STUDIES ON HERPETIC INFECTION IN MICE

III. The Visceral Lesions in Suckling Mice*

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Plate 11

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In the course of a study of the pathogenesis of central nervous system infection after intranasal instillation of herpes virus in suckling mice (1), we have noted not only widespread distribution of the virus in the host, but also the production of specific lesions in a variety of organs and tissues. The extraneural lesions of herpetic infections have been the subject of considerably less investigation than those that are produced by the virus in nervous tissues.

The polycytotropic nature of herpes simplex virus in certain experimental animals is well known. By direct inoculation of various organs of the rabbit, Goodpasture and Teague (2) were able to demonstrate inclusion bodies in tissues derived from all three embryonic layers. On the other hand, the ability of the virus to invade these same tissues of the rabbit by way of the blood stream appears to be decidedly limited. However, Smith (3) was able to produce suprarenal lesions in rabbits by injecting relatively large amounts of the virus directly into the circulation.

The literature contains little information regarding the visceral lesions of herpetic, murine infection, and nothing regarding the visceral lesions produced by the virus in mice during the first weeks of life. Levaditi and Nicolau (4) injected an epithelial tumor of a mouse with rabbit-passaged virus. Thirteen days later, triturated fragments of the tumor proved capable of evoking corneal lesions in rabbits, or encephalitis in rabbits when given by the intracerebral route. Luger and Silberstern (5) noted herpetic inclusions in epithelial cells of a calyx after injection of the virus into the kidney of the mouse. Luger and Lauda (6) described herpetic inclusions in mouse liver after intrahepatic inoculation. Andervont (7) found the virus to be present in kidney, spleen, and rarely in liver, as well as in the brain, in murine infection by various routes, but reported no histopathologic observations of these organs.

Materials and Methods

The 100th passage of the HF strain of herpes simplex virus maintained by brain-to-brain inoculation in mice was used. A description of the methods of handling the virus, inoculating animals, etc., may be found in a previous report (8). In another communication (1) we have recorded observations of the pathogenesis of central nerv-

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ous system infection by herpes virus instilled intranasally in 14-day-old Swiss mice. It was stated in that report that certain of the viscera of each of 2 of these mice were fixed in Zenker's formalin solution at the same intervals at which the central nervous system was removed for examination: every 24 hours from 1 to 4 days after intranasal inoculation. Single sections were made of each of these viscera, the histopathology of which forms the basis of the present report. Sections were cut at 7μ and stained with hematoxylin-eosin.

In addition, 3 two-week-old mice were allowed to die after intranasal infection and their viscera removed and treated in the same fashion. Finally, 3 two-week-old mice were given 0.1 ml. of 10 per cent mouse-brain antigen intraperitoneally. After death, their tissues, including sections at various levels of the brains and spinal cords, were fixed for examination.

OBSERVATIONS

It was found by preliminary experiment that following aspiration into the nares of a tiny drop (0.005 ml.) of a virus suspension, the virus appears promptly in the lungs of 2-week-old mice, that it can be detected in the blood within 24 hours, and that thereafter it is constantly present in the circulation until the death of the animals from 96 to 120 hours after inoculation. The experiment was repeated several times with uniform results.

The criterion for identifying lesions in our material as herpetic was the intranuclear inclusion body. The inclusion ranges somewhat in its appearance from the more frequently occurring, finely granular, distinctly acidophilic variety which is usually located centrally in the nucleus, and exhibits a clear, unstained zone between itself and the nuclear membrane, to a variety occurring less frequently but not rarely in which the inclusion is more homogenous, faintly basophilic, and fills the entire nucleus. In both varieties the nuclear chromatin and the nucleolus, if one be visible, are marginated.

In prenecrotic lesions in several tissues, the cytoplasm of cells, nuclei of which contained inclusion bodies, was also altered. The cytoplasm of such cells was swollen, and unstained. Although discernible in almost every tissue in which an early lesion was present, the cytoplasmic change was well marked in stromal cells of the lungs.

Results of Intranasal Inoculation

Lungs.—Virus aspirated into the lungs produced a focal, interstitial pneumonia (Fig. 1). A mononuclear exudate becomes apparent 24 hours after inoculation. Inclusion bodies appear at 48 hours chiefly in stromal cells (Fig. 2). It is strange that despite the rather marked involvement of the lung parenchyma, inclusions occur but rarely in the epithelium of the bronchi and bronchioles. That the pneumonia results from aspiration of the virus is shown by the fact that none of the mice injected by the intraperitoneal route developed lesions in the lungs.

Liver.—Lesions are apparent in all the animals examined 48 hours and later

after infection. The earliest lesions seen consist of inclusion bodies in the nucleus of a Kupffer cell (Fig. 4) and in those of several adjacent cord cells. It would appear, therefore, that virus in the blood stream attacks visceral endothelium from which it spreads to the parenchyma. Inclusion bodies were found occasionally in the nuclei of the endothelium of portal venules.

Later, lesions in the liver consist of the familiar foci of necrosis (Fig. 3), some of which are hemorrhagic. The virus, once it has attained the parenchyma, appears to spread from cell to cell along the cords. In some mice a very considerable portion of the hepatic substance is necrotized. Even wandering cells, both mononuclear and polymorphonuclear, are involved by the necrotizing process.

Spleen.—Splenic lesions were present in 4 of 6 mice examined 48 hours after infection and later. The reticulum is principally attacked. Unlike early lesions elsewhere, the initial cellular response is predominantly polymorphonuclear. As elsewhere, the end-result is necrosis.

Suprarenal.—Lesions were seen in 2 of 6 glands from 6 mice examined 48 hours after infection and later. In both instances, the lesions were confined to the cortex (Fig. 7). In some of the mice allowed to die of herpetic infection, almost the entire gland was necrotic, indicating that the medulla is not immune.

Kidney.—A single kidney showed herpetic involvement. It was taken from one of the 2 mice killed 96 hours after inoculation, and contained but a single lesion situated in the medulla. There was inclusion-body formation and necrosis of the epithelium of a portion of a few collecting tubules. Although adjacent capillary endothelium contained herpetic inclusions, the parenchymal lesion was the more advanced, thus making it seem probable that the primary infection of the kidney was uriniferous rather than blood-borne. All the sections of kidney were searched for evidence of glomerular involvement, but none was found.

Lymph Nodes.—Lesions were seen with a fair degree of consistency in cervical, mediastinal, and retroperitoneal lymph nodes. As in the spleen, it appeared to be the reticulum that was primarily involved. In several nodes, necrosis was present only along marginal sinuses. Lymphocytes were involved by the necrotizing process, but none was seen to contain an inclusion body.

Gastro-Intestinal Tract.—Lesions of the epithelium of the upper esophagus were seen in several instances, the result, no doubt, of swallowing the virus.

Single sections of the stomachs of all mice were examined, but no lesions were found.

Heart.—No lesions present.

Thymus, Thyroid, and Hypophysis.—No lesions present.

Results of Intraperitoneal Inoculation

The distribution of lesions following intraperitoneal inoculation is not substantially different from that following infection by the intranasal route,

the notable exception being the absence of pneumonic lesions. In a single mouse, an ovary showed herpetic inflammation, but this seemed to have taken place by direct extension from the peritoneum. In this same animal, a section taken through a rib showed rather extensive necrosis of the marrow (Fig. 5). A few of the better preserved marrow cells contained inclusion bodies (Fig. 6). In none of a considerable number of sections through vertebrae, ribs, and skull of the mice inoculated intranasally was involvement of the marrow noted. One mouse injected intraperitoneally showed a medullary renal lesion similar to that already described.

GENERAL DISCUSSION

A study of the visceral lesions allows making certain added deductions in regard to the pathogenesis of herpetic infection following intranasal instillation of the virus in suckling mice. The virus inoculated intranasally under ether anesthesia is aspirated into the lungs where it causes interstitial inflammation. It reaches the blood stream in less than 24 hours, probably from both the lungs and the nasopharynx, and, disseminated by this route, produces lesions in several of the solid viscera. Lymph, draining infected areas, carries the virus to regional nodes where characteristic lesions are again produced. Anderson (9) has demonstrated the capacity of herpes simplex virus to produce visceral lesions in the chick embryo. She has also shown that these lesions are the result of infection of vascular endothelium by virus in the circulating blood. Observations included in the present report lend support to her finding of endothelial invasion as a mode of metastasis. It is undoubtedly important that in our material visceral lesions resulting from blood-borne infection occur, for the most part, in organs in which the endothelium is highly phagocytic. This finding provides a probable explanation for the fact that the brains of mice inoculated by the intranasal route are infected only along neural pathways from the nasal membrane, and are utterly devoid of evidence of blood-borne metastasis (1). It might be added that in the sections taken at various levels through the brains of the mice allowed to die after infection by large doses of the virus given by the intraperitoneal route, there was a complete lack of histopathological evidence of encephalitis or of vascular damage.[1]

The immediate goal of the present studies has been achieved. In view of the susceptibility of nervous tissue to herpes virus, the discovery that a passive immunity consistently protects suckling mice against a lethal dose of the virus

[1] There was evidence of myelitis in these mice. In some instances, involvement of the spinal cord could be traced directly to a paravertebral, sympathetic ganglion. It thus appears that the virus first establishes itself in visceral foci and ascends autonomic nerves to reach the spinal cord. This course of events has recently been shown to take place in the rabbit following the introduction of herpes virus into the circulation (10).

instilled intranasally (8) seemed to be significant. A study of the pathogenesis of herpetic infection of the central nervous system following primary intranasal infection (1) has established that significance. The finding that herpes virus pursues the olfactory pathway (in addition to other nerve routes) from the nasal mucous membrane to the brain makes intranasal infection of the suckling mouse a critical test of the efficacy of a purely passive immunity. One has only to recall the anatomical arrangement in the nasal membrane of the olfactory receptor cells and their processes in order to appreciate this.

Most of the previous experimental work on the prophylactic value of immune serum in virus diseases of the nervous system, resulting from primary infection of a peripheral focus, has been concerned with prevention of infection by the virus of poliomyelitis. Many years ago, Flexner and Lewis (11) presented limited evidence to show that passive prophylaxis was effective against poliomyelitis in the monkey when the virus was given by the intranasal route. A number of years later, Schultz and Gebhardt (12), as the result of a more extensive study, concluded that the general trend of their experimental results indicated that immune serum is far from being a dependable prophylactic agent against poliomyelitis. However, many of their animals were infected by direct, intracerebral inoculation of the virus. There were included in their experiments 26 animals in which the virus was introduced intranasally; of these, 57.7 per cent survived. Of 15 control animals similarly inoculated, 13.3 per cent survived. Theiler (13) in a consideration of some of the aspects of poliomyelitis did not reject the presence of antibodies as important in the mechanism of defense against this disease. Finally, Howe and Bodian (14), as a result of their studies of experimental poliomyelitis, have stated: "We have offered reasons for believing that humoral mechanisms may be more significant in protecting the host at the portal of entry, before virus has entered into insulated combination with nervous tissues."

It would be injudicious to attempt to translate the present findings too literally into terms of naturally occurring virus diseases of the nervous system. However, considering the constancy of the principles of immunity, it does seem to be a reasonable forecast to say that it will prove possible to prevent other experimentally induced infections of the nervous system by means of passive immunization, providing the prospective host acquires sufficient antibody, and the primary infection is initiated peripherally, in a manner not too foreign to the genesis of the naturally acquired disease.

SUMMARY

Intranasal instillation of herpes virus in suckling mice results in specific lesions widely distributed in the viscera. The lungs are infected by aspiration of the virus. Virus disseminated by way of the blood establishes itself in endothelium in certain situations where parenchymal lesions result by direct

spread from the vascular foci. Evidence of blood-borne infection was found frequently in the liver and spleen, less frequently in the suprarenals, and, in one instance, in the bone marrow. Renal infection appeared to be uriniferous. Lymph carriage of the virus also occurs, and lymph nodes draining infected areas were often found to contain herpetic inclusion bodies. Herpes virus seems incapable of invading the central nervous system of suckling mice by the vascular route.

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EXPLANATION OF PLATE 11

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

FIG. 1. Lung. Focal, interstitial pneumonia due to aspiration of herpes virus. 48 hours after intranasal instillation of the virus. × 100.

FIG. 2. Same as Fig. 1. Herpetic, intranuclear inclusion bodies in stromal cells of the alveolar interstices. The cytoplasm of the affected cells is greatly swollen. × 1500.

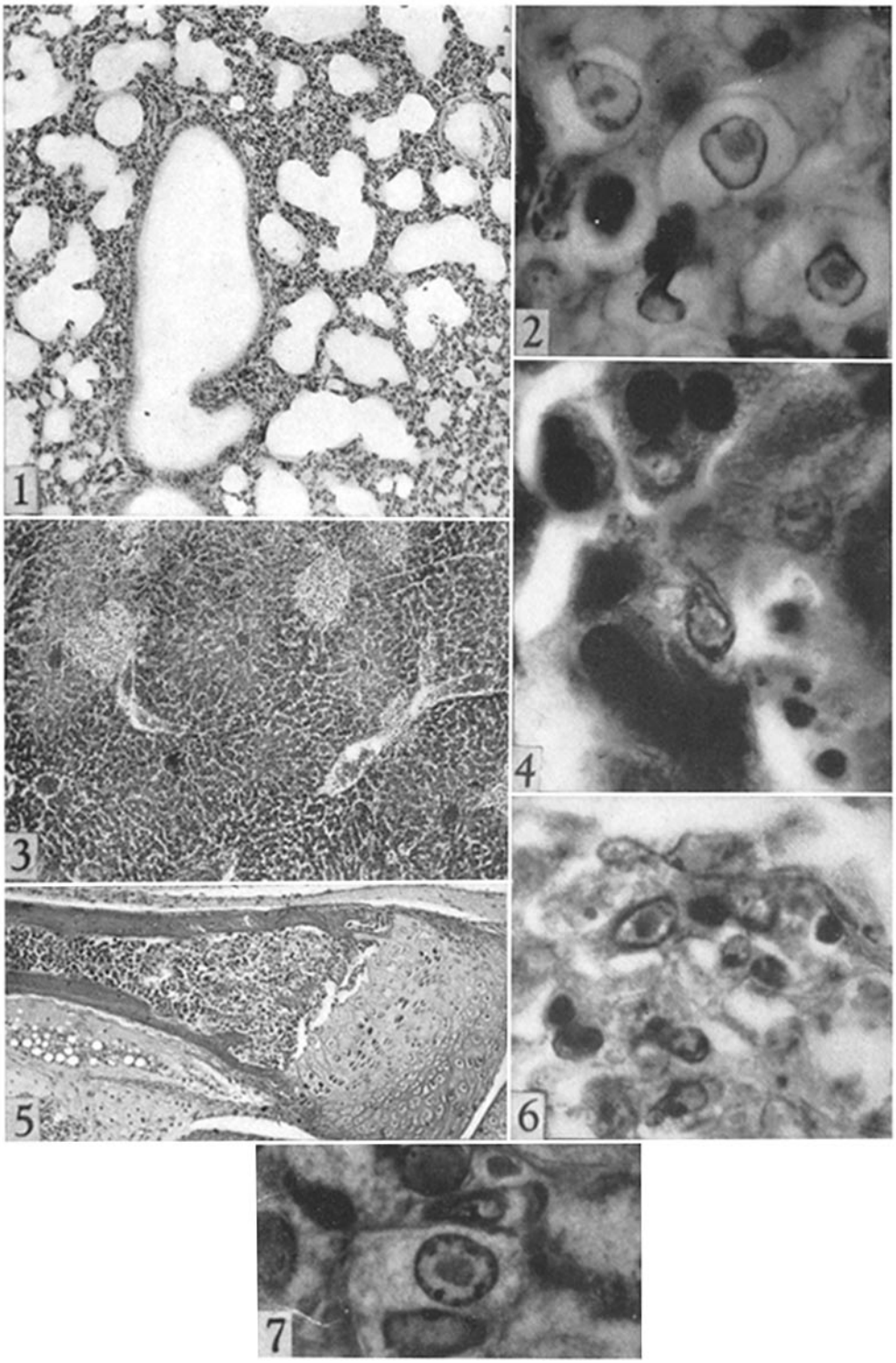
FIG. 3. Liver. Focal necrosis due to blood-borne herpes virus. 72 hours. × 60.

FIG. 4. Liver. Very early lesion showing herpetic inclusion body in the nucleus of a Kupffer cell. 48 hours. × 1500.

FIG. 5. Rib. An extensive portion of marrow adjoining the epiphysis is undergoing necrosis. Animal allowed to die following intraperitoneal inoculation. × 100.

FIG. 6. From same specimen as Fig. 5. Herpetic inclusion body in marrow cell. × 1500.

FIG. 7. Suprarenal cortical cell containing an herpetic inclusion body. 48 hours after intranasal instillation of the virus. × 1500.



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