza virus. The brain was slightly congested. Portions of brain and lung were put in 50 per cent glycerine-saline and kept in a refrigerator for fourteen days before transmission to the Wellcome Research Institute, together with the patient's serum, and with these materials inoculations were made in England into mice, guinea-pigs, and a rhesus monkey.

The patient's serum was inoculated intracerebrally into two guinea-pigs in doses of 0·05 c.c. The first guinea-pig showed a rise of temperature to 104·5° F. on the seventh day after inoculation and remained between 104·0° F. and 105·7° F. during the next eight days. The temperature then sank to 97° F. and the guinea-pig was killed when very weak. The animal had become emaciated and at death the lungs showed considerable areas of
consolidation. The second guinea-pig's temperature rose to 104·8° F. on the sixth day. It was killed five days later when weak and emaciated; post-mortem the lungs were consolidated. Blood removed from both animals on the first day of fever was bacteriologically sterile and inoculated intracerebrally into mice, which died with spastic paralysis symptoms typical of lymphocytic choriomeningitis in six to nine days after injection. The brains of both guinea-pigs removed post-mortem produced lymphocytic choriomeningitis symptoms on intracerebral injection into mice and on subcutaneous injection of guinea-pigs. The original guinea-pig's brain was inoculated intracerebrally into a rhesus monkey, which showed a rise of temperature eleven days after injection and died suddenly on the thirteenth day. The brain inoculated intracerebrally into a guinea-pig produced fever, emaciation and death, and inoculation of the guinea-pig brain into mice produced death with symptoms of lymphocytic choriomeningitis. Of 12 mice inoculated intracerebrally with the original guinea-pig brain two died, while three others were subsequently immune to intracerebral inoculation with the virus of lymphocytic choriomeningitis.

The lung of the original guinea-pig was injected intracerebrally into two guinea-pigs, which showed fever on the seventh and thirteenth days respectively after inoculation; at death, areas of consolidation were present in the lung. Blood and brain from these guinea-pigs were bacteriologically sterile and on subcutaneous injection into guinea-pigs caused similar symptoms to those already described; on intracerebral injection of mice spastic paralysis and death occurred in from six to nine days.

Mice immune to an English strain of lymphocytic choriomeningitis virus were inoculated intracerebrally with the R strain and failed to develop symptoms.

The lesions produced in mouse and guinea-pig brains by the R strains of virus consisted of mild meningeal infiltration with marked involvement of the choroid plexus, changes identical with those caused by other strains of lymphocytic choriomeningitis.

The virus isolated from the guinea-pig material received in England and that obtained from the patient's own serum appeared to be immunologically identical.

The fact, therefore, that the virus of lymphocytic choriomeningitis was isolated from the patient's blood-serum and cerebrospinal fluid in two separate laboratories leaves little doubt that the patient was infected with this virus, which was the cause of his meningitic symptoms.

METHODS OF TRANSMISSION.

The first clue as to the means of spread of the disease was due to the finding by Traub (1935) that a stock of laboratory mice in America were infected with the virus. Later, Findlay, Alcock and Stern (1936) reported that the virus was present in mice in Great Britain, while Lépine and Sautter (1936)
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Similarly found laboratory mice infected in France. Since then evidence has been brought forward of the presence of the virus in Ireland (Collis, 1935), North Africa (Laigret and Durand, 1936), and Japan (Kasahara et al., 1937). Once strains of mice have become infected, they may harbour the virus for many years. Thus Traub (1939) records continued infection in a mouse colony for four years, while in England a strain of mice has retained the virus for more than five years. In mouse colonies infection may be transmitted either in utero or by direct contact. These observations suggest that the virus may be the cause of an enzootic among mice and that from mice infection may be transmitted to man. Support for this view is provided by the observation of Armstrong and Sweet (1939) that wild mice were found infected in two houses in which cases of lymphocytic choriomeningitis had occurred. In addition, the virus of lymphocytic choriomeningitis is excreted in the urine of mice while, as shown by Findlay and Stern (1936) in the case of mice and monkeys, findings confirmed by Dalldorf (1939), for monkeys, and Shaughnessy and Milzer (1937), and Shaughnessy and Zichis (1939) for guinea-pigs, the virus may pass through the lightly scarified skin or even through the apparently normal skin. If the skin forms the portal of entry for the virus of lymphocytic choriomeningitis, the disease has certain analogies with leptospiral jaundice, where also the infective agent Leptospira icterohaemorrhagiae is excreted in the urine of rats and infects through small abrasions of the human skin.

In the case of lymphocytic choriomeningitis, however, other possible means of transmission must be considered. At least one institutional outbreak has been described in America (Gilliam, 1938, and Wooley et al., 1939), while MacCallum and Findlay (1939) isolated the virus from the nasal washings as well as the cerebrospinal fluid of a patient. There was no evidence that the patient, H. R., had been in contact with wild mice, but respiratory infections had been occurring in the ward in which he was confined when he developed meningitis.

In a spontaneous outbreak involving monkeys in the laboratory animal house, infection of two rhesus monkeys was traced to the fact that hay and sawdust which had been in contact with infected guinea-pigs had fallen into the monkeys' cages. In the case of a laboratory worker described by Lépine and Sautter (1938), the virus entered through the conjunctiva. It is, therefore, possible that the virus may be transmitted directly from man to man by droplet infection, the portal of entry being the mucosa of the nasopharyngeal tract or through the conjunctiva.

A third possibility is that infection may, under certain circumstances, be transmitted by biting arthropods which take up the virus as it circulates in the peripheral blood-stream. Coggeshall (1939) for instance, found that Aedes aegypti was able to transmit infection by bite from monkey to monkey for from four to fourteen days after feeding on an infected monkey, while Shaughnessy and Milzer (1939) obtained infection with the excreta of the tick Dermacentor andersoni Stiles. Preliminary experiments with lice,
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Pediculus humanus var. corporis, have shown that after feeding on an infected monkey these arthropods may retain the virus for at least some hours as shown by their capacity to infect guinea-pigs when ground up and injected subcutaneously. Normal lice of the same strain, for which our thanks are due to Prof. P. A. Buxton, caused no symptoms when similarly injected in guinea-pigs.

The most probable method of infection would seem to be from mouse to man through abrasions of the skin or mucous membrane; in view, however, of the occurrence of institutional or small familial outbreaks, the possibility of direct transmission from man to man cannot be ruled out.

Although the available evidence points to the mouse as the chief source of the virus, there is a suggestion that certain other animals may act as a reservoir. Dogs, when injected intracerebrally with lymphocytic choriomeningitis virus, show no symptoms although they subsequently become immune (Findlay and Stern). In 1937, Dalldorf, Douglass and Robinson in America, found that certain specimens of canine distemper vaccine prepared from dog spleens caused a fatal infection when injected intracerebrally in rhesus monkeys; later (Dalldorf, 1939,) it was found that the fatal infection was due, not to the virus of canine distemper, but to that of lymphocytic choriomeningitis, the virus of which had been present in the dogs' spleens. Unpublished observations have shown that in England also pooled sera from dogs may occasionally show the presence of immune bodies to lymphocytic choriomeningitis, while on one occasion the virus was actually isolated from the spleen of an apparently normal dog. Efforts to show the presence of immune bodies in the serum of cats or to isolate the virus from cats' spleens have so far proved negative.

**Diagnosis.**

In view of the wide range in symptomatology varying from a mild influenzal syndrome to one closely simulating poliomyelitis which can be produced in man by the virus of lymphocytic choriomeningitis, clinical diagnosis is of comparatively little value. The disease must be borne in mind as a possibility in cases clinically resembling meningococcal meningitis. However, the examination of the cerebrospinal fluid often reveals changes which may be of use in diagnosis. The fluid is usually slightly turbid and under increased pressure. At the peak of the infection there are as a rule about 500 to 1,500 cells, predominantly lymphocytes, per c.mm. The protein content is raised and the sugar lowered in a typical case. The fluid is bacteriologically sterile.

The most reliable method of diagnosis consists in the isolation of the virus from the blood or cerebrospinal fluid by injection into mice or guinea-pigs.

In cases where the acute symptoms have passed, three laboratory methods of diagnosis have been employed. These methods are:
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(1) Demonstration of virucidal immune bodies.
(2) Complement fixation.
(3) Precipitin tests.

Neutralizing antibodies in the blood-serum are not always present in high titre, though they may persist for a considerable period. Armstrong and Wooley (1937) found specific neutralizing antibodies in 11 per cent of 1,248 normal sera, in 32 per cent of sera from persons who had a history of acute aseptic meningitis and in 28 per cent of sera of persons recently recovered from an upper respiratory infection. These data lend support to the view that the virus may be of greater importance as a cause of human infection than the occasional cases of involvement of the central nervous system would indicate.

Complement fixation was first attempted by Howitt (1937), and has since been extensively employed by Lépine, Mollaret and Sautter (1938), and by Smadel, Baird and Wall (1939). The antigen most commonly employed is made from the lung or spleen of guinea-pigs dying of the disease. More recently Smadel, Baird and Wall (1939) have employed as antigen a soluble specific factor obtained from infected guinea-pig spleen. With this soluble specific factor and immune serum, Smadel, Wall and Baird (1940) have been able to obtain a specific precipitin reaction.

THE CONTROL OF LYMPHOCYTIC CHORIOMENINGITIS.

From what has been said of the possible means of spread of the disease, it is obvious that precise steps cannot be laid down for its prevention.

In view, however, of the presence of the virus in the urine of mice, steps should be taken to keep down the number of these rodents in all buildings occupied by large numbers of men. All cases of non-bacterial meningitis, with lymphocytes in the cerebrospinal fluid, should be regarded as possible sources of infection.

Specific immunization with formolized virus was attempted by Traub (1938). A vaccine prepared from infected guinea-pig lung tissue immunized guinea-pigs, but vaccines from infected mouse tissue were incapable of immunizing guinea-pigs owing to the presence of an excess of foreign antigen. More promising results have been obtained with formolized vaccine prepared from virus grown in a serum Tyrode mixture containing chick embryo tissue.

Neither prontosil rubrum nor sulphanilamide has any curative action in man, although in experimental infections in mice certain observers have claimed that prontosil rubrum has a slight effect in large doses when the infective dose of the virus is small.

The fact that the patient, whose case is here described, had been treated with sulphapyridine immediately before the onset of his nervous symptoms, may be taken as evidence that this compound is also of little value in the treatment of lymphocytic choriomeningitis.
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CONCLUSION.

A case of lymphocytic choriomeningitis is described. The diagnosis and method of spread of the disease are discussed.

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REFERENCES.

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