THE HISTOPATHOLOGY OF NUTRITIONAL ENCEPHALOMALACIA OF CHICKS

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Plates 36 to 39

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In a recent paper, Pappenheimer and Goettsch (1) described a nutritional disorder of growing chicks, characterized by striking alterations in the brain. While the general features of the lesions were pointed out in this article, further experiments have afforded abundant material for a more detailed and complete histopathological study. The application of neurohistologic methods has brought out features not disclosed by the routine technique, and has thrown light upon the pathogenesis of the lesions and upon the course of their development.

Technique

In addition to such routine stains as hematoxylin-eosin, phosphotungstic acid hematoxylin, Masson's trichrome and Scharlach R for fat, the following neuropathological methods have been employed:—Spielmeyer's method for myelin sheaths, Rio del Hortega silver carbonate method for microglia and oligodendroglia, Cajal's gold sublimate method for astrocytes, Bielschowsky's method for axis cylinders, and Laidlaw's reticulum stain.

Histopathology of the Brain Lesions

The salient features originally noted may be summarized as (1) edema, with separation and disruption of the cellular and fibrillar elements; (2) degeneration and necrosis of the Purkinje cells, and of the small cells constituting the granular layer of the cerebellum; (3) small hemorrhages scattered through the cortical white matter, or within the cortical zones; (4) hyaline capillary thrombi in and about the necrotic areas. Emphasis was placed in the previous paper upon the cerebellar lesions, although reference was made to two chicks in which there

occurred lesions of essentially the same character in the cerebrum. The material which has accumulated has taught us that cerebral localization of the lesions is of not infrequent occurrence, and that similar changes may be also found at times in the medulla and midbrain. The relative frequency with which the lesions have been found in the different portions of the brain is shown in Table I.

Since the brains were not serially sectioned, these figures cannot be regarded as very accurate, but they indicate correctly the relative sensibility of the different portions of the brain to the disease, and the predominance of the cerebellar lesions. Lesions of the cerebrum, unaccompanied by cerebellar lesions were found in but thirteen chicks, and isolated lesions of the medulla or midbrain in but three. Only once were lesions found in the optic lobes.

TABLE I

		per cent
Total No. of chicks	169	
Cerebellar lesions	153	90.6
Cerebral lesions	48	28.4
Medullary or midbrain lesions	19	11.3

The earliest and mildest recognizable lesions point very definitely to a circulatory disturbance as the initial factor in the production of the lesions. Thus, in the cerebellum, there is seen engorgement of the pial vessels, and of the capillaries of the molecular and granular layers, and of the central white matter. Not all the vessels are filled with erythrocytes; some are dilated or empty. This is seen especially in the white matter, less often in the granular layer, and only occasionally in the lower portion of the molecular layer. Numerous small hemorrhages occur into the pia and the cerebellar layers, but are most conspicuously seen in the lower portion of the molecular layer. A mild edema separates the fibers of the white matter.

A further step in the development of the lesions is seen in the presence of definite edema in the Purkinje cell layer, which becomes strikingly rarefied and spongy (Fig. 6). The Purkinje cells and the Bergmann astrocytes are spread apart, and often displaced away from the granular layer towards the pia. In Bielschowsky preparations, the ascending fibers of the Golgi cells, the basket fibers, and the collaterals of the Purkinje axones which form the plexus infra-ganglionaris, are seen to be spread apart. In Cajal gold sublimate preparatons, the large astrocytes of the Bergmann type, instead of forming two or three regular layers at the Purkinje cell level and sending out parallel rows of fibers at right angles to the pia to form

the external glial membrane, are found irregularly arranged and separated by the edema.

The Purkinje cells, Golgi cells, and the small cells of the granular layer are seen undergoing degeneration of the type described by Spielmeyer as "ischemic necrosis" (Fig. 7). A similar change is later seen in the basket cells.

The Purkinje cells lose their Nissl substance, become angular and narrow, and their nuclei pycnotic. Intracellular fibrillae disappear. The other large ganglion cells react similarly. The nuclei of the Bergmann cells are swollen and hydropic (Fig. 8).

The small granular cells show marked pycnosis and shrinkage of their nuclei followed in later stages by karyorrhexis into small spherical fragments. The internuclear protoplasmatic islands stain very faintly. These changes are patchy. A few affected Purkinje cells may be seen in an edematous area, with the adjacent granular layer showing a small group of degenerating cells, or these lesions may be found separately. The extent of the change in the granular layer may vary from a small focus to an area involving the entire granular zone.

At this point, small collections of rod cells appear, chiefly in the molecular layer, but also occasionally in the white matter. Stained by Hortega's silver carbonate method, these are seen to be groups of microglia cells. As compared with the normal, their cell body is thickened and elongated, the nucleus lengthened, and the processes short, reduced in number, distorted, clubbed, and without spines (Fig. 1). The increase in the normal number of these elements is first observed in the lower half of the molecular layer, and later diffusely through this entire zone (Fig. 9). The microglia cells arrange themselves as on a trellis along the outlines of the dendrites of the Purkinje cells (Spielmeyer's "*Strauchwerk*"), probably functioning as neuronophages.

At this, and at earlier stages, the oligodendroglia, as stained by a modification of Hortega's silver carbonate method, lose their processes, but do not undergo the mucoid degeneration which has been described in early degenerative lesions of the mammalian brain (Figs. 2 *a* and 2 *b*).

The alterations of the fibrillar astrocytes are not particularly striking. There is an apparent increase in the number of the Bergmann cells, and probably of their fibers. With the disappearance of the Purkinje cells, the Bergmann fibers, as shown in the Cajal gold sublimate preparations, become concentrated. The pial surface may become sunken over such an area. In the healing stages, the fibers may become incrusted with calcium (Fig. 10).

In the central white matter, especially in the vicinity of the fourth ventricle, one occasionally observes multiplication of the fibrous astrocytes with the formation of many new glia fibers (Fig. 11). In such areas, the myelin sheaths are shown in Spielmeyer-stained sections, to have undergone destruction, and the Bielschowsky preparations bring out the fact that the neuraxons have also disappeared (Fig. 3).

At this stage, the ganglion cells have been almost entirely destroyed, only nu-

clear, protoplasmatic, and fibrillar fragments remaining to indicate their original position. Lipoid droplets are found within these degenerating remnants.

Interesting alterations are seen in the capillaries. The endothelial cells become greatly swollen, and grow actively, often with lateral sprouting, into the necrotic tissue. Mitotic figures are frequently encountered.

From this point on, the astrocytes and oligodendroglia degenerate and disappear (Fig. 4). The tissue becomes more and more spongy, and contains numerous compound granule cells, which in the Scharlach R stain are seen to be laden with fat (Fig. 12). The hyaline thrombi, as described in the previous paper, are present in and about the lesions from the very first appearance of the degenerative changes in the ganglion cells (Fig. 13).

As a final stage in the process, one finds that the malacic areas become more or less organized by the ingrowth of mesodermal tissue. Reticular fibers are seen in increasing abundance in and about the walls of the cortical blood vessels, often constituting compact, tubercle-like nodules. Small focal areas may be organized in this way, or one or several entire lobules. From these, fibers pass out and interlace through the area to form a loose spongy network (Fig. 5). In this are found varying numbers of phagocytes, containing brownish yellow pigment, some of which gives an iron reaction.

It is not to be assumed that the lesions throughout the cerebellum are of necessity all at the same stage of evolution. On the contrary, it is not unusual to find early acute degenerative changes in some of the lobules, while in other lobules or areas, various stages of repair are in progress. One may assume from this fact that the brain has been subjected to repeated or continued insult. On the other hand, in many of the chicks that were killed soon after the onset of symptoms, only the early lesions are discoverable; and if one may draw deductions from the clinical behavior, most extreme changes may develop in a very short period—certainly within 6 hours or less.

The study of the lesions in the cerebrum and medulla adds nothing of importance to what has already been described. Both the degenerative and the reparative processes are essentially identical with those occurring in the cerebellum.

In those cases in which both cerebrum and cerebellum are affected, it has repeatedly been noted that the cerebral lesions are recent in their appearance, while the cerebellar lesions are in the healing or reparative stage. Clearly, the injury to the cerebellum in these cases antedates that in the cerebrum.

DISCUSSION

It was stated in the previous paper that the lesions in the chicks were probably initiated by vascular disturbances, and further morphological study tends to confirm this view. Summarizing the changes described above, we find a degenerative lesion, characterized by edema, rapid necrosis of the neural elements, and later of the astrocytes and oligodendroglia. As reactive changes, one may list the multiplication and local increase of the microglia, with subsequent transformation into compound granular cells, the proliferation of endothelium with the formation of new vascular ingrowths into the degenerated areas, and finally, the partial mesodermal organization of the softened tissue. This series of changes is characteristic of the encephalomalacic areas which are produced in the central nervous system by functional or anatomic vascular disease.

In the disease of chicks, changes in the larger vessels may be excluded as a possible cause of the lesions. While hyaline and fibrin thrombi are almost invariably to be found within and about the degenerated areas, not all the capillaries are thus occluded and we have not been able to bring proof that the capillary thrombosis is a primary cause of the ensuing necrosis. Indeed, it may well follow upon a prolonged vasoconstriction, or vasomotor paralysis, or the first followed by the second.

As an indication of the functional nature of the disturbance, we have found marked symptoms without detectable histological lesions in thirteen chicks. There have been noted tremors, ataxia, retropulsive movements, and in a few chicks, retraction of the head. In the absence of complete serial sections, one cannot be entirely certain that small lesions may not have been present.

Spielmeyer (2) has shown that exactly similar lesions to those here described may occur, not only as a result of gross vascular disease, but in cases in which no anatomic changes are demonstrable and in which one is forced to assume a functional circulatory disturbance. Ricker (3) has long held the view that various factors, mechanical or chemical, may affect the vasomotor nerves, producing vasoconstriction and dilatation of sufficient duration to bring about local necrosis of tissue.

In his study of epilepsy, Spielmeyer (4) has come to the conclusion

that the local lesions often found in that condition, are the result of functional vascular disturbance. He cites the observations of Horsley, Hartwell and Kennedy, Foerster, and others who have actually seen constriction of the cerebral vessels during epileptic seizures. He points out the similarity between the lesions in the Sommers sector of the hippocampus found in epilepsy, unassociated with alterations of the vessel walls, and those which result from gross disease of the vessels supplying that area. It is particularly interesting that Spielmeyer's description of the early cerebellar lesions occurring in epilepsy, applies in all respects to the lesions which we have noted in the chicks.

As an explanation of the greater susceptibility of the cerebellum to circulatory injury, Spielmeyer calls attention to the rather sharp right angled turn of the vessels at the Purkinje cell level, as described by Pfeifer (5). In the cerebellar lesions of the chicks, the early changes are most frequently detected in and about the Purkinje layer, and it seems not unreasonable to believe that this preferential localization may be explained in this way.

CONCLUSION

Whatever may be the nature of the nutritive error or deficiency which in chicks is responsible for encephalomalacia, the immediate cause must be looked for in some agent or condition which impairs the capillary circulation of the brain. The essential lesion is an ischemic necrosis, followed, if the animal survive, by reparative organization of the dead tissue.

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EXPLANATION OF PLATES

PLATE 36

FIG. 1. Increase in number of microglia cells, with elongation of bodies and nuclei and loss and distortion of cell processes. Del Rio Hortega's silver carbonate stain for microglia. Obj. 16 mm. Oc. 10.

FIG. 2 *a*. Section through cerebellar cortex showing normal oligodendroglia.

FIG. 2 *b*. Similar section from encephalomalacic chicks, showing changes in oligodendroglia. Loss of cell processes. Del Rio Hortega's silver carbonate stain for oligodendroglia. Obj. 16 mm. Oc. 10.

FIG. 3. Degeneration of neuraxones in white matter. Bielschowsky stain. Obj. 4 mm. Oc. 10.

PLATE 37

FIG. 4. Destruction of astrocytes in degenerated area without any reaction. Cajal gold-sublimate stain. Obj. 4 mm. Oc. 10.

FIG. 5. Connective tissue replacement of degenerated lobule with formation of nodular areas of condensation in cortex. Laidlaw stain. Obj. 4 mm. Oc. 10.

PLATE 38

FIG. 6. Edema and rarefaction of Purkinje cell layer. Degeneration of cells of granular layer. Perivascular hemorrhages. Hematoxylin and eosin stain. × 138.

FIG. 7. "Ischemic" necrosis of Purkinje cells. Pycnosis of nuclei of granular layer. Edema. Hematoxylin and eosin stain. × 618.

FIG. 8. Swollen and hydropic nuclei of Bergmann cells. Hematoxylin and eosin stain. × 618.

FIG. 9. Marked increase in microglia cells in molecular layer. Hematoxylin and eosin stain. × 138.

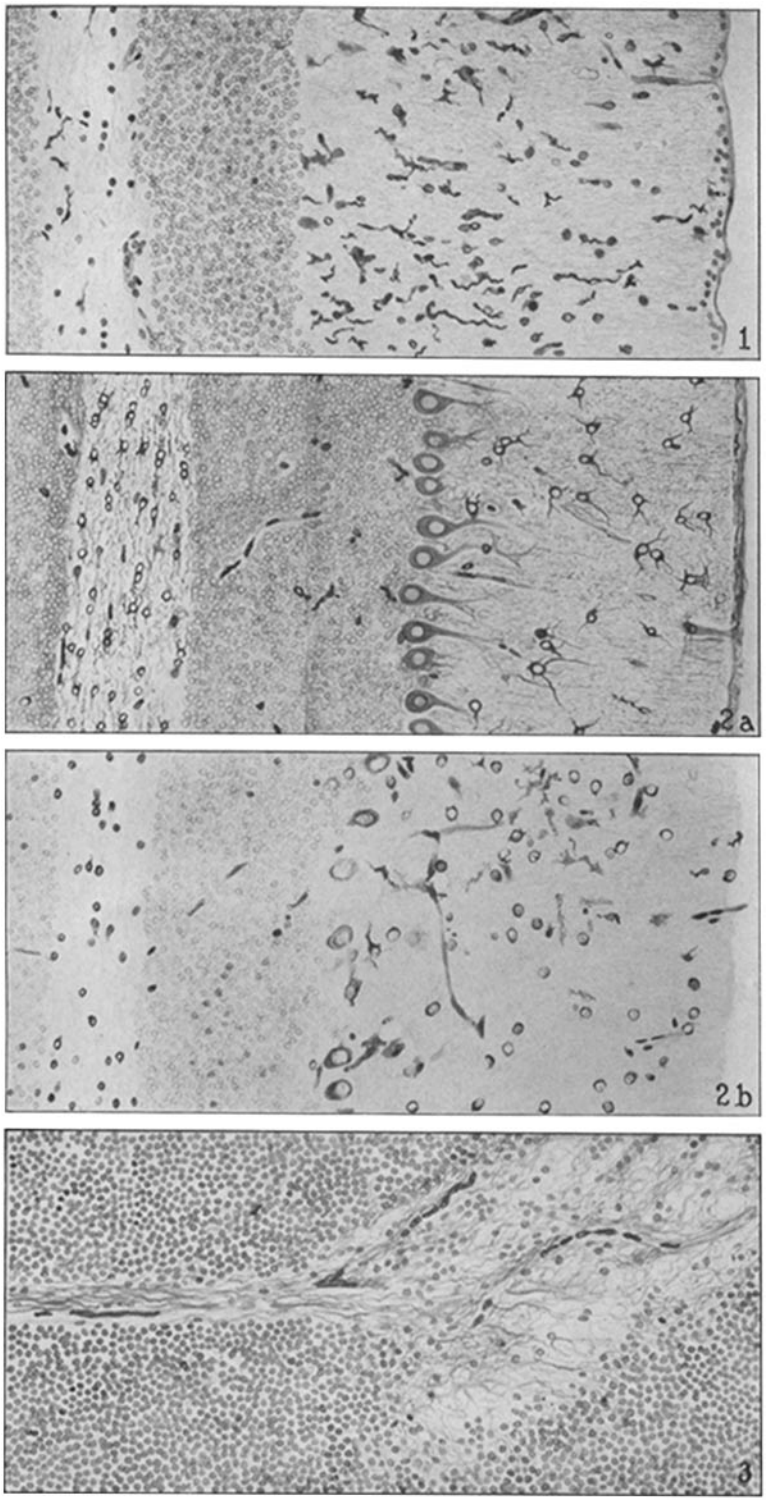
PLATE 39

FIG. 10. Incrustation of Bergmann fibers with calcium. Hematoxylin and eosin stain. × 618.

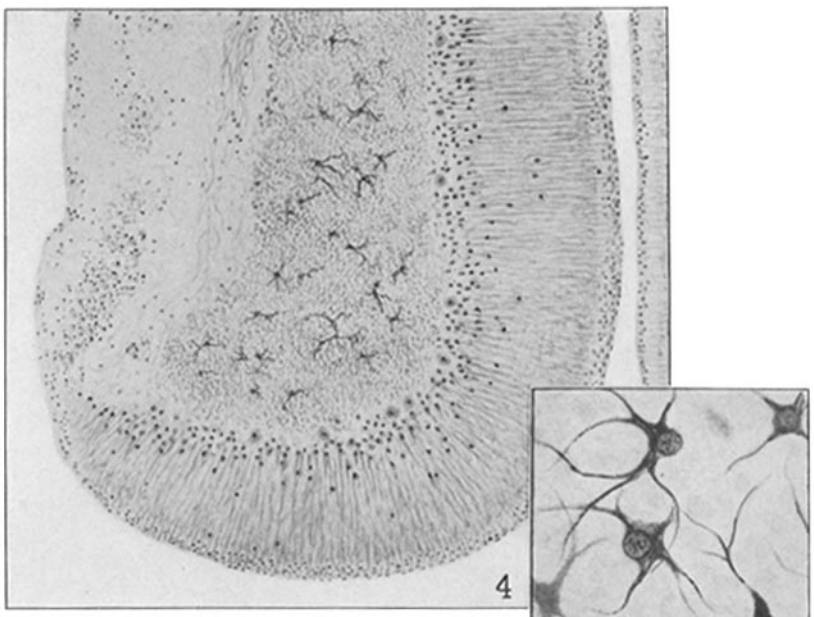
FIG. 11. Increase of astrocytes in white matter near ventricle. Note also destruction of granular and accumulation of microglia in molecular layer. Hematoxylin and eosin stain. Low power. × 138.

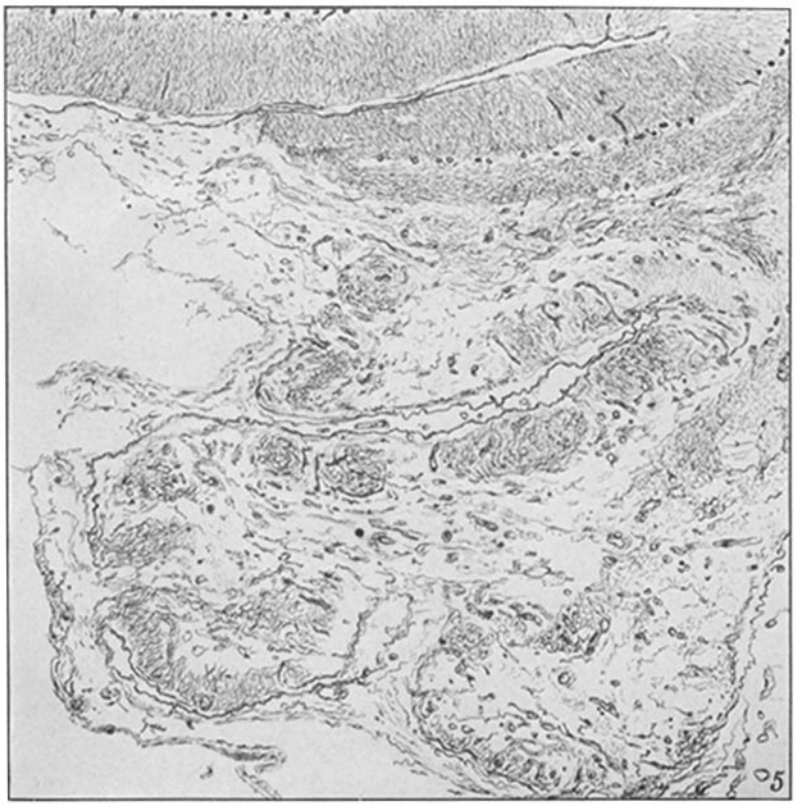
FIG. 12. Atrophy of lobule with appearance of large numbers of lipoid-containing phagocytes. Hematoxylin and eosin stain. × 138.

FIG. 13. Hyaline thrombi in capillaries in degenerated area. Hematoxylin and eosin stain. × 138.



(Wolf and Pappenheimer: Nutritional encephalomalacia of chicks)





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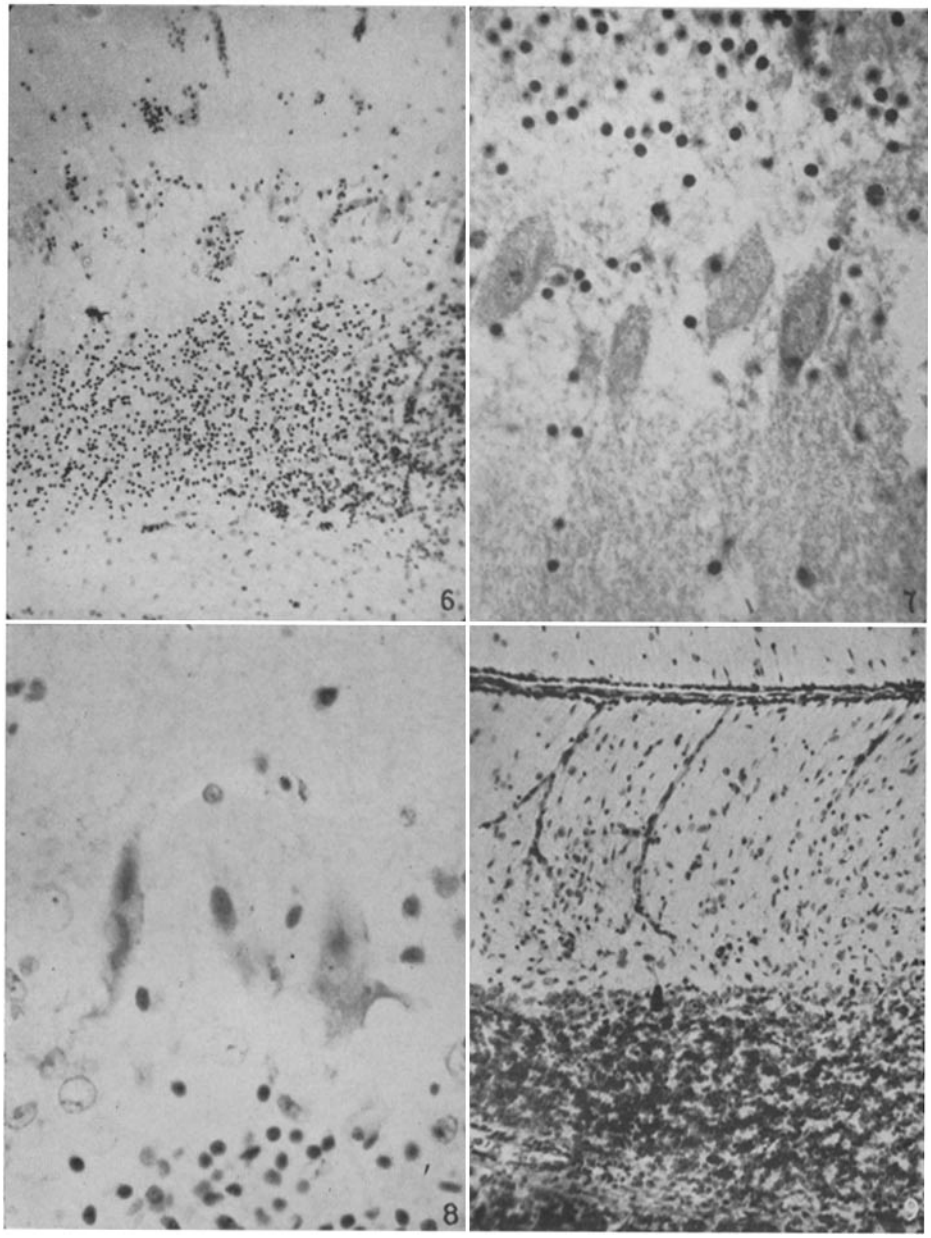

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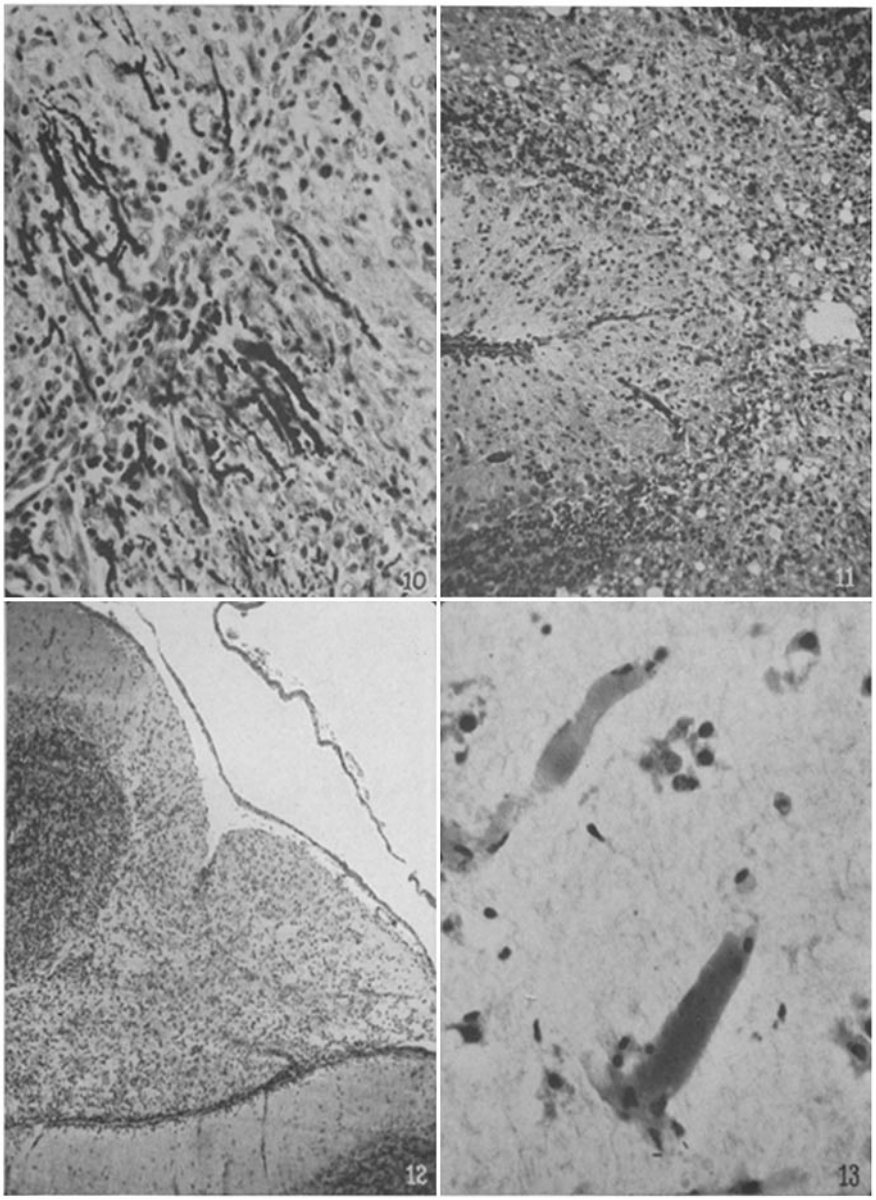

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