STUDIES ON EASTERN EQUINE ENCEPHALOMYELITIS

V. Histopathology in the Mouse

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Plates 6 and 7

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Previous writers on equine encephalomyelitis have devoted scant attention to the histologic features in the mouse, and the characteristic details have not been heretofore described. Certain unusual features warrant separate description.

Methods and Material

In the study of the pathology about 80 mice were examined. Half of these were studied in serial sections, cut at 10 microns, with every 15th section mounted and stained. The remainder were studied in non-serial sections. Hematoxylin-eosin and phloxin-methylene blue were used as routine, with silver and myelin stains where indicated. When infant mice were studied, the entire skull and brain were fixed and decalcified before sectioning. With adults, the brain was removed.

Findings

As in the guinea pig (1) two distinct types of reaction can be distinguished, which may be called inflammatory, and degenerative or destructive. There is no necessary relation between these two modes.

In infant mice, the earliest change in the brain is clearly inflammatory. After peripheral inoculation of virus, as intraperitoneally, polymorphonuclear leucocytes may be observed free in the brain tissue as early as 36 hours after injection. Such cells are generally scarce at first, and, with serial sections, only a few scattered areas may be found. By 48 hours, however, the inflammation may be very widespread and may, in some instances, involve most of the cerebrum. There is naturally individual variation in regard to the time factor. After intracerebral inoculation in infants the pathologic changes may develop much more rapidly, so that by 36 hours inflammation may be significant in amount.

Fig. 1 from an infant mouse shows the type of change. Leucocytes are scattered through the tissue rather sparsely, with infrequent accumulations. Only rarely are

there focal accumulations as intense as are found in the guinea pig. Such massing of cells as may be present is often in relation to blood vessels. The vessels themselves frequently show severe alterations, with necrosis of the wall, early thrombosis, and perivascular hemorrhage. Fig. 2 illustrates such an area. Thrombotic material in the lumen is not distinct in the photograph. There can be no question of the hemorrhage being artificially produced, as Hurst (2) has suggested for the guinea pig, for the head of this infant mouse was fixed entire without manipulation of the brain. As may be readily seen from the photographs, the inflammatory change is at first unaccompanied by any form of nerve cell damage detectable by the microscope.

In the adult mouse, and even in young animals that have progressed beyond the stage of infancy, the inflammatory changes are in general much less pronounced than in the infant. In the adult the incubation period of the disease is considerably longer than in infants, especially after peripheral inoculation. Yet inflammation is sometimes insignificant, in spite of advanced degenerative and necrotic changes.

The pathology of the ganglion cells and intercellular tissue is quite distinct from any exudative changes. Ganglion cell and associated alterations may develop in areas where there is preexisting inflammation, or, on the contrary, may appear independently and become marked where inflammation is either absent or quite insignificant. This latter is especially true in adults.

The characteristic change affects intercellular tissue first, and nerve cell bodies only subsequently. The constitution of the tissue between the cell bodies is markedly altered.

The very earliest degree of change is illustrated in Fig. 3. This photograph from the neocortex of an adult mouse was taken on a metallographic plate and emphasizes the background at the expense of cellular clarity. The sole change from normal consists of irregular areas of spongy change in the ground substance, with a slight degree of color difference in reaction to eosin, very difficult to render in black and white. There is no morphological change in nucleus or cytoplasm of any of the cells. There is no inflammation.

A much more advanced degree of this process is seen in Fig. 4, taken from the olfactory bulb of an infant mouse. There is a bubbly, fine meshed vacuolation of the affected area, giving the intercellular tissue a honeycombed appearance. The change involves the molecular and mitral layers markedly, and the internal granular layer to a less degree. In this photograph attention was centered on the injured tissue, with some resultant overexposure of the normal areas on the left.

Especially to be noted is the preservation of the ganglion cells in the affected area. There are several pycnotic nuclei, of granule and glial cells, but the mitral cells show distinct preservation of their nuclei with a cytoplasm that is somewhat pale and granular. The pycnotic nuclei clearly represent necrotic cells, but it is obviously incorrect to call the large mitral cells necrotic. The damage is primarily in the tissue between the cell

bodies. Morphological change in the neurones appears later. Rather unusually for an infant brain, there is no inflammatory reaction in the affected area.

Other examples of this change are presented in Figs. 5 and 6. Fig. 5 shows a low power view of the brain after an intracerebral inoculation. The needle track is located between the arrows. The altered ground substance stains markedly lighter than the other portions, and especially at the margins the fine vacuolation of tissue is pronounced. The symmetry of the damage should be especially noted.

The portion of Fig. 5 marked by a rectangle is shown under higher power in Fig. 6. The photograph was taken to show cellular detail at the expense of the background. Here again a very few cells are pycnotic, but the great majority are not. The cytoplasm of the neurones is swollen, and partly disintegrated, with its affinity for basic dyes largely lost, but the cytoplasm nevertheless does not stain with eosin. The nuclei, however, which are the final criteria of cell viability, show but little change. Some hydropic change may be evident with slight dispersion of the nuclear contents in some cells, but the degree of damage is surprisingly slight.

The cerebella of infant mice are almost constantly affected by this type of change. Fig. 7 illustrates the disintegration of the ground substance in the granular layer. The nuclei are in many instances pycnotic, but for the most part are well preserved. The cytoplasm of the cerebellar granule cells is not demonstrable by ordinary histological methods. In these foci in the granular layer, inflammatory changes are constantly absent, although in the roof nuclei inflammation may be pronounced. The necrosis in any single cerebellar lesion is very rarely more intense than that pictured in Fig. 7, but the number of such foci may vary widely.

Elsewhere in the brain, however, the end stage of this process can be much more severe than is here pictured. The vacuolar degeneration of the ground tissue sometimes gives way to a completely granular appearance, in which all parenchymal cells are necrotic. Leucocytes that are present frequently survive the destructive process.

Occasionally patchy areas of neuronal necrosis are scattered through the brain in which the ground and intercellular substances are minimally affected. In such instances the cells show typical ischemic necrosis with deeply pycnotic nuclei and homogeneous cytoplasm that is generally acidophilic, sometimes basophilic. Fig. 8 illustrates this type of change, with a few of the necrotic nerve cells indicated by arrows. The alteration in the ground substance is very slight, and leucocytes are numerous. This distinctly uncommon change is presumably a much more fulminating attack on the tissue than is shown in the other photographs.

The destruction of axis cylinders and of myelin is proportional to the damage to the tissue as a whole. But it is noteworthy that with this virus there is no evidence of neutral fat formation, nor of the transformation of microglia and mononuclear cells into gitter cells. After intracerebral injections, the needle track usually shows a few such gitter cells in its course, but the areas of typical virus damage never do.

DISCUSSION

As in the guinea pig, the earliest detectable change from normal is an inflammatory reaction. This is most noticeable in infant mice. The reasons why inflammation in the adult is much less constant than in infants are at present not clear.

The type of necrosis appearing in the mouse is rather unusual in neuropathology, although other viruses may undoubtedly cause a similar change. Especially in infant mice this damage appears later than the inflammatory reaction. But in adults as well as in infants it can be seen that the two types vary quite independently of each other. The exudative changes are not merely a response secondary to the necrosis, but represent an independent variable.

The term necrosis is used only for conformity to current usage and for lack of a better term. In the ultimate stages there may indeed be complete necrosis of all elements, but in the early and middle stages the alteration should not be so designated. The initial change is clearly in the ground tissue between the cell bodies. The composition of this tissue is not altogether clear. There are the myriads of cell processes demonstrable only with Golgi stains, as well as numerous myelinated and unmyelinated nerve fibers, and the processes of glial cells. In addition there is considered to be an intercellular matrix (the cerebral gray matter of Nissl), most recently considered by Taft (3). There is as yet no evidence concerning the site of action of the virus, whether it be inside the nerve cell or in extraneuronal elements; if within the neurone, whether in the cell body primarily or exclusively, or indiscriminately in the cell body and all the fine processes. Consequently the different types of lesions cannot yet be correlated with different modes or loci of virus action. But it seems unjustifiable to maintain that the primary damage is nerve cell necrosis.

The peculiar vacuolation and destruction of the ground tissue described above must be distinguished from a vacuolation of tissue occurring when nerve cells disappear. This latter is readily seen in the Purkinje cell layer of the cerebellum and the pyramidal cell lamina of the hippocampus in a variety of conditions, and is quite different from what has been described in this paper. Still different is the reaction to cerebral softening, as from vascular occlusion, with its vigorous gitter cell mobilization. The relation to the *état criblé* of the cerebral cortex seen in some cases of paresis is not clear. It is worth noting that a reaction somewhat similar to that in the mouse has been clearly described by Courville (4) in nitrous oxide asphyxia in human beings. Dr. Courville kindly sent the author some of his material for examination. Comparable changes in the brain have also been observed by the author in some instances of vascular disorder.

This type of disturbance has not been seen in guinea pigs inoculated with equine encephalomyelitis virus (1). Nerve cells may exhibit disintegration of cytoplasm with persistence of nuclei, but the tissue between the nerve

cell bodies is not affected. It seems likely that the difference between the two hosts is a difference in species reaction rather than a fundamental difference in pathogenesis.

SUMMARY

In mice affected with equine encephalomyelitis, the first pathological disturbance in infant animals is an inflammatory reaction, which is usually less pronounced in adult animals. A characteristic type of parenchymal damage appears to be independent of the inflammation. In such foci of injury there is initially a vacuolation of intercellular tissue. Neurones in such areas are at first intact, later show cytoplasmic changes, and finally nuclear alterations. Complete disintegration of tissue and all its elements may be the end result.

BIBLIOGRAPHY

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EXPLANATION OF PLATES

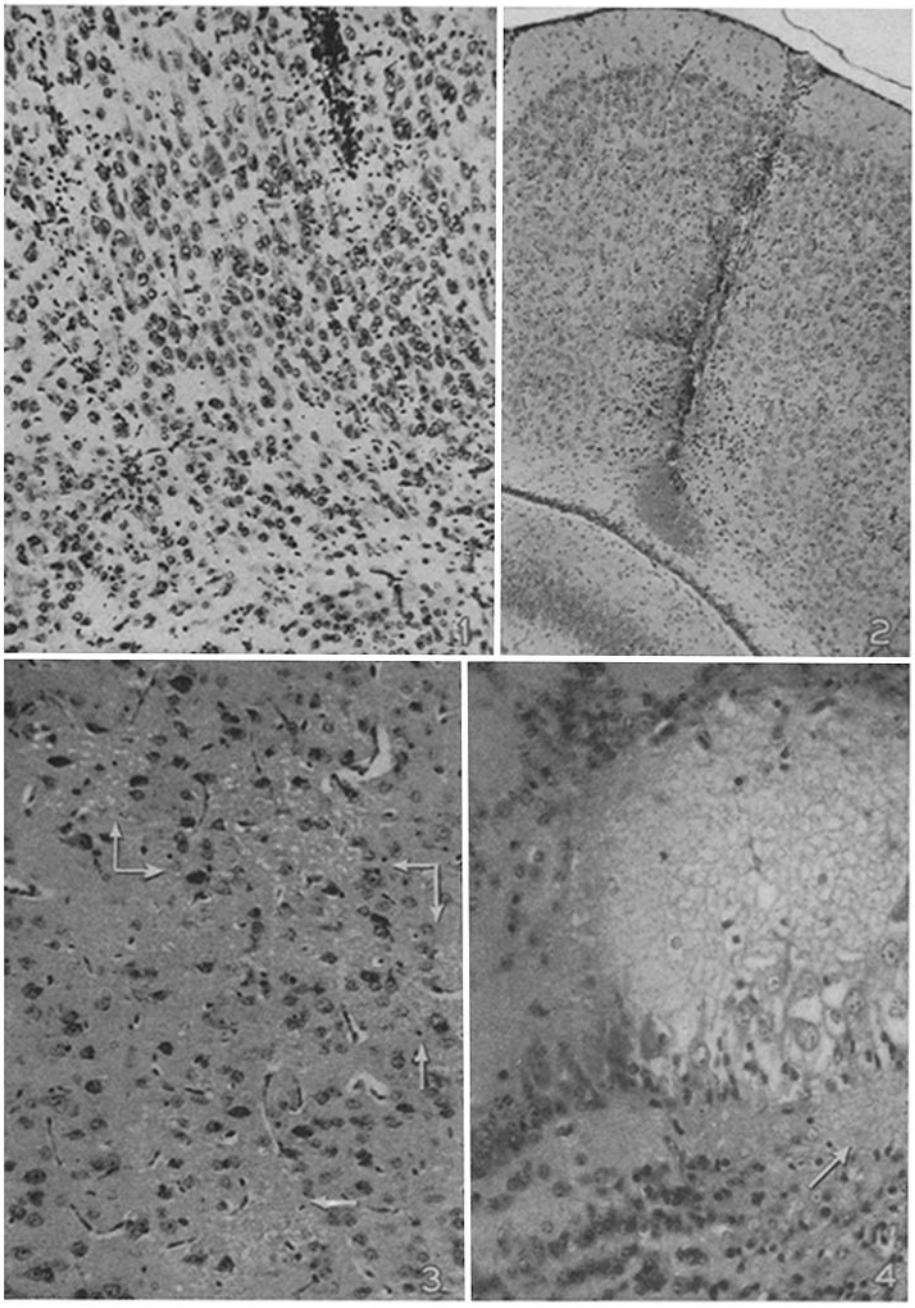
PLATE 6

FIG. 1. Cerebral cortex of infant mouse inoculated intracerebrally. There is a diffuse scattering of polymorphonuclear leucocytes through the tissue, but the nerve cells are intact. × 137.

FIG. 2. Cerebral cortex of infant mouse. Intraperitoneal inoculation. The blood vessel shows partial thrombosis, with necrosis and inflammation of its walls. There is considerable perivascular extravasation of blood, and leucocytic infiltration of the parenchyma. × 75.

FIG. 3. Cerebral cortex of adult mouse inoculated intraperitoneally. Although the nerve cells are intact, the intercellular ground tissue shows a spongy vacuolation, some of which is indicated by arrows. × 182.

FIG. 4. Olfactory bulb of infant mouse after intracerebral inoculation. On the right is a marked degree of the vacuolation of ground substance, involving molecular, mitral, and granular layers. A few small cells are necrotic, as shown by their pycnotic nuclei but the large mitral cells are quite well preserved. The arrow points to an earlier stage of the vacuolation than is present elsewhere. Inflammation is absent. An identical change is seen if virus is given intraperitoneally. × 255.



Photographed by J. A. Carlile

(King: Pathology of equine encephalomyelitis)

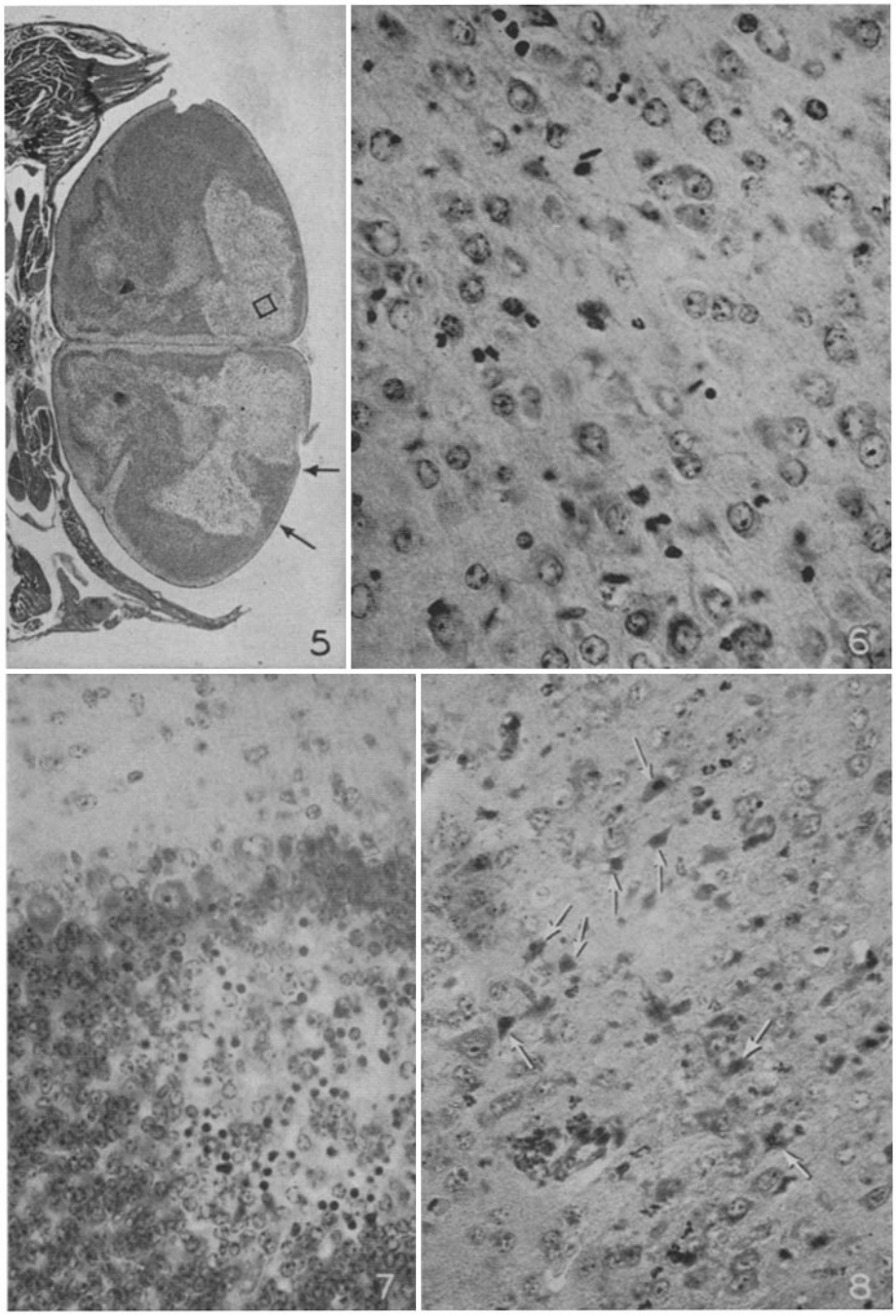
PLATE 7

FIG. 5. Brain of infant mouse injected intracerebrally. The needle track can be seen in the portion between the arrows. There are symmetrical areas of damage to both hemispheres, characterized chiefly by an injury to the intercellular tissue, which stains less deeply with eosin than the remaining tissue. The area within the rectangle is reproduced in Fig. 6. × 10.7.

FIG. 6. Portion of Fig. 5, within rectangle, shown under higher magnification. The nuclei of the nerve cells are excellently preserved in spite of some cytoplasmic changes. A few leucocytes are scattered through the tissue. × 345.

FIG. 7. Cerebellar cortex of infant mouse. Virus instilled into the nares. The granular layer shows a focus of destruction, involving primarily the ground substance, which appears markedly lighter than the normal intercellular tissue. Many nuclei are pycnotic, but many are entirely normal. The Purkinje cells are normal. There is no inflammation. × 357.

FIG. 8. Cerebral cortex of infant mouse after intraperitoneal inoculation. There is minimal change in the intercellular tissue, but many of the neurones are acutely necrotic with pycnotic nuclei and shrunken, eosinophilic, homogeneous cytoplasm. A few such cells are indicated by arrows. Most of the nerve cells are entirely normal. Some inflammation is present. × 320.



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