CONTRIBUTIONS TO THE PATHOLOGY OF EXPERIMENTAL VIRUS ENCEPHALITIS.

I. An Exotic Strain of Encephalitogenic Virus.

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INTRODUCTION.

The experimental study of encephalitis has entered upon a new phase. Opened to exploration by the investigations of Strauss[1] and his coworkers on epidemic encephalitis and by Grüter[2] and Löwenstein[3] on experimental febrile herpes, the generalizations adopted by them or their immediate successors are now in process of revision. In view of the seriousness of the situation arising out of the epidemic invasions of encephalitis between 1917 and almost the present date, it was perhaps to have been expected that, given any degree of success attending the experimental studies on encephalitis, they should have led to premature conclusions.

It is only recently that the results of these studies have come to be considered critically. Hence many of the earlier deductions have been found excessive and in need of rectification. The sources of some of the main errors have become evident from a more precise examination of the experimental material—the rabbit chiefly—employed.[4] Another error, which perhaps should have been more effectually guarded against, arose from lack of due consideration being accorded the evidences which suffice to establish relationship of cause and effect between a given microorganism and a recognized pathological process or disease.

[1] Strauss, I., Hirshfeld, S., and Loewe, L., *New York Med. J.*, 1919, cix, 772.

[2] Grüter, W., *Klin. Monatsbl. Augenheilk.*, 1920, lxv, 398.

[3] Löwenstein, A., *Münch. med. Woch.*, 1919, lxvi, 769; *Klin. Monatsbl. Augenheilk.*, 1920, lxiv, 15; 1920, lxv, 399.

[4] Flexner, S., *J. Am. Med. Assn.*, 1923, lxxxi, 1688, 1785.

The founders of bacteriology were confronted with this problem and, of course, with the pitfalls inseparable from this class of experiments. Out of the safeguards erected by them there emerged the so called postulates of Koch which, until recently, dominated the conceptions of bacteriologists working in the etiological field. These postulates were, moreover, formulated at a period in which bacteriologists were dealing with microscopically visible organisms which also could be secured in artificial cultures. If, therefore, these elaborate precautions were found necessary then, how much more so, it may be inquired, are they demanded now that we are seeking to connect the far more subtle and elusive, invisible, so called filterable, parasites with particular kinds of specific disease. Our experimental methods should, under these circumstances, have become more rigorous, which has not, in fact, always been the case.

Hitherto there have been described two varieties or classes of encephalitis-inducing virus: one supposedly obtained from cases of epidemic encephalitis,[1,5] the other definitely obtained from the vesicular contents of febrile herpetic eruptions.[6] As these two kinds of virus have been more and more studied, they have been shown to possess common properties and to be indistinguishable by any biological tests now available.

The so called virus of encephalitis has been secured under circumstances not always free from suspicion of doubt. Of the, on the whole, small number of specimens of this virus described, the larger part has been derived from inoculations either of nasopharyngeal secretions or of brain substance taken at autopsy. The former material, even when filtered, cannot be regarded as a simple inoculum, and the latter has oftener than not been taken from organs removed in the usual course at autopsy and thus subject to gross contamination.

The case is different with the reported successful inoculations of the cerebrospinal fluid withdrawn during life from cases of encephalitis in man. This fluid is readily obtained free of extraneous microorganisms. There can be no doubt that in certain instances the

[5] Levaditi, C., and Harvier, P., *Compt. rend. Soc. biol.*, 1920, lxxxiii, 354; *Ann. Inst. Pasteur*, 1920, xxxiv, 911.

[6] Grüter, Löwenstein, and many others.

injection of cerebrospinal fluid into the brain of rabbits sets up an encephalitis which is transmissible in indefinite series from rabbit to rabbit. In this instance everything depends on the interpretations put upon the results achieved and whether they are held to be due to the action of the true virus of encephalitis or some other agent capable of inducing an inflammation of the brain in the rabbit.

The experiments to be reported in this paper derive their particular value from the significant fact that a virus, on the one hand indistiguishable from the so called virus of encephalitis, and on the other hand, from the virus of febrile herpes, has been obtained from the cerebrospinal fluid of a patient suffering from vascular and neural syphilis, who had been under continuous observation for more than 2 years, and who never presented symptoms of epidemic encephalitis.[4]

History of the Case Yielding the Exotic Virus.

The patient, J. B., from whom the virus was obtained was a male, 51 years old, treated at the Presbyterian Hospital, New York. We are indebted to the physicians of the hospital for the cerebrospinal fluid of this and of many other cases, employed in our experiments. The patient had been ambulatory since February, 1921. He attended the dispensary, would enter the hospital for intraspinal, salvarsan medication, and then return to his work. The Wassermann reaction was several times found positive in the blood and cerebrospinal fluid. The patient was still under observation in September, 1923. At no time in this long period had symptoms other than those referable to the syphilitic infection been complained of or observed.

Scope of the Investigation.

The experimental reproduction in animals of a condition resembling epidemic encephalitis has engaged our attention from 1919 onward. When the first fatalities caused by the disease occurred in New York, we secured, through the kind cooperation of thepathologists of several hospitals, portions of the brain or the entire organ as removed at autopsy for the purpose of inoculating animals. In view of the successful use of *Macacus rhesus* in the experimental study of poliomyelitis, our first inoculations were made on this species. No unequivocal successful result was secured. Following the announcement of the successful use of rabbits by Strauss and his coworkers, this species was employed by us but again without success.

The method we pursued was to select material from the basal ganglia in as clean a manner as possible. When the whole or half the brain came into our possession it was wrapped in sterile towels and transported at once to the laboratory. As a rule the exposed outer portions of the brain could be sliced away, exposing the ganglia, the chief seat of the inflammatory lesions, which often proved sterile on culture tests. The fragments were emulsified and injected subdurally or intracerebrally into the anesthetized animals, and portions were, at the same time, placed in glycerol and also in fixing fluids, the last for histological preparations.

As Strauss and his coworkers continued to report successful inoculations of rabbits, especially with filtered washings of the nasopharynx and of filtered extracts of nasopharyngeal mucosæ, removed at autopsy, and finally with cerebrospinal fluid, our efforts to secure transmission of a virus were made to include all those materials but again without a single unequivocal result. The confirmatory reports of the work of Strauss by Levaditi, and then by Doerr[7] led us to extend our search much more widely, for which purpose we employed the cerebrospinal fluid from a large number of clinical cases diagnosed as epidemic encephalitis as well as other cases of varied nervous and other affections. For the opportunity to carry out this wider study we are indebted to the clinical staffs of the Presbyterian Hospital and the Neurological Institute. In the course of this comprehensive study one of us (H.L.A.) became connected with the Johns Hopkins Hospital, Baltimore, where he carried out similar inoculations. Finally, an outbreak of epidemic encephalitis arose at Winnipeg, Canada, and Dr. A. J. Douglas courteously sent specimens of cerebrospinal fluid, enclosed in thermos bottles, from eight cases. Except the last specimens, which were put into rabbits about 12 days after the collection, all the fluids were injected within 24 hours of their withdrawal.

Procedures.

Two methods of injection were followed in this inclusive investigation. At least two anesthetized rabbits received the injection of 0.35 cc. of each cerebrospinal fluid into the forebrain through a trephined opening, and at least two others received 1 cc. intracranially by simple lateral needle puncture through the suture of the skull behind and above the level of the outer canthus of the left eye in the manner employed by Strauss, Hirshfeld, and Loewe.[1] Very few rabbits were lost from pressure. The fluids were always tested for sterility in deep bouillon tubes and for globulin by the Noguchi method. A given specimen of the cerebrospinal fluid was injected into from two to five rabbits. For the miscellaneous fluids two rabbits were used;

[7] Doerr, R., and Schnabel, A., *Schweiz. med. Woch.*, 1921, ii, 469; *Z. Hyg. u. Infectionskrankh.*, 1921, xciv, 29.

for the fluids coming from supposed cases of epidemic encephalitis, five or more were injected. The inoculated rabbits were kept under observation for many weeks. They were individually inspected twice a day. Any animal showing symptoms, no matter how trifling, was closely watched: the rectal temperature was taken, it was tested for impaired movement, excitation, salivation, etc.

The miscellaneous collection of cerebrospinal fluids included cases of syphilis (many), brain tumor (several), general nervous affections, including multiple sclerosis (a number), acute infections—pneumonia, epidemic meningitis, influenza, etc. (a number).

The number of deaths in the large series of rabbits inoculated was small. In a single instance only was a disease encountered which was identified with the condition described in the literature as "experimental epidemic encephalitis." As will appear presently, our view is that the disease does not experimentally reproduce epidemic (lethargic) encephalitis, but that it is induced by a living agent or virus and hence we have preferred to designate the condition "virus encephalitis." Our belief is also that this virus encephalitis is indistinguishable from the experimental form of encephalitis induced in the rabbit by the virus of febrile herpes, with which it may be and probably is, identical. Aside from this one instance of fatal virus encephalitis, all the fatalities among the inoculated rabbits arose from injury (fighting), snuffles, or other secondary diseases. All the rabbits *in extremis* were etherized and submitted to complete autopsy and bacteriological examination. Particular attention was directed to the central nervous organs, which were also submitted to histological study. In all doubtful instances reinoculation of brain material into other rabbits was made immediately in order to guard against the loss of any virus strain possibly present, and because, as is well known, the first implantation of virus into the rabbit is difficult, while the subsequent transfers are readily effected.

The total number of specimens of cerebrospinal fluid injected into rabbits was 100, of which 27 were derived from patients diagnosed or suspected as being cases of epidemic encephalitis, and 73 from cases of miscellaneous character.

EXPERIMENTAL RESULTS.

On Jan. 4, 1923, two rabbits were given 0.35 cc. of cerebrospinal fluid, from patient J. B., by the intracranial route. One of the rabbits failed to show symptoms. On Jan. 13 the other was noted to be tremulous, to turn in circles to the left (side of inoculation), to move the head rhythmically backwards and forwards, and to grind the teeth (gnashing). The rectal temperature was 105.6°F. There was no salivation. Death occurred the next day. The autopsy showed congestion of the brain and meninges and normal-appearing other organs. Three rabbits were injected intracranially on Jan. 14 with brain suspension of this animal. Other portions of the brain were preserved in 50 per cent glycerol and taken for histological examination.

A second specimen of the cerebrospinal fluid of J. B. was injected into five rabbits, by the intracranial method, on Feb. 2 and a third specimen into four rabbits (two into the brain, two into the eye after scarification of the cornea) on Sept. 20, but without results.

The strain of virus from the rabbit succumbing on Jan. 14 was transferred to other rabbits, in indefinite series, with ease and in them produced regularly the train of symptoms and the pathological effects to which the name of experimental encephalitis is applied. This strain of virus has been employed in a comparative study of the effects of the so called virus of epidemic encephalitis and the virus contained in febrile herpes vesicles, and it has been found indistinguishable from them by any of the known biological tests.

Series A. Intracranial Inoculation.

On Jan. 14 three rabbits were injected intracranially with a 10 per cent suspension of the brain (virus) of the rabbit succumbing to the inoculation of the cerebrospinal fluid of patient J. B.

Rabbit A received 0.25 cc. of the suspension. Jan. 16. The animal was slow, showed a rectal temperature of 106.5°F., gnashing of teeth, salivation, tremor, and later convulsions. Jan. 17. Etherized. The brain was congested; urinary bladder distended. Ordinary cultures showed no bacteria.

Rabbits B and C were given respectively 0.3 and 0.35 cc. of the same brain suspension. 24 hours later there were noted fever, rapid respiration, spasmodic contractions of the abdominal muscles, attended by cough, then prostration, convulsions, and urine retention. One died and the other was etherized when moribund on Jan. 17. The brain was sterile for ordinary bacteria.

The results of the inoculation of the above three rabbits show the J. B. virus to possess high activity or virulence, and to be capable of inducing the train of clinical events to which the term experimental virus encephalitis is applicable. A long series of experimental inoculations, extending over nearly 3 years, has been carried on with

the J. B. virus and in that time no falling off of potency has been noted. On the other hand, the virus has shown itself glycerol-resistant, active by means of paper and Berkefeld filtration, and by other modes of inoculation than the intracerebral. Some protocols of animals injected with paper and Berkefeld filtrates follow. In respect to glycerolation, it may be stated that samples of the brain which were kept at 4°C. in glycerol for more than 6 months showed undiminished activity.

May 21, 1923. Two rabbits received 0.05 and 0.025 cc. of a paper filtrate of a 5 per cent emulsion of fresh virus (brain). Both animals developed characteristic symptoms, including fever, salivation, muscular agitation, convulsions, and urine retention. Death occurred on the 7th and 8th days respectively.

Oct. 28. Two rabbits were given 0.35 cc. of a Berkefeld filtrate prepared from a 10 per cent suspension of brain (virus), glycerolated for 6 weeks. Typical symptoms appeared on the 4th, and death followed on the 9th and 10th days respectively.

Series B. Corneal Inoculation.

That the brain tissue taken from rabbits succumbing to inoculation with the so called encephalitis virus is capable of inducing a kind of herpetic keratitis in the rabbit was pointed out by Levaditi and Harvier[8] after the demonstration of the similar effect of the herpes virus on the cornea of rabbits by Blanc and Caminopetros.[9] That the J. B. virus can excite a corresponding inflammation of the cornea is shown by the following protocols.

On Jan. 14, 1923, the cocainized eye of Rabbit G was incised with a cataract knife previously dipped into a suspension of the brain of the original rabbit succumbing to the J. B. virus. 48 hours to 4 days later there were noted marked suppuration and keratoconjunctivitis. The local effects progressed and became very severe, and on Jan. 22 were attended by general symptoms, consisting of tremor, gnashing, falling to the side of the inoculated eye (left), and fever (106°F.). Death took place on the 9th day.

The brain from this animal was used for intracranial inoculation of another rabbit which developed, within 48 hours, high temperature and paralysis of the extremities. On the 5th day convulsions and death occurred. From this animal Rabbit H was inoculated into the cornea. Within 72 hours a severe kerato-

[8] Levaditi, C., and Harvier, P., *Compt. rend. Soc. biol.*, 1921, lxxxiv, 300.

[9] Blanc, G., and Caminopetros, J., *Compt. rend. Soc. biol.*, 1921, lxxxiv, 629.

conjunctivitis occurred, followed on the 7th day by tremor, head turning, salivation, urine retention, convulsions, and death.

On Sept. 9, 1924, two rabbits were given intracorneal inoculations of J. B. virus taken from a fresh brain which came from the animal succumbing to injection of the 6 months glycerolated specimen of virus. Both rabbits developed severe keratoconjunctivitis and typical symptoms of encephalitis and succumbed on the 8th and 9th days respectively.

These protocols could be considerably increased in number. They suffice, however, to show that the J. B. virus possesses definite and pronounced affinity for the rabbit cornea in which it multiplies, provoking a severe keratoconjunctivitis, whence it regularly invades the brain, setting up a fatal encephalitis. The virus is transmissible in series from cornea to cornea, from cornea to brain, and from brain to cornea.

In the course of a long series of inoculations into the cornea, the fact was noted that glycerolation tends to diminish numerically the viable microorganisms constituting the virus. That is, if the long glycerolated virus is implanted directly upon the cornea, a milder keratoconjunctivitis results, from which recovery may occur, attended by subsequent immunity; while if the same glycerolated specimen is introduced into the brain, a fatal encephalitis results. The cornea inoculated from the brain of such an animal invariably develops severe inflammation which, in turn, leads to encephalitis and death. These facts indicate not a mitigation of virulence through the action of the glycerol but a partial destruction of the organisms within the brain tissue. And in conformity with this effect experiments could be cited in which an occasional specimen conserved in glycerol has lost entirely its power to infect rabbits, even when injected directly into the brain.

Series C. Skin Inoculation.

Just as the so called virus of encephalitis has been shown capable of inducing keratitis in the rabbit, Levaditi, Harvier, and Nicolau[10] have shown it to possess, in common with the virus of febrile herpes, power to excite a vesicular dermatitis in this animal. As the fol-

[10] Levaditi, C., Harvier, P., and Nicolau, S., *Compt. rend. Soc. biol.*, 1921, lxxxv, 287.

lowing examples attest, the J. B. virus acts on the skin of rabbits in an identical manner.

On Jan. 23, 1924, Rabbit I was shaved over the left side of the abdomen and the skin abraded with two scratches 1 inch long. The area was then smeared with fresh brain from a third transfer of J. B. virus. Suppuration and vesiculation, most marked along the scratches, followed on the 6th day. General symptoms appeared and death took place on the 11th day. A second rabbit (J), treated in the same manner, reacted in the same general way. On the 16th day tremor and paralysis of the extremities were notable; and death occurred on the 17th day.

With the brain tissue of Rabbit I, Rabbit K was given a skin inoculation which was followed by vesicular dermatitis, ataxia, urine retention, and death on the 10th day.

J. B. virus attacks the skin surface as it does the corneal tissue, and, as in the case of the latter, tends invariably to pass from the skin into the interior of the nervous system and to excite a fatal encephalitis. The glycerolated J. B. virus acts on the skin in a manner in all essential respects identical with the fresh (brain) virus.

Series D. Blood Inoculation.

The herpes and so called encephalitis viruses are believed to pass by way of the peripheral nerves to the central nervous organs. This view has been especially developed by Goodpasture and Teague[11] and employed by them to explain the head and body turnings. The same general considerations apply to the J. B. virus. Moreover, it has been found that when the herpes and encephalitis strains of virus are injected into the blood encephalitis also follows. An identical effect is produced by the intravenous inoculation of the J. B. virus.

On May 10, 1924, Rabbits L and M received respectively an intravenous injection of 2 and 3 cc. of a suspension of fresh brain tissue taken from a rabbit developing encephalitis 5 days after an intracerebral inoculation of J. B. virus. Both developed general symptoms consisting of fever (106°F.), tremor, gnashing, salivation, muscular excitement, including rhythmical erection of the body, and retention of urine. Death resulted on the 17th day.

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[11] Goodpasture, E. W., and Teague, O., *J. Med. Research*, 1923–24, xliv, 139.

Series E. Nasal Inoculation.

The power to penetrate external surfaces, including the nasal mucous membrane, is possessed by the J. B. virus in common with the other varieties of virus referred to.

On Jan. 23, 1924, there was inserted into the left naris of Rabbit N a cotton plug carrying fresh brain pulp taken from a rabbit succumbing to J. B. virus encephalitis. This animal developed the usual train of general symptoms, including fever, tremor, circling movements (to the left), and urine retention. Death occurred on the 7th day.

Series F. Testicular Inoculation.

The discovery by Noguchi that the testicle is a favorable site of multiplication of vaccine virus led to the employment of the testicle for the cultivation of other kinds of virus. Levaditi'[12] showed that this organ suffices also for the class of viruses we are dealing with and that from the testicle an invasion of the brain took place.

On May 13, 1924, Rabbit O received an intratesticular injection of 1 cc. of a 10 per cent suspension of fresh (brain) J. B. virus. May 23. The organ was swollen and edematous. 0.3 cc. of exudate was aspirated and injected into the testicle of Rabbit P. In addition to the inflammation of the testicles, both animals developed general symptoms, including fever, salivation, tremor, palsy, and convulsions, and died on the 10th and 17th days respectively. The virus was transmitted further from these two animals both by testicular and by intracranial inoculation.

The intratesticular mode of inoculation differs in one respect from the other, perhaps more direct means of inoculation of the virus employed, in that remission of symptoms may be observed. After a first period of fever there is a return to normal, and then after a further interval of several days, a return of the fever, the appearance of general symptoms, and the occurrence of death.

Histology of Virus Encephalitis.

In keeping with the local effects following the direct inoculation of the virus into the eye, skin, and other parts, histological lesions,

[12] Levaditi, C., Ectodermoses neurotropes. Poliomyélite, encéphalite, herpès, Monographies de l'Institut Pasteur, Paris, 1922, 132, 138.

more or less profound, are encountered. These have been described repeatedly for the herpes and encephalitis viruses; they do not differ in respect to the J. B. virus. In this section, therefore, attention will be centered on the microscopical changes met with in the nervous system, the brain chiefly, of the inoculated rabbit. It would naturally be supposed that with the symptoms of inflammation of the brain so severe and striking there would appear correspondingly profound lesions in that organ. As a matter of fact, what is found is a wide disparity of lesions and no close relationship between their intensity and the severity of the symptoms.

The mode of inoculation affects the character of the lesions, which tend to be more marked when the intracranial injection of virus has been made. In this instance several factors cooperate: trauma, positively chemotactic tissue elements, and virus. Usually, therefore, a meningoencephalitis results, in which the polymorphonuclear leucocyte takes a considerable part. The injection of a Berkefeld filtrate instead of a tissue suspension diminishes but does not abolish the local reaction. To determine, therefore, the effect of the virus itself, it is necessary to study the brain of rabbits into which the virus has penetrated from the skin, eye, blood, etc.

It might be expected that the examination of a suitable series of brains from this class of animals would yield quickly a definitive result. Such is, however, by no means the case; at least it has not been the case with our animals. It is true that the local traumatic and other effects present in intracerebrally inoculated rabbits are wanting. But the lesions in general met with are subject to wide fluctuations of situation and severity. This disparity has proved puzzling in the past and has led certain experimenters into error and the making of false inferences.

The difficulty arises, in part, from the nature of the experimental animal—the domestic rabbit—itself. At least this is true of the American laboratory rabbit, and certain evidences point to the same being true for the rabbits employed by continental investigators. The apparently healthy domestic rabbit exhibits not infrequently a variety of encephalitis—inflammation of the brain—in the preinoculation condition, which leads to a degree of confusion in interpreting

the actual virus lesions, often not to be resolved.[13] The circumstance that wide and profound indications of encephalitis are often found in the rabbit in which no recognizable symptoms have appeared, is notable because, when the virus form of encephalitis arises, it is attended by symptoms of great severity and gravity. Hence it would seem that the inflammatory lesions alone cannot be held to account for the symptoms, from which it follows that beside provoking perivascular and interstitial mononuclear cell invasions the virus attacks nerve cells directly, either stimulating them to overfunction, or paralyzing their activity altogether. The clinical symptoms observed in virus encephalitis clearly show these two opposite effects.

With the stocks of rabbits employed by us, it has often not proven possible to tell when one variety (old) and the other variety (new) of lesions began and ended. The old variety of lesions, it has now been shown, is induced in part by a microsporidial parasite, *Encephalitozoon cuniculi* (Levaditi),[14] first observed in 1922 by Wright and Craighead,[15] and rediscovered recently by Levaditi and Nicolau. Since, in the meantime, this parasite has been shown to localize in the kidney, in which it regularly induces inflammation, and since it is only accidentally and incidentally deposited in the brain, where its growth excites a varying degree of cellular reaction, it is of dubious propriety to emphasize in its name the encephalitogenic property.[13] Among the preinoculation brain lesions are many in which no parasites of any kind have been detected; they are regarded as scars or residues, perhaps, of a microsporidial infection and possibly of still other kinds of infection. It is this class of lesions from which the main difficulty arises in determining the effects to be attributed, on the one hand, to the virus and, on the other hand, to preexisting infection.[16]

However, so far as this discussion concerns the manner of action of the J. B. virus on the brain of rabbits, it may be stated that no distinction can be made between the effects of this virus, the herpes

[13] Smith, T., and Florence, L., *J. Exp. Med.*, 1925, xli, 25.

[14] Levaditi, C., Nicolau, S., and Schoen, R., *Compt. rend. Acad.*, 1923, clxxvii, 985.

[15] Wright, J. H., and Craighead, E. M., *J. Exp. Med.*, 1922, xxxvi, 135.

[16] McCartney, J. E., *J. Exp. Med.*, 1924, xxxix, 51.

virus, and the so called encephalitis virus. And this is true, not only as regards the histological changes themselves, but also as respects the minute, chromatic, intracellular bodies described by Lipschütz, Da Fano, and others.[17] There is little probability that these bodies are microorganisms; they are more probably degeneration products. It is of interest to record that Parker[18] failed to find corresponding bodies in the human brain from cases of epidemic encephalitis.

Although the mouse[19] occasionally shows the microsporidial parasite in the brain, the guinea pig and white rat are free from a similar infestation. The three species are, however, subject to virus encephalitis, and respond, on intracerebral inoculation, with profound inflammatory lesions and severe symptoms. Unfortunately these animals do not respond with encephalitis to virus inoculation elsewhere in the body than the brain; hence the traumatic and virus effects are not readily disentangled.

Hitherto, what may be spoken of as the direct action of the virus on the nerve cells of the brain has been considered little, if at all. Attention has been directed especially to the perivascular and other infiltrative lesions. But since, as we have seen, this latter class of lesions may prevail on a large scale without provoking symptoms, it is probable that in virus encephalitis another kind of action, involving more directly the nerve cells themselves, comes into play. Since the symptoms of virus encephalitis provide indications of the parts of the brain especially affected, the minute study of nerve cells in the affected regions is something open to exploitation, and is, indeed, in progress.

[17] Lipschütz, B., *Arch. Dermatol. u. Syph., Orig.*, 1921, cxxxvi, 428. Da Fano, C., *Brit. Med. J.*, 1921, i, 153; 1921, ii, 652; *J. Path. and Bact.*, 1923, xxvi, 85. Levaditi, C., Ectodermoses neurotropes. Poliomyélite, encéphalite, herpès, Monographies de l'Institut Pasteur, Paris, 1922, 164. Goodpasture, E. W., and Teague, O., *Proc. Soc. Exp. Biol. and Med.*, 1922–23, xx, 400. Cowdry, E. V., and Nicholson, F. M., *J. Exp. Med.*, 1923, xxxviii, 695.

[18] Parker, F., Jr., *J. Med. Research*, 1923–24, xliv, 289.

[19] Cowdry, E. V., and Nicholson, F. M., *J. Am. Med. Assn.*, 1924, lxxxii, 545; *J. Exp. Med.*, 1924, xl, 51.

Immunity Reactions.

It is admitted that the virus of herpes and the so called virus of encephalitis produce in the rabbit identical immunity effects. When an animal infected with these viruses recovers it becomes, after the lapse of a few weeks, insusceptible to reinoculation in any manner to either of the two varieties of virus. In process of identification of the J. B. strain of virus suitable tests were carried out to determine the immunological reactions it would display.

J. B. vs. J. B. virus.—Mar. 20, 1923. Rabbits 1 and 2 were inoculated upon the scarified skin and scarified cornea respectively with glycerolated J. B. virus. The former showed no effect; the latter developed keratoconjunctivitis. The inoculations were repeated on Apr. 24 with no effect in either animal. On May 29 both animals received an intracranial injection of 0.1 cc. of a paper filtrate of a 5 per cent suspension of fresh (brain) J. B. virus. Rabbit 1 succumbed typically to virus encephalitis; Rabbit 2 proved immune.

It is obvious that the glycerolated J. B. virus used was too weak to induce infection in Rabbit 1, which therefore remained susceptible, while Rabbit 2, which had recovered from a mild keratoconjunctivitis, was protected against the intracerebral inoculation of the active virus.

Through the kindness of Dr. Levaditi we came into possession of a specimen of his encephalitis virus, variety (*souche*) C. The next protocol records a cross-test with the J. B. and Levaditi strains of virus.

J. B. vs. Levaditi Virus.—Jan. 2, 1923. Rabbits 3 and 4 received skin inoculations of Levaditi virus. Mild inflammation, vesiculation, and desquamation followed in both. On Mar. 29, Rabbit 3 received an intracerebral injection and Rabbit 4 a corneal inoculation of J. B. virus. The control animal developed typical virus encephalitis; neither of the others showed any effects whatever.

J. B. vs. Herpes Virus.—Feb. 26, 1923. Rabbit 5 was given a skin inoculation with herpes virus (strain Stewart). Inflammation with desquamation, followed by recovery, ensued. Apr. 28. Rabbit 6 had left naris plugged with cotton carrying Stewart herpes virus. Mild general symptoms with recovery resulted. On Sept. 18 both rabbits and a control received a corneal inoculation of J. B. virus. The control succumbed characteristically to virus encephalitis, while the other rabbits were unaffected.

The preceding set of tests shows conclusively that the J. B. virus is immunologically indistinguishable from a strain of so called encephalitis virus and a strain of herpes virus. In this respect, as in all other respects, as experimentally determined, the J. B. virus corresponds with the other varieties of encephalitogenic virus.

Action on Other Rodents.

The usual strains of herpes and encephalitis viruses are effective when injected into the brain of the guinea pig, mouse, and rat, and they are ineffective in the pigeon and usually in the monkey. We have injected the J. B. virus into the brain of pigeons, young dogs, and *Macacus rhesus* monkeys without success. The guinea pig, mouse, and rat, when so inoculated, respond with severe and fatal encephalitis. The brain of these animals shows, on microscopical study, marked, acute, widespread inflammation.

Guinea Pigs.—Two animals were given intracerebrally 0.1 cc. of a 10 per cent suspension of J. B. virus (rabbit brain). 4 days later both were tremulous, ataxic, gnashing, and salivating, and one turned in circles to the inoculated side (left). Convulsions set in and death occurred on the 5th and 6th days respectively.

Rats.—Two white rats were injected on the same day and in the same manner. They developed typical symptoms of encephalitis and died on the 4th day.

Mice.—Two white mice received each 10 drops of the same suspension of virus. Both developed the typical symptoms and died on the 3rd day. One mouse showed remarkable rhythmic, rapidly circling movements, followed by convulsions, prostration, and death.

SUMMARY AND CONCLUSIONS.

In this paper is given an account of an inoculable virus disease produced in the rabbit with cerebrospinal fluid taken from a case of vascular and neural syphilis.

The study which yielded the results presented was undertaken in the course of an investigation into the etiology of epidemic or lethargic encephalitis.

Twenty-seven samples of cerebrospinal fluid, derived from cases of epidemic encephalitis, were tested by us upon rabbits without positive result. The one successful instance in which an inoculable disease was produced arose from the injection of one of three speci-

mens of the cerebrospinal fluid taken from the case of syphilis. Following this success, two subsequent injections of the fluid, taken from the same patient, were made unsuccessfully.

Although certain American and European investigators have reported securing a virus from the cerebrospinal fluid of cases of epidemic encephalitis, we have consistently failed in our endeavors to confirm their results. However, we believe that the finding of the J. B. virus may serve to clarify the obscurity and confusion now enveloping the so called virus of encephalitis.

It had previously been shown that no biological differences could be detected between the herpes and the encephalitis strains of virus. The former, as is well known, is readily secured by inoculating rabbits with the contents of herpes vesicles, while the latter has, at best, been obtained with great difficulty.

The J. B. virus agrees biologically with the herpes and encephalitis strains of virus. It is our opinion that the J. B. virus is merely a herpes virus which has gained access to the cerebrospinal fluid and, at the time of inoculation of the rabbits, was present in a concentration sufficing to induce virus encephalitis.

The fact, if fact it is proved to be, that the herpes virus may find its way into the cerebrospinal fluid opens to question all the supposed instances of successful implantation of a virus of epidemic encephalitis upon the rabbit. It is indeed highly probable that, in so far as such a virus has been found at all in the cerebrospinal fluid, it also is a specimen of the herpes virus.

Our studies lead us to suppose that at best it is an infrequent event for the herpes virus to occur in demonstrable form in the cerebrospinal fluid. Perhaps a more delicate means of detection than the rabbit inoculation would serve to reveal the presence oftener. It is known that strains of herpes virus of greater or less intensity of action for rabbits exist. It is, of course, possible that we discover, by present methods, only the highly active strains and those only when chancing to be present in a certain concentration. We inoculated 100 specimens of cerebrospinal fluid and obtained in a single instance the virus infection of the rabbit.

In all respects the J. B. virus agrees in intensity of effect, in mode of attack upon the cornea, skin, and brain, and in immunization re-

sponses, with the true strains of herpes virus and the so called strains of encephalitis virus.

If, as the above statements indicate, all the virus strains of the class considered are examples of the herpes virus, it follows that the etiology of epidemic encephalitis remains entirely unresolved. It is highly improbable that the ubiquitous herpes virus plays the kind of part in human pathology which it has been shown to play in experimental rabbit pathology. While the active strains of that virus possess a strong affinity for the brain structures of the rabbit, the virus has not in the past shown any selective affinity for the brain of man. To ascribe epidemic encephalitis in man to particular and peculiar varieties of the herpes virus is, with our present knowledge, unwarranted.[20]

The wide variations in histological lesions described in the brain of rabbits succumbing to herpes or virus encephalitis raise the question of the essential manner of action of the virus upon the brain tissues. Hitherto it has been the cellular infiltrative lesions which have been emphasized. We have, however, learned that very extensive infiltrations about blood vessels and in the brain substance may exist independently of even mild symptoms of disease.[16] The question is propounded, therefore, whether the herpes virus does not attack nerve cells directly, affecting them quantitatively in such ways as at one time to produce stimulation and at another time paralysis. The manifold symptoms of virus encephalitis in the rabbit are open to this interpretation. In order, however, to base this notion on microscopical findings, a more subtle technique than hitherto widely employed is required. A histological restudy of the subject is being made with this view in mind.

The name virus encephalitis is proposed for the experimental disease produced in rabbits by the inoculation of the herpes and allied viruses.

[20] Levaditi, C., and Nicolau, S., *Compt. rend. Soc. biol.*, 1924, xc, 1372. Levaditi, C., Nicolau, S., and Poincloux, P., *Compt. rend. Soc. biol.*, 1924, xc, 1376.