more gradual; and we now know from the sequelæ which have developed in the interval that the infection may occur without producing any inconvenience that could reasonably be ascribed at the time to encephalitis. In 1924 some cases were so misleading as to determine a definite diagnosis of disseminated sclerosis. In one instance Dr. Marshall and I demonstrated on several occasions, as a typical example of disseminated sclerosis (ankle clonus, positive Babinski, absence of abdominal reflexes, nystagmus), a case which afterwards became lethargic, and which, on *post-mortem* examination, revealed the definite evidence of encephalitis in the basal nuclei and in the posterior cornua. There was no histological evidence of disseminated sclerosis. I have four *post-mortem* records of similar cases. It is important to note that the optic nerves were normal in each case. There has been no epidemic since 1924, and in the interval I have not seen a single acute case in which the diagnosis of lethargic encephalitis was unequivocal.

(For discussion, *vide* p. 776.)

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## *The Pathology of Epidemic Encephalitis.** By J. Godwin Greenfield, M.D., F.R.C.P.

### 1. *Ætiology: The Virus of the Disease.*

Epidemic encephalitis resembles many of the common infectious diseases in being caused by a virus which no one has seen, or has been able to grow in an artificial medium. Whether it has been possible even to transmit the disease to animals is still a matter of dispute, although the most recent work tends to confirm rather than to discredit the assertions of those who claim to have done so. This work is of the greatest interest, and it is of special importance in that it clarifies some of the difficult clinical problems which the disease presents. In particular, it seems to shed light on the question why the more acute forms of the disease are less often followed by progressive sequelæ than those in which it begins more insidiously.

Those who have claimed to infect animals with the disease must be divided into two groups. The protagonists of the first group are Loewy and Strauss of New York, and Kling and his associates in Sweden. Some of those who obtained the earliest positive results in this country must also be included in this group. These

* Being one of the opening papers of a discussion at the Annual Meeting held at Edinburgh on July 21, 1927 (conjointly with the Sections of Neurology and Mental Diseases of the British Medical Association meeting).

workers used either brain pulp, or cerebro-spinal fluid, blood, naso-pharyngeal secretion, urine, or fæces from cases of encephalitis for injections into rabbits. The animals showed no symptoms, but when they were killed and their brains examined microscopically, about 50% were found to show lesions similar to those of the human disease; in particular, foci of lymphocytic exudate and perivascular infiltration. This disease could be transmitted from rabbit to rabbit indefinitely, but only about half of the rabbits became infected. The claims of these workers were shown to be invalid by McCartney, who examined 372 stock rabbits at the Rockefeller Institute, and found similar lesions in the brains of 55%. Following on the work of Bull and Oliver he was able to trace the ætiology of the encephalitis in these animals to a protozoon which Levaditi has named the *Encephalitozoon cuniculi*. This comparatively harmless parasite had obviously caused an epizootic among the experimental animals, which chanced to resemble encephalitis lethargica in its histological characters. These results must therefore be completely discounted.

The other group of workers who have transmitted encephalitis to animals claim that the ætiological agent is identical with that of herpes febrilis, differing from it only in possessing a greater affinity for the nervous system. The first to transmit this virus from the human disease were Levaditi and Harvier, who in 1920 produced the symptoms of acute encephalitis in a rabbit by the subdural injection of brain-tissue obtained from a case of encephalitis lethargica. It is noteworthy that the patient from whom this material was taken had facial herpes at the time of death. Following on this, Doerr, Schnabel and Berger in Bale, Luger and Lauder in Vienna, and Perdrau in London, have produced encephalitis in rabbits by the inoculation of either brain-pulp or cerebro-spinal fluid. All these workers state that it is extremely difficult to infect animals, and that only a very few of the human cases of encephalitis examined yield a virus which is pathogenic for animals. But when encephalitis is produced in the rabbit, it can usually be transmitted as a rapidly fatal disease from animal to animal.

It has been known, since the pioneer work of Grüter and its confirmation by Kraupa and Löwenstein, that fluid obtained from the vesicles of dendritic ulcers or of labial herpes contains a virus which, when inoculated on to the scarified cornea of rabbits, produces a keratitis, accompanied by sero-purulent conjunctivitis, and often followed by rapidly fatal encephalitis. Levaditi and Harvier, in ignorance of this work, inoculated the virus which they had obtained first into the anterior chamber of the eye, and later on to the scarified cornea of rabbits, and in both instances produced not only local

lesions, but an encephalitis from which the animals died in ten to fourteen days after inoculation. It only remained for Blanc, working in the Pasteur Institute at Athens, to compare the virus obtained from these two different sources, and it was later proved that they were identical by crossed immunity experiments. It is true that certain differences are noticeable, not only between encephalitis and herpetic virus, but also between different strains of encephalitic virus. Some of the latter constantly cause both keratitis and encephalitis; others produce a less severe keratitis, but when injected into the brain produce a fulminating encephalitis. On the whole, the herpetic virus is more virulent for the cornea, and less virulent for the brain, whereas the encephalitic virus may be comparatively avirulent for the cornea. But when keratitis has been produced by a virus of either kind, it is impossible to reinfect that cornea with virus of any other strain, although it still remains sensitive to inoculation by other viruses, such as that of vaccinia.

The only serious criticism brought against the theory of the identity of the virus of herpes febrilis with that of encephalitis lethargica, is that in the few instances in which encephalitis has been caused in rabbits by the inoculation of human material the virus of herpes febrilis had been accidentally included. This may well have been so in Levaditi's first case, in which herpetic vesicles were present at death. In fact, Flexner obtained an apparently identical virus from the cerebro-spinal fluid of a convalescent case of neurosyphilis. Herpes is a very common and wide-spread disease among mankind, and the fluid from herpetic vesicles is constantly pathogenic to rabbits. Not only so, but saliva and nasopharyngeal washings of those who are susceptible to herpes are frequently sources of herpetic virus. It would, therefore, not be surprising if occasionally virus of this kind reached the cerebro-spinal fluid during life, or the brain after death. This opinion is strengthened by the observations of Teissier, Marinesco and others, that patients suffering from encephalitis lethargica are not more but less immune to herpes inoculated cutaneously than are the generality of mankind. Levaditi himself thought to cure encephalitis lethargica by injecting the virus of herpetic encephalitis into the lumbar canal of patients, and produced in many of them facial or labial herpes. There is therefore no such crossed immunity in the human subject as exists in rabbits. But it is notorious that human immunity to herpes febrilis is very slight. Even in the rabbit immunity to herpes does not last long and shows many peculiarities. It is definitely a tissue immunity and not a humoral immunity. Mixing the serum of an immune rabbit with virus does not reduce its virulence, although the addition of immune brain-pulp sometimes does so.

Perdrau found that the brain-pulp of rabbits immunized by cutaneous inoculation contained aggressins in addition to immune bodies, and that when kept in glycerin from two to three weeks the aggressin remained active, whereas the immune bodies deteriorated. Acting on these observations, he fortified the virus contained in human brain emulsion by adding to it before inoculation twice as much brain-pulp from a recently immunized rabbit. In this way he produced encephalitis in rabbits with material which, when injected alone, proved ineffective. He also made use of the "negative phase" of immunity by performing his intracerebral injection the day after a dermal inoculation, or even by giving on four successive days two dermal and two intracerebral inoculations. In this way he was able to produce encephalitis in rabbits with material from each of three fatal cases of encephalitis lethargica. His work is of the utmost importance, since it seems to indicate why so many of the earlier experiments had failed. He showed in the first place that the most virulent human virus, which, if preserved in glycerin long enough to rid it of the accompanying immune bodies, produced in rabbits a fulminating encephalitis, fatal in four days from the time of inoculation, and might, if injected fresh and without the help of aggressins, produce no symptoms at all. And secondly, that less virulent material, especially that obtained from less acute cases of the disease, might produce in rabbits a subacute form of encephalitis with lethargic symptoms. These symptoms were quite unlike those of the ordinary herpetic encephalitis, but were exactly similar to those seen if herpes virus were inoculated intra-cerebrally into immunized animals at a time when their immunity was passing off. In both cases the brains of these animals did not yield any virus which could be transmitted to other animals. From these observations he concluded that in the human disease one of three things may happen:

"(1) The development of a local cellular immunity which overcomes the infective agent and leads to a complete recovery. (2) The development of a state of cellular immunity which only partially overcomes the infection, the final issue being either a fatal one or a chronic infection. (3) Failure of the development of any immunity and a quickly fatal result."

Cases of type (3) would yield a virus which, being free from immune bodies, would be easily transmissible to rabbits; but the majority of cases were of type (2), and material from them would be either completely non-infective, or might produce a subacute encephalitis from which no further active virus could be obtained. If, however, it were freed from immune bodies by prolonged glycerination, or if its virulence were increased by the addition of

"aggressins," such material might produce a typical herpetic encephalitis transmissible to other rabbits. Now the experimental work with herpes has shown that the immunity obtained by the inoculation of a weak virus is less complete and more evanescent than that given by a strong virus. It seems, therefore, likely that in the human subject an infection by a comparatively non-virulent strain might fail to produce sufficient cellular immunity to kill off the virus completely, and that it might spread in the brain, attacking only those cells which have less power of resistance. It may be, as Perdrau has suggested, that certain cell-groups are less resistant than others, and possibly the melanin-bearing cells of the substantia nigra and substantia ferruginea are particularly susceptible. If so, it is easily understood why cases in which the initial cerebral symptoms are of the slightest character frequently go on progressively to a state of post-encephalitic Parkinsonism.

### 2. *Clinical Pathology: the Cerebro-spinal Fluid.*

The only laboratory examination which gives any assistance in arriving at the clinical diagnosis is that of the cerebro-spinal fluid. Here the evidence is sometimes negative rather than positive, as, even in the early stages, the fluid may be quite normal or may contain a slight excess of glucose, which is always of doubtful significance. But usually in the first few weeks of the disease there is a lymphocytosis of 10 to 100 or even more cells per cubic millimetre. In my experience, as well as in that of others, the cells are usually all mononuclear, but occasionally the presence of a varying proportion of polymorphonuclear cells has been reported. In the past I have been inclined to doubt the diagnosis when any considerable proportion of the cells was polymorphonuclear. But in one case in which I found 10% polymorphonuclear cells, the onset of typical sequelæ has established the diagnosis; and there appears to be no reason for doubt in Douglas's Sheffield cases, where as high a proportion as 44% was sometimes found. In a case examined histologically by Da Fano, as also in one of von Wiesner's, the inflammatory exudate in the brain was largely polymorphonuclear in character, and this is the case also in the herpetic encephalitis of rabbits during the more acute stages. In view of these facts I am inclined to alter my earlier opinion, and to agree that polymorphonuclear cells may sometimes occur in the cerebro-spinal fluid. It is characteristic of encephalitis that the rise in the cell-count is not usually associated with any great increase either in the total protein or the globulin, and sometimes a large cellular excess is associated with a normal protein percentage—the so-called cell-protein dissociation. Excess of protein is exceptional, and a coagulum

practically never forms. Yellow or hæmorrhagic fluids are sometimes obtained when there has been meningeal hæmorrhage, but such cases appear to be rarer now than they used to be. Not uncommonly, the colloidal gold reaction gives curves of the luetic type. Some workers have obtained such reactions in every case examined, others only in 15 to 50% of cases, but all are agreed that some change in the colloidal gold is characteristic of the disease. Occasionally fairly strong curves of the paretic type have been obtained, but are rare. The colloidal gold reaction is of special importance in the post-encephalitic states, as it may give the only evidence of any abnormality in the fluid. The reactions obtained at this stage are usually so weak that the other colloidal reactions may fail to demonstrate them.

### 3. *Morbid Histology.*

The histological picture of the disease is so well known that it would be unnecessary to add anything to it, were it not that there has lately been a tendency to focus attention on the mid-brain, forgetting the cerebral cortex, and to think more of the cellular exudate than of the damage done to the neurons. It has now been definitely established that post-encephalitic Parkinsonism is due to the destruction of the cells of the substantia nigra, and certain evidence, both clinical and pathological, appears to justify the assumption that sometimes the disease lingers on in this region of the brain after it has died out elsewhere. But there can be no doubt that destruction both of these melanin-containing cells and of nerve-cells elsewhere in the brain may, and does in fact, take place at a very early stage. I have examined the brain of a case of lethargic encephalitis which terminated on the twenty-third day of the disease, in which most of the cells of the substantia nigra had already disappeared.

Now it is easy by any of the ordinary staining methods to establish the disappearance of melanin-bearing cells, as the granules of melanin lie about free in the tissues for some time afterwards. But it is very much more difficult to tell when there has been destruction of some of the cells of the cortex or of the basal ganglia. In the case of the latter organs cell-counts have occasionally been done, and have shown that a certain small proportion of the neurone has disappeared. General shrinkage of the basal ganglia has rarely been taken into account, although it is well known that this may occur and may confuse the cell-count. In the cortex comparative cell-counts are extremely difficult. But decay of the neurons of the cortex and basal ganglia may be demonstrated in a large number of

cases by the collection of lipoid granules around the walls of the small vessels in those regions. The majority of these granules appear to consist of the lipochrome of nerve-cells, although a certain number may result from the destruction of myelin. Compound granular corpuscles, however, are very rarely seen, and demyelination of the cortical layers is never prominent. There is evidence that lipochrome is not easily metabolized in the tissues, and its appearance in large amounts in the walls of the small cortical vessels after a few weeks of disease may, I think, be taken as showing at least severe damage to, if not destruction of, a large number of cortical neurons.

I have recently examined the brain of a case of encephalitis which ended in mania about four weeks after the onset of diplopia and paræsthesia. In all the regions of the cortex examined the accumulation of lipoid, not only in the nerve-cells, but also around the walls of the vessels, was very striking. It was not everywhere of equal intensity, but was usually greatest at the bottom of the sulci, and from here spread outwards in an irregular fashion towards the surface of the brain. Similar collections of lipoid pigment were also present in the putamen and caudate nucleus, but were less noticeable in the pallidum and optic thalamus. Unfortunately I did not have an opportunity of examining the mid-brain in this case. In a post-encephalitic case in which, in addition to Parkinsonian bradykinesia, there was pronounced lethargy, similar perivascular collections were found in the cortex, especially in the occipital poles. In the frontal cortex there was, in addition, a definite disappearance of nerve-cells.

We are therefore justified in considering encephalitis lethargica as a disease in which there may be wide-spread and severe destruction of nerve-cells, not only in the brain-stem, but also in the cortex; and it seems clear that this neuronal degeneration is quite independent of any inflammatory cellular reaction that may be present. It is of interest to note that such skilled observers as Bouman and Bok and Flexner and Amoss have demonstrated a similar direct action by the virus on the nerve-cells in the herpetic encephalitis of rabbits. This conception of the pathogenesis of the disease is supported by the observations of McNalty, Boyd and others, that in the most rapidly fatal cases there may be little or no inflammatory exudate or perivascular cuffing. It is not a new conception, but it is perhaps a useful one to keep in mind when we try to visualize the pathological substratum of the mental sequelæ of lethargic encephalitis.

(For Dr. Greenfield's comments on his opening paper and his further remarks and the general discussion, *vide* p. 736.)