STUDIES ON HERPETIC INFECTION IN MICE

II. The Pathways of Invasion of the Central Nervous System after Intranasal Instillation of Virus in Suckling Mice*

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Plate 10

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Evidence presented in a previous report (1) showed that a passive immunity is capable of protecting suckling mice against herpes virus instilled intranasally. Because of the well known neurotropic potentiality of this virus in various host species, it seemed desirable to determine whether the central nervous system (CNS) of suckling mice is invaded by virus entering the organism by the intranasal route, as well as the pathway, or pathways, which might be elected by the virus in such an invasion.

At the time this study was undertaken, the literature contained no report of investigations of the pathogenesis of herpetic encephalitis in mice following infection by the intranasal route. While the work was in progress, Burnet and Lush (2) reported that herpes virus instilled into the nares of the mouse" ..... may reach the central nervous system through the fifth or one of the lower cranial nerves, but the olfactory bulbs are never infected." Using a different method than they, we have been able to confirm their results in part, and also to show that the virus may progress from the nasal mucous membrane to the brain in association with the olfactory nerves. It is of interest to note that the Australian workers employed the same strain of virus (HF) that we have used in all our experiments, and that both their substrain and ours were maintained by brain-to-brain passage in mice. On the other hand, they used only adult mice in investigating the pathogenesis of intranasal infection.

Materials and Methods

Mice, virus, and methods of handling the virus have been described in the previous report (1). The 100th mouse-brain passage of the HF strain was used in the present experiment.

Under ether anesthesia, 11 two-week-old mice were given intranasally 0.005 ml. of a 10 per cent suspension of mouse-brain antigen freshly prepared in Locke's solution. The mice were allowed to aspirate the material after it had been dropped upon

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the nares. At 24 hour intervals, from 24 to 96 hours inclusive, 2 mice were killed with ether and their tissues fixed for histological section. The skin was removed from the head, the mandible disarticulated, and the skull with its contents fixed *in toto*. Also fixed for section were the entire spinal column, thymus, heart, lungs, liver, spleen, kidneys, suprarenals, and stomach. Fixation was carried out in Zenker's formalin solution, the material embedded in paraffin and sectioned at 7 μ. The skull and spinal column were decalcified for a few hours in a mixture of dilute lactic and nitric acids before being embedded. The skull was sectioned serially, every 25th section being mounted and stained for study. Ribbons were saved for future reference. Sections of the spinal column were taken through the low cervical, high dorsal and mid-dorsal regions, and through the lumbar portion of the spinal cord. Single sections were made of each of the viscera. Sections were stained with hematoxylin-eosin. Visceral lesions are described in the third paper of the present series (3).

Of the 11 mice, 8 were killed and used for histological study. Each of the remaining 3 was dead on the 5th day, somewhat less than 120 hours after instillation of the virus; cultures of the brains and spleens for bacteria were sterile.

Pathogenesis of Nervous System Infection

Nasal Mucosa.—The primary attack of the virus after it has been instilled into the nares is upon the epithelium of the nasal mucous membrane. Involvement of the membrane is of a focal rather than a diffuse nature, and is not restricted either by the type of epithelial cell or its location. Lesions of the squamous epithelium of the anterior nares and of cylindrical cells in many situations are consistently seen. Moreover, portions of the olfactory mucosa are always attacked by the virus.

The earliest unequivocal change in the nasal membrane is the formation of intranuclear inclusions in squamous cells of the anterior nares 24 hours after the administration of the virus. At the end of 48 hours, the involvement of the membrane is more extensive with foci of varying size scattered indiscriminately. Some of the foci show desquamation of epithelium that is undergoing necrosis. Within the nasal passages there is considerable mucus in the meshes of which desquamated epithelial cells, some of them containing inclusion bodies, are not infrequently seen. Even at this stage the assault is not always superficial, and necrosis may extend to involve the glands and connective tissue of the submucosa. At the end of from 72 to 96 hours, the herpetic rhinitis is still more extensive (Fig. 1). Areas of ulceration have become larger and more numerous, submucous involvement is more marked, and additional foci of more recent occurrence are appearing. This last observation can be accounted for only on the basis of autoinfection; unquestionably the nasal discharges after the 48 hour period contain large quantities of the virus. The appearance of fresh lesions at from 72 to 96 hours in the nasopharynx, pharyngeal recesses, larynx, and upper esophagus also lends support to this conclusion.

Ganglionic Connections of the Nasal Mucosa.—The first histopathological

evidence of involvement of the nervous system does not appear until 72 hours after the virus has been instilled into the nares. At this time, inclusion bodies are seen in neurons in the Gasserian, cervical sympathetic (Fig. 3), and sphenopalatine ganglia (Fig. 4), and there is herpetic inflammation in one or both olfactory bulbs. The nature of the latter will be considered separately. In all 4 mice examined 72 hours or later after infection, either one or both Gasserian ganglia, and either one or both sphenopalatine ganglia showed herpetic involvement. Three of the 4 showed unilateral or bilateral involvement of the cervical sympathetic ganglia. There is a definite correlation between the absence of lesions in one nostril and the absence of ganglionic infection on the same side.

In the earliest lesions seen in the ganglia mentioned, the affected neurons usually constitute a small group, or groups, of adjacent cells. Although the presence of inclusion bodies in the nerve cells of the ganglia is the most striking initial finding, beginning disintegration and occasionally necrosis of a few neurons are apparent. In addition, a few glial cells in the immediate neighborhood of damaged neurons may also contain inclusion bodies. In animals examined 96 hours after infection, ganglionic lesions may have progressed to the point of necrosis (Fig. 2). The appearance at this stage often indicates that the infection has spread from cell to cell within the ganglia. There may be at this stage a localized meningitis overlying the affected Gasserian ganglia.

The extradural portions of the sensory branches of the trigeminal nerves were examined with care. They exhibit an unaltered appearance.

Olfactory Bulbs.—The lesions of the olfactory bulbs appear simultaneously with those seen in the ganglia. They were present in one or both olfactory bulbs of all 4 mice examined 72 hours or later after intranasal inoculation. They appear to be related to lesions of the olfactory portion of the nasal mucous membrane. Lesions are not seen in this membrane until 48 hours after instillation of the virus, and are somewhat more extensive 24 and 48 hours later. Histopathological evidence of herpetic infection of the olfactory membrane is found both in anatomical recesses and upon freely exposed surfaces.

In several situations, olfactory neuritis, as evidenced by round cell infiltration and necrosis, was seen in the extracranial portions of some of the olfactory rootlets. Intranuclear, herpetic inclusions could be identified in the neurolemma of these rootlets.

The lesions seen in the olfactory bulbs themselves are of a uniform type. They consist chiefly of mononuclear cell infiltration, varying degrees of necrosis, and the presence of occasional herpetic inclusions principally in cells of the neuroglial type. These evidences of inflammation are most prominent in the glomerular and external fibrous layers (Fig. 5). Where necrosis is conspicuous, polymorphonuclear leukocytes are more or less numerous. In 2 of the 4 brains examined after 72 hours, these changes were confined solely to the superficial portions of the olfactory lobes, and the mitral cells were completely spared.

The other 2 brains (one 72 hours and the other 96 hours after infection) both showed necrosis of the mitral cell layer, but in the 72 hour brain it was of limited extent. Inclusion bodies were seen in mitral cell nuclei in both these brains (Fig. 6). A localized mononuclear cell meningitis is usually seen overlying the diseased portion of the olfactory bulbs. It is of greatest intensity in the regions where an affected olfactory rootlet penetrates the meninges.

Other Lesions of the Brain.—Other than alteration of the olfactory bulbs, the only lesions seen in the brain are obviously the result of extension from the Gasserian ganglia. They make their appearance 96 hours after nasal infection, and are distributed along the 5th nerve pathway in the brain stem. Rather extensive necrosis of the sensory, trigeminal nuclei was seen in both the 96 hour brains. The specificity of the lesion was again attested by the finding of herpetic inclusions in affected areas.

Spinal Cord.—In our material, it was not possible to identify spinal cord lesions which could with certainty be attributed to extension of the virus from the cervical sympathetic ganglia. A section through the dorsal region of one of the cords examined 96 hours after infection presented, however, an interesting lesion. Portions of the corresponding ganglia of the thoracic sympathetic chain were included in this section. One one side, the sympathetic ganglion was unaltered; several of the neurons of the contralateral ganglion contained herpetic inclusions. At this level, there was obvious herpetic inflammation of the lateral horn of the cord on the same side as the deranged ganglion. Without much question, the lesion had resulted from establishment of the virus in a viscus and neural ascent to the cord *via* the sympathetic ganglion.

DISCUSSION

Before remarking the significance of the findings described in the foregoing paragraphs, it might be well to consider briefly some of the observations which have been presented by other observers on the pathogenesis of nervous system infection after intranasal introduction of herpes and "related" viruses into various hosts.

Gaviati (4), while investigating herpetic encephalitis following corneal inoculation in rabbits, found rather extensive lesions in the olfactory bulbs of animals allowed to die of the infection. Having noted that the rabbits frequently developed nasal discharge in association with the herpetic keratitis, he postulated that virus from the infected cornea reached the olfactory mucosa by way of the nasolacrimal duct and then pursued the olfactory nerves to the brain. He did not demonstrate the presence of virus in the nasal discharges. Gaviati's assumption, although ingenious, lacks experimental proof, for it is not possible to ascertain whether lesions present in the olfactory bulbs at death are the result of virus reaching them by way of the olfactory

nerves or by way of some focus previously established within the brain. An observation such as that of Da Fano (5) invalidates any assumption that might be made regarding the route of infection of the brain based on examination of the olfactory bulbs of rabbits allowed to die of encephalitis following intranasal introduction of herpes virus. Of one animal, which succumbed 12 days after receiving herpes by this route, he states: "Meningeal, perivascular, and parenchymatous infiltrations were present in every part of the brain from its oral end to the medulla oblongata." This difficulty was appreciated by Levaditi (6), who also noted lesions in the olfactory bulbs of rabbits allowed to die of encephalitis, but who found that surgical extirpation of the bulbs did not prevent the virus when given intranasally from reaching the brain. He suggested that the virus probably utilized the trigeminal and cervical sympathetic pathways in reaching the CNS from the nasal mucosa. In a subsequent publication (7), Levaditi was able, by sacrificing the animals before they showed clinical evidence of encephalitis, to demonstrate virus in the Gasserian ganglia at a time when it was not present in the olfactory bulbs. He concluded that the route preferred is that afforded by trigeminal nerves. Finally, Sabin (8), working with pseudorabies virus in 15-day-old mice, was able to present histopathological evidence that this virus pursued the trigeminal and cervical sympathetic pathways as well as that provided by the sphenopalatine ganglia in reaching the CNS. The recently published observations of Burnet and Lush (2) on herpes virus in mice have already been cited.

With these statements in mind, the finding that herpes virus in mice is able to progress from nose to brain in association with the olfactory nerves came as a distinct surprise. As inferred in the introduction to this report, the discrepancy in this respect between our observations and those of Burnet may be due to the differences in the age of the animals used in his experiments and ours. The natural resistance of adult mice brings this possibility to mind. Whereas of 64 adult mice given virus intranasally by Burnet, 17 per cent died, the mortality rate in suckling mice in our experiments (1) is close to 100 per cent. In just what manner aging might modify the olfactory pathway so as to prevent infection of the bulbs is at present unanswerable. It is possible, of course, that some other factor than the age of the host may be concerned.

Demonstration that the strain of herpes virus used in our experiments is encephalitogenic, that in suckling mice it reaches the brain from the nasal mucosa by neural routes, and, finally, that it is able to utilize the olfactory pathway adds considerable significance to the finding that a purely humoral immunity is capable of protecting mice of this age against virus given by this route. Experiments to determine whether mice can be passively protected against viruses which pursue the olfactory pathway exclusively are under way.

SUMMARY

Instilled intranasally into suckling mice, a mouse-passaged strain of herpes virus (HF) reaches the brain by both the trigeminal and olfactory pathways.

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EXPLANATION OF PLATE 10

All sections fixed in Zenker's formalin fluid and stained with hematoxylin and eosin.

FIG. 1. Anterior nares. *A*, herpetic vesiculation of stratified ephithelium. *B*, necrosis and desquamation of the epithelial lining of an adjacent paranasal sinus. 96 hours after intranasal instillation of the virus. × 100.

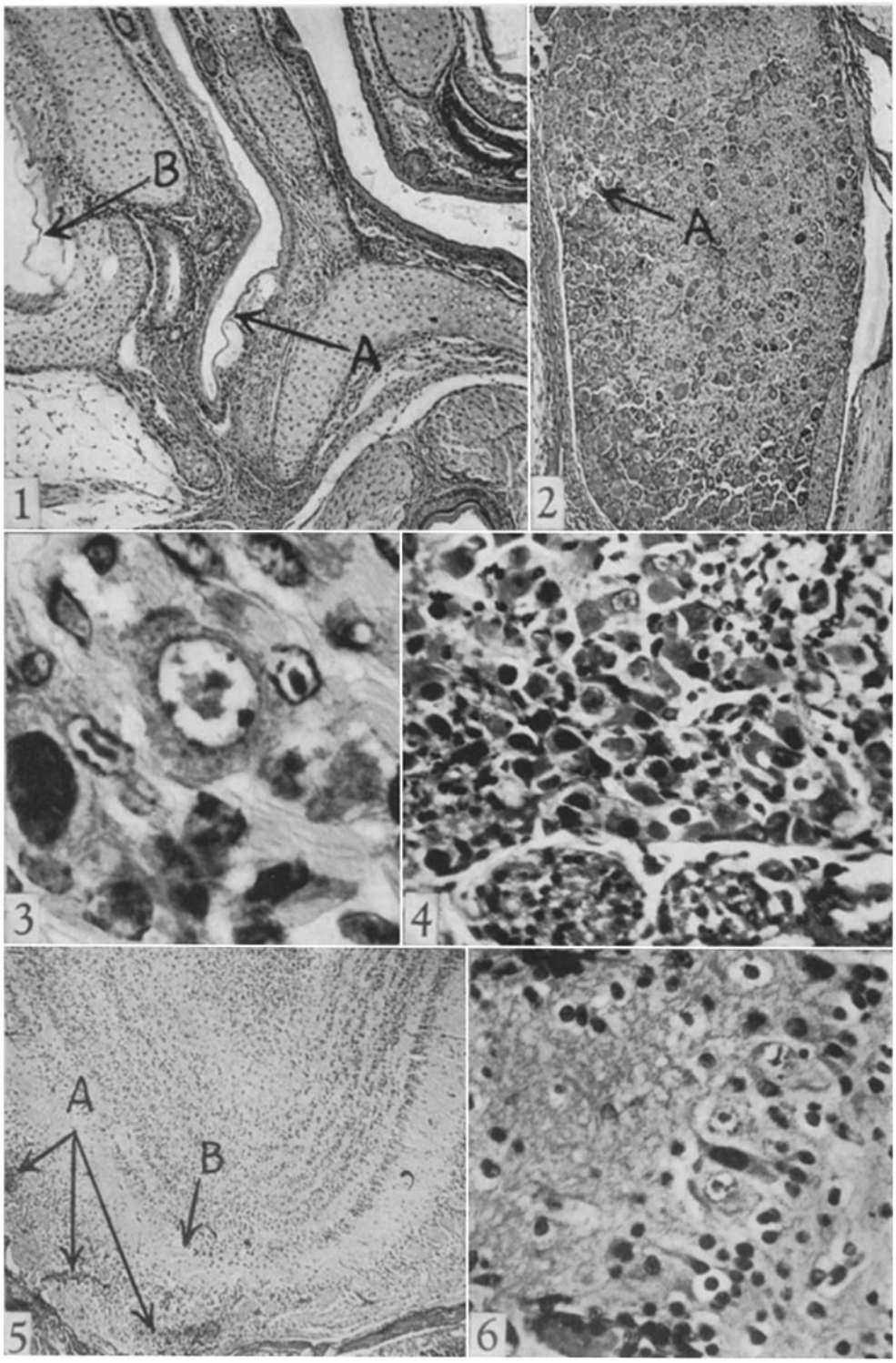
FIG. 2. Gasserian ganglion. *A*, necrosis of a small group of nerve cells. 96 hours. × 100.

FIG. 3. Cervical sympathetic ganglion. There are typical herpetic inclusion bodies in the nuclei of a nerve cell and of two adjoining glial cells. 72 hours. × 1500.

FIG. 4. Sphenopalatine ganglion. Several nerve cells and glial cells contain herpetic intranuclear inclusion bodies. Necrosis and cellular infiltration is already in evidence. 72 hours. × 430.

FIG. 5. Olfactory bulb. *A*, cellular infiltration of external fibrous and glomerular layers. *B*, dissolution of some of the mitral cells. 96 hours. × 60.

FIG. 6. Olfactory bulb. Herpetic inclusion bodies in mitral cells. 72 hours. × 430.



(Slavin and Berry: Herpetic infection in mice. II)