CONTRIBUTIONS TO THE PATHOLOGY OF EXPERIMENTAL VIRUS ENCEPHALITIS.

IV. RECURRING STRAINS OF HERPES VIRUS.

By SIMON FLEXNER, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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Anyone wishing to inform himself of the present state of our knowledge of the subject of virus encephalitis would do well to read the two comprehensive reviews of Doerr. While these reviews deal chiefly with herpes virus, they touch on the related subject of so-called encephalitis virus as well. In this way, the etiology of epidemic encephalitis is also brought under consideration. While Doerr inclines to the view that epidemic encephalitis in man is a form of herpes virus infection, yet he presents an impartial because quite complete discussion of the many sides of this important topic of controversy.

Our studies, to which those to be recorded in this paper relate, have dealt with the encephalogenic and other properties of the herpes virus, because Flexner and Amoss were led to an investigation of this virus through experiments regarding the etiology of epidemic encephalitis. The conclusions reached were in conflict with those previously announced by Doerr and by Levaditi. The studies on the etiology of encephalitis have been continued and the present papers are intended to record the results of the more recent experiments. These later studies support the view previously put forward, namely that herpes virus encephalitis and epidemic encephalitis are definite pathological affections, etiologically distinct.

4 Levaditi, C., L'herpès et le zona, Paris, 1926.
 Persistent Strains of Herpes Virus. 

The tendency for simple or febrile herpes to recur in certain individuals has often been noted. In these susceptible subjects, relatively trifling causes bring on a herpes of the lips or other parts. This circumstance has led to discussions and experiments concerning herpes virus carriage in normal persons, regarding which beliefs are still divided. What does not seem to have been attempted is the determination of the quality of the herpes virus present at widely separated intervals in the herpes-susceptible individuals. In other words, no one has undertaken to answer the question as to whether one or more strains of herpes virus occur in these persons.

We have studied one habitual herpetic subject from this point of view. The subject is a victim of frequent common colds, with attendant labial herpes. The first examination, carried out 4 years before the one now to be reported, has already been recorded in previous papers. The first H. F. strain of herpes virus was isolated by rabbit inoculation in March, 1922. The vesicular fluid, sterile for ordinary bacteria, was injected subdurally into etherized rabbits and gave rise to violent symptoms of virus encephalitis on the 6th day of incubation, followed by death. The brain of this rabbit provided the material for the series of experiments already described. This strain of H. F. virus was highly potent; of fifteen rabbits receiving eye instillations, either with or without corneal scarification, all developed severe keratoconjunctivitis accompanied by symptoms of virus encephalitis, terminating in death. In four of the fifteen instances, glycerolated virus was inoculated.

The second examination of the subject was made in March, 1926, or after an interval of 4 years. The virus isolated came in this instance also from a labial vesicle, but it was secured through corneal and not from a subdural inoculation. Two rabbits received a salt solution dilution of the clear vesicular fluid, free from ordinary bacteria, in the cocainized, scarified right eye. Inflammation quickly ensued; the
eyes were closed and vesicles appeared along the incisions within 24 hours. By the 4th or 5th day, the temperature reached 41.7°C., and was accompanied by circling movements to the right. As the local symptoms subsided, these movements ceased. Recovery of the animals followed and was complete. The virus thus obtained was used in the subsequent experiments and designated H.F. II.

Corneal Passages.

Cornea to cornea inoculation was carried out in a series of eight rabbits. The inoculum was taken from the inflamed eyes on the 4th or 5th day of the keratoconjunctivitis. The course of the infection and inflammation was severe and uniform in the first four rabbits of the series, and moderate in the second four of the series. Circling and high temperatures occurred only, or markedly only, in the first four passages. All the inoculated animals recovered completely and, as will appear, were tested later for immunity. In one instance only did the symptoms point to more profound involvement of the brain. The protocol of this experiment follows.

Rabbit.—Apr. 9, 1926. Right eye coxainized and cornea scarified with cataract knife dipped in salt solution containing scrapings from cornea of rabbit of 2nd passage. Apr. 11. Eye closed; much purulent secretion; vesicles along incisions. Apr. 14. Temperature 41.2°C.; head turns to the right side. Apr. 16. Temperature 40.7°C.; animal circles and falls to the right side. Eyelids open, and inflammation subsiding. Apr. 18. Temperature 39.8°C. Animal still circles and falls. Eye clearer. Apr. 20. Circling is less; animal does not fall. The inflammation of the cornea has subsided and the eye is clear. From this date on improvement continued until recovery was complete.

Comment on this series of experiments will be limited for the present to a few obvious points. It appears, and we had previously observed the fact, that contrary to Doerr's view,7 fever does attend the eye inflammations, even when there is but moderate brain involvement. But fever and intensity of the ocular inflammation are associated. In order to follow the temperature changes reliably, daily measurements of the rectal temperatures are to be made in all inoculated rabbits. The protocol given shows that recovery may occur from

severe secondary herpetic cerebral inflammation, and the successive inoculations in series indicate that cornea to cornea passages of the H.F. II virus lead to moderation, not to intensification, of its pathogenic action.

Brain and Cord Passages.

In order to compare the mode of action, or virulence, of one strain of herpes virus with another, it is desirable to submit the strains tested to essentially the same treatment. We have observed that a strong, but not a weak, strain of herpes virus when obtained from the brain of a succumbing rabbit is more likely to produce fatal encephalitis on corneal inoculation than the same virus derived from mere corneal passages. Why this difference should arise, is not known; and whether it is merely a quantitative effect, or has to do with a qualitative change in the strain, has not been determined. It will be recalled that the original H.F. I strain of virus, obtained by subdural inoculations of the rabbit, when subsequently instilled into the eyes produced keratoconjunctivitis followed by fatal virus encephalitis in all of the fifteen rabbits inoculated.

The H.F. II virus injected subdurally in rabbits regularly induces stormy symptoms of virus encephalitis and brings about death in from 4 to 6 days. Characteristic histological lesions and inclusion bodies are found on microscopic examination of the brain tissue. But when the infected brain is used for corneal inoculation, a difference appears in that, contrary to the fatal effects of H.F. I virus under these circumstances, recovery ensues from the inflammation induced by H.F. II virus.

Brain to Cornea Passage.—Three protocols are given, in order to show that while the injection of H.F. II vesicular fluid directly into the brain induces fatal virus encephalitis, the brain to cornea passage of the virus which induces severe keratoconjunctivitis sets up only transient brain symptoms.

Rabbit I.—Mar. 30, 1926. 0.1 cc. of salt solution suspension of herpes vesicular fluid injected intracerebrally. Mar. 31. Temperature 41.8°C.; urine reten-

All operations were performed under ether anesthesia.
tion. Apr. 1. Temperature 41.4°C.; tremor. Apr. 3. Tremor increased. Apr. 4. Salivation; gnashing; convulsions; prostration; death.


This experiment may be taken as indicating a difference in quality in two herpes virus strains derived from a supposed virus carrier, that is, a subject of recurrent labial herpes, taken at an interval of 4 years. The next experiment, in which indirect brain, spinal cord, and corneal passages were made, brings out the same general fact, although it shows that the H.F. II virus is capable at times, in passing from the cornea to the brain in sufficient concentration, to set up fatal virus encephalitis.

Brain to Brain to Cornea Passages.—Although the direct passage of the H.F. II virus from brain to cornea failed to produce keratoconjunctivitis followed by encephalitis and death, yet if the virus is passed successively through the brain, it is capable of setting up fatal encephalitis upon corneal inoculation. For example, after the fourth cerebral passage, in which death occurred respectively on the 4th, 6th, 4th, and 4th days, the symptoms having in all instances been characteristic of virus encephalitis, a rabbit was inoculated into the cocainized right eye, the cornea of which had been scarified. The protocol of the test follows.


Indirect Brain, Cord, and Corneal Passage.—The protocols which follow relate to skin to spinal cord, cord and brain to cornea, and cornea to cornea passages.


With the results of the foregoing experiments before us, we are prepared to place the H.F. II virus among the strains of herpes virus which are dermatotropic, rather than neurotropic in property. This strain of virus is of medium pathogenicity. It tends to produce local inflammation of the cornea and conjunctivae, and to invade the brain to a slight degree only; and yet, when implanted on the scarified skin and especially when passed successively through the brain, it acquires neurotropic properties of such invasive power as to suffice to induce fatal encephalitis on corneal implantation. Skin inoculation of the virus gives rise to myelitis, with diffusion of the virus throughout the central nervous organs. Although the test is not conclusive on the point, yet the concentration of the virus in the lumbar cord appeared to be greater than in the pons under these circumstances.

A comparison of the activities of H.F. I virus obtained in 1922 with H.F. II virus secured in 1926 from a subject of recurrent labial herpes shows quite conclusively that the pathogenic activity as measured in the rabbit differs. H.F. I virus is predominantly neurotropic, while H.F. II virus is predominantly dermatotropic. Perhaps the isola-
tion and study of a third strain of the virus may throw light on the nature of the variation.

II.

In addition to the tests carried out with the H.F. II strain of herpes virus, two other specimens of virus from subjects of recurrent herpes were studied. One, called T. strain, was derived from a labial vesicle of a female adult suffering from common cold, and the other, called F. strain, from a child of 4 years who passed at intervals of a few months through attacks of slight fever attended by obscure, nervous disturbances slight in degree, and labial herpes. As will appear, the T. and F. viruses stand at extreme scales of pathogenic action, the former being highly and the latter hardly at all neurotropic for the rabbit.

Corneal Passages, T. Strain.

Three passages from cornea to cornea were carried out with the T. strain, as shown in the following protocols. The course of the pathological process was essentially the same in all, consisting of severe keratoconjunctivitis, encephalitis, and death.


Brain to Cornea Passages, T. Strain.

A parallel series of inoculations to the former was carried out with the T. virus, in which the original, diluted vesicular contents were inoculated intracerebrally into Rabbit I, and the brain virus thus secured was used to inoculate the cornea of Rabbit II. Rabbit I developed virus encephalitis and succumbed on the 9th day; Rabbit II first developed keratoconjunctivitis, then showed signs of encephalitis on the 6th day, and succumbed to the latter on the 9th day. A third rabbit was inoculated in the cocainized, scarified eye from the brain virus of Rabbit II of the cornea to cornea series. The succession of events was typical: gradual development of keratoconjunctivitis, involvement of the brain, with symptoms of tremor, circling, falling, salivation, and death on the 12th day.

The strain of F. virus was studied in detail, as will appear from the summary of experiments to follow, in the course of which a certain number of disputed points were dealt with and perhaps elucidated.

Cornea to Cornea Passages, F. Strain.

A salt solution suspension of the contents of the labial herpetic vesicle was inoculated by the usual method, that is, by dipping the cataract knife into the suspension and then scarifying the cocainized right cornea of two rabbits with it. Keratoconjunctivitis developed promptly in both animals, and ran the usual course attended by recovery. No symptoms of brain involvement appeared; the highest temperature recorded was 40.7°C., which was reached in both animals.

At the height of the local inflammation (3rd day), exudate was used to inoculate the next two rabbits, constituting the second passage of the series. The events resembled those of the first passage, except that in both animals circling to the right appeared on the 3rd and 4th days respectively. About the 9th day after inoculation the circling abated or ceased, after which recovery was uninterrupted.

In all, ten cornea to cornea passages were made. In no instance was a fatal virus encephalitis induced, and in no case did other signs of brain involvement than that of circling appear. When both eyes were inoculated no circling took place, although the inflammation
produced was typical and severe. Temperatures as high as 41.1°C. arose independently of cerebral symptoms. In the later passages, seventh to tenth, the local reaction was less severe, the temperature lower, and recovery more rapid. Although one eye only was inoculated, no circling occurred.

The conclusions we have drawn from this series of inoculations are:
1. The typical herpes virus keratoconjunctivitis in the rabbit may be attended by fever.
2. Circling to the inoculated side may appear without any other signs of cerebral invasion of the virus.
3. Circling does not appear when the two eyes are inoculated.
4. The virus diminishes in activity in successive corneal passages, as indicated by feebler inflammatory reaction, shorter duration of the inflammation, and absence of circling movements.

**Brain and Spinal Cord to Cornea.**

The F. virus, as stated, is a weak strain. Tests were carried out in order to determine whether intracerebral injections induced virus encephalitis. It was found as a rule that fatal encephalitis ensued when eye exudate or brain virus was thus injected, but that recovery might occur after intracerebral inoculation.

**Rabbit.**—Mar. 15, 1926. Animal received intracerebral inoculation of fresh rabbit brain virus. Mar. 19. Temperature 41.1°C.; tremor; retention of urine. These symptoms persisted for 3 days, then abated, and recovery followed.

Experiments were also made in order to determine whether the F. brain virus introduced into the cornea induced both keratoconjunctivitis and frank encephalitis. As a matter of fact, the inflammation of the eye induced is severe, while the effect on the brain is mild only, and if permitted, recedes leaving the rabbit to all intents and purposes normal. And yet if the affected rabbit is killed at the right moment, the virus is detectable by inoculation in the brain tissue.

**Rabbits.**—Two rabbits, A and B, were given eye inoculations with the fresh F. rabbit brain virus on Sept. 8, 1927. In both, severe keratoconjunctivitis followed. Rabbit A showed fever (40.6°C.) and the head turned to the inoculated (right) side on Sept. 13. On Sept. 15, circling was noted. Killed on this date,
and two rabbits, C and D, were given intracerebral injections of suspension made from the pons.

Rabbit B developed similar symptoms to Rabbit A and was permitted to recover.

Rabbits C and D were given cerebral inoculations with the pons of Rabbit A on Sept. 16. Typical virus encephalitis with characteristic symptoms arose on the 5th and 6th days, and death resulted in both instances on the 9th day.

Since the F. virus is a weak strain, the preceding experiment does not always succeed. In one example, two rabbits were given eye inoculations with material taken from an eye on the 4th day of the keratoconjunctivitis. Severe inflammation attended with circling developed. On the 6th day of the circling, one of the rabbits was killed and the brain used to make a corneal inoculation in one animal, and cerebral inoculation in two animals. No effects followed. At this later period in the encephalitic process, the virus was no longer demonstrable by the inoculation test.

The conclusion to be drawn from these tests is that the F. virus is only weakly neurotropic, and while capable of penetrating to the brain of rabbits, tends to be suppressed there. In this respect, the rabbit treats a weak virus in a manner resembling the way in which the guinea pig also deals with a weak virus. When the quantity of a weak strain reaching the brain of the rabbit is not excessive, it can be destroyed; and yet, this same weak strain when injected in quantity, is capable of producing fatal encephalitis. As is to be expected, a stronger virus, such as the H.F. II strain, is even more readily detected in the pons after eye infection.

**Skin to Spinal Cord.**

Although the F. virus does not pass from the cornea to the brain in sufficient concentration to produce fatal virus encephalitis, yet it is capable of passing from the skin to the spinal cord in a way to induce paralysis and probably death.

*Rabbit.*—Mar. 9, 1926. Right side of body shaved, scratched, and covered with 30 per cent suspension of rabbit brain virus taken 5th day after inoculation. Mar. 12. Mild dermatitis. Mar. 15. Dermatitis and formation of vesicles. Temperature 40.9°C. Mar. 17. Curvature of spine; hind legs paralyzed; reten-

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tion of urine. Mar. 18. Temperature 40.8°C.; moves fore legs; almost prostrate; etherized.


Immunity.

The experiments with H.F. II virus yielded a number of rabbits in which recovery took place from the corneal inflammation. The animals were subjected to immunity tests in a twofold manner, namely by way of corneal and of intracerebral inoculation. It developed that while the previously uninoculated cornea proved to be but partially protected from infection and inflammation, the brain test showed complete immunity of that more sensitive organ, a result in conformity with observations made by Rose and Walthard. The protocols will be given in pairs, in order to bring out this interesting fact. The brief interval elapsing between the two protection tests is, according to usual measures, insufficient to produce general immunity. As the reinoculations in each set were made on the same day, single control rabbits sufficed for each. Thus it can be stated in advance that the control for the corneal tests developed severe keratoconjunctivitis followed by symptoms of severe virus encephalitis, while the control for the intracerebral tests succumbed to typical virus encephalitis on the 6th day.


Test II. Rabbit.—In this instance the interval between the two corneal inoculations was 2½ months. July 19, 1926. Left, coccinized, scarified cornea inocu-


In order to complete the account, it remains to mention the simultaneous tests by intracerebral inoculation of two rabbits which had received corneal inoculations 3½ to 4½ months earlier respectively. The 3½ month animal succumbed to virus encephalitis; the other remained free of symptoms.

Besides the H.F. II animals tested for immunity, five rabbits which had been given corneal inoculations of the F. virus 5 or 6 months before were, along with a control, reinoculated in the left (unused) eye. The point of interest is that while all six rabbits developed keratoconjunctivitis from which they recovered, only the control animal showed the circling symptom.

SUMMARY.

In this paper, three strains of the herpes virus have been dealt with. The H.F. II strain was obtained from the subject H.F. 4 years after the H.F. I strain was secured. H.F. is a victim of recurrent herpes. If the subject is also a chronic carrier of the herpes virus, then it is not one, but two or more strains which are persistently carried. The H.F. II strain is of mitigated pathogenic action for the rabbit, as compared with the H.F. I strain; it is to be classed as dermatotropic rather than neurotropic. And yet, in the subject there was no indication that the attack of herpes provoked was different from the other attacks associated with the H.F. I virus.

The other two herpes strains derive their interest from the fact that they came also from persons who suffer from repeated attacks of labial herpes. One strain proved highly neurotropic, resembling in this respect the H.F. I strain; the other was hardly neurotropic at all, but was none the less definitely dermatotropic. It may be possible
at a later date to secure other samples of virus from these individuals for comparison. The dermatotropic F. strain penetrates to the central nervous system far more readily and certainly from the skin than from corneal surfaces.

The recovered inoculated rabbits showed only relative protection to reinoculation of the herpes virus. A notable difference appeared in the degree of protection acquired, on the one hand by the cornea and on the other by the brain. While the one was partial, the other was complete. The complete resistance of the brain was shown (a) by the complete failure of the intracerebral inoculation, and (b) by the absence of circling movements following corneal inoculation.

CONCLUSIONS.

Subjects of recurrent labial herpes may yield more than one strain of the herpes virus.

While the H.F. I strain is notably neurotropic, the H.F. II strain, obtained 4 years later, is slightly neurotropic and strongly dermatotropic for rabbits.

The neurotropic property of the H.F. II virus is somewhat increased by brain passage.

Dermal inoculation of the H.F. II strain leads to myelitis, with extension of the virus to the brain. The concentration of the virus in the lumbar cord seems greater than in the pons.

The T. specimen of the herpes virus is apparently of maximal neurotropic potency for rabbits.

The F. specimen of the herpes virus is of low neurotropic and moderate dermatotropic activity. Passage from eye to eye tends to diminish the effect of the virus. When the F. strain is inoculated into one eye, circling occurs; when into both eyes, circling does not occur. None of the corneally inoculated F. rabbits succumbed to virus encephalitis. And yet, the F. virus exists in the brain of the corneally inoculated rabbits and can be detected there, by cerebral inoculation, on the 1st or 2nd day, but not on the 6th day of the circling. When the F. virus does not reach the brain in excessive amounts, it is suppressed there; when injected in large quantity, it induces fatal encephalitis.
The rabbit brain possesses the power of destroying weak strains of the herpes virus in a manner not dissimilar to that possessed by the guinea pig brain.

Immunity tests showed that in rabbits previously inoculated into the cornea, the opposite cornea is only partially, while the brain is wholly, protected against reinoculation effects. The partially protected rabbits developed on corneal reinoculation local lesions, but unlike the control animal, did not show circling movements.