THE PATHOGENESIS OF HERPES VIRUS ENCEPHALITIS

I. Virus Pathways to the Nervous System of Suckling Mice Demonstrated by Fluorescent Antibody Staining

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The way in which viruses gain access to the central nervous system (CNS) in natural infection and after extraneural inoculation has interested pathologists and virologists since experiments with rabies in the early 19th century. Various theories of hematogenous and neural pathways have been proposed; the latter being the more popular until the recent reassessment of the pathogenesis of poliomyelitis gave impetus to theories of blood-borne CNS infection. The many studies on this problem now clearly establish that some viruses enter the CNS from the blood and others by centripetal movement in nerves. However, the "blood-brain barriers" to viruses are largely undefined, and the structures of peripheral nerve through which viruses move remain unknown. Regarding the neural route Burnet (1) has said, "... it is quite impossible to review the literature without accepting the existence of such movement and almost equally impossible to believe in its physical reality."

More precise studies of pathogenesis are now possible utilizing techniques of immunofluorescence by which specific cellular infection during the incubation period can be determined. However, this method has received only limited application in the study of the pathogenesis of encephalitis. With fluorescent antibody staining Liu and Coffin (2, 3) found canine distemper virus antigen in the cytoplasm of endothelial cells of small cerebral veins and capillaries, and in encephalitic cases, infection of cerebral parenchyma followed this vascular involvement. Similar findings, which could be interpreted as growth through endothelium of small cerebral vessels, have recently been reported with West Nile virus infections in mice (4). However, with tick-borne encephalitis virus Albrecht (5) found no fluorescence in vascular endothelium in experimental murine encephalitis, and to date no study has implicated this mechanism of hematogenous infection with a virus which is a major cause of human encephali-

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tis. Furthermore, no fluorescent antibody studies have been reported defining the pathway of spread to the CNS along nerves.

Herpes simplex virus was selected for study because it is an important cause of severe human encephalitis (6) and because different investigators have suggested that it may reach the CNS by either hematogenous or neural routes. This study combines fluorescent antibody staining with conventional histologic and titration methods to demonstrate the vascular and neural routes by which herpes virus enters and spreads within the CNS of immature mice.

Materials and Methods

Virus.—The HFEM strain of herpes simplex (7), originating from the classical Rockefeller HF strain (8), was used.

Mice.—Multicolored outbred mice of the Hall Institute strain were used. For maximal susceptibility to extraneural inoculation the youngest mice suitable for inoculation and histologic sectioning were selected; 4- to 5-day-old suckling mice proved most satisfactory and were used throughout this study.

Inoculations.—The standard diluent was 0.5 per cent gelatine in borate-buffered saline. Intracerebral and subcutaneous injections in 0.03 ml volumes and intraperitoneal injections in 0.05 ml volume were used. Intranasal inoculation was performed by dropping 0.025 ml of inoculum over the nares and holding the mice until the drop was inhaled. End points were calculated by deaths using the method of Reed and Muench (9) after daily observation of mice for 20 days.

Titrations.—Organ titrations were performed daily on pooled specimens from 3 animals. Mice from different litters were killed by exsanguination and organs removed aseptically. Whole blood and organs were prepared as 20 per cent suspensions and titrated in 10-fold dilutions on chorioallantoic membranes of 11-day-old embryonated eggs, using 4 eggs per dilution. Titers are expressed as pock-forming units (pfu) per gram of tissue or milliliter of whole blood.

Fluorescence Microscopy.—Tissues for fluorescent antibody staining were frozen in containers in a bath of liquid nitrogen and stored at −20°C. Sections (6 μ) were cut on a rocker microtome at −20°C. Peripheral nerves were examined in cross-sections of whole limbs; spinal cords were examined in cross-sections of vertebral column and adjacent muscles, so that the relationships of cord, ganglia, and nerve roots were preserved. After intranasal inoculation heads were skinned and lower jaws disarticulated before freezing, and sagittal sections of the entire heads were made. To facilitate sectioning, lymph nodes, nerves, and other small tissues were mounted in gelatin capsules (10) or liver before freezing.

Sections on glass slides were dried at room temperature for 1 hour, fixed in acetone for 10 minutes, and redried for 10 minutes; fixation in either formalin or methyl alcohol (at −60°C) and unfixed sections proved less satisfactory. Fluorescent antibody staining was done by the indirect method (11). Sections were treated with human herpes-immune serum for 20 minutes, washed in 2 changes of buffered saline for 20 minutes, and stained for 20 minutes with fluorescein-labeled goat anti-human γ-globulin (Microbiological Associates, Bethesda). Rhodamine-conjugated bovine albumin was added to the fluorescein conjugate as a counterstain. After a final washing for 20 minutes in two changes of buffered saline, sections were mounted in neutral glycerol and examined with a Zeiss microscope equipped for fluorescence observations and illuminated with an Osram HBO 200 high pressure mercury lamp.

Numerous human sera and antisera prepared in rabbits and roosters were tested before a serum giving adequate fluorescence was found. This quality was unrelated to neutralizing antibody titers as determined by pock neutralization tests. Several human and rabbit sera,

which were satisfactory for identifying infected cells in HeLa cell monolayers, did not give sufficiently clear definition of single infected cells against the non-specific fluorescence encountered in suckling mouse brain sections. Direct conjugates prepared from the test serum also failed to yield sufficient contrast in staining; and, therefore, the more sensitive (12) but more time consuming indirect method was used. This serum-conjugate system stained only cytoplasmic antigen, and there was no antecedent development of nuclear fluorescence as has been described with a different herpes-immune serum system (13). Specificity of staining was determined by staining normal tissues and by indirect staining of infected tissues with non-immune human serum. With experience non-specific staining was easily differentiated and actually proved a helpful adjunct in anatomical localization.

For confirmation of the cytology of infected cells fluorescence was recorded in photomicrographs with Ilford HP3 film, coverslips were floated off in 10 per cent formalin, and the sections were stained with hematoxylin and eosin or toluidine blue.

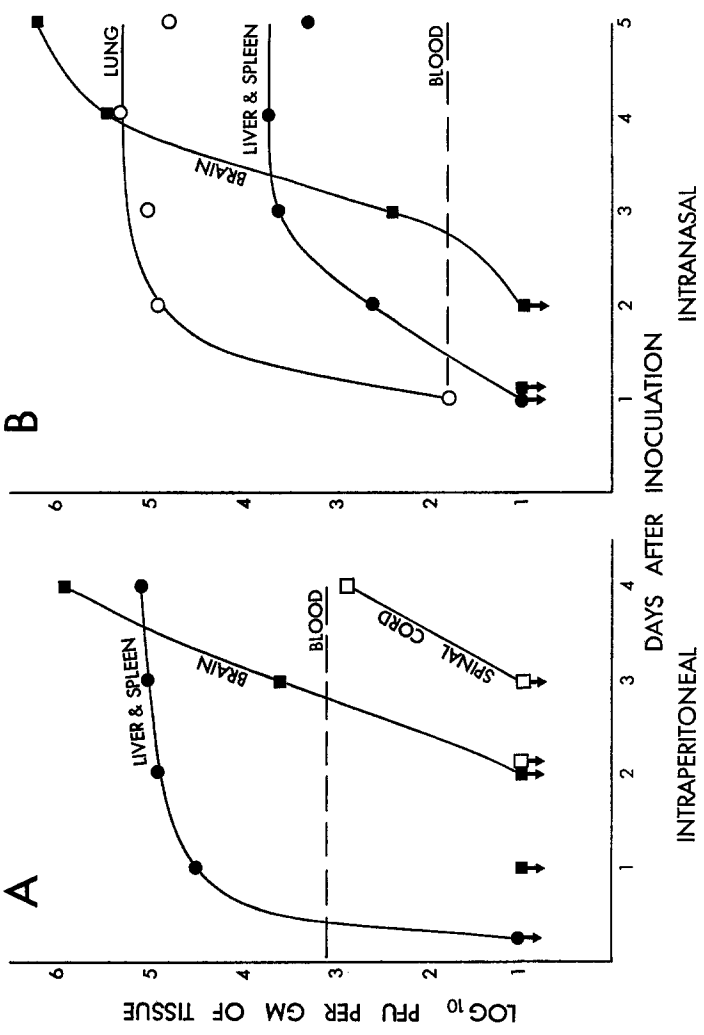
RESULTS

The pathogenesis of infections with the HFEM strain of herpes simplex virus in suckling mice proved different with varied routes of inoculation. Results with intracerebral, intraperitoneal, subcutaneous, and intranasal inoculation are, therefore, presented separately.

Intracerebral Inoculation.—Suckling mice were highly susceptible to intracerebral inoculation with HFEM, the LD_{50} being equal to only 3 pfu. After intracerebral injection of 100 LD_{50} mice remained well for 2 days. On the 3rd day signs of encephalitis developed, and mice died within a few hours. Virus titers rose rapidly in the brain to about $10^{6.7}$ pfu/gm by the 3rd day. There was no significant extraneural growth, although a few pfu of virus were irregularly recovered from blood, liver, or spleen.

Fluorescent antibody staining revealed virus antigen only within the CNS. Twenty-four hours after inoculation numerous cells in the leptomeninges (Fig. 1) and a few ependymal cells showed specific fluorescence. The rapid dispersion of virus in the cerebrospinal fluid (CSF) was shown by the infection of meninges of the caudal spinal cord at 24 hours. Infection of the cerebral meninges and and ependyma was extensive by 48 hours, and a few underlying parenchymal cells were fluorescent. At the end of the incubation period (72 hours) these subependymal and submeningeal foci had enlarged with widespread infection of both neural and glial cells. The cerebellum was less involved than the cerebrum despite confluent infection of the invaginations of leptomeninges between them. The choroid plexus was consistently free of infection. Only rarely were cells with specific apple-green fluorescence found near the needle track at the site of injection, although this track was surrounded by a green-orange non-specific fluorescence due to a concentration of cells from hemorrhage and inflammation. This non-specific fluorescence was present in control mice inoculated with diluent.

Thus, intracerebral inoculation resulted in dispersion of virus *via* the CSF with initial multiplication in the mesenchymal cells of the meninges and ecto-



TEXT-FIGS. 1 *A* and 1 *B*. Growth curves of HFEM strain of herpes simplex virus in 4- to 5-day-old suckling mice after intraperitoneal inoculation of 100 LD_{50} (1400 pfu) are shown in Fig. 1 *A* and after intranasal inoculation of 20 LD_{50} (50,000 pfu) are shown in Fig. 1 *B*. Mean virus titers per milliliter of whole blood are shown by broken lines. Although the similarity of growth curves suggests similar pathogenesis, the route of virus spread to the CNS was hematogenous in Fig. 1 *A* and primarily centripetal infection along cranial nerves in Fig. 1 *B*.

dermal lining of the ventricles. Infection then spread directly into the underlying CNS involving neurons and glial cells.

Intraperitoneal Inoculation.—Suckling mice were almost as susceptible to intraperitoneal as to intracerebral inoculation, the LD_{50} being only 14 pfu. After injection of 100 LD_{50} intraperitoneally mice remained well until the 4th day, when they developed signs of encephalitis and died within 24 hours. Viremia developed between 2 and 5 hours after injection and continued until death with titers ranging from $10^{2.5}$ to $10^{3.7}$ pfu per ml. Rapid virus multiplication occurred in liver and spleen, but virus was not detectable in the CNS until the 3rd day after inoculation (Text-fig. 1 *A*). This sequence of growth in viscera and viremia preceding encephalitis suggested blood-borne CNS infection, as has been shown with arthropod-borne viruses and polioviruses (14–16). However, Cooke *et al.* (17), using intravenous injection of herpes virus in rabbits, presented evidence that virus spread from infected viscera along afferent nerves to the spinal cord. Therefore, spinal cords were examined in the present study, and virus was found to appear later in the cord than in the brain (Text-fig. 1 *A*).

Fluorescent antibody staining showed antigen first in free macrophages washed from the peritoneal cavity 6 hours after inoculation. At 24 hours a few serosal cells on the hepatic and splenic surfaces and a few Kupffer cells and splenic red pulp cells were fluorescent. By 48 hours infection of the serosal surfaces of liver and spleen was almost confluent, and there were many foci of infection in the hepatic cells and in the red pulp of the spleen. These lesions were massive by 72 hours with central necrosis and loss of central fluorescence coincident with the leveling off of infectivity titers (Text-fig. 1 *A*). Foci of infection were occasionally found in kidneys, lungs, and adrenal cortex, but retrosternal lymph nodes draining the peritoneal cavity remained free of infected cells.

Foci of fluorescent neural and glial cells were found in the brain at 96 hours. Unlike infection after intracerebral inoculation, meninges and ependyma were not involved, and foci were randomly distributed deep in the cerebrum and cerebellum unrelated to neural tracts.

The initial growth in viscera, the late involvement of the spinal cord, and the distribution of cerebral lesions all suggested hematogenous spread of virus to the CNS. To confirm this hypothesis suckling mice were given 100 LD_{50} of virus intraperitoneally. Seventy-two hours later, under ether anesthesia, the vena cava was cut, and India ink was injected into the left ventricle under minimal pressure. By this method blood was replaced with India ink, and the capillary system was outlined. Multiple horizontal sections of brains were prepared. In 2 mice small foci of fluorescent cells were found, and in both, these were associated with small cerebral vessels (Fig. 2). These were the only foci found in the brains of these mice establishing that the initial virus growth in the CNS occurred in or around the endothelium of small cerebral blood vessels.

In view of reports that herpes virus penetrates the spinal cord *via* afferent nerves following intraperitoneal inoculation (17–19), cords and ganglia were examined. In 1 mouse with multiple hematogenous foci of infection in the brain and no lesions in the spinal cord, infected neurons in a thoracic dorsal root ganglion were found. Although the ganglion lesion may have been caused by blood-borne infection, the possibility exists of an alternate neural route of spread taking place at a slower rate than the more important hematogenous dissemination.

In summary, following intraperitoneal inoculation viremia developed within 5 hours and continued until death. Initial virus growth was found in free peritoneal macrophages and then along the serosal surfaces and within the parenchyma of liver and spleen. Infection of the CNS was blood-borne with virus antigen appearing first around small cerebral vessels.

Subcutaneous Inoculation.—Approximately 100 times as much virus is required to produce disease in suckling mice by subcutaneous inoculation as by intraperitoneal inoculation. Doses of 50,000 pfu were given subcutaneously in the hindfoot-pad to produce 100 per cent mortality. No local lesions developed in the foot, and the mice remained well for about 5 days. Then they became unable to walk and lay supine with back flexed and hindlegs kicking. Forelegs appeared normal and, unlike the encephalitis after intracerebral and intraperitoneal inoculation, no lethargy or convulsions developed. Disease lasted for 1 or 2 days terminating with hindleg paralysis and abdominal distension.

Viscera and blood obtained daily after inoculation failed to yield virus. Brains from moribund mice contained little if any virus. Fluorescent antibody staining of liver, spleen, and brain showed no virus antigen, and popliteal nodes dissected from 6 mice showed a small group of infected cells in only 1 node. No focus of local virus multiplication occurred at the injection site, but scattered elongated fluorescent cells were present in the subcutaneous tissue. In the same area small subcutaneous nerve fibers contained fluorescent cells. This infection of endoneural cells could be followed on serial sections to the main trunk of the sciatic nerve and into the corresponding dorsal root ganglia. The majority of these lumbar ganglion cells on the side of inoculation were fluorescent; many endoneural cells in the dorsal roots and a few cells in anterior roots were infected. The femoral nerve, which does not supply the inoculation site, contained no fluorescent cells.

Initial infection of the lumbar spinal cord was most marked in the ipsolateral posterior column corresponding to the entry zone of afferent fibers, but as infection spread within the spinal cord, neurons and glial cells in all quadrants were infected (Fig. 3). Fluorescent cells were more numerous toward the periphery of the cord, but no widespread involvement of meninges or central canal occurred to suggest spread *via* CSF.

Subcutaneous inoculation of foot-pads of the forelegs resulted in a similar

spread of infection through cellular elements of the nerves to the dorsal root ganglia and cervical spinal cord. Within the cord, infection extended caudally as well as rostrally. The early involvement of cervical cord and medulla in these mice resulted in a more acute disease, and death occurred 1 day earlier than in mice inoculated in the hindleg.

In summary, subcutaneous inoculation in foot-pads resulted in infection of a few subcutaneous cells (probably histiocytes) and the endoneural cells of subcutaneous nerve fibers. No viremia or visceral infection developed, and virus reached the CNS solely by centripetal infection of endoneural cells.

Intranasal Inoculation.—Susceptibility of suckling mice to nasal inoculation was similar to subcutaneous inoculation; inhalation of a droplet containing 50,000 pfu was necessary for 100 per cent mortality. Mice remained well for 4 or 5 days; then encephalitis developed with median time of death at 5 days. Organ titrations were similar to those obtained after intraperitoneal inoculation, but viremia was of lower titer ranging from less than $10^{1.3}$ to $10^{2.9}$ pfu per ml (Text-fig. 1 *B*). Initial rapid multiplication in the lungs was followed by hematogenous spread to the liver and spleen on the 2nd day. Again virus was not detectable in the CNS until the 3rd day after inoculation.

Fluorescent antibody staining showed foci of infection in the lungs, but the extent varied greatly from mouse to mouse. Although a portion of inoculum was undoubtedly swallowed, no antigen was found in mucosa of stomach or intestines. By the 4th and 5th day fluorescent hepatic and red pulp cells were present, but these hepatic and splenic foci never attained the size or frequency of those following intraperitoneal inoculation.

Stained sections of heads taken 2 and 3 days after inoculation showed areas of fluorescent nasal mucosa and fluorescent cells in the submucosa and in terminal nerve fibers. Fluorescent cells were not found within the CNS before the 4th day. Semiserial sections of heads of 9 mice with encephalitis were examined. Seven had extensive infection of cells in 1 or both trigeminal nerves, and in 3 this infection extended centrally into the brainstem, 5 showed direct viral invasion of the olfactory bulbs or their meninges, and 2 had multiple deep foci of CNS infection suggesting blood-borne infection. Of these latter mice, 1 had lesions in the ventral nucleus of the thalamus and in a cerebellar folium without involvement of cranial nerves or olfactory bulbs; the other had, in addition to infection of the olfactory bulbs, several foci in the cerebellum and one in the area postremata, despite the absence of other brainstem or trigeminal involvement.

The pattern of fluorescent cells showed two types of spread into the olfactory bulbs. In 3 mice infection extended directly from nasal mucosa and submucosal tissue through the meninges into the subarachnoid space; this gave rise to widespread infection of meninges similar to that seen after intracerebral inoculation. In 2 mice, however, there was infection of the olfactory bulbs without meningitis. In 1 of these mice fine lines of fluorescent cells were found traversing the

cribriform plate and meninges in association with olfactory nerve fibers (Fig. 4); in this mouse mitral and glomerular neuron layers showed more extensive infection than other cells of the olfactory bulbs (Fig. 5).

The many sagittal sections of heads prepared during the incubation period allowed a detailed study of the histology and chronology of infection along the trigeminal nerve. Antigen appeared to be limited to cells within the nerve (Fig. 6). After recording fluorescence on photomicrographs sections were restained with conventional stains to identify fluorescent cells; Schwann cells were primarily involved, but perineural, and possibly endoneural, fibroblasts were also infected. During the incubation period a centripetal progression of infection occurred with individual infected cells seldom more than a millimeter proximal to the area of major fluorescence. Fluorescence was not found in gasserian ganglion or in brainstem until the tide of infection reached these structures. Once the brainstem was penetrated, infection spread caudally and rostrally and even into the adjacent cerebellum.

Cross-sections of infected trigeminal nerves showed fluorescence scattered throughout the nerve and extending into the perineurium (Fig. 7). By superimposition of ultraviolet light and phase contrast photomicrographs, it was found that fluorescence was absent within visible myelin sheaths (Fig. 8); that is, no virus antigen was present in the large axons. Fluorescence often partially encircled the myelin sheaths further identifying infected cells as Schwann cells. Axons remained free of antigen even after extensive fluorescence was present in the perikaryon of the corresponding ganglion cells.

Thus, after intranasal inoculation virus gained access to the CNS by multiple neural pathways and by blood-borne infection. Direct invasion of the subarachnoid space with dispersion of virus in the CSF similar to the spread after intracerebral inoculation, infection of cells along nerves (both olfactory and trigeminal) similar to the spread after subcutaneous inoculation, and hematogenous infection similar to the spread after intraperitoneal inoculation were found. This diversity of pathogenesis may account for the apparently conflicting results obtained in previous studies of herpes virus encephalitis following intranasal inoculation (19–22).

DISCUSSION

These studies establish that herpes simplex virus can penetrate the CNS of suckling mice by both hematogenous and neural routes. Blood-borne infection was associated with virus multiplication in or around endothelium of small cerebral blood vessels; virus moved centripetally within nerves by infection of endoneural cells. The primary factors determining the pathway were the route of inoculation and initial site of virus growth, but often more than one pathway was utilized. Since these findings are contrary to many widely held theories on the pathogenesis of encephalitis, they will be discussed briefly in relation to previous studies.

A previous study employing fluorescent antibody staining in experimental herpetic encephalitis has been reported by Lebrun (13). Following intracerebral inoculation of mice, she described fluorescence along the needle track and in ependyma and failed to find infected neurons. Concentration of infection along the needle track would not be anticipated, since it has been shown that intracerebral inoculum is not deposited in cerebral tissue but dispersed in CSF with overflow into the blood (23, 24). After intracerebral inoculation of poxviruses and Murray Valley encephalitis virus, Mims specifically noted absence of fluorescent cells along needle tracks (24). In the present study infected cells were limited to the meninges and ependyma during the first growth cycle and were only rarely found along needle tracks thereafter. Contrary to Lebrun's findings, fluorescence of neurons was present throughout the CNS. CNS cells supporting virus growth vary with different viruses; fluorescent antibody staining in mice has shown that poxviruses multiply solely in meninges and ependyma (24), arthropod-borne encephalitis viruses only in parenchymal cells (4, 5, 24–26), and the highly selective fixed rabies virus only in certain neurons (27). Herpes simplex virus multiplies in all CNS cells, with possible exception of choroid plexus epithelium, and thus is similar to neurotropic strains of influenza virus (28, 29).

Fluorescent antibody staining has not previously been used to study the pathogenesis of herpes virus encephalitis following extraneural inoculation, but in the past 40 years many studies have been made with other methods. Doerr and Vöchting (30) first produced experimental herpetic encephalitis by corneal inoculation of rabbits. Since intravenous injection of virus produced similar encephalitis, they concluded that virus passed from cornea to brain *via* the blood. This theory, however, has received little support except for Anderson's (31) finding of inclusions in cerebral capillary endothelium in infected chick embryos and Field's (32) suggestion that vasomotor reflex might account for segmental CNS localization of lesions after extraneural inoculation of herpes virus. In the present study hematogenous spread of herpes virus to the CNS of suckling mice after intraperitoneal inoculation was clearly established by demonstrating the initial CNS infection around small cerebral vessels.

Theories of pathogenesis of viral encephalitis were long dominated by the concept that the CSF was the nutritive fluid of the brain and that substances must enter the CSF before gaining access to cerebral tissue; deep lesions within the brain would not, therefore, be blood-borne. Even after this theory was discredited, the dye studies showing permeability of cerebral capillaries in the area postremata, neurohypophysis, pineal, choroid plexus, and intercolumnar tubercle led pathologists to look for lesions in these areas if hematogenous infection was suspected; but no virus was found selectively causing lesions in areas of permeability. Hurst (33) first suggested that virus might "grow through" cerebral capillaries, a mechanism which would lead to a scattered or diffuse infection of the brain. Subsequent quantitative studies on inclusion

bodies in the capillary endothelium in cases of fox encephalitis supported this theory (34); and Coffin and Liu's (3) fluorescent antibody studies with canine distemper showing antigen in endothelial cells gave it graphic confirmation.

Further questions regarding the viral "blood-brain barrier" arise from the failure of most neurotropic viruses to cause encephalitis when inoculated intravenously (35). A period of extraneural growth appears to be prerequisite. This might be explained if virus giving rise to encephalitis were attached to or within cells, which then settle in small vessels and infect endothelium or pass through endothelium by diapedesis. In the present study the majority of herpes virus in blood after intraperitoneal inoculation was recovered from plasma, but small quantities were also recovered from washed blood cells. Studies to determine the relative role of blood cell–borne virus are needed to better understand the pathogenesis of hematogenous CNS infection.

Three years after Doerr and Vöchting's report of experimental herpetic encephalitis Goodpasture and Teague (36) and Marinesco and Draganesco (37) reported evidence for the neural transmission of herpes to the CNS, and this became the accepted theory of pathogenesis. Many of the early experiments are of questionable validity, since either very gross titrations of tissues or solely pathologic or clinical criteria were employed to determine pathways. Wildy (38) recently presented the first unequivocal evidence of centripetal spread of herpes virus within nerves by titrating nerves after foot-pad inoculation of mice.

How virus moves within nerves has remained a perplexing problem. Goodpasture and Teague (36) inoculated rabbits by varied routes and in different sites and found pathologic changes in corresponding CNS segments; they concluded that virus spread centripetally within axon cylinders, "not in sense of passive transport but by active reproduction." Herpes virus is now known to enter the nucleus before replication can take place, making this theory untenable (39). Sabin (40) working with the two related herpes viruses, B virus and pseudorabies, found no evidence of replication in the axons. Nevertheless, he concluded that virus may travel up the long axis cylinders before any multiplication occurs. The argument might be advanced that Schwann cell infection seen in the present study only mirrored this axonal progression, with cells being infected from non-replicating virus within the axon. However, the failure to find fluorescence in ganglion cells until the tide of infected endoneural cells reached them and the random spread of virus after reaching the spinal cord both militate against any transport within axoplasm. Indeed, above the cord segments initially infected, the ipsolateral dorsal column, which contains all of the presynaptic fibers from the infected peripheral nerve, was at times relatively free of infected cells (Fig. 3). Thus, there appeared to be no relationship between axons and the pathway of neural infection.

Although the theory of axonal spread has been popular for many years, it, like other theories, was not based on positive evidence but on the exclusion of

other pathways. The neural lymphatics were first incriminated in Marinesco and Draganesco's original study (37). However, subsequent work has shown neural lymphatic flow to be centrifugal unless the abdominal-subarachnoid pressure gradient is reversed by highly unphysiological procedures (41).

Another theory of neural spread has been that virus ascends *via* tissue spaces between nerve fibers. Wright (42) has presented convincing evidence that tetanus toxin penetrates the CNS *via* these interspaces and has concluded that viruses also may utilize this pathway. He refers to 3 possible *conduits*: axons, lymphatics, and tissue spaces. He rules out axons because of the viscous nature of axoplasm and lymphatics because a reverse flow of lymph would be needed. He, thus, implicates tissue spaces by exclusion, despite his own evidence that sclerosis of these spaces, which prevents tetanus, actually hastens the onset of herpetic myelitis in the rabbit. The present study clarifies this problem by showing that no *conduit* is necessary; cells within the peripheral nerve are infected, and infection spreads from cell to cell probably both directly and *via* the interspaces.

Although this mechanism of neural spread has not previously been proposed in the pathogenesis of herpes simplex virus infections, ascending infection of neural cells has been suggested in the pathogenesis of pseudorabies. With this closely related virus Hurst (43) found numerous inclusion bodies in Schwann cells and postulated an ascending interstitial infection rather than penetration *via* the axoplasm. Immunofluorescence studies have demonstrated infected endoneural cells with ectromelia (10) and tick-borne encephalitis virus (5) infections in mice; however, these findings were unrelated to pathogenesis of CNS infection since infection of olfactory nerve cells after inhalation of ectromelia did not lead to CNS infection and the generalized fluorescence of endoneural cells with tick-borne encephalitis virus infection followed CNS involvement and appeared to result from centrifugal infection.

It is of interest that Goodpasture (44), who founded the theory of axonal spread, raised the possibility of "ascending herpetic neuritis by propagation of the virus through cells of the neurolemma;" but he discarded this idea because pathologic changes were not prominent in the nerve. Restaining of highly fluorescent nerves in the present study confirmed the paucity of inflammatory reaction in infected nerves and absence of inclusions in infected Schwann cells, but this simply demonstrates the potential weakness of routine histologic methods in the study of viral pathogenesis.

SUMMARY

The pathogenesis of herpes simplex virus encephalitis and myelitis was studied in suckling mice using routine titration procedures and fluorescent antibody staining for the identification of infected cells. After intracerebral inoculation virus was shown to disperse rapidly in the cerebrospinal fluid (CSF),

multiply in meninges and ependyma, and then invade the underlying parenchyma infecting both neurons and glia.

Following extraneural inoculation virus gained access to the central nervous system (CNS) by both hematogenous and neural pathways. After intraperitoneal and intranasal inoculation virus was found to multiply in viscera and produce viremia; foci of CNS infection then developed around small cerebral vessels.

After subcutaneous and intranasal inoculation neural spread of virus was demonstrated along corresponding peripheral and cranial nerves. This spread resulted from the centripetal infection of endoneural cells (Schwann cells and fibroblasts). Antigen was not found in axons even after infection of the corresponding ganglion cell perikaryon. Subsequent spread within the CNS was unrelated to neural tracts, and there was no evidence of axonal spread of virus in the host-virus system studied.

These findings are discussed in relation to previous and current theories of the viral "blood-brain barrier" and neural pathways of infection.

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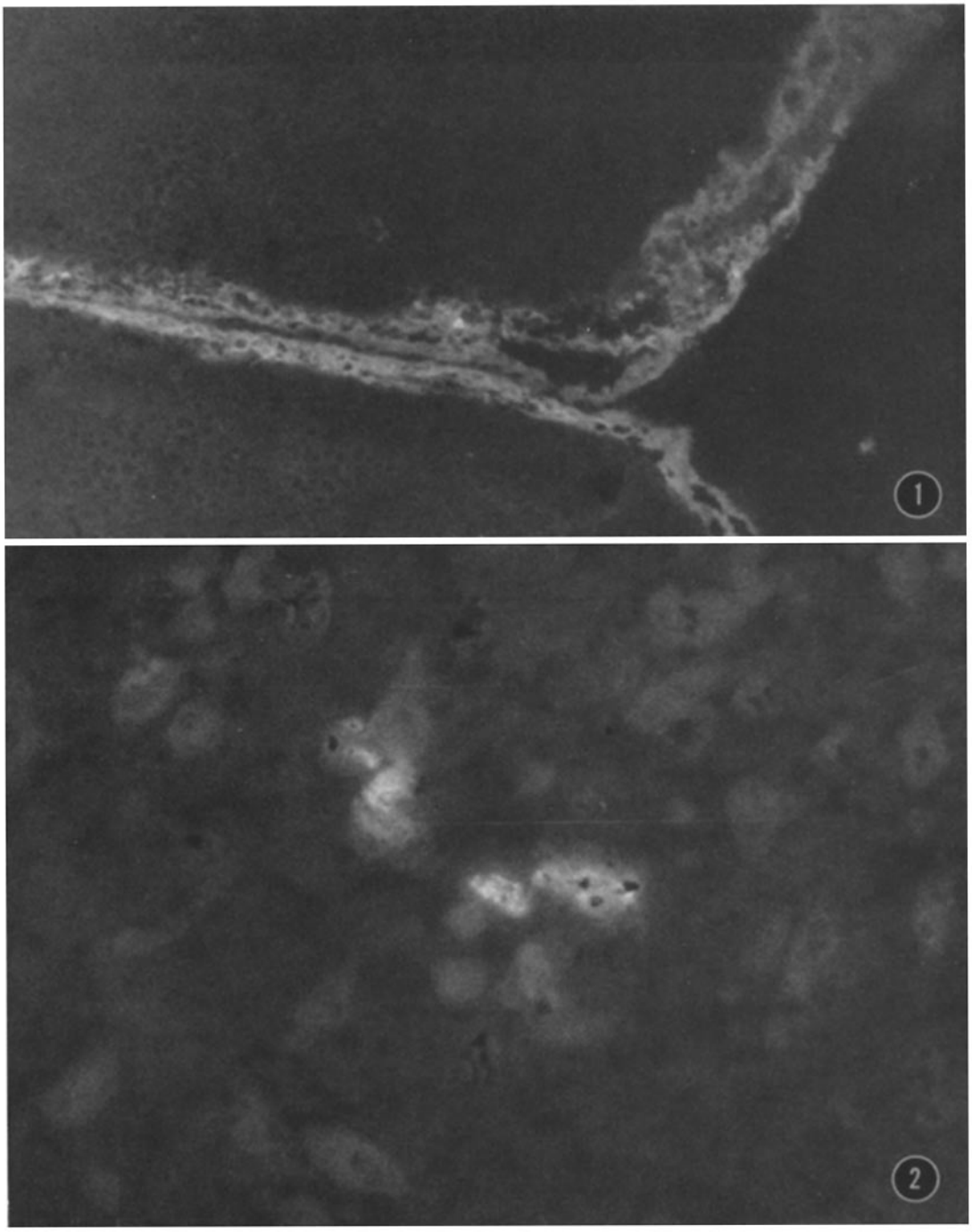
EXPLANATION OF PLATES

PLATE 37

FIG. 1. Horizontal section of brain of suckling mouse 24 hours after intracerebral inoculation of herpes virus. Fluorescence is limited to meningeal cells of the olfactory bulb (above) and frontal lobe (below); infection has not yet spread to underlying parenchymal cells. × 40.

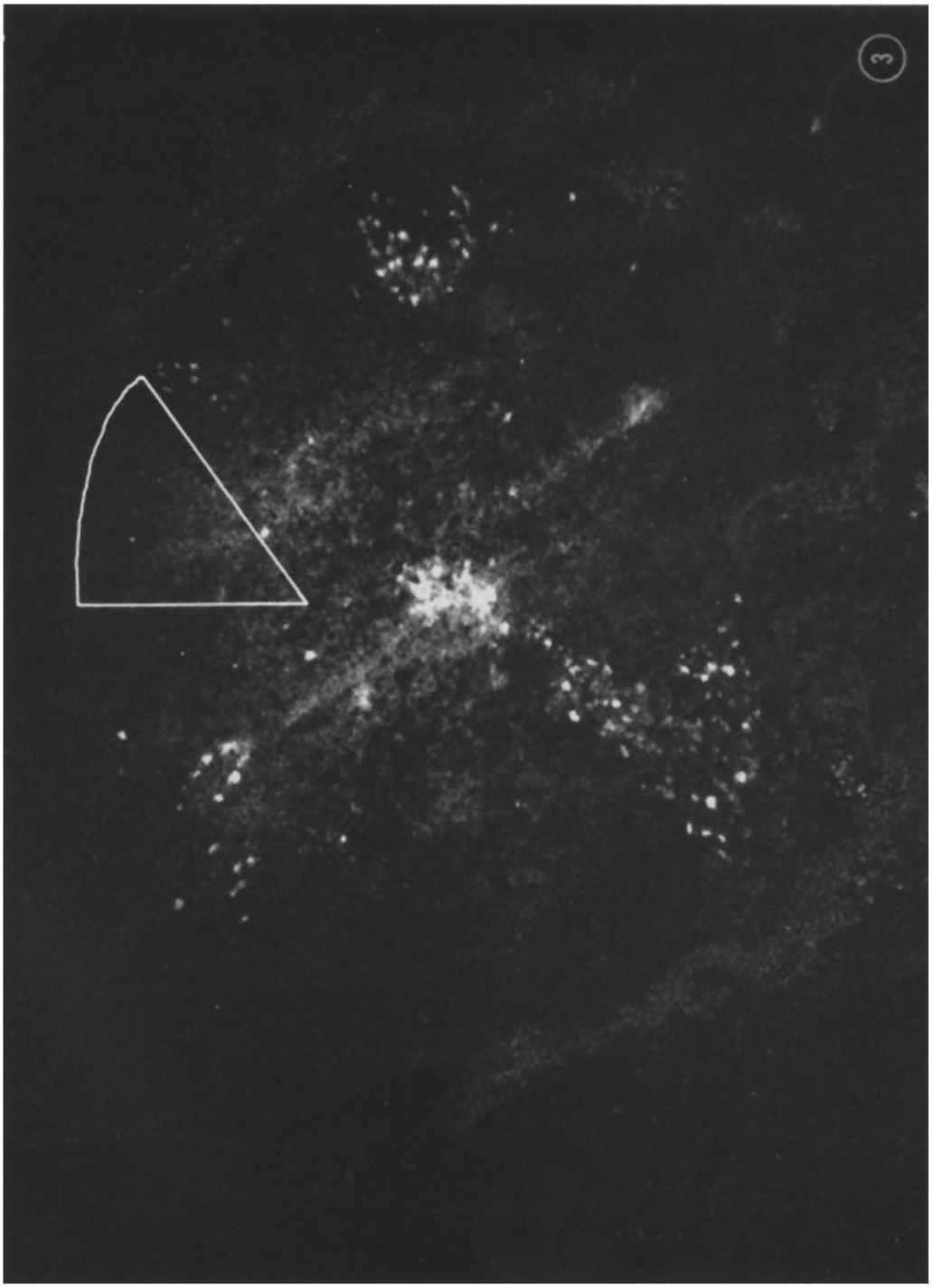
FIG. 2. Section of cerebral white matter 72 hours after intraperitoneal inoculation. Fluorescence is around small cerebral vessels which have been filled with India ink. No other fluorescent cells were found in the CNS of this mouse. × 520.



(Johnson: Pathogenesis of herpes virus encephalitis. I)

PLATE 38

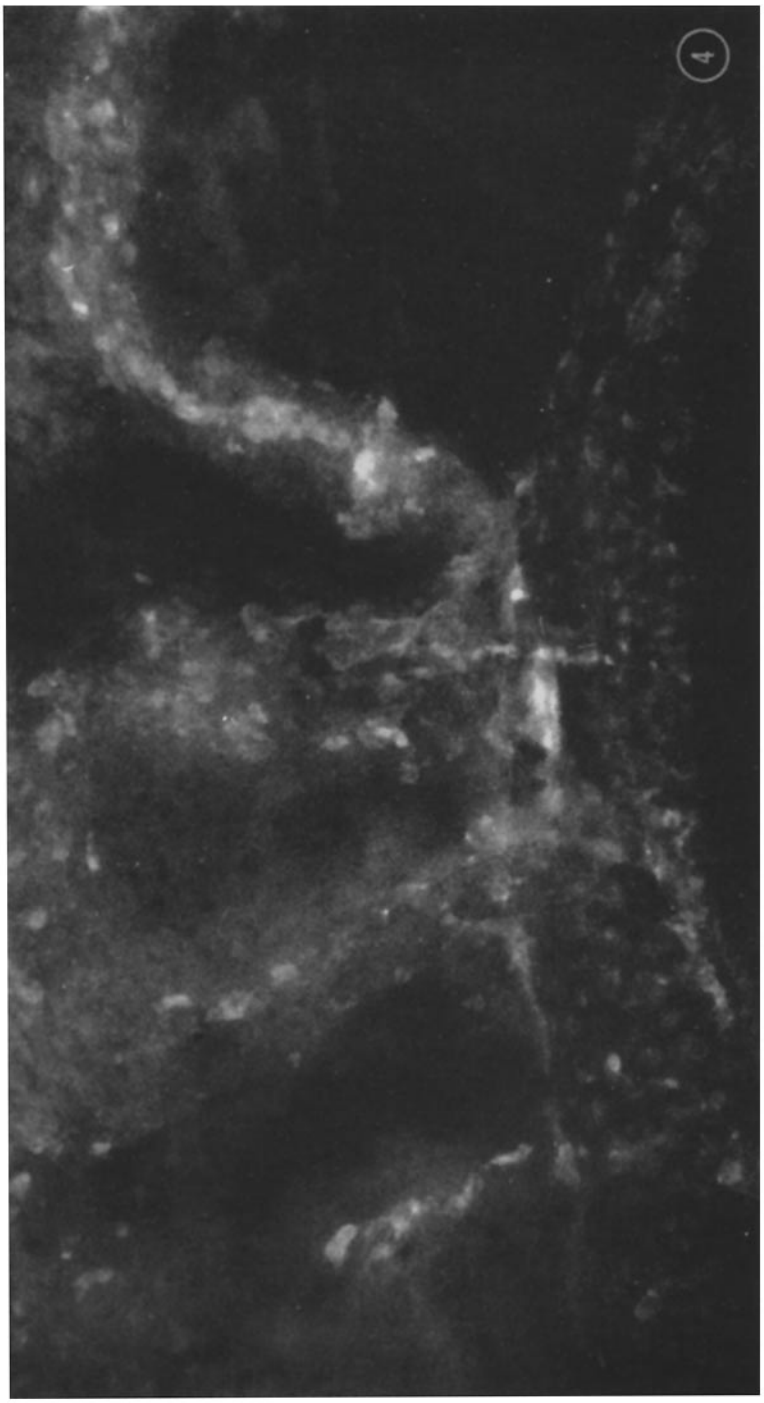
FIG. 3. Cross-section of thoracic spinal cord of suckling mouse 5 days after subcutaneous inoculation in the left hindfoot-pad. Fluorescent cells are present in all quadrants but are most numerous in the central grey matter and in the right anterior horn and white matter (lower left). The left dorsal column, the only tract of ascending presynaptic fibers, is outlined and is relatively free of fluorescent cells. Meninges are not infected. × 78.



(Johnson: Pathogenesis of herpes virus encephalitis. I)

PLATE 39

FIG. 4. Sagittal section across cribriform plate (below) and olfactory bulb (above) of suckling mouse 4 days after intranasal inoculation of herpes virus. Fluorescent endoneural cells are shown within the olfactory fibers traversing the cribriform plate and entering the bulb. × 200.

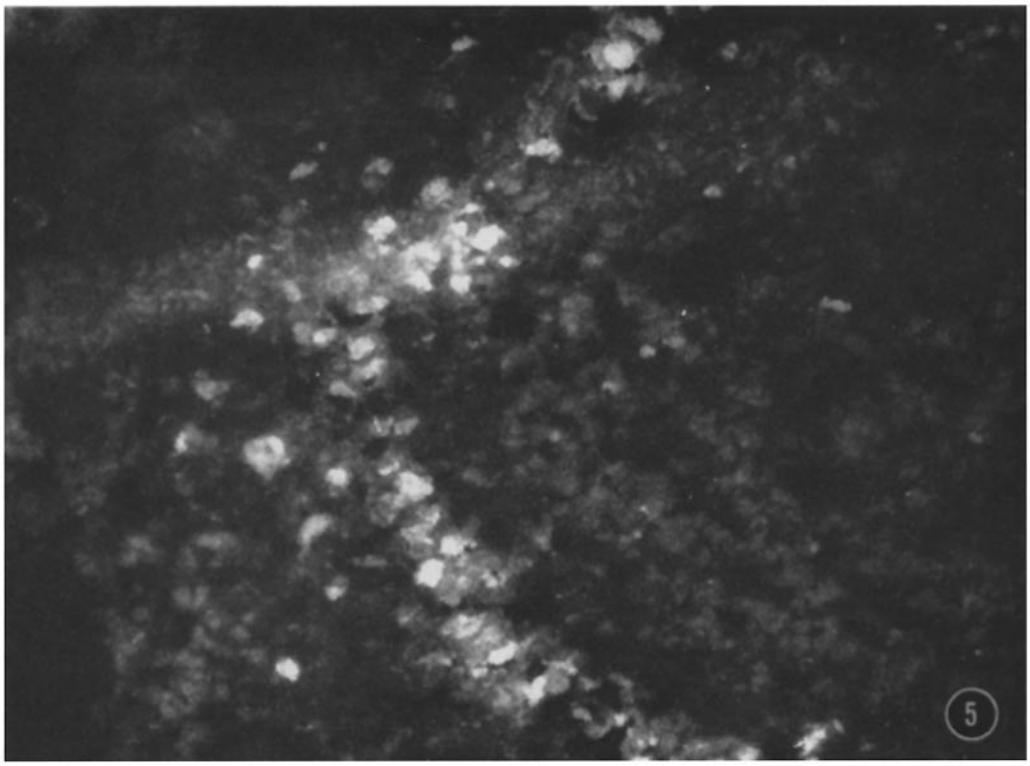


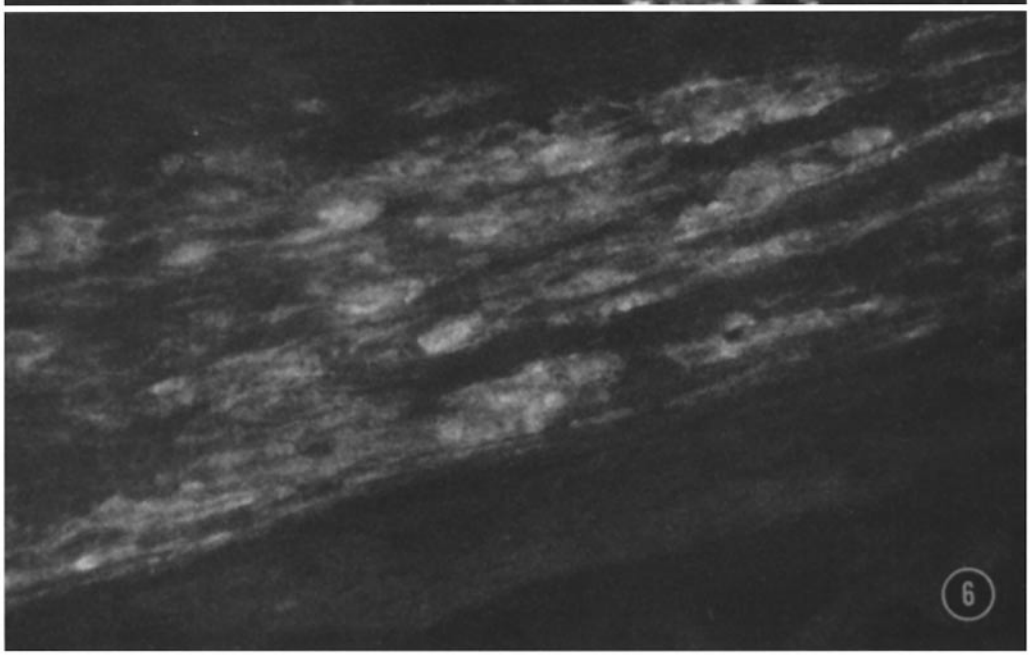
(Johnson: Pathogenesis of herpes virus encephalitis. I)

PLATE 40

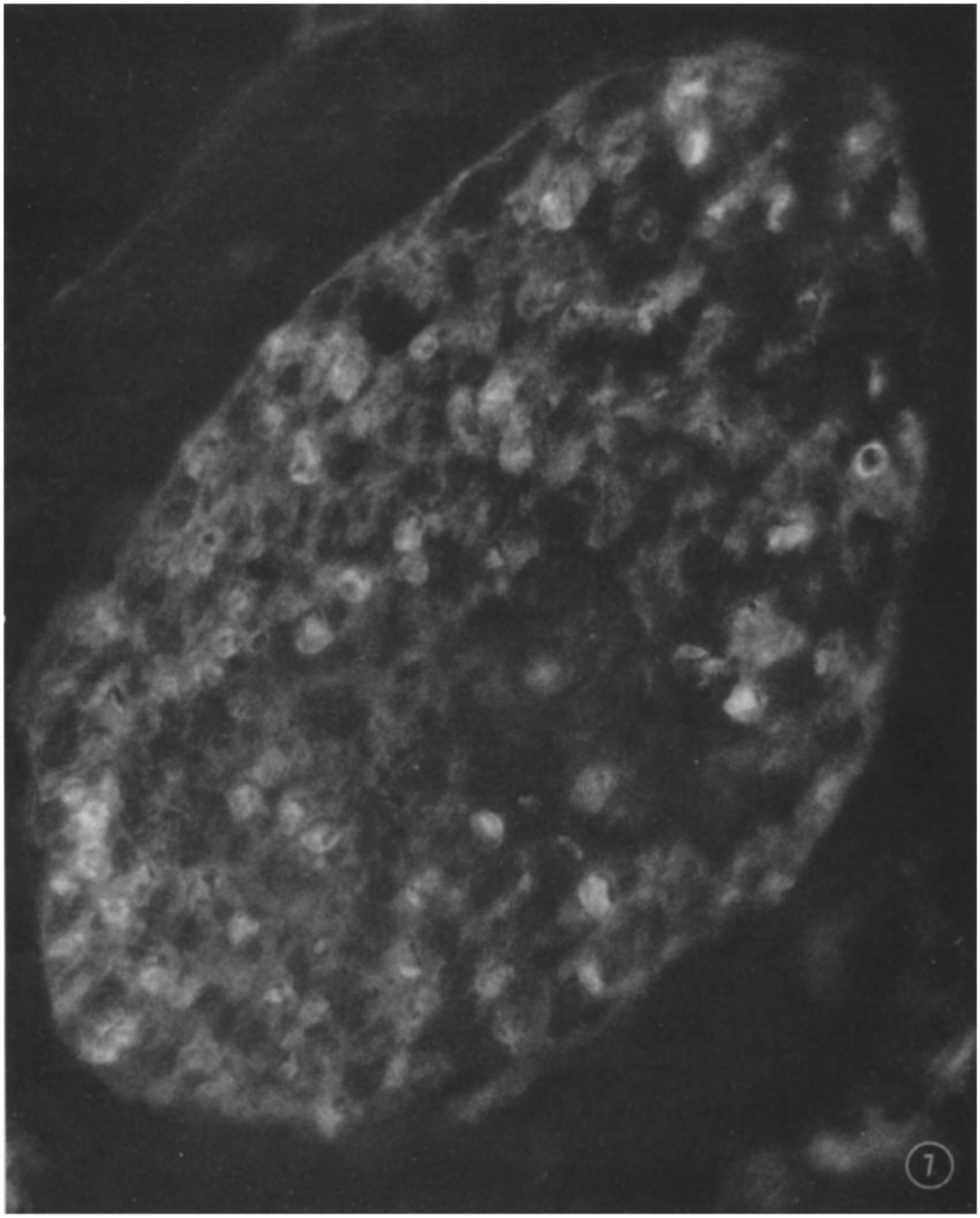
FIG. 5. Sagittal section of olfactory bulb of same mouse. Fluorescent cells are in the glomerular and mitral layers of neurons. × 200.

FIG. 6. Longitudinal section of trigeminal nerve proximal to the gasserian ganglion 5 days after intranasal inoculation. Fluorescence is shown in many endoneural cells; this appearance extended from the submucosal fibers to the brainstem. × 560.





(Johnson: Pathogenesis of herpes virus encephalitis. I)



(Johnson: Pathogenesis of herpes virus encephalitis. I)

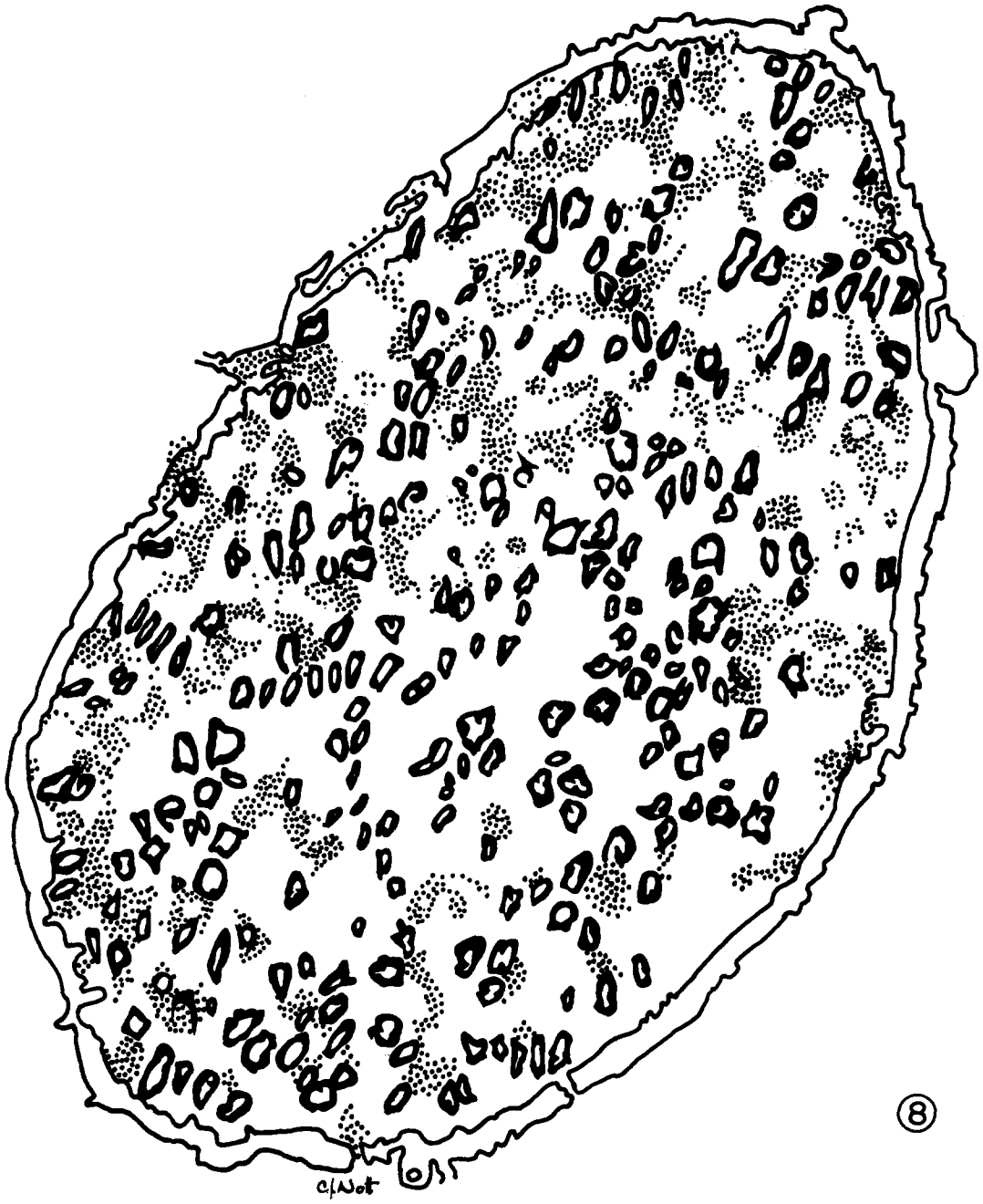


PLATE 41

FIG. 7. Cross-section of branch of trigeminal nerve distal to the gasserian ganglion 4 days after intranasal inoculation with herpes virus. Many fluorescent endoneural cells are shown. × 800.

PLATE 42

FIG. 8. Drawing from superimposition of facing photomicrograph with phase contrast photomicrograph of the same field. Myelin sheaths are shown by solid lines and areas of bright fluorescence by stippling. Fluorescence is shown to be outside of myelin sheaths but often partially encircling sheaths in the location of Schwann cells. Clear areas within fluorescent patches were identified by phase contrast as cell nuclei. Fluorescence can be seen to extend into perineurium along left side of field.