TRANSMISSION OF THE VIRUS OF HERPES FEBRILIS ALONG NERVES IN EXPERIMENTALLY INFECTED RABBITS *

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A strain of herpetic virus (virus M), which we obtained in February, 1923, from a typical vesicle of herpes febrilis on the lip of a patient with lobar pneumonia, when inoculated upon the scarified cornea of a rabbit, produced within forty-eight hours a purulent kerato-conjunctivitis followed by herpetic encephalitis from which the animal died on the nineteenth day. This virus has been passed from rabbit's eye to rabbit's eye every two or three days for six months and has without fail produced herpetic encephalitis in adult rabbits.

This manifestly strong affinity for nervous tissue has lent itself usefully to a study of the way in which the virus reaches the central nervous system, and of the lesions there produced.

Doerr and Vöchting first described the peculiar nervous symptoms which follow inoculation of the rabbit's cornea with certain strains of virus of herpes febrilis, the most prominent being a continual turning and finally a twisting of the head to the inoculated side. They correctly attributed this symptom to herpetic encephalitis, the brain of such an animal having been shown to contain the virus, and to present acute lesions, histologically attributable to herpetic infection. They believed a generalized infection through the blood stream followed the keratitis with a localization of virus in the brain. This seemed the more credible from their success in producing encephalitis by intravenous injection of virus from an infected eye.

Levaditi confirmed these authors' observation that encephalitis followed inoculation of herpetic virus into the rabbit's cornea, and noted the turning of the head to the inoculated

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side. He demonstrated virus in the brain of such rabbits, and described inflammatory lesions at the base of the brain. He was not successful, however, in producing an encephalitis by intravenous injections of virus from the eye, and explained the encephalitis as being due to a passage of virus directly from an inoculated cornea along the optic nerve to brain. Zdansky also holds the same opinion regarding transmission of virus along optic nerve, claiming that he had observed transmission of the infection through retina and optic nerve to the Regio subthalamica in histological preparations of the brain of a rabbit inoculated upon the cornea.

The symptom of turning the head to the inoculated side is constant in rabbits developing encephalitis following herpetic infection of a cornea. This fact in itself would make a unilateral lesion of the nervous system seem most probable and really excludes the possibility of its production solely by infection of the brain by way of the blood stream. Yet in the now numerous studies of this experimental disease, we find no mention of a unilateral lesion of the central nervous system in encephalitis following inoculation of the cornea and no suggestion that it occurs. On the contrary, Levaditi describes an elective zone at the base of the brain which is attacked by the virus, as he seems to believe, almost exclusively.

Previous work has thus demonstrated the essential fact that herpetic virus may be transmitted from an infected cornea to the brain, the two routes suggested as possible modes of transit being the blood stream and the optic nerve.

Our experiments with a strain of virus from human herpes febrilis have conclusively proved that this virus may be readily transmitted from a peripheral focus of infection directly to the central nervous system along sensory, motor, or sympathetic nerve fibers depending on the locus of infection, and that such transmission has invariably occurred following inoculation of the adult rabbit's cornea with our strain over a long period of frequent inoculations from cornea to cornea without a single passage through the brain.

The latter fact is of importance in comparing our experience with that of previous workers, since the virulent "neurotropic"

property of the virus we used may be essential to the results herein recorded and the conclusions drawn.

Since previous workers have utilized the rabbit's cornea as a means of propagating the virus experimentally, an index of virulence and "neurotropic" property of various strains may be gained by a comparison of the behavior of the infection in this situation.

While probably any strain of virus from lesions of human herpes febrilis would produce a purulent kerato-conjunctivitis when inoculated upon the scarified cornea of a rabbit, there is extreme variation in their ability to produce a fatal encephalitis following such inoculation. In the experience of Levaditi with several strains, the variations in this respect were so pronounced that he adjudges the exceedingly neurotropic character of a virus obtained from the brain of a case of human epidemic encephalitis the essential property distinguishing it from virus obtained from human lesions of herpes febrilis. He states that the viruses are the same, barring this one feature, and a correspondingly greater affinity of herpetic virus for epidermis. We have had considerable experience with two strains of virus from human herpes febrilis propagated through the cornea of rabbits for several months with passages at two or three day intervals, and they are perfectly opposed with respect to their ability to produce encephalitis following corneal inoculation. The first, strain F, has never caused an encephalitis subsequent to corneal inoculation; the second, now propagated for six months, has never failed to produce encephalitis in adult rabbits, and has little affinity for normal rabbit's epidermis.

It was with our highly "neurotropic" virus that the following experiments were executed. By the use of it we have been able to prove conclusively that, following inoculation upon the cornea, the virus is transmitted along sensory fibers of the fifth cranial nerve to the pons, where a local unilateral acute inflammatory lesion histologically characteristic of herpes is produced in the proximal end of this nerve and within the pons and medulla in the region corresponding to the distribution of its descending or sensory fibers. The local, circumscribed and

unilateral character of the cerebral lesion has been graphically demonstrated by means of vitally staining the animal with trypan blue and subsequently clearing the brain by the method of Spalteholz, when the local lesion is stained a deep blue in contrast to unstained brain tissue elsewhere. The details of this procedure will be described in a separate paper.

The directness with which the virus progresses to brain from a local site of infection, and the equal facility of its transmission along motor and sensory fibers, has been demonstrated by multiple injections of the virus into the right masseter muscle with the subsequent production of an acute herpetic lesion occupying the region of the corresponding motor nucleus of the fifth cranial nerve. We have shown that when the virus is inoculated into the vitreous humor, a herpetic retinitis is produced and herpetic lesions may be traced to midbrain along the corresponding optic nerve, and through the optic tracts to midbrain; inoculated upon tracheal mucosa on the left side, it follows the corresponding vagus producing a local herpetic lesion involving the site of termination of this nerve on the corresponding side; from the skin it produces a dorsal myelitis on the same side at the entrance of sensory root fibers; virus from a lesion in striated muscle proceeds to cord or brain by way of motor nerve fibers; when inoculated into the liver, spleen, adrenal, peritoneum, ovary, and nerve, it also produces a local infection and a myelitis involving the spinal cord on the level of distribution of nerve fibers to the infected tissue, passing readily along sympathetic fibers, as well as those of motor and sensory nerves. A subcutaneous or subconjunctival inoculation causes infection neither locally nor in central nervous system, nor does it confer immunity to subsequent corneal inoculation. Inoculations into the salivary gland and kidney have given no evidences of infection.

In a previous paper we have shown that the presence of intranuclear bodies of Lipschütz is a reliable histological criterion by which to establish the specificity of a lesion produced by virus of herpes febrilis, and in these experiments we have used this as a means of identifying herpetic lesions at the site of inoculation and in the nervous system, and in certain instances

where the bodies could not be demonstrated as in the sciatic nerve, virus has been disclosed in lesions by direct inoculation upon the cornea or into the brain.

Our experiments not only prove conclusively the passage of herpetic virus along nerves, and more specifically along certain groups of fibers in a single nerve, but they suggest very impressively that the virus passes by way of axis cylinders from the periphery to the central nervous system. Evidence as it relates to this problem will be presented in the discussion.

Individual experiments illustrating the behavior of herpetic infection, following inoculations in rabbits in various localities, are presented below.

Endemic Spontaneous Encephalitis of Rabbits. In investigating experimental encephalitis in rabbits histologically or in determining the presence of a given virus by inoculation into rabbit's brain, it is necessary to recognize and take due account of a widely distributed, highly contagious, spontaneous disease, lesions of which may be present in the brain and cord of a large proportion of these animals gathered from various sources and used for experimental purposes. The presence, in the brain, of lesions of this disease was noted by Bull in 1917, described more in detail by Oliver in 1922, and recently studied by Twort and Archer, who recognize a generalized infection affecting, in certain rabbits, more especially the kidneys. The lesions in the brain are focal in character, miliary in size, irregularly distributed, usually chronic and mild in adult animals, and causing few or no recognizable symptoms. These miliary lesions consist of a compact accumulation of mononuclear cells with pale irregular nuclei resembling the epithelioid cells of a tubercle; or in apparently older lesions they are large, polygonal, and with granular cytoplasm. There may be in the center the remains of necrotic cells. The lesion is nearly, if not always, intimately associated with a small blood vessel to the side of which it is situated. This blood vessel and others are usually accompanied by a mantle of small mononuclear cells. No polymorphonuclear leucocytes have been observed to participate in the inflammatory reaction. When this disease is present in the brain there is always an excess of mononuclear leuco-

cytes in the meninges, particularly at the base of the brain. These are large and small cells of the lymphocytic series, and with them plasma cells are frequently seen. We have often noted the presence of such cells in the meninges where sections of the brain have shown no focal lesion; yet they probably indicate the presence of the disease in the brain and, as focal lesions are not infrequently few in number, a larger percentage of infection as determined by the presence of miliary cerebral foci would have been found, we feel, had a greater number of sections been examined from each brain. By examination of sections from one to ten areas including the entire brain in cross section from olfactory lobes to cervical cord, we have found typical lesions of this spontaneous encephalitis in 25 per cent of cases in a series of thirty adult rabbits.

The presence of this spontaneous disease among the animals used in our experiments has been recognized from the beginning, and we have regarded no lesion in the brain as proven herpetic unless it were acute and contained typical intranuclear bodies which are characteristic of herpetic lesions when they occur.

There is no difficulty in differentiating chronic lesions of the spontaneous disease in the brain from acute herpetic lesions by the character of the exudate, but as we are not at the present time fully acquainted with the acute manifestations of the rabbit's disease, we have relied entirely in our histological studies on the presence of intranuclear bodies of Lipschütz to confirm the diagnosis of herpes.

Material used and Methods of Inoculation. The virus used in the following experiments was obtained from herpetic keratitis by inoculations upon the cornea of rabbits. Twenty-four hours after inoculation the eye shows little change grossly. There is usually no pus, little congestion, and a clear cornea. On close inspection clear vesicles can be seen, and in sections there are abundant intranuclear bodies of Lipschütz. The surface and secretions are relatively free of bacteria. For inoculations internally as into adrenal and ovary, the twenty-four-hour material was used and obtained by anaesthetizing the animal, lightly scraping the corneal surface with a needle and washing

with a small quantity (½ to 1 c.c.) of saline, finally drawing the washings into the syringe. Fractions of this amount were used in most of the injections. Inoculations upon surfaces, as on conjunctiva, skin and trachea, were made by applying a drop of pus which had accumulated in the conjunctival sac forty-eight hours after inoculation. This material is thick and can easily be rubbed without diluting directly into superficial scarifications.

Experiments

The following experiments are presented in three groups demonstrating passage of herpetic virus (1) along sensory nerves, (2) along motor nerves, (3) along sympathetic nerves.

1. Transmission Along Sensory Nerves

Experiment I. *Inoculated upon right cornea, virus of herpes febrilis passes along sensory fibers of the right fifth cranial nerve, producing a local unilateral lesion in the pons and medulla on the right side corresponding to the distribution of sensory fibers of this nerve.*

Inoculation upon the scarified cornea has been performed in a large series of rabbits in the passage of virus from eye to eye every two or three days during the past six months, and with very uniform results. The animal is anaesthetized with ether, the cornea exposed, usually by evulsion of the eyeball by pressing the handle of a scalpel beneath the lower lid, and scarified by making several rather deep incisions crosswise so that the entire corneal area is traversed. A large drop of pus from an eye inoculated forty-eight hours previously is then removed on a knife-blade and rubbed and patted over the roughened cornea so as to force some of the pus into the incisions. At the end of twenty-four hours the inoculated eye shows little change on casual observation. The conjunctiva is slightly congested and the lids moist. There is little or no pus. The cornea is transparent, but by reflected light one may detect minute dewdrop elevations between the healing lines of scarification, and similar elevations at the ends and to the sides of the incisions. These are clear vesicles, and on histological examination epithelial

cells in and about such areas are found to contain intranuclear bodies characteristic of the infection. At the end of forty-eight hours the lids are stuck together and the conjunctival sac filled with a large quantity of thick white pus. The conjunctiva is deeply congested and the cornea cloudy, especially along the scarified lines, which are distinctly gray and depressed by ulceration.

Usually on the fourth or fifth day (occasionally on the third), as the rabbit sits quietly in his cage, it will be noted that its head gradually turns toward the inoculated side and is quickly drawn back again to its correct position. This movement is slight at first and may be repeated several times a minute. The appetite is good and the animal otherwise alert and active. The turning movement, always to the inoculated side, increases markedly in extent and duration in the succeeding twenty-four to forty-eight hours, until the head is held rigidly twisted to the inoculated side and a loss of equilibrium, with a tendency to turn the entire body and to fall to this side, becomes evident. There is a rise in temperature to 105°–106° F., the animal is restless, and he attempts to brace himself against the cage with the inoculated side applied to the wall and the opposite extremities outstretched in an attitude of propping or pushing. During the next day or two, equilibrium is completely lost and he lies in the cage on the inoculated side apparently unconscious but with frequent movement of extremities. There is no apparent paralysis. Sometimes in this stage there is abundant salivation. Death occurs usually in seven or eight days after inoculation. We have seen no recovery in adult rabbits inoculated on the cornea with strain M virus, with which all these experiments were performed.

At autopsy no gross lesion may be evident, or small hemorrhages may be found in the pons and medulla, always on the inoculated side and along the distribution of sensory fibers of the fifth cranial nerve from its entrance in the pons to the caudal extremity of the medulla. This lesion is always unilateral and invariably has the above distribution. Its extent and situation with respect to the fifth nerve has been clearly demonstrated in gross preparations by the method of staining the animal

acutely with trypan blue, after the first symptom of turning, and subsequent fixation and clearing according to the process of Spalteholz. In such preparations an intense blue staining begins at the entrance of the fifth cranial nerve and extends beneath the corpus trapezoides, then superficially along the side of the medulla where it broadens in the mid-portion, finally tapering toward the cervical cord. From entrance in the pons the line gradually curves dorsally and may extend along a dorso-lateral line as far as the first cervical nerve. In these grossly stained preparations there is no other stained focal area in the brain. In animals permitted to live until later stages of the disease, there may be a diffuse staining of the ventral surface of the telencephalon, including Ammon's horn, confined to the inoculated side and a bluish tint about the infundibulum. Microscopic examination has shown that these areas may be secondarily infected, as will be described later.

The initial, and in earlier stages the only, gross lesion of the brain following inoculation upon the right cornea, then, is situated in the intra-dural portion of the fifth cranial nerve in its portio major, extending after entrance into the pons, beneath the corpus trapezoides and spreading out superficially along the latero-dorsal surface of the medulla and tapering to an end toward the cervical cord. This in general is the distribution of the descending root of the fifth cranial nerve, in which is distributed sensory fibers through the Gasserian ganglion from the inoculated cornea and conjunctiva. Microscopic sections of extra-dural portions of the sensory division of this nerve have shown no lesion which can with certainty be attributed to herpes. There may be small accumulations of mononuclear cells between medullated fibers, though similar foci may be found also in portions from the uninoculated side. Similarly, in sections through the Gasserian ganglion one usually finds foci of mononuclear leucocytes alone or surrounding a degenerating ganglion cell. It has appeared that such areas are more numerous on the inoculated side, but since similar cellular accumulations are found also in the ganglion from the unaffected side, little weight can be attached to this impression. Changes in ganglion cells themselves are certainly more marked on the in-

oculated side, though not strictly confined to this side. They consist in shrinking of the nucleus and of the cell as a whole; a more acidophilic staining reaction and a more homogeneous appearance to both nucleus and cytoplasm, the latter losing to a great extent its tigroid mottling. These cells do not appear to be undergoing necrosis. No intranuclear bodies, the distinctive lesion of herpes, have been found either in the ganglion or extra-dural portions of the nerve. Whether the change observed is brought about by peripheral influences or is the effect of the more central lesion within the dura has not been determined. From sections of the extra-dural portions of the fifth cranial nerve, we cannot say there is any alteration indicating the passage of herpetic virus along its fibers from the cornea. There is, however, a very different appearance beginning quite sharply on entrance of the nerve through the dura. The intra-dural portion of cerebral and spinal nerves has a different structure from the extra-dural portion, in that the sheath of Schwann is wanting in the former, and neuroglial cells are present between the fibers of the latter. It is in the nuclei of the neuroglial cells that the specific lesion of herpes is found, that is, the acidophilic bodies of Lipschütz, which we have described as occurring in various lesions of rabbits, produced by inoculation or injection of the virus of herpes febrilis. The earlier the lesion the more numerous are these intranuclear bodies; and it is to be observed in herpetic lesions of the central nervous system, as in other situations like the cornea, that they precede the necrotizing and inflammatory lesions, and are less numerous in more advanced stages, none being found during repair of central nervous lesions. In the case of the lesion in question in the fifth nerve, pons, and medulla, however, the animals die so promptly that both intranuclear bodies and the other changes usually occur together. Beginning sharply with the intra-dural portion of the fifth nerve there is found necrosis of cells, partly neuroglial cells and partly polymorphonuclear leucocytes, appearing as pyknotic nuclear fragments; abundant hemorrhage both diffuse and about fairly large blood vessels; fibrin in dense masses about blood vessels and in a delicate meshwork in the surrounding lepto-meninx. The cellular exudate consists of

both polymorphonuclear and large mononuclear leucocytes. The former are aggregated into small groups in edematous areas, where nerve fibers are ruptured and separated. Many of them are disintegrating. The large mononuclears with irregular nuclei are scattered throughout the lesion, and in later stages are filled with fat vacuoles. Such a lesion, varying in intensity, is continuous throughout the area indicated by staining in the gross preparation. Microscopically as well as grossly the lesion is strictly unilateral and peripheral. In early stages it is less violent and passes through the medulla situated in the ventral limb of the descending root of the fifth nerve. In more advanced cases it extends to the periphery, after passing beneath the corpus trapezoides, involving the roots of the seventh and eighth cranial nerves, and extending along the medulla, destroying a good part of the tractus spino-cerebralis dorsalis. Destruction of ganglion cells is as a rule not observed, the virus apparently attacking glial cells and nerve fibers along the bands of nerves constituting and external to the fifth nerve root. As it involves the caudal end of the medulla laterally, roots of the spinal accessory nerve are also affected, and the lesion was once observed to extend into the dorsal root of the first cervical nerve.

Over this entire area and extending both ventrally and dorsally to and often beyond the midline, is an acute meningitis with a hemorrhagic, fibrinous, serous, and cellular exudate. The cells present are both polymorphonuclear and mononuclear varieties.

The symptom which is the constant and striking clinical manifestation of this lesion during life is turning of the head to the affected side with later loss of equilibrium attended by tendency to fall toward this side. Doerr first associated this behavior with an encephalitis, and his observations were confirmed by Levaditi and others, but no attempt was made to associate it with any particular lesion found. Owing to the presence of the lesion described, we would attribute the loss of equilibrium to direct injury to the cochlear division of the eighth cranial nerve, for there is no apparent paralysis, and the rotat-

ing movement of the head is a coördinated motion. Injury to the roots of the spinal accessory nerve may also play a part in holding the head rigidly to the inoculated side.

The infection in the brain does not long remain confined to the lesion produced in the pons and medulla at the site of entrance through the fifth cranial nerve, but if the animal lives a few days longer it spreads on the same side to the base of the telencephalon and about the infundibulum. The animal dies shortly after such an extension, and the lesions found are very acute. They consist in a widespread infection of ganglion and neuroglial cells in this portion of the brain, indicated by the presence of intranuclear bodies in great numbers, some focal necroses with local accumulations of polymorphonuclear and large mononuclear leucocytes. There is little or no peri-vascular infiltration, but an extensive basal meningitis with fibrin, polymorphonuclear and mononuclear leucocytes. Intranuclear bodies may be found to a lesser extent on the side opposite that inoculated and in parietal cortical ganglion cells of the affected side. The more extensive involvement of the inoculated side bespeaks a direct passage of the virus to this portion of the brain, and this, we believe, takes place, though it has not been possible to trace evidences of such progress through intervening midbrain. There is no evidence in our material of a passage of virus to the midbrain and telencephalon from an eye inoculated upon the cornea by way of the corresponding optic nerve and optic tracts, as claimed to have been proved by Levaditi, and as Zdansky later maintained could be seen in his preparations. Sections of optic nerves and optic tracts show no lesion in our animals, and, as will be observed in a following experiment, it is quite easy to trace the course of infection along this path following inoculation into vitreous humor and infection of the retina. Furthermore, we have produced a train of symptoms and identical lesions in pons and medulla by scarifying and inoculating the conjunctiva after the eye-ball had been removed, thus eliminating the optic nerve, as in the following experiments.

Experiment II. *After removal of left eye-ball, later inoculation of the conjunctiva is followed by purulent conjunctivitis and acute encephalitis involving the left medulla through the corresponding fifth cranial nerve.*

R. 368. Left eye-ball removed and conjunctiva sutured. Four days later the conjunctiva contained a little pus but otherwise appeared clean. It was scarified and inoculated with virus in forty-eight hour pus from eyes of Rs. 366 and 379. Two days after inoculation there was purulent conjunctivitis, and six days after inoculation the head was turned to the left and temperature was 106° F. In ten days salivation was marked and the animal was killed.

Microscopic sections of the pons and medulla show an acute herpetic encephalitis involving the area of distribution of sensory fibers of the fifth cranial nerve, demonstrating the passage of virus to the brain along this nerve in the absence of continuity of the optic nerve on the same side.

Our virus can readily enter the brain through the optic nerve, but the lesions produced are quite different from those following inoculation upon the cornea, yet care must be taken to avoid confusion, for an infection of the conjunctiva may follow inoculation into vitreous humor, as we have demonstrated, and give rise to lesions in pons and medulla from invasion through the fifth nerve. In such an event, an acute herpetic lesion in the pons and medulla will be found.

Experiment III. *Inoculation of herpetic virus into vitreous humor of the left eye causes a herpetic retinitis, optic neuritis on the same side, and an encephalitis involving both optic tracts, the Ganglion geniculatum laterale of the right, and corona radiata, centrum ovale and Area parietalis on the left side.*

R. 415. Vitreous humor aspirated from left eye-ball and twenty-four hour virus from cornea of R. 407 injected. Killed three days later.

1. *Left Cornea and Conjunctiva.* Cornea shows no herpes bodies. There is a purulent conjunctivitis most intense at margin of cornea where areas of ulceration of conjunctiva are present. Further out there are microscopic pustules in conjunctival

epithelium and general infiltration by polymorphonuclear leucocytes. No herpes bodies found.

2. *Left Retina.* Cells of retina in great numbers contain typical herpes bodies.

3. *Left Optic Nerve.* There is a mononuclear exudate in the meninges over the optic nerve. Within, there are a few minute areas where mononuclear leucocytes with irregular nuclei are accumulated, and here are a few necrotic cells. Uniformly throughout the cross section of nerve are numerous cells probably neuroglial containing typical herpes bodies.

4. *Left Fifth Cranial.* Shows no evident lesion.

5. *Frontal Lobes. Anterior to Optic Chiasm.* Cellular exudate in meninges of base, both small and large mononuclear leucocytes. No plasma cell type.

6. *Cerebrum through Optic Chiasm.* At base of chiasm there is a moderate cellular exudate in meninges composed of both mono- and polymorphonuclear leucocytes. In the optic tract on *left* no herpes bodies are found. They are first seen at the point of decussation in the center. To the right they are very numerous, and on right at base are areas of necrosis with mononuclear infiltration. Herpes bodies are found throughout right optic tract in this section, most numerous at base. At base on either side of third ventricle, brain cells contain numbers of herpes bodies. None found elsewhere.

7. *Cerebrum through Infundibulum.* Very slight increase in mononuclear cells in meninges at base. No infiltration of brain tissue. In *left* optic tract herpes bodies are numerous in cells; and small areas of necrosis with large mononuclear cell infiltration are found.

In the right optic tract no herpes bodies are observed.

On the *left* side numerous herpes bodies are found within nuclei of corona radiata and centrum ovale. They are present in abundance also in the cortex of this side but restricted to a definite area, that is in the area parietalis immediately adjacent to the fissura sagitallis lateralis. No herpes bodies are found in similar situations on right or elsewhere.

8. *Cerebrum and Midbrain through Third Ventricle and Pineal Gland.* In the optic tract on the *right* side as it overlies

part of the pes pedunculi cerebri there are numbers of *herpes bodies* and a few foci of necrosis with nuclear fragments and few mononuclear leucocytes. The bodies are not found in the optic radiation, being sharply delimited from it. In the dorsal ganglion of the ganglion geniculatum laterale on this side, nearly every nucleus contains herpes bodies, and there are foci of necrosis containing numerous nuclear fragments and a few mononuclear cells with irregular nuclei. No polymorphonuclear cells are found.

Other localities inoculated with herpetic virus and followed by an infection of the central nervous system through sensory nerves include tracheal mucosa, vagus nerve, skin and peritoneum. We have already demonstrated the local presence of intranuclear bodies and herpetic lesions in the tracheal mucosa and skin following scarification and inoculation, but following inoculations into nerve and peritoneum we have not been able to observe this diagnostic criterion of herpetic infection. However, a local lesion does occur; and we now know from subsequent experiments that herpetic lesions, typical in every other respect and containing active virus, but in which we have found no intranuclear bodies, do occur. Such lesions may be in the epidermis itself. Consequently the absence of intranuclear bodies does not exclude infection, and in the cases of nerve, peritoneum, and voluntary muscle we are assured of a local infection not only by the presence of an inflammatory lesion, but by the development of an encephalitis or myelitis at the termination of neural fibers in the central nervous system supplying the inoculated area, and these lesions contain typical nuclear inclusions.

Experiment IV. *Inoculation of the tracheal mucosa on the left side produces an acute herpetic encephalitis at the entrance of the left vagus nerve in the medulla and in the corresponding vagus nucleus.*

R. 255. Under ether anaesthesia the trachea was opened by an incision between two cartilagenous rings perpendicularly to the long axis, and the left side toward the lung scarified lightly with a needle. A drop of pus from herpetic keratitis in R. 252 forty-eight hours after inoculation, was rubbed into the scari-

fied spot. The tracheal wound was then sutured. Five days later the temperature rose to 105° F. and continued until the tenth day after inoculation, when it was 106°, and there was a distinct turning of the head to the left side. The animal was killed and the brain injected through the carotids with Helly's fluid, removed, and fixed in this solution.

At autopsy there was a thin fibrino-purulent exudate over the area of scarification and incision in the trachea, and small hemorrhages were in the adrenals. Otherwise no lesions were found except in the brain. Cross section through the medulla showed macroscopic hemorrhage in the region of entrance of the left vagus nerve.

Microscopic sections of entire cross-areas of brain were as follows:

1. *Parietal through Infundibulum.* There is slight infiltration about infundibulum and neighboring blood vessels by mononuclear leucocytes. No nuclear inclusions seen.

2. *Pons through Entrance of Fifth Nerves.* No lesion at entrance of these nerves or elsewhere in this section.

3. *Medulla at Entrance of Vagus Fibers.* There is extensive hemorrhage and necrosis of medullary tissue on the left side, along the entrance of vagus fibers. A large area of tissue including portions of descending root of fifth nerve and Tractus lateralis is destroyed and replaced by serum, red blood cells, fibrin, necrotic fragments, and an abundant exudate of large mononuclear cells many of which are vacuolated. About this area there is a peri-vascular infiltration by small and large mononuclear leucocytes. A meningitis with cellular exudate is present here and on the right side at entrance of vagus fibers. The medulla just at the entrance of right vagus fibers has a moderate accumulation of mononuclear leucocytes. The left vagus nucleus shows a diffuse and peri-vascular cellular infiltration and destructive changes in ganglion cells. Similar changes are present also in the right corresponding nucleus. No nuclear inclusions are found.

The lesion on the left extends superficially dorso-laterally along groups of fibers to the first cervical segment, where it has destroyed outer portions of the left dorsal horn.

While the lesion in the medulla is almost entirely confined to the left side, the presence of slight injury at the point of entrance of the right vagus and the cellular infiltration of the right tenth nucleus indicate that virus entered also on this side, which was to be expected from probable infection of tracheal epithelium in the incision which crossed the mid-line to the right. A lesion confined to the left side and involving more specifically the corresponding tenth nucleus was produced in the following experiment by injecting a small amount of virus directly into the left vagus nerve.

Experiment V. *Injection of herpetic virus into the left vagus nerve produces an infection of the left tenth cranial nucleus with necrosis of ganglion cells.*

R. 459. Twenty-four hour virus in saline washings from herpetic keratitis of R. 458 was injected into the left vagus nerve. The animal died suddenly four days later. Autopsy about two hours post-mortem. Brain fixed in Helly's fluid. No gross lesions discovered.

Microscopic sections were as follows:

1. *Mid-Cerebrum through Infundibulum.* There are a few cells around the infundibulum which contain typical herpetic intranuclear bodies, but the lesion is so early that there is no cellular reaction.

2. *Pons Anterior to Entrance of Fifth Nerve* shows no lesion.

3. *Pons through Entrance of Fifth Nerve* shows no lesion.

4. *Medulla.* Sections through entrance of vagus nerve and through vagus nucleus show a superficial area of necrosis and cellular infiltration in the vagus as it enters the medulla, and involving medullary fibers for a short distance about. In this area herpetic intranuclear inclusions are abundant. The lesion is confined to the left side. In bundles of fibers passing toward the vagus nucleus at the floor of the fourth ventricle are several intranuclear bodies within neuroglial cells, but no exudate. The entire left vagus nucleus is the site of an acute herpetic inflammation. Ganglion and neuroglial cells in great numbers contain intranuclear bodies. There are many small focal areas of necrosis with polymorphonuclear and large mononuclear

cell exudation. A moderate peri-vascular infiltration is present. In certain sections the herpetic lesion extends for a short distance beyond the mid-line just at the floor of the fourth ventricle.

5. *First Cervical Segment* shows a small focus of herpetic infection with intranuclear bodies in the posterior commissure next to the central canal.

The sudden death of this animal was probably due to an involvement of a vital center.

Experiment VI. *Inoculation of herpes virus on skin of right hind leg after inflaming with croton oil sixteen hours leads to paralysis first of this leg, later of the opposite one, and an associated acute herpetic myelitis of lumbar portion of cord with gross lesions along left dorsal region at entrance of sensory root fibers.*

R. 307. Fifty per cent croton oil in olive oil applied to shaved skin of right thigh. After sixteen hours the indurated and inflamed skin was scarified and inoculated with pus from herpetic keratitis (three day) of R. 303. The next day it was reinoculated with forty-eight hour pus from herpetic keratitis. On tenth day temperature 106° F., and beginning paralysis of left hind leg appeared, progressing to complete paralysis on this side in the next five days and partial on the opposite side. Killed on fifteenth day. Central nervous system injected with fixative. Microscopic sections were as follows:

1. *Mid-Cerebrum through Infundibulum.* At the base of the brain about the infundibulum is a cellular infiltration of the meninges with mononuclear and polymorphonuclear cells with occasional areas of hemorrhage and fibrinous exudate. Within the brain tissue in and immediately about the infundibulum is a dense infiltration of leucocytes, polymorphonuclear cells predominating. Here there are extensive areas of necrosis in which are nuclear fragments from disintegrated leucocytes. Glial and nerve cells in these areas and immediate vicinity in great numbers contain *herpes bodies* in their nuclei. There is a moderate cellular exudate in the meninges elsewhere over the base, but no herpetic lesion in the brain substance anywhere.

In dorsal and lateral cortex is a moderate infiltration about blood vessels and one or two miliary foci characteristic of chronic spontaneous encephalitis of rabbits.

2. *First Spinal Segment.* Moderate cellular infiltration of meninges. Few polymorphonuclear cells present. Considerable peri-vascular infiltration about vessels on right side, particularly in dorso-lateral region. Cellular infiltration in dorsal septum. No herpes bodies found.

3. *Fifteenth Spinal Segment.* Cellular infiltration of meninges especially on left side. Peri-vascular infiltration. No herpes bodies.

4. *Twenty-Fourth Spinal Segment* at level of posterior ganglia. Considerable cellular exudate in meninges, hemorrhagic and fibrinous as well, over right dorsal horn. Throughout cord there is a moderate peri-vascular infiltration, particularly marked about posterior commissure. The right dorsal horn is replaced by hemorrhage and large mononuclear cells containing clear vacuoles. Myelin sheaths and many axis cylinders as well as nerve cells are replaced. Deeper in the gray matter on this side, ganglion cells are degenerating showing chromatolysis, and there is a diffuse infiltration with mononuclear cells between dorsal and ventral horns. On the left side near ventral mid-line and midway of medullated tracts is a focal area filled with large vacuolated mononuclear cells replacing myelin sheaths and axis cylinders. Otherwise left side shows little injury. The dorsal root ganglion on the right shows no definite pathological change, though the sensory nerve entering it contains focal infiltration with mononuclear leucocytes. No change noted in left ganglion. *No herpes bodies* found in section. Central canal filled with leucocytic exudate.

5. *Section between Twenty-Fourth and Twenty-Fifth.* Shows more infiltration of left anterior horn with leucocytes and more extensive peri-vascular infiltration, otherwise quite like above.

6. *Twenty-Fifth Spinal Segment.* About the same degree of cellular, hemorrhagic and fibrinous exudate in meninges over right dorsal root. Hemorrhagic and cellular infiltration to either side of right dorsal horn. No herpes bodies found. Central canal filled with leucocytes. Clear spaces in lateral col-

umns on right, some of which contain round or oval, sometimes scalloped bodies which appear to be forming corpora amylacea.

Experiment VII. *Scarification and Inoculation of left peritoneal wall are followed by a dorsal myelitis of the sixteenth, seventeenth, and eighteenth spinal segments.*

R. 256. A laparotomy was performed and the left peritoneum about midway the lateral abdominal wall scarified in two places over an area about 1 cm. in diameter and inoculated by rubbing in pus from the eye of R. 252, inoculated forty-eight hours previously. On eighth day temperature 105.4° F. On fifteenth day temperature 106.6° F., rabbit very excitable, grinding teeth at times. Lateral curvature of spine with convexity to left in mid-dorsal region.

That virus from the inoculated peritoneum traversed sensory nerve fibers and entered the spinal cord is shown by sections of the cord at segments of entrance of these nerves. Later the virus reached the brain, producing an acute encephalitis of the telencephalon. Sections of brain and cord are as follows:

1. *Mid-Cerebrum.* There is an abundant cellular exudate over the base of the brain, not more extensive about infundibulum. The cortical cells of the base of telencephalon in large numbers contain typical herpes intranuclear bodies. There is in these areas very little cellular infiltration. Here and there are small foci without relation to blood vessels, within which are nuclear fragments and mononuclear leucocytes with indented nuclei. There is only an occasional polymorphonuclear cell. In these regions there is a moderate peri-vascular infiltration.

2. *Ninth to Fifteenth Spinal Segments.* Sections show slight cellular exudate in meninges but no alteration of cord.

3. *Sixteenth Spinal Segment.* On the left side dorsally just median to the left dorsal horn is an area, occupying almost the whole dorsal half of the cord median to horn, in which there is destruction of myelin sheaths and partial replacement by large phagocytes filled with vacuoles. About the vessels in immediate contact there is a peri-vascular infiltration by large mononuclear leucocytes. No herpes bodies found. There is a considerable cellular infiltration of meninges over this area.

4. *Seventeenth Spinal Segment.* Presents similar condition. Cellular reaction most abundant.

5. *Eighteenth Spinal Segment.* Lesion same but has shifted outward and covers completely left dorsal horn.

6. *Nineteenth to Thirty-First Spinal Segments.* Show no lesion other than slight cellular exudate in meninges.

II. Passage of Herpetic Virus Along Motor Nerves

That virus of herpes febrilis can pass from a peripheral focus of infection along motor nerve fibers to the brain or cord has been demonstrated by directly injecting the virus into voluntary muscles such as the masseter and muscles of the hind leg, and into the sciatic nerve. Although no nuclear inclusions have been demonstrated in the local lesions produced, these bodies have been found in abundance in nerve cells of the resulting lesions of the central nervous system.

Experiment VIII. *Injection of herpetic virus into the right masseter muscle causes an encephalitis localized in the pons and medulla, with infection in and destruction of ganglion cells in the motor nucleus of the fifth cranial nerve.*

R. 449. Saline washings from the eyes of Rs. 439 and 435 inoculated twenty-four hours previously were injected into several places in the right masseter muscle. Six days later there was fever of 105.6° F. and marked salivation but no turning of the head. The animal died on the seventh day, and the brain was injected with Helly's fluid. In gross no lesions were to be observed. Cross sections of the brain from the following areas were studied:

1. *Mid-Cerebrum through Infundibulum.* There are several miliary foci and areas of peri-vascular infiltration as well as a mononuclear cell exudate in meninges, all considered to be due to the spontaneous disease of rabbits. There are no lesions of herpes about the infundibulum nor in Ammon's horns, and no nuclear inclusions are found in the section.

2. *Pons above Entrance of Fifth Nerve.* The motor nucleus of the fifth nerve on the right is definitely outlined under low-

power lenses as a clearer, vacuolated area dotted with cellular exudate. In this area there are typical herpetic nuclear inclusions in both neuroglial and ganglion cells. Many of the latter are completely destroyed and are undergoing phagocytosis by polymorphonuclear leucocytes. The entire area is richly infiltrated with both large mononuclear and polymorphonuclear leucocytes in groups and scattered diffusely. There is another area of acute herpetic inflammation peripherally near the entrance of the fifth nerve. There is a moderate peri-vascular infiltration about blood vessels both on the right and on the left near the fifth motor nucleus, but on the left, ganglion cells are normal and there is no acute exudate. A moderate acute meningitis is present over inferior portions of the pons.

3. *Right Fifth Cranial Nerve at its Entrance into Pons* shows a violent acute herpetic inflammation of its intra-dural portion fairly sharply contrasted with the extra-dural portion which shows little cellular exudate and only for a short distance. The intra-dural portion is almost completely replaced by perivascular hemorrhage, necrosis, and leucocytic exudate, polymorphonuclears preponderating.

4. *Medulla.* Sections through various levels of the medulla show a diffuse herpetic infection and acute inflammatory reaction in the central portion on both sides of the mid-line median to the descending roots of the fifth nerve, which is not involved. Herpetic nuclear inclusions are present in characteristic form in nuclei of ganglion and neuroglial cells, and there is a diffuse and focal infiltration with polymorphonuclear and large irregular mononuclear leucocytes.

Experiment IX. *Injection of herpetic virus into muscles of left hind leg is followed by paralysis first of this extremity, later of the other, and an accompanying herpetic neuritis on the right, and meningomyelitis of the lumbar portion of spinal cord.*

R. 358. Saline washings from eyes of R. 334 inoculated with herpes virus twenty-four hours previously was injected intramuscularly into left hind leg. Six days later the temperature was 105.6° F., on the seventh day 107° F. On the eighth day there was complete paralysis of the left hind leg and partial

paralysis of the right, and the rabbit was killed. The brain and spinal cord were fixed by injecting Helly's fluid intra-arterially.

1. *Parietal Region through Infundibulum.* There is considerable cellular infiltration in meninges, many of the cells being of plasma cell type. In substance of brain are miliary lesions typical of spontaneous encephalitis in rabbits.

2. *Pons.* Slight cellular infiltration of meninges and peri-vascular infiltration throughout. Several miliary lesions of spontaneous encephalitis. No evidence of herpes.

3. *Fifth Spinal Segment.* Slight mononuclear infiltration of meninges. No evidence of herpes.

4. *Fifteenth Spinal Segment.* Considerable infiltration of meninges with mononuclear leucocytes, especially about blood vessels of the membranes.

5. *Twentieth Spinal Segment.* Meningeal cellular exudate more abundant and now contains a few polymorphonuclear leucocytes. There is moderate peri-vascular infiltration about vessels in the cord itself.

6. *Twenty-Fourth and Twenty-Sixth Spinal Segments.* Abundant cellular, fibrinous, and hemorrhagic exudate in meninges, especially ventrally. Small cellular focus in left dorsal horn just beneath and apparently extending from meninges. No herpes bodies.

7. *Left Sciatic Nerve Near Cord* shows an extensive diffuse acute inflammation. There is an abundant accumulation of mononuclear leucocytes, and a few small hemorrhages are present about blood vessels. Some axis cylinders are destroyed.

8. *Right Sciatic Nerve Near Cord* shows an acute peri-neuritis with serous, fibrinous, and cellular exudate. There is little involvement of nerve fibers themselves in contrast to the opposite side.

In the absence of destructive changes in anterior horn cells, the paralysis on the left would seem best explained by the neuritis. The virus evidently passed from motor fibers in the muscle to the sub-dural space, producing an acute meningitis which extended outward peri-neurally on the opposite side. Several sections through the cord may have shown areas of necrosis in motor ganglion cells as well.

That a neuritis may occur with passage of the virus into the cord causing herpetic infection and destruction of anterior horn cells was shown by injecting the virus directly into the left sciatic nerve.

Experiment X. *Injection of herpetic virus into the sciatic nerve produces an acute neuritis, and an acute herpetic meningo-myelitis destroying anterior horn ganglion cells.*

R. 388. Twenty-four hour herpetic virus in saline washings from eye of R. 386 was injected into the left sciatic nerve. Five days later, temperature was 106.2° F. and there was weakness in left hind leg. The following day this leg was completely paralyzed and there was little ability to use the opposite leg. In afternoon of same day there was complete paralysis of both hind legs, but the animal could drag itself forward with front legs. There was tremor of entire body, and paralysis of urinary bladder. The rabbit was killed and sciatic nerves and spinal cord fixed.

Sections of cord and nerve are as follows:

1. *Twentieth to Twenty-Fifth Spinal Segments.* There is a very severe acute meningitis with a thick membrane composed of fibrin, white and red corpuscles entirely surrounding the cord. The exudate extends into the cord by way of the ventral septum, along blood vessels, and on both ventral surfaces along nerves. The exudate about blood vessels and within the gray matter is more marked on the left side, though along ventral nerves it is about the same on the two sides. Near the surface of the right ventral portion of cord is an area of hemorrhage. Leucocytes of exudate consist of both mononuclear and polymorphonuclear forms, the former predominating. Within the gray matter the most intense cellular infiltration, consisting almost entirely of polymorphonuclear cells, some of them necrotic, is at the point of contact with ventral septum and about the central canal. There is extensive injury and destruction of ganglion cells in both ventral horns, some of which have been entirely removed, their spaces being occupied by polymorphonuclear cells. Typical intranuclear herpes bodies are present in numbers of ependymal, neuroglial, and ganglion cells.

Ganglion cells show chromatolysis, shrunken pyknotic nuclei, and other evidences of degeneration.

In the lower segments there is penetration of the cord by cellular exudate completely around its circumference, and there is considerable injury to the left posterior horn, and degeneration of myelin sheaths median to it. Infiltration and destruction is in all sections most marked on the left side, though ventral horn ganglion cells on both sides contain herpes bodies and others have been phagocyted and replaced by polymorphonuclear leucocytes. Herpes bodies are numerous in glial cells of white as well as gray matter.

2. *Sciatic Nerve Left.* The sciatic nerve from the site of injection to the cord shows extensive injury and inflammatory reaction. The branches of the nerve distal to site of injection were not studied.

At the site of injection there is hemorrhage between nerve fibers. Axis cylinders are swollen, fragmented, undergoing granular disintegration, and sometimes completely destroyed. Myelin sheaths which appear intact may be greatly distended in a fiber showing disintegration of its axis cylinder. There is an abundant infiltration by mononuclear leucocytes generally, but most marked about small blood vessels. A few polymorphonuclear cells partake in the exudate. There is also destruction of myelin sheaths in places, with infiltration by large mononuclear cells with vacuolated cytoplasm. No intranuclear bodies found.

III. Transmission of Virus of Herpes Febrilis along Sympathetic Nerve Fibers

By demonstrating intranuclear bodies of Lipschütz and active herpetic virus in local lesions following injections of virus, we have been able to show that a herpetic infection may be produced in the liver, adrenals, ovary, and testes. Following inoculation into the liver, adrenal, and ovary, paralysis has resulted from an acute herpetic myelitis at the level of the spinal cord where sympathetic fibers from these localities reach the central nervous system through rami communicantes. A myelitis less

extensive but situated at the level of entrance of sympathetic fibers has followed similar inoculation into the spleen.

In order to reach the cord through the dorsal roots at these levels, it is necessary that the virus pass along sympathetic fibers from the site of inoculation, sometimes, as in the case of the ovary, for quite a distance, and enter the spinal canal with sensory fibers through the communicating branches; and in each of these instances there has been a lesion in the dorsal horn on the inoculated side, though the infection has in some instances become very widespread within the cord at the level of entrance.

Experiment XI. *Virus of herpes febrilis inoculated into the liver produces complete paralysis of the lower dorsal region as a result of acute herpetic myelitis.*

R. 370. Herpetic virus in saline washings from twenty-four hour keratitis of R. 366 was inoculated into the liver on the left side. Five days later temperature 106° F. Six days after inoculation there was partial paralysis of hind legs. In twenty-four hours, abdominal wall was completely paralyzed and there was complete disuse of hind legs though they could be moved. The rabbit was killed on the ninth day. Brain and spinal cord injected with Helly's fluid.

Cross sections of central nervous system were as follows:

1. *Mid-Cerebrum through Infundibulum.* There is slight cellular infiltration in brain substance and meninges about infundibulum. At one point on right side near infundibulum is a focal area in brain substance beneath meninges consisting of mononuclear cells with irregular nuclei. An occasional *herpes body* is seen.

2. *Pons.* Moderate cellular infiltration in meninges. No pathological change noted in substance of medulla.

3. *Fifth Spinal Segment.* No pathological change noted.

4. *Seventeenth Spinal Segment.* Considerable cellular exudate in meninges, and moderate peri-vascular infiltration throughout cord. The central canal is almost completely destroyed and tissue immediately about in anterior and posterior commissures is thickly infiltrated with both mono- and

polymorphonuclear leucocytes. A few neuroglial cells contain herpes bodies. Ganglion cells on right side show extensive chromatolysis, degeneration, and shrunken nuclei. Less on left side.

5. *Twentieth Spinal Segment.* Considerable cellular exudate in meninges. There is severe injury to right side of cord both dorsally and ventrally, less on left.

In left dorsal region just ventral to dorsal horn is destruction of myelin sheaths and replacement by mononuclear cells, some of which are large and vacuolated. Similarly a large area ventro-laterally is injured and infiltrated with cells. In the gray matter the region of greatest injury and reaction is between dorsal and ventral horns on right. Central canal on this side almost completely destroyed. Chromatolysis of ganglion cells in right ventral horn. Some peri-vascular infiltration on left. On left there is an area of cellular infiltration and destruction of myelin just median to dorsal horn. Few herpes bodies found.

Experiment XII. *Injection of herpes virus into left adrenal produces complete paralysis below lower dorsal region of spinal cord, and corresponding acute herpetic myelitis.*

R. 371. Saline washings from twenty-four hour herpetic keratitis of R. 366 injected into left adrenal. Four days later temperature 106.2° F. On fifth day there was paralysis of abdominal muscles, lateral curvature of spine with convexity to left, and total disuse of both hind legs although they could be moved voluntarily with difficulty. Killed on fifth day, and central nervous system injected with fixative. Cross sections microscopically were as follows:

1. *Cerebrum through Infundibulum.* Meninges over base and over cortex generally infiltrated with moderate number of mononuclear cells, many of which are of plasma cell type. Thin mantle of small mononuclear cells about many blood vessels on the left. Minute circumscribed foci appearing like miliary tubercles. *No herpes bodies* found. Meningitis and miliary lesions due to *chronic spontaneous encephalitis.*

2. *Medulla* shows moderate increase in mononuclear cells,

many of them plasma cells in meninges. Focal tubercle-like lesion on right side, in and about which are amorphous orange pigment granules having the appearance of hemosiderin. No noticeable peri-vascular infiltration. *No lesions of herpes.*

3. *Sixth Spinal Segment.* There is an abundant cellular exudate in the meninges entirely about the cord and following the membranes along penetrating blood vessels. The cells are for the most part large mononuclear leucocytes, some with irregular nuclei and few of them of plasma cell type. Polymorphonuclear cells are frequent. Situated on the right side, in a latero-ventral position, is an area of cellular infiltration extending into the cord about one third the lateral width of the lateral columns. The area involves a bundle of motor fibers emerging from the cord, and an adjoining blood vessel is surrounded by cellular exudate. Under high power the lesion is obviously acute; the cellular exudate is composed of mononuclear leucocytes with irregular nuclei and polymorphonuclear cells in about equal proportion. Similar cells surround the blood vessels and overlying meninges. Medullary sheaths and axis cylinders are partly replaced by exudate. Several glial cells have nuclei almost completely filled by a mass of *inclusion characteristic of herpes*. There is a small hemorrhage in the pia overlying the lesion. The lesion is confined to the white matter of the cord, but the large ventral ganglion cells on the same side are shrunken so that a clear space surrounds them; their nuclei and cytoplasm are dense and deeply staining. Nissl substance is diminished in some, and the aggregates difficult to distinguish in others. No herpes bodies in these nuclei. They contrast strikingly with corresponding cells in opposite ventral horn. The lesion is characteristic of herpes and seems to be invading the cord from the meninges along nerve roots and perhaps about blood vessels.

4. *Seventeenth Spinal Segment.* There is an abundant cellular exudate in the meninges entirely around the cord, thickest over ventral surfaces where on the left side it forms a dense membrane with white, and a few red, blood cells embedded in a meshwork of fibrin. In this membrane there are many necrotic cells as indicated by scattered nuclear fragments. Under low

power there are seen to be extensions of inflammatory exudate collected in foci and along blood vessels into the substance in dorsal, lateral, and ventral positions restricted to the left half of the cord. The larger areas are in the entering dorsal root, ventral root, and laterally at a point where blood vessels enter from the meninges. Smaller scattered foci are present both in white and gray matter on this side. There is a complete circle of cellular exudate about the central canal, widest in the posterior commissure. Under high power the exudate in each of these foci is seen to be composed of large mononuclear, with indented nuclei, and polymorphonuclear leucocytes. *Very numerous typical nuclear inclusions* are found irregularly distributed in glial cells, ependyma of central canal, and in ventral motor ganglion cells on the right side. The bodies are very numerous in ependyma. On the left all motor ganglion cells are pale with complete chromatolysis, cytoplasm vacuolated and shrunken. Nuclei are pale and appear to be undergoing lysis. On the right a few intact ganglion cells are found; others show chromatolysis, and still others contain in nuclei large lobulated masses characteristic of herpes inclusions. On this side there is little cellular exudate except immediately around the central canal.

5. *Eighteenth Spinal Segment.* Presents a picture very similar to seventeenth spinal segment except that the injury and reaction are considerably more marked and occur on both sides of the cord still less extensive on right side. There is much fibrin in meningeal exudate, and small hemorrhages about central canal. An area of cellular infiltration in right dorsal cord at entrance of sensory root fibers. Apparently this is an extension from dipping meninges. A similar area in ventral tracts. Gray matter extensively infiltrated on both sides, more on left. All ganglion cells injured; chromatolysis and neuronophagia by polymorphonuclear leucocytes. Few nuclei of ganglion cells contain herpes bodies. Numerous herpes bodies in glial and ependymal cells. Desquamation of ependymal cells of central canal and exudation into canal. On left, areas of injury and infiltration appear to follow sensory and motor nerve fibers into cord.

6. *Nineteenth Spinal Segment.* More extensive destruction of gray matter on both sides of cord, especially around central canal, which shows almost complete desquamation of ependymal cells, the dilated lumen being filled with cellular exudate. Neuronophagia more marked. Extension of inflammation along sensory and motor nerves into the cord, more extensive on left. Meningeal exudate somewhat less abundant. *Herpes bodies* in nuclei of posterior root ganglion cells on left.

7. *Twentieth Spinal Segment.* Injury and inflammation much less intense. Exudate in cord more marked on left, following dorsal and ventral nerves, but foci of exudate in ventral bundles are present near gray matter. *Herpes bodies* in motor ganglion cells in both horns especially large. Definite *herpes bodies* in dorsal root ganglion cells on left.

8. *Twenty-fourth Spinal Segment.* Considerable cellular exudate in meninges entirely around the cord. Slight perivascular infiltration in cord on left and about central canal. Few inflammatory cells in central canal. No other exudate or injury of cord observed.

Experiment XIII. *Injection of herpetic virus into left ovary is followed by complete paralysis of caudal half of body and an acute herpetic myelitis of mid-thoracic spinal cord.*

R. 317. Early pregnancy. Twenty-four hour virus in saline washings from herpetic keratitis of R. 313 injected into left ovary which contained corpora lutea. Seven days later temperature 105° F. On eighth day there was diarrhoea and partial paralysis of posterior half of body, progressing rapidly to complete paralysis on the next day. Killed on ninth day. Dead embryos in utero. Sections of central nervous system as follows:

1. *Cerebrum through Infundibulum.* There is a slight exudate of mononuclear leucocytes in the meninges over the base of the brain. Most of these are small, with distinct rim of cytoplasm and round or oval nuclei. Miliary lesion of *chronic encephalitis* in left hemisphere. No noticeable peri-vascular infiltration. *No evidence of herpetic lesion* nor of intranuclear inclusions.

2. *Medulla.* On the ventral surface of left side of medulla there is considerable cellular exudate in meninges, large mononuclear and polymorphonuclear leucocytes. Much less on right ventral surface. Following peripheral layer of white matter one third circumference of left side from mid-ventral point, the substance of the medulla is infiltrated with mononuclear leucocytes with indented nuclei and by polymorphonuclear cells. Peri-vascular infiltration with similar cells is marked. Much necrosis of leucocytes as indicated by quantity of nuclear fragments. Typical herpetic lesion superficial. Numbers of neuroglial cells containing nuclear inclusions. No ganglia appear to be affected.

Meningitis is present but less marked on right ventral surface. Near mid-line in right ventral portion of medulla is a local herpetic inflammation with cells containing inclusions, among which are a few ganglion cells. On mid-dorsal surface of left side is a small area of cellular infiltration under the surface in brain substance, composed of mononuclear cells. No herpes bodies found here. Moderate peri-vascular infiltration in ventral half of right side.

3. *Fifth Spinal Segment.* There is a slight infiltration of the meninges, left dorsal root, and blood vessels throughout the cord by mononuclear leucocytes; many of these are of plasma cell type. In the left dorsal root ganglion, left ventral horn and posterior commissure there are several empty spaces, many of which appear by size and shape to have been occupied by ganglion cells. In the ventral portion of left ventral horn is a small area in which are accumulated a number of mononuclear cells with irregular, indented nuclei and a few polymorphonuclear cells. Several glial cells here contain *inclusion bodies*, and ganglion cells in the area are vacuolated and degenerating.

4. *Tenth Spinal Segment.* Cellular infiltration of meninges is more marked than at fifth segment, but is only slight about blood vessels of the cord. Many mononuclear, with folded and indented nuclei, and a few polymorphonuclear leucocytes partake in the meningeal exudate. Throughout the gray matter, particularly of ventral horns and between ventral and dorsal horns, about equally on each side, is a slight infiltration of dif-

fusely scattered mononuclear cells with indented nuclei. In the same distribution are numerous neuroglial cells containing typical *herpes bodies* and on the right side between dorsal and ventral horns are ganglion cells containing in their nuclei *herpetic inclusions.*

5. *Fifteenth Spinal Segment.* Meningeal exudate about the same as in tenth spinal segment. Peri-vascular infiltration more marked on left side of cord. In mid-left lateral region is a band of cellular infiltration moderate in amount, extending from the surface to gray matter, and a similar infiltration in gray matter between dorsal and ventral horns. Cells of exudate are mononuclear with indented nuclei. Few *intranuclear bodies* in neuroglia of lateral tracts, none found in gray matter. There is a slight diffuse infiltration with large mononuclears in gray matter especially in ventral horns. In left ventral horn are several polymorphonuclear cells. No herpes bodies in gray matter.

6. *Sixteenth Spinal Segment.* Moderate infiltration of meninges with mononuclear cells. Peri-vascular infiltration most marked in left dorsal. There is complete destruction of the central canal. Its outline is obliterated, lumen filled with leucocytes and fragmented epithelial lining. Dense infiltration about with large mononuclear cells. Degree of injury and reaction about same on either side. Chromatolysis of ganglion cells in ventral horns. Diffuse infiltration of gray matter of ventral horns and between ventral and dorsal horns by mononuclear cells. Glial cells containing *herpes bodies* numerous. Posterior horns less affected than remaining gray matter. Very few polymorphonuclear leucocytes in exudate. Chromatolysis of anterior horn ganglion cells more marked on right side.

7. *Seventeenth Spinal Segment.* General appearance about the same as in sixteenth, except that injury and destruction about central canal is less marked. The greatest injury in this section is in the gray matter between dorsal and ventral horns on the right side. Destruction and chromatolysis of ventral horn ganglion cells marked on right. On right lateral surface of cord, opposite point of greatest destruction of gray matter, is a small focus of large mononuclear cells within substance of

cord. No definite lesion found in posterior root ganglion on right side.

8. *Eighteenth Spinal Segment.* Injury and inflammation everywhere less marked than in seventeenth. There is some destruction of cells and infiltration of mononuclears on the left side of central canal.

9. *Nineteenth Spinal Segment.* Abundant meningeal exudate especially over dorsal and ventral surfaces. Small focus of cellular infiltration on mid-lateral surface on left side within substance of cord. Extensive destruction and cellular infiltration in middle of gray matter extending through both sides. Mononuclear cell exudate predominates. A good many polymorphonuclear cells participate. Several typical *herpes bodies* in glial cells in gray matter, but relatively few in proportion to destruction and exudation. Chromatolysis and necrosis of ganglion cells in both ventral horns.

10. *Twentieth Spinal Segment.* Slight cellular infiltration of meninges. Band of slight infiltration of left dorso-lateral region of cord extending from surface to gray matter; these latter contain some infiltration. Central canal filled with mononuclear leucocytes. Some chromatolysis of ganglion cells in left ventral horn. No other injury to gray matter. No herpes bodies found. Moderate peri-vascular infiltration in cord.

11. *Twenty-Third Spinal Segment.* Slight meningeal infiltration. Small focus in mid-lateral gray matter infiltrated with mononuclears with irregular nuclei. No *herpes bodies.* Otherwise cord appears normal.

Experiment XIV. *Injection of herpetic virus into spleen is followed by encephalitis and a left dorsal herpetic myelitis in mid-thoracic segment of spinal cord.*

R. 347. Saline washings from herpetic keratitis of R. 341, twenty-four hours in duration, were injected into the spleen. On the fourth day temperature 105° F. Seventh day marked salivation, pawing movements with both front feet, attempts to jump out of cage or against its sides as if blind. Eighth day lying on belly, very excitable. No one-sided symptoms observed. Died, and central nervous system injected with Helly's fixative. Sections of central nervous system were as follows:

1. *Mid-Cerebrum through Infundibulum.* There is a rich cellular exudate in meninges over base of brain, consisting almost entirely of mononuclear cells. It is more marked over the left base of telencephalon, where the cellular exudate is present also beneath meninges in brain substance along the surface. There is also a moderate cellular exudate including polymorphonuclears on left about infundibulum. Numbers of brain cells, neuroglial and nervous, contain herpes bodies on both sides, much more numerous on left where many cells are undergoing degeneration, least about infundibulum. Both Ammon's horns are affected. Ependymal cells of left lateral ventricle in this region are extensively affected showing great numbers of characteristic bodies, while cells of choroid plexus in intimate contact show none. *Herpes bodies* are found throughout the brain on both sides, especially in cortex, over which there is little or no meningitis. It is difficult to determine whether they are more numerous on one side. At base of median longitudinal septum they are especially numerous, with areas of necrosis and cellular infiltration. Here also cellular exudate in meninges is present.

2. *Medulla.* In the rubro-spinal tract on the left side is a small area infiltrated by a few mononuclear leucocytes with folded nuclei. In several glial cells in this area typical herpes bodies are found. There is a slight accumulation of mononuclear leucocytes in the meninges about the medulla.

3. *Sixth Spinal Segment.* Slight accumulation of mononuclear leucocytes in meninges.

4. *Eleventh Spinal Segment.* Same.

5. *Fourteenth Spinal Segment.* Same.

6. *Seventeenth Spinal Segment.* The left posterior horn is densely infiltrated with mononuclear leucocytes with folded nuclei, and pyknotic nuclear fragments are numerous. Several typical herpes bodies are found in nuclei in this area. Abundant cellular infiltration about left posterior root as it enters cord. In addition to cellular infiltration of meninges and ventral septum, there is a fibrino-hemorrhagic exudate in meninges particularly marked over the right side.

7. *Twentieth Spinal Segment.* Considerable cellular exudate

in meninges, some peri-vascular infiltration, but no lesions of the cord observed.

8. *Twenty-Third Spinal Segment.* Same.

IV. Production of Generalized Encephalitis without Local Lesion in Brain Associated with a Peripheral Nerve

It is to be noted that in the above described experiments, in addition to a local acute herpetic lesion of brain or spinal cord at the site of entrance of nerves supplying the peripheral areas inoculated, there was frequently an acute herpetic encephalitis involving the base of the brain. In animals inoculated upon the cornea or into the masseter muscle, stained vitally with trypan blue and killed late in the disease, it can be readily seen that the diffuse lesion of the base of telencephalon is almost, and in some cases entirely, unilateral and on the side in which the lesion at the entrance of the fifth cranial nerve is present, that is, on the inoculated side. It is difficult to explain this distribution otherwise than by assuming a direct passage of virus along nerve tracts through the mid-brain. It is possible that the virus reaches the base of the brain directly through the meninges, as there is always an accompanying meningitis, and then spreads through the basal cortex. While this mode of extension may play a part in the dissemination of virus, it can hardly be considered the only method of extension.

The unilateral distribution at the base precludes the possibility of the infection arising through the blood stream. However, experiments with our strain of virus have demonstrated the possibility of producing an encephalitis by direct inoculation of virus into the blood stream, as was accomplished also by Doerr.

Experiment XV. *Injection of herpetic virus into the blood stream produces a diffuse bilateral herpetic encephalitis.*

R. 369. Pus suspended in saline from the eye of R. 361 with herpetic keratitis of forty-eight hours' duration was injected into a vein of the ear. There was high fever in forty-eight

hours, which continued until the day before death on the eighth day. Autopsy was negative in gross.

Microscopic sections of the brain were as follows:

1. *Mid-Cerebrum through Infundibulum.* There is considerable cellular exudate in the meninges, most marked over the base. Here polymorphonuclear cells participate to a considerable extent. There is cellular infiltration of brain tissue about the infundibulum and of telencephalon on both sides. Polymorphonuclear leucocytes are numerous in this exudate. Numbers of herpes bodies are found in nuclei, especially in Ammon's horns. In these regions there is considerable necrosis of nerve cells, with more or less focal accumulations of leucocytes. Herpes bodies are found both in glial and ganglion cells, more numerous in the latter. Although the lesions are more marked at the base, numerous *herpes bodies* without much cellular infiltration are found irregularly distributed throughout the brain substance of this section, especially in gray matter of cortex on both sides.

2. *Sections of Cord* show no evidence of herpes.

Following inoculation of herpetic virus into the testicle we have noted no evidences of infection in the spinal cord, but after a relatively long time the animal succumbed to a generalized encephalitis. In such an instance as this it is impossible to determine whether the virus reached the cord and indirectly infected the brain, or entered the brain directly through the blood stream, the possibility of which the last experiment illustrated.

Experiment XVI. *Herpetic virus inoculated into the testicle produces a generalized encephalitis, but no evidence of myelitis.*

R. 348. Left testicle was inoculated through the skin with twenty-four hour virus in saline from the eye of R. 341. The animal gradually became greatly emaciated. There was high fever on the eleventh day, and death occurred on the sixteenth day. Autopsy showed no gross lesion.

Microscopic sections of the central nervous system are as follows:

1. *Mid-Cerebrum through Infundibulum.* There is an acute

encephalitis throughout the brain in this section, particularly marked at base on both sides and in gray matter of entire cortex on right. In these regions under low power one can see a dense cellular infiltration which is due to accumulation of polymorphonuclear leucocytes with extensive necrosis of ganglion cells. There is a corresponding abundant cellular exudation in meninges. In right cortex and elsewhere in brain substance there is little cellular exudate but *herpes bodies* in great numbers in glial and ganglion cells. In the inflamed areas they are exceedingly numerous. In such areas peri-vascular infiltration is slight and composed of both mononuclear and polymorphonuclear leucocytes.

2. *Spinal Cord.* There is some peri-vascular infiltration in meninges of lumbar region, but no lesion of cord.

V. Inoculation of Herpetic Virus into Subconjunctival or Subcutaneous Tissue, Salivary Glands and Kidney is not Followed by Evidences of Infection either Locally or in Central Nervous System

Two rabbits received injections of herpetic virus subcutaneously in the hind legs, and one rabbit was similarly inoculated under the conjunctiva. The material and dosage employed was the same as was used to produce a successful infection with transmission to the spinal cord when injected intra-muscularly. None of these rabbits showed any rise in temperature or other indication of infection, and one month after receiving the injection each was inoculated upon the cornea to test their immunity. Had a small local lesion been produced, we felt sure an immunity partial or complete would have developed in this time. In a previous experiment with the same virus, in which a local infection of the skin was induced, a substantial immunity to corneal inoculation was subsequently demonstrated. The three rabbits, however, inoculated subcutaneously and subconjunctivally, showed no more immunity than normals. They developed typical purulent kerato-conjunctivitis and later, encephalitis. In the case of the subconjunctivally inoculated rabbit, the corresponding eye was inoculated.

Histologically we have been unable to demonstrate intranuclear bodies at the site of injection of herpetic virus into salivary glands and in the kidney. Correspondingly animals thus inoculated have shown no symptoms of infection with virus in dosage which readily induced myelitis when injected into adrenal, ovary and even spleen.

The facts that no herpetic nuclear inclusions were found in local lesions of muscle following injection of virus, and that the virus readily passed along the sciatic nerve to cord, suggest the possibility of a primary growth of virus within sarcoplasm without nuclear change, or within the nerve-filaments of end-plates. The strongly selective neurotropic quality of the virus led us to suspect that an intact nerve supply might be an essential for a "take" in muscular tissue, and the following experiment was undertaken to test this point, by cutting a sciatic nerve and inoculating muscle supplied by it after a suitable period for degeneration of axis cylinders.

Experiment XVII. *Intra-muscular injections after cutting the sciatic nerve.*

On June 29, 1923, the left sciatic nerve was sectioned and a piece 1 cm. long removed in Rs. 483 and 484.

On July 15 (sixteen days later) twenty-four hour herpes virus "M," collected in saline from both eyes of R. 0, was inoculated into the muscles of the left hind leg of Rs. 483 and 484 and a normal rabbit as a control, each receiving approximately one third of the saline washings. The injections were made at three or four different places in the leg muscles.

The control normal rabbit lost the use of the left hind leg on July 22, the seventh day, and on the next day the posterior part of its body was paralyzed.

Temperature	7–18	1 P.M.	102.4
	7–19	11 A.M.	102.2
	7–21	3.40 P.M.	105.4
	7–22	11.30 A.M.	108.1
	7–23	1.30 P.M.	104.2

Killed. Cord and brain saved for sections.

R. 483 has shown no symptoms as a result of the inoculation. A temperature of 104 was recorded on the sixth day at 3.40 P.M. At other times it has varied between 102.6 and 103.6.

R. 484 showed no symptoms as a result of the inoculation. On July 21, six days after inoculation (killed), a portion of the inoculated muscles was excised, ground up in a mortar, suspended in saline and inoculated into the right hemisphere of the brain of

R. 11. This rabbit showed a rise of temperature for a few days, apparently due to an infected and swollen submaxillary gland, and has since that time appeared normal.

The above experiment shows that the virus did not cause a local infection following section of the sciatic nerve supplying the muscle injected, after a lapse of time sufficient for degeneration of the peripheral axis cylinders. This indicates that the axis cylinders and filaments are necessary for a local "take" in muscle, and the view expressed in the discussion that virus passes along the axis cylinders of nerves by active growth is in conformity with the facts thus established.

Discussion

It is obvious from the foregoing experiments that the strain of virus of herpes febrilis which we used is an unusually virulent one for rabbits and its property of invading the central nervous system through peripheral nerves is very pronounced. For six months it has been passed from eye to eye in rabbits every two or three days and has uniformly reached the brain, causing an encephalitis in adult animals. This fact in itself is sufficient to prove the untenability of Levaditi's distinction between virus obtained from herpes vesicles and that which he regards as the cause of epidemic encephalitis, so far as a more predominantly "neurotropic" character claimed for the latter is concerned. In addition our virus has readily reached the central nervous system through nerves from peripheral sites of inoculation other than the eye, producing, when these nerves entered the spinal cord, an acute herpetic myelitis, which Levaditi was unable to demonstrate even though an animal inoculated

into the sciatic nerve with his virus of epidemic encephalitis caused a fatal encephalitis. We cannot believe that the virus obtained by Levaditi from the brain of a human case of epidemic encephalitis therefore is distinguishable from virus of herpes febrilis on the basis which he alleges.

We have been able to prove conclusively that the virus of herpes febrilis can pass from a peripheral site of inoculation along corresponding nerves to spinal cord or brain, and produce at the site of entrance and along the central distribution of these nerves an acute herpetic lesion demonstrable grossly and histologically to be directly related to the nerve along which it passed. The transmission of the virus may be along sensory, motor or sympathetic fibers, depending on the nerve supply to the inoculated area.

Our experience indicates that an injury to cells is a necessary preliminary to a local infection, and that an extension of herpetic virus along nerves to the central nervous system will not occur unless there is a local infection where sufficient virus may be generated.

In most of the peripheral sites inoculated, we have found in the lesion conclusive evidence of a "take" in the form of characteristic nuclear inclusions of Lipschütz. These inclusions have been found locally following inoculations of the cornea, conjunctiva, retina, skin, trachea, liver, adrenal, ovary and testis. This criterion has not been demonstrated in local lesions following injections into muscle and nerve, though there is an acute inflammatory reaction in both, indicative of local infection; and in segments of sciatic nerve central to the site of inoculation we have demonstrated active virus by inoculation into the brain of a rabbit which later succumbed to herpetic encephalitis. In these latter situations we believe the local "take" is in the nerve fibers and endings themselves, with local proliferation of virus and rapid extension along neural paths. That a local infection is necessary to neural transmission and involvement of the central nervous system, is indicated also by the negative results following injection of large doses of virus subcutaneously and subconjunctivally. The inoculated virus in these experiments was not passively carried in lymphatic spaces, to pro-

duce a lesion in the more favorable environment of the central nervous system.

Our observations thus far indicate that the virus of herpes febrilis is better adapted for growth in an intracellular rather than in an extracellular environment. There is no evidence that growth will take place primarily in body fluids, while there is the anatomical evidence that the intranuclear bodies so characteristic of an infection represent proliferation of this virus within nuclei. In order to induce a primary local infection in various places, we have found it necessary to cause a local injury, as in scarification of the cornea. Local injury we regard as a preparation of the cells rendering them more permeable to the virus. If the contention of Lipschütz and ourselves is correct, that the intranuclear bodies are essentially masses of virus, we must assume a preliminary passage of the virus through the cytoplasm and most probably growth there before the nucleus is penetrated. Invasion of the nucleus would then be only an incident in the growth of the virus, and an intranuclear environment not an exclusive one for such growth. This seems to be the case, for following injections of virus into voluntary muscle and into nerves, we are not able to demonstrate any nuclear inclusions, yet there is a lesion which must be attributed to activity of the virus; and as shown in Experiment XVII, virus will not grow in muscle of the leg after section and degeneration of the sciatic nerve. In the case of injection into the sciatic nerve we have proof of the presence of virus locally.

There are certain cells which appear to be particularly easily penetrated by the virus. These are cells of the central nervous system, both ganglion cells and neuroglial cells, as indicated by the ready development of characteristic nuclear inclusions. This we interpret to mean that the protoplasm of these cells is easily permeable to this agent and is a suitable medium for its growth. Since axis cylinder processes within nerves are merely attenuated projections of cytoplasm of the nerve cells, we may believe that they also offer a favorable environment to the virus when it is introduced into them by injections which injure their protecting sheaths. Likewise the abundant arborizations of terminal filaments in the end-plates of voluntary muscle

would be exposed to an invasion following injection of the virus into muscular tissue. Having gained entrance into this assumed favorable medium, the virus may extend centripetally or centrifugally along an axis cylinder, not in the sense of a passive transportation but by active reproduction. Since sections of some nerves along which the virus must pass show no certain evidence of its presence in the form of intranuclear bodies or inflammatory change, we may assume that under some circumstances the virus passes directly to the intra-dural space without penetrating the myelin sheaths, for the indications are that, did it thus penetrate, a local reaction in the nerve would follow. This is apparently what occurs when the virus is injected into muscles of a hind leg. Sections of the corresponding sciatic nerve to the cord show an acute neuritis, with destruction of axis cylinders and an acute inflammatory reaction. Following direct inoculation of a sciatic nerve, there is a similar though more violent neuritis. When peripheral nerves reach the subdural space it is evident that contained virus becomes liberated, for there is a local acute meningitis and a myelitis or encephalitis spreading to neighboring tissues. Within the dura, it seems that the axis cylinders are not so well protected as extra-durally, for they have no sheath of Schwann. This fact may permit of a more abundant escape of virus.

While this abrupt diffusion of virus in central nervous tissue is evident, we must still conclude that the nerve fibers direct the course of infection in the brain and cord after their entrance. This is particularly evident in the case of motor nerve fibers, as is indicated by herpetic infection of ganglion cells in the motor nucleus following injections of virus into a masseter muscle, and an almost exclusive invasion of the vagus nucleus with its motor ganglion cells following direct inoculation of the vagus nerve; and a similar destruction of motor ganglion cells of ventral horns when the sciatic nerve is inoculated.

This directing influence of nerve fibers on the extension of herpetic infection within the brain is well demonstrated also by inoculation into the vitreous humor of one eye and infection of the retina. Spread of the virus along the optic nerve and within optic tracts may readily be followed in sections before

there is any microscopic evidence of inflammation, by the presence of nuclear inclusions within neuroglial cells between the optic fibers. While the presence of these bodies within neuroglial cells must mean that the virus is outside axis cylinders, the lesion remains so confined to the nerve and optic tracts, as they pass through the brain to terminations in ganglia of the midbrain, that it seems more rational to assume that there is an escape of virus from axis cylinders here and there, than that there is a diffuse growth of this infectious agent in all the surrounding fluid. Infection of ganglion cells of neural terminations in a restricted area in the superior geniculate ganglion also points to the same assumption.

In our experience there does not seem to be, as assumed by Levaditi, a "zone elective" in the brain where the virus finds an optimum environment. The virus will grow readily in any part of the central nervous system to which it gains access in sufficient amounts, either in nerve, spinal cord or brain, and while we recognize that the base of the brain about the infundibulum and in Ammon's horns is the first area infected, secondary to a lesion elsewhere in central nervous tissue, we rather attribute this to a more easy access of the virus to this site than elsewhere, though at present we have no evidence as to how this is brought about.

The weight of evidence at hand is in favor of a transmission of the virus of herpes febrilis, as exhibited in these experiments, along axis cylinders of nerves by active growth within these cytoplasmic branches of the nerve cell, which thus offer a direct portal of entry to the central nervous system. The other alternative of a passage by way of peri-neural spaces has not, however, been positively excluded.

By virtue of its ability to produce a specific lesion within a nerve and locally at its central origin or terminus, the neural transmission of the virus of herpes febrilis in rabbits has been definitely proved.

That this is the only way in which herpetic virus can enter the central nervous system from a peripheral inoculation is in doubt, owing to the fact that an encephalitis may be produced by intravenous injection of the virus. This fact, however, does

not exclude the possibility that under the conditions of this experiment virus was admitted to the brain through the medium of nerves, for we know that, after an inoculation into the spleen, virus reaches the cord through neural channels, and after intravenous injection, small blood vessels supplied with nerves may be the site of emboli or thrombi, whence an undiscovered local lesion developed with extension through involved nerve terminals to the brain. Under any natural conditions of infection in a rabbit with this virus, we believe the central nervous system would be first and probably only infected through peripheral nerves.

SUMMARY

1. The virus of herpes febrilis in experimentally infected rabbits enters the central nervous system through the pathway of nerves from a peripheral focus of infection.

2. Within the brain and the cord the virus produces a characteristic acute herpetic lesion having a definite relation to the nerve through which it entered.

3. The virus will traverse sensory, motor or sympathetic nerves to the brain or the cord, depending on the nervous supply to the peripherally infected area.

4. The view is expressed that the mode of transit is by way of axis cylinders, rather than peri-neural spaces, and not by passive transportation but by invasive proliferation.

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DESCRIPTION OF PLATES VII–X

Plate VII

Fig. 1. Rabbit inoculated on left cornea in last stages of resulting encephalitis. There is complete loss of equilibrium, the animal lying on the left side with head turned to the left.

Fig. 2. Rabbit inoculated into liver, showing complete paralysis in dorsal region and total disuse of hind legs.

Fig. 3. Rabbit inoculated into left adrenal gland, showing complete paralysis of abdominal muscles and total disuse of posterior extremities.

Fig. 4. Rabbit inoculated on skin of right hind leg, showing paralysis of same extremity.

Plate VIII

Fig. 5. Pons of rabbit inoculated on cornea of left eye, showing lesion of herpetic encephalitis peripherally on this side along descending portion of fifth cranial nerve.

Fig. 6. Section of spinal cord through lumbar region in rabbit inoculated on skin of left hind leg. Acute herpetic myelitis with lesion in left dorsal horn and dorsal tract on same side.

Plate IX

Fig. 7. Fifth cranial nerve, showing upper or intra-dural portion acutely inflamed and lower extra-dural portion containing little exudation. Lesion following multiple injections into corresponding masseter muscle.

Fig. 8. Section of sciatic nerve, showing acute cellular exudation following inoculation into muscles of corresponding leg followed by paralysis of this extremity.

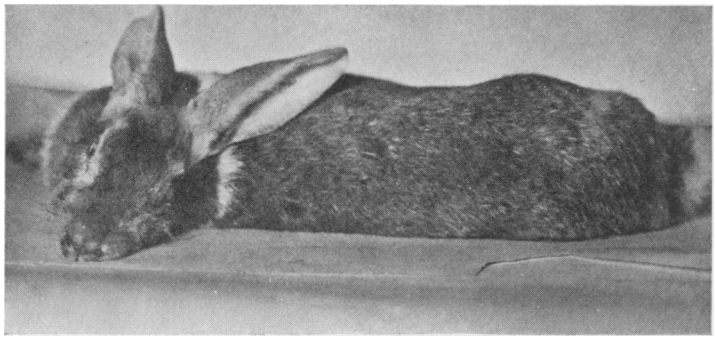
Fig. 9. Section of brain near infundibulum, showing acute herpetic lesion with numerous intranuclear bodies in neuroglial cells.

PLATE X

Fig. 10. Low power of right ventral horn of lumbar region of spinal cord, showing cellular exudate and destruction of ganglion cells.

Fig. 11. High power of 9, showing neuronophagia of motor ganglion cells, and intranuclear body, indicated by arrow, in neuroglial cell.

Fig. 12. Rabbit inoculated on right cornea, later developing encephalitis and stained with trypan blue. Brain cleared by Spalteholz's method shows distribution of herpetic lesion along fifth cranial nerve on right side.



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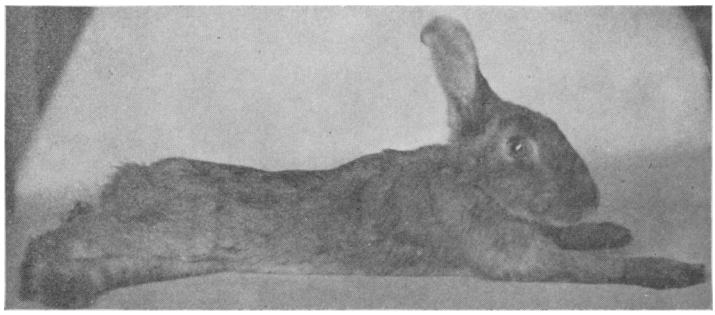

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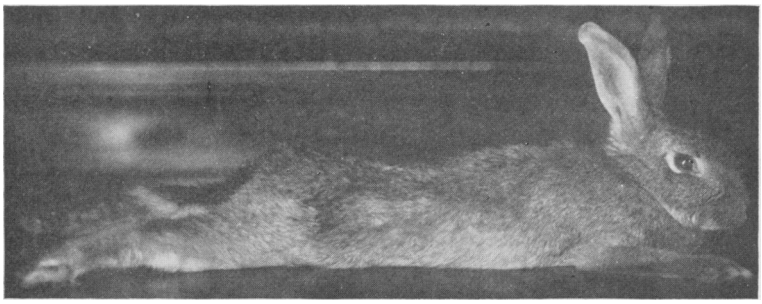

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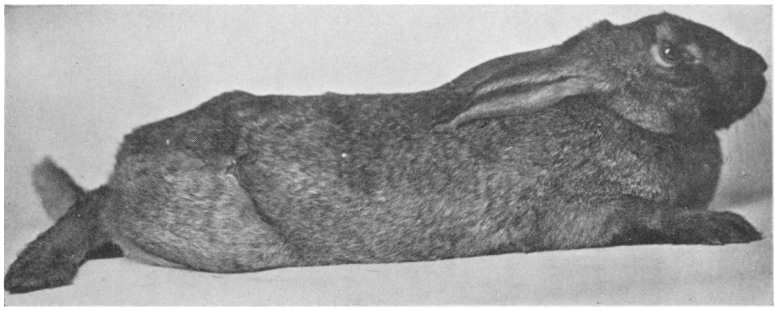

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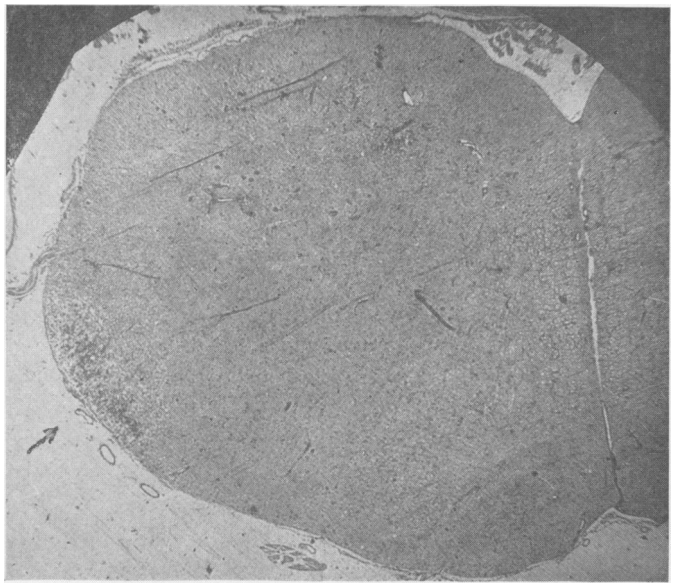

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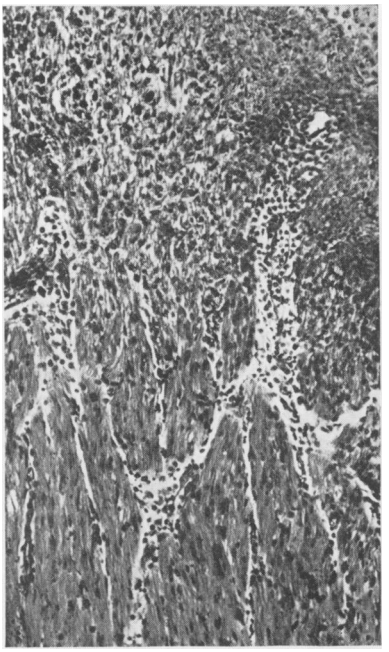

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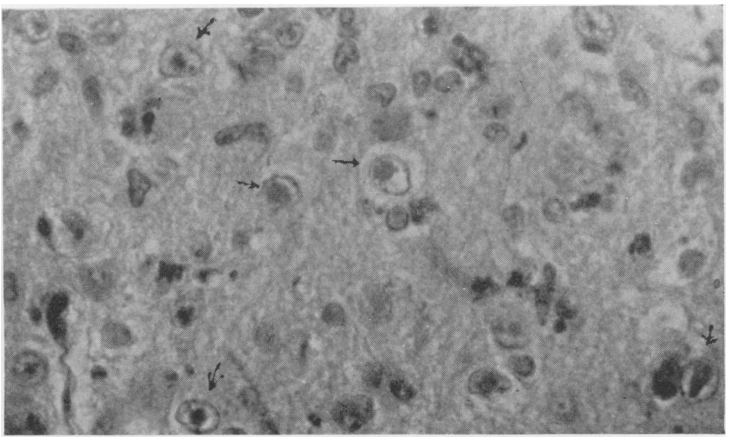

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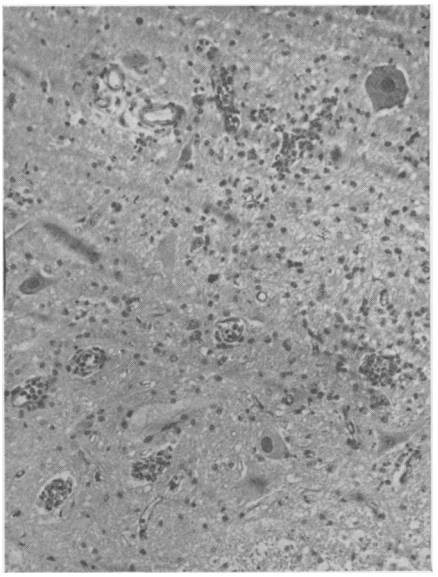

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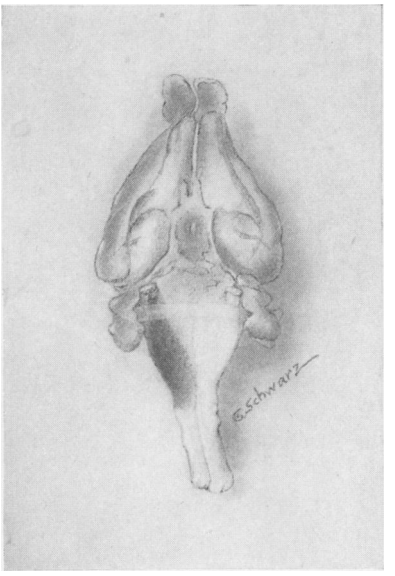

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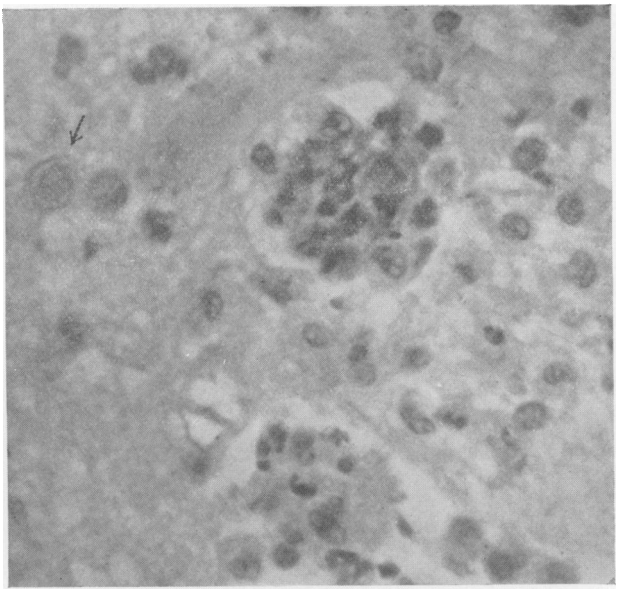

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