EPIDEMIC ENCEPHALITIS AND SIMPLE HERPES.

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The pandemic of encephalitis which encircled the world between 1916 and 1920 brought a new problem into epidemiology and pathology. Taking its beginning inconspicuously, probably in Austria in 1916–17, the disease first named lethargic encephalitis (von Economo), was observed next in France and England in 1918 and later (1919–20) in the United States and other more remote countries (1). The indications are that the disease appeared in mass first in Eastern Europe, passing thence to Western Europe, America, and the Far East. While the disease has died down in, although it has not disappeared from European countries and America, it has prevailed in force in Eastern countries within the past 2 years. Japan was visited by an epidemic of encephalitis in 1924, in which 6000 or more cases were reported (2). This large recent outbreak was characterized by a high mortality (reaching 70 per cent), while the mortality of the earlier European and American malady was about 25 per cent. In the absence of an established specific cause for the epidemic outbreaks, the question remains whether all were of the same nature, or whether several distinct diseases have, for unknown reasons, been attended by a high proportion of brain complications or sequels.

The wide prevalence of an epidemic disease presenting novel clinical features gave rise at once to speculations such as, (a) the previous occurrence, and (b) the nature and causation or etiology. While encephalitis, or brain inflammation, is known to attend certain definite diseases, among which are epidemic influenza, ulcerative endocarditis, and epidemic cerebrospinal meningitis, yet as such attendants, the number of cases arising is relatively not large. Along with the other peculiarities of the epidemics of encephalitis of this period are
to be mentioned, therefore, the wide occurrence, the large number of cases, and the absence of close correlation with epidemics of influenza.

The earlier literature does not contain records of parallel outbreaks of encephalitis. Perhaps the most frequent precursor or concomitant of small prevalences of encephalitis, so far as the records show, is epidemic influenza. A pandemic of epidemic influenza of extreme severity swept over the world between 1917 and 1920. The question arises immediately, therefore, whether the two epidemic maladies—influenza and encephalitis—have, in this instance, been connected in a causal way.

In endeavoring to supply a definite answer to this question, we are in the difficult position of having no certain knowledge of the nature of the microbic incitant of influenza. Were we in possession of this much sought knowledge, we should probably be able to state immediately whether influenza and encephalitis are induced by the same microbe, acting chiefly on different parts or organs of the body. In the absence of this knowledge, the answer to be given to the question must be indirect and hence perhaps not final.

This significant question has been examined in detail elsewhere (1), and the conclusion reached is: “The pandemic of lethargic encephalitis of the second decade of the twentieth century is quite unprecedented in recorded medical history, and is probably not merely an unusual nervous manifestation of epidemic influenza, but is rather an independent affection, etiologically considered, to be compared in its specific nature with other defined pathologic entities, such as typhoid fever, tuberculosis, poliomyelitis and epidemic meningitis.”

Encephalitis.

The kind of inflammation of the brain to which the name encephalitis is applied is a not uncommon pathological condition in man and other mammals. In man, the commonest varieties are met with in African sleeping sickness, a disease incited by trypanosomes; and in syphilis, a disease incited by the spiral organism Treponema pallidum. Encephalitis is also an attendant of poliomyelitis in man, although in this disease the brain is less often affected than the spinal cord, and the high degree of affection of the one usually obscures the slighter involvement of the other. Aside from the particular and specific forms
of encephalitis in man there occur the cases already mentioned as complicating epidemic influenza, endocarditis, and epidemic meningitis, as well as certain other grouped cases of still uncertain nature, as represented by the so called Australian X disease (3).

Other mammals, namely horses, dogs, rabbits, and doubtless still other species, are subject to particular kinds of encephalitis. The prevailing form in horses, often reaching epidemic proportions, is the so called Borna’s disease; while many domestic stocks of rabbits show encephalitis, partly incited by the protozoan parasite Encephalitozoon cuniculi, and partly perhaps by other microbes (4). Encephalitis is not infrequently detected in dogs succumbing to distemper and to rabies.

This pathological condition, widely present in mammals, shows a striking uniformity of microscopic characters in all the species affected. Two classes of lesions are met with: degenerative and infiltrative. The first consists of various destructive changes in nerve cells and supporting (glia) tissues. The second consists of cellular invasions most conspicuous about small blood vessels and in the adjoining nervous tissues. The nerve cells consist chiefly of the mononuclear elements called lymphocytes. They tend to be heaped about the blood vessels and to form small aggregations in the tissues themselves. This peculiar distribution of the invading lymphocytic cells gives a striking character to the microscopic appearances, especially revealed in African sleeping sickness and lethargic encephalitis in man, and in Borna’s disease and the so called spontaneous encephalitis in rabbits (5).

**Experimental Encephalitis.**

The method followed in investigating epidemic encephalitis experimentally was to inoculate rabbits and monkeys with brain tissue and cerebrospinal fluid taken from cases of the disease in man. The inoculations were usually made by intracerebral injections. These animals withstand such injections very well and show no symptoms referable to the inoculation, unless infection occurs. The infection which may occur arises (a) from specific organisms, as in experimental poliomyelitis; or (b) from accidental secondary, usually pyogenic organisms. The two resulting conditions are readily distinguishable.
There is general agreement among investigators that the monkey is not subject to inoculation with materials taken from the central nervous system of cases of epidemic encephalitis. The rarely successful inoculation reported may be interpreted as produced by an extraneous cause (1).

An active controversy, not yet wholly composed, has centered about the result of the inoculation of rabbits. While certain investigators succeeded in producing in rabbits encephalitis which they identified with epidemic encephalitis in man, other equally experienced ones failed wholly to obtain unequivocal results. A part of the disparity has been explained by the discovery of the frequency of spontaneous encephalitis in the domestic rabbit; in this way the early, supposedly successful experiments of Loewe, Hirshfeld, and Strauss (6) may be accounted for. Another part of the disparity may be explained by the discovery that the clear contents of herpes vesicles in man contain an active substance, believed to be a living, submicroscopic organism, so called virus, which is inoculable in rabbits, in which animals an encephalitis, usually fatal, is often induced (7).

Experimental Herpes.

Simple or febrile herpes is a common and innocent affection in human beings. The eruption consisting of vesicles to which this name is given usually appears on the lips. Certain persons are so subject to the eruption that very slight maladies, simple colds, etc., frequently produce an outbreak. On the other hand, the rabbit as far as is known is not naturally subject to a corresponding affection. And yet, when the clear, non-bacteria-containing, vesicular contents are inoculated into rabbits, profound effects are produced. If the contents are inoculated into the scarified cornea, severe kerato-conjunctivitis arises; if into the scarified skin, vesicular dermatitis follows; if into the brain, fatal encephalitis results. In some instances the corneal inoculations are followed by the nervous symptoms and fatal effects of an encephalitis; the skin inoculation by symptoms and effects of a myelitis and subsequent encephalitis; and injection of the virus into the testis also by a fatal encephalitis.

When the symptoms and effects on the brain of the experimental
herpes rabbits are compared with the few instances of actual successful inoculation of rabbits with nervous materials taken from human cases of epidemic encephalitis, it has been found that the two sets of conditions are indistinguishable. And in keeping with this observation it is also found that the so called virus of encephalitis, as contained in the brain of rabbits, is inoculable upon the cornea and the skin of these animals, in which it produces vesicular inflammations corresponding accurately to those set up by the herpes virus. Hence two questions at once suggest themselves: (a) Are the herpes virus and the supposedly encephalitis virus identical; and (b) if identical, is epidemic encephalitis merely a manifestation of the location in and action of the common herpes virus upon the brain of man, made possible by particular and thus far undiscovered conditions which have prevailed fitfully in different parts of the world from 1916 up to the present time (8)?

Two points of view are being entertained regarding these questions: one represented by Levaditi and to a less extent by Doerr (9), according to which epidemic encephalitis is a manifestation of the action of special strains of herpes virus acting on the prepared, susceptible nervous system of man; and the other represented by Flexner and Amoss (10), who hold that the true virus of epidemic encephalitis remains undiscovered, and that the small number of successful inoculations of human encephalitic materials reported can be explained on the supposition that there was present in these an accidental admixture of herpes virus.

That this kind of admixture actually may occur is shown by an instance in which herpetic encephalitis was induced in the rabbit by the injection of the cerebrospinal fluid taken from a case of neurosyphilis showing at no time symptoms of epidemic encephalitis. This strain of herpes virus produces all the inoculation effects and is biologically indistinguishable from the ordinary herpes and the so called encephalitis virus (11).

**Recent Experimental Findings.**

The discussion regarding the etiology of epidemic encephalitis and the relationship existing between herpes and the so called encephalitis viruses, has recently centered about certain anomalous ex-
periments made on guinea pigs as reported by Rose and Walthard (12) and by Dmitrieff (13). In order to follow the views of these authors, it is necessary to recall the fact, established by many investigations, that while the rabbit is highly susceptible to inoculation with the herpes group of viruses, the guinea pig, the rat, mouse, and other rodents, are far more resistant. Other species—dog, cat, monkey—are practically wholly insusceptible.

Although the rabbit is so sensitive to the inoculation, yet in order to implant a virus on this animal from materials taken from cases of epidemic encephalitis, the rule is to inject several animals, so that the differences in individual sensitivity naturally existing may be compensated. When the inoculation has succeeded, which has rarely been the case, usually one animal in the series develops symptoms. As has already been stated, the great majority of investigators have not succeeded in obtaining any transmission whatever to the rabbit.

The reported experiments with guinea pigs are supposed to explain this anomaly. In effect they are as follows: When a strain of the herpes-encephalitis virus is injected intracerebrally into guinea pigs, after an incubation period of several days, an encephalitis arises. The symptoms and effects of the encephalitis produced resemble those of the rabbit, although they tend to be less severe. Rabbits when inoculated intracerebrally with an active strain of virus not only develop encephalitis, but regularly succumb to the disease. The guinea pig, on the contrary, is said to tend to recover from the encephalitis and animal to animal transmission to be impracticable. Indeed, according to Rose and Walthard and to Dmitrieff, even when encephalitis is produced in the guinea pig, the introduced virus is rapidly destroyed. It is this rapid destruction within the susceptible brain tissue which led the authors to venture the opinion that similarity exists between the effect of the herpes-encephalitis group of viruses on the brain of man and of the guinea pig, and to see in this supposed similarity support for the belief that epidemic encephalitis in man results from infection with a virus of the herpes-encephalitis kind.

The observations reported by Rose and Walthard and by Dmitrieff should as such be accepted as a fact, although the number of experiments performed is not large. In Dmitrieff's case the number is indeed very small. The question which the experiments do not an-
answer and which is yet decisive is, whether the results obtained apply to one or two strains only, or to all strains of the herpes-encephalitis virus. Flexner and Amoss (14) have shown that, as measured by rabbit inoculations, what may be called strong and weak strains of the virus exist. It is noteworthy that the Levaditi and Doerr strains of the virus, supposedly of encephalitic origin, belong to the weak class (15). Rose and Walthard used a Doerr strain in their tests. Hence it becomes important to ascertain what happens when a strong or highly virulent strain of the virus is injected intracerebrally into guinea pigs.

We possess in the H. F. strain such a strong herpes virus (14). With this virus we have infected guinea pigs by intracerebral and intra-corneal inoculations through a series of passages which, at the termination of the 10th cerebral transfer, showed no diminution of activity and no lengthening of the incubation period. If anything extraordinary occurred in the course of these passages, it was an adaptation of the virus to the guinea pig, which regularized its activity by making the inoculation effects more uniform and certain as well as of shorter duration. The cerebral injections were performed in pairs, and while at first the two pigs might develop symptoms a few days apart, in the later passages they often developed them simultaneously; unless sacrificed for experimental purposes, these pigs succumbed.

The symptoms were typical of experimental herpes virus encephalitis and included high temperature (up to 106.5°F.), paralysis, and salivation. The fact should be emphasized especially, since the observation is a new one, that the inoculation of the cornea not only induced kerato-conjunctivitis, but also in a number of instances a fatal encephalitis. The following is a protocol of an experiment of this kind.

Protocol.

Nov. 4, 1926. Right eye of guinea pig cocainized and scarified with cataract knife dipped in a 10 per cent suspension of fresh guinea pig brain of animal which reacted to intracerebral inoculation of H. F. virus. Nov. 6. Beginning kerato-conjunctivitis; temperature 104.6°F. Nov. 7. Opacity and small vesicles on cornea; inflammation increased; temperature 105°F. Nov. 8. Eyelids glued together; temperature 106.2°F. Nov. 10. Temperature 105.2°F.; turns to right. Nov. 11. Tremor, ataxia, falls easily. Nov. 12. Temperature 106°F.;

That it is possible to excite a severe kerato-conjunctivitis in the guinea pig by eye to eye inoculation is shown by the pig inoculated from the eye of the above named animal on Nov. 8. This animal developed typical progressive inflammation of the cornea and conjunctiva, and on Nov. 13, 24 hours after the temperature had reached 106.2°F., began turning to the right side (side of inoculation). Tremor, ataxia, convulsions, and salivation ensued, and death occurred on Nov. 18, or 10 days after the inoculation.

Penetration of the virus from the eye to the brain of guinea pigs did not always occur. The behavior of the corneally infected pigs was often typical of the rule, namely the inflammatory reaction would be less severe than is observed in rabbits, and with its abatement the eye would return to normal. The cornea tended not to become opaque and the eyeball shrunken.

The employment for the inoculation of guinea pigs of a strain of herpes virus shown by rabbit tests to be strong, permits of the drawing of entirely different conclusions from those reached by Rose and Walthard and by Dmitrieff, both of whom used weaker strains. What has been described by Rose and Walthard and by Dmitrieff are only special instances of the action of such weak strains of the virus in guinea pigs. Moreover, a comparison of the experiments of Rose and Walthard with those of Dmitrieff suggests that the strain of virus used by the latter was definitely weaker than that employed by the former, and more quickly suppressed by the guinea pigs. Indeed, in view of the history of many cases of epidemic encephalitis in man in which the disease pursues a progressively degenerative course, it would seem almost inevitable that the inciting microbic agent, far from being quickly destroyed, actually possesses the power of continuous, slow multiplication, because of which the pathological processes fail to be arrested.

Contradictions in Etiologic Findings.

Epidemic diseases show, as a rule, identical microbic incitants, irrespective of the time and place of their prevalence. Once the microbe has been discovered and shown beyond doubt to be the incitant, then the epidemic disease, whether appearing in Europe, America, Asia, or Africa, has always been accompanied by particular
microbes, the biological properties of which are indistinguishable in all essential respects.

If this test of correspondence is applied to the agents obtained during outbreaks of epidemic encephalitis, it fails to hold. Attention has already been drawn to the fact that in only three certain instances (Levaditi, Doerr, Schnabel) (1) has an herpes-encephalitis strain of virus been obtained from cases of epidemic encephalitis, while many more investigators have failed altogether in their search for this virus. On the other hand, still other investigators have reported the isolation of kinds of virus which differ from the herpes-encephalitis variety, and these exceptional specimens fail to agree with one another. The outstanding exceptional findings are those of Kling of Sweden and of Takaki of Japan.

In both instances, disease was induced in rabbits through injecting materials derived from cases of human encephalitis. The experimental disease described by Kling differs wholly from that described by all other successful investigators, in that it is not an acute, but a chronic pathological process. A critical examination of Kling's results has been made elsewhere (1). It remains to examine the results obtained by Takaki (2).

In the summer of 1924, about 6000 cases of epidemic encephalitis were reported from different parts of Japan. Transmission experiments were undertaken, and both failure and success were reported. Perhaps the most notable instance of success is that of Takaki, who reports 6 transmissions of a virus disease to rabbits with autopsy material from 6 fatal cases. The virus cannot be cultivated artificially; it is inoculable by way of the cornea, brain, and other organs. The eye effects, however, differ from those of the herpes virus effects; the general symptoms, which include paralysis, but not excitement, also differ from those of the herpes virus effects. The symptoms, therefore, as exhibited by rabbits do not correspond to the symptoms produced by inoculation of the herpes-encephalitis group of viruses. Moreover, comparison of the Japanese virus with the herpes-encephalitis virus through immunity tests and reactions, shows it to be dissimilar (2).

In view of this discordant finding, the question arises whether the Japanese and the European epidemic diseases are pathologically the
same. Fortunately this question can be answered, and apparently in the affirmative. The clinical and pathological descriptions which have been published show close similarity. Through the kindness of Professor Kimura, of the Imperial University in Sendai, I have been enabled to examine specimens taken from the brain of fatal cases. These specimens show pathological changes closely resembling those found in the brain of Europeans and Americans who have succumbed to epidemic encephalitis. The changes or lesions are of two sorts: monocellular (lymphoid) infiltrations of the blood vascular sheaths and brain tissue, and degeneration of ganglion and glia cells. The distribution of the lesions is also typical. Special attention may be drawn to the lesions of the substantia nigra which are prominently present in the Japanese, as well as in the European cases of the disease.

There is no doubt that the Swedish cases of epidemic encephalitis are identical with the other European and the American cases. From what has just been stated, there are strong reasons for believing that the Japanese epidemic disease is of the nature of the European and American disease. The essential differences relate to the microbic incitants described by Kling and by Takaki. As tested by these discrepancies, the epidemics would have to be regarded as distinct. The fundamental question raised by the discrepancies is, therefore, whether the experimental findings are not open to the suspicion of not revealing the real incitant of the epidemic disease. What must also be taken into account is the possibility of other circumstances coming into play, such as unrecognized, preexisting disease of the rabbit, or of the operation of contaminating organisms which produce effects in the inoculated animals and yet play no part in the human epidemic disease.

Native Animal Viruses.

The employment of the rabbit for the experimental investigation of epidemic encephalitis has led to a state of confusion not yet terminated. That the earlier observations of Loewe, Hirshfeld, and Strauss (6), Kling (16), and some others were vitiated by the presence of an unrecognized, preinoculation form of encephalitis in these animals, is now either proven or probable. Nishibe (17) has recently
shown that the domestic rabbit of Japan likewise suffers from preinoculation encephalitis, and hence is unsuited for the study of the perivascular and other infiltrative lesions of the brain. It may be queried whether Nishibe is more fortunate in citing as evidences of experimental infection, the more subtle lesions of nerve cells of the rabbit's brain, which he regards as indicative of this condition. In emphasizing these lesions and in discarding the infiltrations as indications of a state of encephalitis, he is introducing not only new considerations in respect to the experimental disease, but is also setting up a class of lesions as criteria which has not yet been described for the human affection.

The difficulties encountered by the use of the rabbit and some other animals for the experimental investigation of epidemic encephalitis do not end here. There can be small doubt that in certain instances, what appear to be transferable virulent materials, differing from ordinary bacteria, have been obtained in the course of the inoculation of rabbits and of dogs with human nervous tissue. These active materials, called by their discoverers "viruses," and believed by them to be concerned with the production of encephalitis, are not all, as has already been pointed out, of one kind. The virus of Koritschoner (18) certainly, and that of Kobayashi (19) probably, are merely strains of rabic virus, while the virus called Eck (20), by reason of the fact that it was recovered from dogs in which an Eck fistula had been produced, has not yet been identified with a known variety. One can only speculate on the source of these virulent agents. Where dogs are employed, it is always possible that a latent rabic virus may come to be isolated in the course of the transfer of nervous tissue. Where autopsy material from human cases is used for inoculation, something may depend upon the way it was removed from the body and how it was treated in order to guard against even gross contamination.

However this may be, it would seem that these explanations do not entirely cover the observations of Takaki (2). The virus obtained by him several times from 6 fatal cases of the Japanese encephalitis, differs apparently from all those hitherto studied. It does not belong to the herpes-encephalitis group, and apparently not to the rabic viruses. Since the Takaki virus was obtained from the cases of epi-
Epidemic encephalitis studied histologically by Kimura,¹ there is no reason to doubt that it came, directly or indirectly, from definite encephalitic material. What is noteworthy, however, is that there is fundamental lack of agreement among the Japanese investigators of the epidemic disease of Japan, just as there is lack of uniformity between their observations and those of European investigators of epidemic encephalitis.

We must leave the clearing up of these discrepancies to further investigations of the native viruses of domestic animals. Thus far almost no heed has been paid to the existence in animals in a state of nature, of virulent agents, possibly true viruses, which remain in a condition of latency until through some simple device they are made to assert themselves. The best example of this class of substances now known is that described by Rivers and Tillett (21) for the rabbit. An analogous example is the one described for the guinea pig by Jackson (22) and by Cole and Kuttner (23). It would seem as if we are at the very beginning of knowledge of this class of potential pathogenic agents; and it would also seem that we shall have to take into account the possibility of a greater number and wider distribution in animals ordinarily called "normal," of these agents in connection with the experimental study by animal inoculation of human diseases, the etiology of which is still undiscovered.

CONCLUSIONS.

The purpose of this paper is to explain the state of our knowledge of the etiology of epidemic encephalitis, and especially to draw a line of demarcation between the established virus of simple herpes and the hypothetical virus of epidemic encephalitis. It had already been shown that the experimental observations on rabbits do not suffice to prove the identity of the herpes with the encephalitis virus. The discussion of the subject in this paper shows that identity cannot be postulated on the basis of the performed guinea pig experiments. Attention has been drawn to the significant fact that there is lack of harmony in the positive results of those investigators who believe that the

¹ We are indebted to Professor Kimura for specimens of human brain tissues which show definitely lesions that are indistinguishable from those occurring in the brain of European and American cases of epidemic encephalitis.
incitants of epidemic encephalitis have been discovered. An attempt has been made to attribute some of the discrepancies reported by these investigators either to accidental and contaminating microbic agents, or to the uncovering of virulent agents preexisting in a latent state in the animals employed for inoculation, the existence of which was not previously known or suspected. Since past experience leads us to believe in a single incitant for widespread epidemic diseases, it is probable that, when certainly discovered, the microbe of epidemic encephalitis will prove to be simple and not multiple. The direct corollary to this point of view is that up to the present, the etiology of epidemic encephalitis has not been determined.

BIBLIOGRAPHY.

   Doerr, R., and Vöchting, K., Rev. gén. ophtal., 1920, xxxiv, 409.