A MURINE VIRUS (JHM) CAUSING DISSEMINATED ENCEPHALOMYELITIS WITH EXTENSIVE DESTRUCTION OF MYELIN

II. PATHOLOGY*

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The mouse encephalomyelitis virus (JHM), described in the preceding paper (1), produces lesions in the central nervous system and elsewhere, which are distinctive, and set it apart from other known neurotropic viruses of mouse or human origin. A characteristic feature which lends it particular interest is its ability to cause widespread demyelination of brain and spinal cord, but not of peripheral nerves. Also, there are found striking lesions in the liver, and occasionally in other tissues.

In this communication, a detailed and illustrated description of the pathological changes in passage material will be presented. The original mice from which the virus derives were not examined histologically, so that our study is based on experimentally infected mice and not upon the spontaneous disease. Indeed, no subsequent examples of the infection have been detected in our mouse colony.[1] During the past 2 years, we have had occasion to examine the brains and spinal cords of many mice infected with one or another neurotropic virus; in none of these have the lesions resembled those incited by the JHM virus.

Materials and Methods

This study is based on the histological study of 228 mice. The routes of inoculation were as follows:—

Intracerebral	158
Intraperitoneal	19
Intramuscular	7
Intranasal	5
Intravenous	20
Various multiple inoculations	19

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[1]The only possible exception is one mouse of the same stock which developed spontaneous convulsions. A first passage mouse, presenting signs of illness on the 9th day, was found to have demyelinating lesions of paraventricular regions of cerebrum and of cervical cord. In further passages, the agent has shown little pathogenicity, and attempts to establish the virus have not yet succeeded.

The material inoculated in most instances was centrifuged brain suspension in dilutions varying from 10^{-1} to 10^{-3}. Suspensions of liver were used both for intracerebral and intraperitoneal inoculations in some animals, and a few have been injected with suspensions of lung, spleen, kidney, filtered intestinal contents, and heparinized blood. A few mice were examined after injection with Seitz filtrate and sintered glass filtrate of brain suspension and with the centrifugal fluid from tissue cultures.

These materials were harvested from various passages as listed:—

Passage	No. of mice examined	Passage*	No. of mice examined
2	1	4A	16
3	2	5A	4
4	71	6A	17
16	1	7A	16
37	1	9A	2
38	4	10A	2
44	2	12A	8
45	13		
46	40	Passage not recorded	10
49 up to 52	18		

* As repeat passage with 2nd passage of original material, maintained in frozen state.

The times elapsing between single inoculations and autopsy were as listed:—

Time	No. of mice				
	Intracerebral	Intraperitoneal	Intramuscular	Intransal	Intravenous
days					
1	1				
2	18				
3	16			3	2
4	26	5	1	2	
5	16	2			1
6	15	1	2		2
7	12	2			
8	7	2	2		2
9	10	1			4
10	3	2			
11	5				
12					4
13		2			
14	1				2
15	1				
16					2
20					
mos.					
1	12				
2	9	2			
3	6				
4	2				

As routine, the brain was dissected out and, together with the spinal cord in its vertebral investment, was fixed in Bouin's fluid. After fixation, the brain was usually sectioned in three or four coronal blocks but in some instances sagittal sections of each hemisphere were made Blocks were taken from four levels of the spinal cord. The abdominal and thoracic viscera were examined in a number of mice. Hematoxylin and eosin was the method employed routinely and the following stains were used on occasion: Mahon's method for myelin, Bodian's method for nerve fibers, Laidlaw's lithium-silver for reticulum, Mallory's phosphotungstic acid-hematoxylin, Giemsa's stain, eosin-thionine, Nyka's stain for demonstration of rickettsia, and Scharlach R for fat (after formalin fixation), Hortega's silver carbonate for astrocytes.

Pathology

Results of a Single Intracerebral Injection.—

Although in some mice there were changes in the liver and other organs, the most constant and distinctive lesions were those of the central nervous system.

Meninges.—Meningitis was a regular accompaniment of the cerebral lesions but varied greatly in intensity and was always patchy in distribution. The cellular composition of the exudate was related to the time elapsing after injection. In the first few days, polymorphonuclear leukocytes were relatively numerous, giving way to large mononuclear and small lymphocytes, the latter in the minority at all stages. Typical plasma cells were not seen. In animals surviving for longer periods, exudate became replaced by plaques of organized connective tissue (Fig. 1). A conspicuous feature was the occurrence of multinucleate giant cells in the exudate and along the pia (Fig. 2). These appeared to derive from connective tissue of the pia, from endothelial cells (Fig. 3), and from fusion of large mononuclear cells. Another striking feature of the exudate seen especially in the early stages was the karyorrhexis of the polymorphonuclear leukocytes with phagocytosis of the chromatin particles. Occasionally the nuclear material was dispersed in the form of minute granules which were difficult to distinguish from elementary bodies. There were no thrombi or other vascular changes in the meningeal arteries or veins other than congestion. The capillaries, however, often showed endothelial swelling and proliferation and multiplication of the capillary pericytes.

Changes of the same kind but usually less severe were present in the spinal meninges. Here one found, at times loose collections of cells in the subdural space, as well as giant cells in dura and pia.

Brain.—Although lesions were found in any or all parts of the brain, there were certain sites of predilection, namely the hippocampus and its connecting fiber tracts, the olfactory lobes, the periependymal tissues, and the brain stem. So regular was this distribution that it could be regarded as characteristic of the reaction to this particular infectious agent. The lesions were of two general types, those of the olfactory lobes and hippocampal region being essentially necrotizing, while those of the brain stem were predominantly demyelinating.

In all but a very few mice, there was a crescentic lesion about the cornu Ammonis, together with necrosis of some (or occasionally all) of the pyramidal cells and of the alveus hippocampi (Fig. 4). Though in a small number of mice, the pyramidal cells of the gyrus dentatus were as much involved as the corresponding cells of the hippocampus, it was usual to find a striking contrast between the normal gyrus dentatus and the severely damaged cornu Ammonis. An integral, and indeed predominant, part of the crescentic lesion was the necrosis of the entorhinal-Ammonical fibers, columna fornicis, and adjacent portions of the corpus callosum and the centrum ovale, including the ependymal and subependymal tissues (Fig. 5). This necrosis had a tendency to split the tissues and to extend further, the longer the lesion was present. For this reason the portions of the centrum ovale mentioned above were only those most uniformly involved, while further extensions were very common, especially in mice autopsied not more than a week after infection. Since this linear lesion appeared as a slit in the sections when examined by

the naked eye, we have spoken of it for convenience as the "slit lesion" (Fig. 6). The necrosis was accompanied in the early stages by an infiltration of inflammatory cells predominantly polymorphonuclear leukocytes, nuclear fragmentation, and phagocytosis of nuclear particles, especially just beneath the ependyma (Fig. 7). Hemorrhage and fibrinoid material were sometimes present in the early lesions. At no stage were compound granular corpuscles conspicuous in the lesion and frozen sections treated with Scharlach R showed practically no stainable lipid. This was confirmed by the lack of birefringence under the polarizing microscope. The necrosis of the ependyma left islands of ependymal cells, sometimes accompanied by the formation of multinucleate giant cells from the surviving ependyma (Fig. 9). Within 10 days after injection, inflammatory cellular infiltration and giant cell formation were less prominent in the lesion and the elements of repair were more clearly evident. New formation of blood vessels was shown by the presence of capillary sprouts and there was a slight but definite astrocytosis. The ependymal cells proliferated as indicated by mitoses, by heaping up into two or three layers, and by extension of flattened cells over the necrotic area. Restitution of the ependymal surface was not complete, however, with the result that cerebrospinal fluid continued to have access to the lesion and was regarded as a factor in further extension of the cavity. In mice autopsied after 2 or more months, the slit lesion had become a porencephalic cavity, occupying half or more of the volume of the cerebral hemishphere and still with an easily demonstrable connection with the ventricular system (Fig. 8). This was lined in part by ependyma and in part by astrocytes, the lining being at first irregular in outline and gradually becoming smooth. Since there was little difference between the cavities in mice autopsied 3 and 4 months after inoculation, this porencephalic cavity is presumed to be the final stage of the slit lesion.

While the olfactory lobes were sectioned in a relatively small percentage of mice, lesions were found in all but one instance of those examined. This, then, seems to be a favored site for lesions. The changes consisted of areas of complete or partial necrosis in any or all layers (Fig. 10). Early there was a polymorphonuclear response followed by a glial cicatrix, which seemed small in comparison to the extent of initial involvement. Since both the olfactory lobes and the hippocampus are part of the olfactory system, this system was regarded as especially vulnerable to JHM virus.

Lesions were often seen in the cerebral cortex and less frequently in other parts of the hemispheres. These were of a rather different type from those described in the olfactory system. They were sharply circumscribed, even when quite large. The ground substance appeared spongy and rarefied (Fig. 11). It also took a lighter stain than in adjacent unaffected areas. The nerve cells by contrast were well preserved. The lesion was accompanied by a minimal amount of inflammatory reaction and only in rare instances by hemorrhage.

In the brain stem, particularly in the pons, there were both demyelinating and necrotizing lesions, the latter affecting the regions immediately adjacent to the ependyma (Fig. 13). The necrotizing lesions showed the same sequences of inflammatory cellular infiltration and repair which have been described in the slit lesion, including the formation of giant cells. It was noted in sections prepared by Bodian's technic that nerve fibers were usually identifiable and free from degeneration, even within lesions which appeared wholly necrotic in sections stained by non-metallic methods (Fig. 12). The demyelinating lesions had the characteristics of those to be described in the spinal cord and seemed more closely related to them than to the changes in the olfactory system.

The white matter of the folia and nuclei of the cerebellum was occasionally involved by lesions of the demyelinating type (Fig. 14). Lesions of the cerebellar cortex were only exceptionally noted. When present, they were circumscribed. The nuclei of the granular layer were shrunken, pyknotic, and separated from one another by edema and the Purkinje cells disappeared within the limits of the lesion. In both pons and cerebellum, isolated microcysts (*état criblé*) were often found in areas otherwise histologically normal.

The blood vessels in the neighborhood of lesions showed similar changes regardless of the

site. At about 5 days after inoculation, exudate was found about some of the capillaries near the zones of reaction. This was shortly followed by proliferation of pericytes, giving the effect of a capillary wall several cells thick. Lymphocytic cuffing made its appearance only in the later lesions and at no time was very extensive. Within 2 weeks after inoculation, all cuffing had disappeared and the blood vessels of the mice autopsied 1 to 4 months after injection were entirely normal. No fibrin thrombi were found in the affected areas at any time, though unorganized hyaline plugs were sometimes seen at the height of the inflammatory reaction. There were no ring hemorrhages in either early or late lesions.

Spinal Cord—The lesions of the spinal cord were distributed most irregularly. The white matter was sometimes involved at all levels examined and at the other extreme the lesions might be confined to a single block of the four studied as routine. There was no preference for any particular level. It was clearly evident that the changes were not of the nature of tract degenerations, for different tracts would be involved at different levels. There were also instances where the cervical and sacral cord contained lesions while the intervening cord was normal. Again, sections from the cervical cord level might be free from change, while different tracts were involved at each of the other three levels examined. The lesions were accompanied by meningitis of the same character and cellular content as that described in the brain, though often less extensive.

The most characteristic feature of the lesions was demyelination. Such changes were found in the ventral, lateral, and dorsal columns and might be so extreme as to involve all the white matter at one particular level or so slight as to appear as a small solitary peripheral patch (Figs. 15–18). The demyelination was usually accompanied by active liquefaction necrosis the degenerated area appearing as ragged cystic spaces (Fig. 19). These lesions of the white matter seemed to start from the pial surface and to extend centripetally until in the larger lesions the grey matter was reached. While the portions of the grey matter immediately adjoining the lesions of the white matter showed a little necrosis and infiltration by inflammatory cells, one of the most striking features of the lesions was the abruptness of transition from severely damaged fiber tracts to essentially normal grey matter. Occasional exceptions to this situation are discussed below. The lesions stopped abruptly at the point where the nerve root emerged from the dura, all portions of the spinal nerves with the histologic structure of peripheral nerves, and the spinal ganglia being wholly unaffected (Fig. 20).

In some instances, the demyelinating process was unattended by any inflammatory cellular infiltration. Often, however, the degenerated areas were early invaded by polymorphonuclear leukocytes and later by mononuclear cells, presumab.y gaining access to the lesions from the pial vessels. Some cells in the region were regarded as microglial in origin. Their nuclei, in comparison with those in the unaffected areas, were pyknotic or lacking. However, fat stainable with Scharlach R was minimal and no material showing birefringence with polarized light could be demonstrated. What the large vacuolated cells sometimes demonstrated in the lesions contained we have not been able to determine, other than that it was not fat. In the most severe lesion some of the neurites were probably destroyed, but it was much more characteristic for them to persist even when other structures were lost or extensively damaged. Longitudinal sections of spinal cord containing advanced lesions were stained with Bodian's technic for nerve fibers. In these, the neurites were found to persist throughout the lesions even though they could not be followed with certainty in preparations made by non-metallic methods. The axis cylinders were merely pushed aside and often clumped in small groups as they passed around the circular or oval spaces left by the liquefaction of myelin and destroyed tissue elements (Fig. 21). They were not thickened, beaded, or granular, having the histological appearance of normal nerve fibers. In Bodian preparations, the sharp line separating the demyelinating lesions in the spinal cord from the intact dorsal and ventral nerve roots was most striking.

It was usual to find the grey matter entirely unaffected, even in sections showing extreme

demyelination of the fiber tracts. In some instances, however, there were lesions of the grey matter ranging from small focal collections of polymorphonuclear leukocytes and microglial cells to large areas of necrosis involving all the supporting elements (Fig. 22). There were a few instances in which almost all the white and grey substance was involved at one particular level, amounting to a complete transection at that point with less extensive involvement at other levels. In general, one is impressed by the resistance of ganglion cells to JHM virus. Even when the ground substance of the grey matter was rarefied, spongy, and infiltrated with wandering cells, the majority of the ganglion cells were intact and possessed normal outlines, nuclei, and Nissl substance. It is clear that the alterations in the white matter are not secondary to ganglion cell degeneration as in acute anterior poliomyelitis. Further proof of this lies in the uniform integrity of the neurites of the motor roots.

The central canal showed in a few instances hyperplasia of ependymal cells with mitotic figures easily demonstrable. In these instances, inflammatory cellular infiltration, fragmentation of nuclei, and chromatophagocytosis were present in mild degree.

The later stages of the spinal cord lesions were characterized by limited ingrowth of connective tissue fibers (impregnated by Laidlaw's method for reticulum) into the demyelinated areas from the pia (Fig. 23) and by a definite but limited proliferation of astrocytes. The spongy appearance persisted for at least 4 months, and by that time no compact glial feltworks had developed. As in the pons and cerebellum, there might be small clear vacuoles in the white matter and occasionally in the grey at points distant from the lesions just described (*état criblé*).

Peripheral Nerves.—The peripheral nerves near their emergence from the spinal cord were available for study in all mice. In some animals, the sciatic and femoral nerves were obtained by sectioning the entire thigh. No lesions of any sort were found.

Liver.—Focal necroses were found in a high percentage of mice injected with the JHM virus. It is possible that some necroses developed in all or nearly all mice since the lesions were small and might not be included in the plane of section. In fact, there were several instances in which necroses were recognized in the gross but not found in one or two blocks saved for histological study.

Upon microscopical examination, the necroses followed a rather uniform pattern. At the periphery of the lesions, the normal liver cells gave way suddenly to cells which were undergoing hyaline degeneration of their cytoplasm, either diffuse or in the form of globular masses. The hyaline material was intensely eosinophilic. The nucleus underwent degeneration later than the cytoplasm and was sometimes partially preserved even when the rest of the cell was completely hyalinized. Eventually, the chromatin broke up into a few irregular strands. Often these hyalinized liver cells became detached and appeared as oval bodies surrounded by a clear space. At the center of the lesions, the liver cells disappeared completely. Here the tissue was composed of the collapsed reticular tissue, many wandering cells, and fat-containing phagocytes possibly derived from Kupffer cells. Polymorphonuclear leukocytes were rather numerous in the early stages but rapidly underwent pyknosis and fragmentation, the rounded masses of chromatin being taken up by phagocytic cells. Fragments of hyalinized liver cells were also ingested by phagocytes. Small multinucleate giant cells were often found and rarely large giant cells originating from liver cells made their appearance (Fig. 24). In later stages, a new formation of delicate reticular fibers was clearly shown in Laidlaw preparations. Occasionally, the necrotic cells became calcified (Fig. 25).

Aside from these focal lesions, the parenchyma was unaffected.

Spleen.—No necroses were seen in this organ or indeed any other change which might be regarded as a specific effect of the JHM virus, unless it be the very rare occurrence of multinucleate giant cells within the Malpighian follicles. These are peculiar structures composed of a large number of closely aggregated vesicular nuclei within a poorly defined cytoplasmic matrix. This description applies also to the giant cells in other lymphatic tissues. The follicles

were often very large, and there might be pyknosis and fragmentation of lymphocytes with phagocytosis by reticular cells. The pulp was congested. Megakaryocytes were extremely numerous and perhaps excessively so, but this could not be stated with certainty since the normal mouse spleen is rich in these elements.

Lymphatic Tissue.—Occasional giant cells were found in peripancreatic lymph nodes and in Peyer's patches (Fig. 26). There were no focal necroses or other striking lesions.

Intestine.—In some animals, the intestine contained dark brownish material suggesting decomposed blood. Sections of the stomachs of these mice showed minute superficial necroses accompanied by an acute inflammatory reaction. In one mouse, there were numerous epithelial giant cells without other changes. The changes in Peyer's patches have been described with those of other lymphatic tissues.

Thymus.—There was accidental involution in the few instances in which this organ was examined, a change common to many infections and not specific for the JHM virus.

Organs without Lesions.—No changes were found in the heart, lungs, pancreas, kidney, adrenal, voluntary muscle, femoral and vertebral bone marrow, or pituitary.

Results of a Single Inoculation by Other Routes.—

Intraperitoneal.—As reported in the previous paper (1), infection is readily accomplished by the intraperitoneal route. Although our material permits us to state that lesions indistinguishable from those proved by intracerebral inoculation result also from intraperitoneal inoculation, it is somewhat difficult to compare the intensity and time of onset because of the relatively small number of mice examined after intraperitoneal inoculation and the fact that mice of different ages and material from different passages were used. It is our impression that the liver necroses appeared early and were very numerous. One mouse receiving a 10 per cent suspension of liver intraperitoneally and autopsied after 8 days developed extensive liver necroses but no changes in the brain or spinal cord. Demyelination was marked after the 8th day but absent or minimal earlier. Two of five mice autopsied on the 4th day showed large areas of necrosis in the ground substance of the central grey matter of the spinal cord with relatively good preservation of the ganglion cells.

Intramuscular.—Five mice which had been injected intramuscularly with brain suspensions proved to have typical lesions of the brain and spinal cord. Demyelination was absent on the 4th day and marked on the 6th.

In striking contrast to the acute myositic lesions produced by certain neurotropic viruses (GD VII, FA, SK, LCM) (2), no local lesions were present other than the non-specific interstitial infiltration resulting from injection of brain tissue.

Intranasal.—Five mice were inoculated by the intranasal route. Sections after decalcification in Bouin's fluid were made through the entire cranium. It could, therefore, be shown that the virus produced no local inflammatory changes in the nasal mucosa. The mice were killed on the 3rd and 4th days, at which time all showed very severe cerebral lesions. The olfactory lobes were extensively affected (Fig. 27). However, this localization was also evident in mice infected by other routes and was probably not due to a direct extension from the

nasal mucosa. The spinal cord in three mice killed on the 3rd day showed no lesions. In each of two of the mice, there was a single small lesion of the retina, consisting of a focus of cellular necrosis with polymorphonuclear leukocytic reaction in the nerve fiber layer (Fig. 28). Sections of the lungs showed no significant changes.

Intravenous.—Twenty-two mice, injected intravenously with doses of brain

TABLE I

Symptoms and Lesions after Intravenous Injection

No.	Date	Days	Dosage*	Symptoms	Brain	Cord	Liver
2469	1/ 3/49	3	100,000	0	0	0	0
2468	1/13/49	3	100,000	0	0	0	++++
2665	2/ 5/49	5	30,000	+	±	±	++++
2776	3/ 4/49	6	1,000	+	Not taken		++++
2777	3/ 4/49	6	1,000	+	+	++++	+
2778	3/ 4/49	6	1,000	+	+++	+++	+
2779	3/ 5/49	7	1,000	+	Not taken		++++
2669	2/ 7/49	8	30,000	0	0	0	0
2694	2/15/49	8	10,000	+	+++	±	+++
2437	12/18/48	9	100,000	0	0	0	0
2782	3/ 7/49	9	1,000	+	+	+++	0
2783	3/ 7/49	9	1,000	+	++++	+	++++
2711	2/17/49	9	5,000	+	++	0	+
2680	2/11/49	11	30,000	0	0	0	0
2534	1/10/49	12	20,000	0	0	0	0
2533	1/10/49	12	100,000	0	0	0	0
2789	3/10/49	12	1,000	+	++	+++	++
2790	3/10/49	12	1,000	+	+++	++	++
2740	2/23/49	15	5,000±	+	++	0	0
2739	2/23/49	15	5,000±	+	+++	±	±
2749	2/24/49	16	5,000±	+	++	+++	++
2797	3/14/49	16	1,000	+	++	++++	0

* I.C. LD_{50} (approximate).

suspension ranging from 1000 to 100,000 LD_{50}, were examined. Six mice remained well, and no lesions were found in central nervous system or liver. The remaining sixteen, which developed symptoms at periods varying from 6 to 16 days, all had lesions of the brain and cord. These differed somewhat from those produced by intracerebral inoculation.

The cerebrum, in general, was not severely affected; there was little or no meningitis; the perihippocampal "slit lesion" was absent, and massive necrosis was exceptional. The lesions when present, consisted of small encephalitic foci scattered through grey and white matter. The brain stem, however, was quite severely affected, and the lesions were characterized by the rarefying vacuolar degeneration of the ground substance seen in animals infected by other routes. In the cord, some animals showed quite typical and extensive demyelination. The grey matter also was frequently involved, and in several animals, the entire cord at one or more levels was almost completely necrotic.

The liver lesions in these mice were particularly interesting, and of an intensity not seen in mice infected by the usual route. While some animals showed only discrete focal necroses of the usual type, others exhibited massive necrosis affecting the entire organ. Grossly, such livers were greatly enlarged, bright yellow, and flecked with opaque whitish spots, which microscopically represented masses of necrotic liver cells which had become calcified. Microscopically, there were comparatively few normally staining liver cells, and of these many contained small fat vacuoles, and had shrunken, deeply staining nuclei. In every lobule, there were large numbers of necrotic cells, devoid of nuclei, staining intensely with eosin, finely vacuolated, and in Sudan IV–stained frozen sections, filled with small fat droplets. The necrosis involved not only the liver cells, but the Kupffer cells as well. There were scattered pyknotic and fragmenting leukocytes through these areas, but the inflammatory reaction was not intense. Large numbers of the necrotic cells stained bluish with hematoxylin, and were shown in Von Kossa preparations to have become impregnated with calcium. In some areas, the necrotic cells had disappeared, leaving the collapsed stroma (Fig. 29).

Table I indicates the individual variation in susceptibility, as well as the lack of correlation between dosage and lesions. Thus mouse 2534, which had been given 100,000 LD_{50}, killed on the 12th day, remained free from lesions and symptoms, whereas mouse 2778, receiving only 1000 LD_{50}, sacrificed on the 6th day, when typically ill, had lesions in brain, cord, and liver. We have at present no explanation for these striking discrepancies in the reaction of individual mice to intravenous injection of the virus.

Multiple Injections.—Sixteen mice surviving intramuscular injection for 13 days were challenged with intracerebral inoculation and examined at periods from 2 to 5 days after the second injection. All had severe and typical lesions and there was no indication that the previous single inoculation had conferred any protection. This was true also of two mice injected subcutaneously and one intravenously followed by intracerebral inoculation 6 and 22 days later.

Comparison of Lesions Produced by Different Passages.—

In the course of these studies, material from the first to the fifty-second passage was used. Repeat passages were also made from second passage material which had been frozen for a 9 months' period. Our material analyzed from this point of view showed clearly that identical lesions were produced, irrespective of the passage from which the material was obtained. However, it was evident that the incubation period on repeated passage had become shortened and this, as might be expected, was associated with a speeding up of the development of the lesions, if one compares the mice of the early and late passages autopsied on the 2nd day. After this time, there was no noticeable difference. The uniformity in the character of the lesions would seem to afford good evidence that no adventitious virus was introduced in the course of the experiments.

Susceptibility of Suckling Mice

Because of the preferential affinity of the JHM virus for white tracts, it was interesting to find most extensive lesions in brain and cord at an age (6 to 8 days)

when myelination is completely lacking in the brain, and only beginning to appear in the dorsal columns of the cord.

The mice died or were killed on the 2nd day after intracerebral inoculation of 0.03 cc. of a 10^{-1} suspension. At this time they showed severe encephalitic symptoms. In addition to intense meningitis, there were large areas of rarefying necrosis in all portions of the brain, with many giant cells. As in older mice, the olfactory lobes were most severely affected. The cord lesion, in addition to meningitis and giant cells, consisted of extensive areas of spongy necrosis of the central grey matter, with comparatively good preservation of ganglion cells.

One may conclude that the virus may establish itself in the central nervous system before myelination has taken place.

The liver, as in the older animals, was the seat of multiple focal necroses.

Lesions Produced by JHM Virus in Other Laboratory Animals

Hamsters.—As observed in the preceding paper, the Syrian hamster proved to be susceptible, succumbing with encephalitic symptoms in 2 or 3 days after intracerebral inoculation.

Only a few animals were available for pathologic study, but the lesions found were essentially similar to those seen in mice dying during the first few days following intracerebral inoculation of the virus. In the brain, there were large sharply circumscribed areas of rarefying necrosis in cortex and midbrain, and especially in the perihippocampal regions, with the characteristic involvement of the ependyma, and lateral dissection of the ventricular cavities. As in the mice the ganglion cells were less affected than the ground substance. The cord lesions were relatively slight, but essentially like those in the mice at a corresponding stage,—namely, small areas of peripheral and inflammatory cellular infiltration in the white matter. In one such area, the swollen hydropic nuclei of the glial cells contained small eosinophilic spherical inclusions, which in Laidlaw preparations stained intensely with fuchsin. Since these were not found in other lesions or animals, their significance remains in doubt.

No necroses were present in the liver. The lungs of all five hamsters examined were grossly edematous and congested, but no definite pneumonic lesions were seen microscopically. Intracerebral inoculation of lung suspensions into mice failed to disclose the presence of virus.

Further experiments with more dilute suspensions of virus will be necessary to show whether it is possible to reproduce extensive demyelination of the cord such as is seen in mice surviving for 6 or 7 days.

When infected hamster brains were passed back to mice either by intracerebral or intraperitoneal injection, the characteristic pathologic changes described above were elicited The meningitis and tendency to formation of giant cells were particularly pronounced. One interesting and hitherto unobserved finding may be briefly recorded. A mouse (2632) injected intracerebrally with a 10^{-1} suspension of hamster brain was sacrificed, when moribund, 24 hours later. The liver was found to be the seat of widespread central necrosis, involving at least the inner half of each lobule. The necrotic cells had lost their nuclei, and stained intensely with eosin. The Kupffer cells were still preserved; there was no inflammatory reaction. Other mice of this same series showed only the usual focal necroses.

White Rats.—The response of white rats to intracerebral injection of the virus, at least in the first passage, is delayed. In fifteen rats injected, symptoms

first appeared on the 13th to 29th days; one developed transient incoordination but recovered, and one remained well. Material from fourteen rats has thus far been studied.[2]

Although a more extensive study is planned, the lesions found in the few available rats are of sufficient interest to merit description at this time (Figs. 30 and 31).

The acute lesions—meningitis, ependymitis, fresh necroses—described in mice, were absent. Instead there were found large circumscribed irregular patches in which the normal structure was replaced by a finely fibrillar and spongy vacuolated tissue containing great numbers of pale, small spherical nuclei, possibly oligodendroglial, and larger nuclei belonging to astrocytes. In myelin stain preparations, the myelin sheaths were found completely lacking in these areas, which, however, were not confined to the white matter. In the brain, such patches were found both in cortex and perihippocampal tissue; the medulla and pons were extensively involved, and in the cord, the white tissue was replaced over large areas. The central grey matter was also affected. The blood vessels showed little change, but a few were surrounded by thin colums of lymphoid cells. In one preparation, a large ependymal giant cell free in the third ventricle testified to the propensity of this virus to evoke such a reaction.

The persistence of virus in this subacute form of the disease, as shown by successful back passage to mice 29 days after the original inoculation, is of interest. It is a point against the idea that the lesions are the product of an allergic reaction against a non-infectious agent.

Guinea Pigs.—As stated in the previous paper, guinea pigs are not susceptible to this agent when inoculated intracerebrally. Two animals which had received 0.06 cc. (20,000 mouse LD_{50}) of 50th passage mouse brain intracerebrally were killed on the 29th day, there being no manifest signs of illness. In the cerebrum of one (guinea pig 5-2) the ependymal lining of the lateral ventricle on one surface was replaced by a glial mat, beneath which were numerous gland-like aggregations of ependymal cells. This was interpreted as indicating a healed ependymitis. No lesion was found in the spinal cord, brain stem, or cerebellum, but the ganglion cells of the alveus hippocampi over a short stretch, were shrunken and degenerated.

In a second guinea pig (No. 5-3), similar changes were seen in ependyma and hippocampus. In addition, it was noted, in a section through the olfactory bulbs, that the central white matter was rarefied and infiltrated with microglia. The spinal cord was normal. In the liver of this animal, which was rich in glycogen, were seen a number of sharply circumscribed areas of necrosis, with complete loss of nuclear staining. There was no inflammatory reaction.

Whether these relatively minor lesions are to be taken as evidence of inapparent infection, cannot be decided without further study. No attempt was made to recover the virus from these animals.

Monkeys.—Several *Macaca mulatta* monkeys have been inoculated intracere-

[2]We have excluded one animal which succumbed on the 16th day, and was found to have bacterial infection of kidney and bladder.

brally with passage mouse material. Only one of these animals has thus far been examined histopathologically, and it seems wiser to defer a description of the effects of the agent in this species to a later publication.

DISCUSSION

The lesions caused by the JHM virus have, as has been pointed out, certain features which differentiate them from those caused by other known "neurotropic" viruses pathogenic for mice. There are many viruses, either originally recovered from mice, or derived from human or other animal sources, which when injected intracerebrally into mice produce encephalomyelitis. The various strains of mouse encephalitis (Theiler's GD-VII, FA, and FV) elicit lesions which, though differing in distribution, are essentially comparable to those of human poliomyelitis in that they affect primarily the grey matter, in contrast to the JHM virus which ordinarily brings about destructive lesions in the white matter and tends to spare the ganglion cells and nerve fibers. There are other differences. The more virulent strains of Theiler's virus (2) bring about intense local myositis on intramuscular injection; this is not true of the JHM virus. Focal necroses of the liver have not been observed in mice injected with GD-VII or other Theiler strains; they occur frequently in JHM-infected mice, whatever the route of inoculation. The tendency to formation of giant cells, not only in the central nervous system but also in lymph glands, intestinal lymphatic tissue, and liver, is not evident in the lesions caused by viruses of the Theiler group. It is unnecessary to point out that the virus of lymphocytic choriomeningitis produces lesions which, as the name indicates, differ fundamentally from those of the JHM agent.

So far as we can find from personal experience and references to the literature, none of the many viruses from various sources which are pathogenic for mice brings about myelin destruction to the same degree as does the JHM. We have studied the central nervous system lesions resulting from infection with the SK Columbia virus of Jungeblut, Eastern equine encephalitis, herpes, LCM, and EK viruses.

Through the kindness of Dr. Koprowski, we have had the opportunity to see Nissl-stained slides from two mice which developed encephalitic symptoms in the course of rabies immunization. The sections suggest a demyelinating process in the cord akin to that seen in the JHM mice, but since no attempts at passing the material were made, it is impossible to form an opinion as to the relationship of their disease to the one we are studying.

A possible exception to this general statement is the myocarditis virus (EMC) isolated by Helwig and Schmidt (3) from cases of spontaneous myocarditis in a gibbon and in a chimpanzee in Florida in 1944. This was successfully passed to guinea pigs, hamsters, and mice. By repeated transfer in mice, it has become highly neurotropic, producing encephalomyelitis by both intracerebral and intraperitoneal injection in

dilutions of 10^{-8}. The myocarditis in mice is found only in animals surviving for 6 days or longer. There is no reference to necroses of the liver. Immunologically, this virus appears to be antigenically related to the MM and Columbia SK strains (4).

In Schmidt's paper on the pathology of this virus disease (5), the lesions in the spinal cord are described as follows: "Sections through the spinal cord of mice (showing paralysis) showed extensive destruction of all the outer neural elements with invasion of mononuclears and a few polymorphonuclear leukocytes which frequently clumped beneath the meninges. The ganglion cells were apparently not involved by the extensive myelitis. In this period and somewhat later foci of encephalomyelitis could be seen in the brain." We are indebted to Dr. Schmidt for sending us slides illustrating these changes. The illustrations to Schmidt's paper include two photographs (Plate 23, Figs. 10 and 11) clearly showing demyelinating lesions of the cord.

Another reference to the occurrence of demyelinating lesions in mice is found in the paper of Margulis, Soloviev, and Shubladze (6). These authors obtained from the cerebral substance of a fatal case of human acute disseminated sclerosis, and also from the blood of a non-fatal case, a filtrable virus which proved pathogenic for puppies, rabbits, guinea pigs, young rats, and mice. Inflammatory lesions, accompanied by myelin destruction, were found predominantly in the brain. After a latent period of 10 days or more, the mice developed tremors, transient convulsions, and forced movements of limbs. Neutralizing antibodies were present in the sera of human patients who had recovered from acute disseminated sclerosis or who had the symptoms of multiple sclerosis.

Since the principal interest in this new mouse disease lies in the fact that it may prove a valuable tool in the study of the demyelinating process in general, one should discuss briefly other examples of demyelinating disease in human beings and laboratory animals.

Demyelination is a salient feature of the following diseases and conditions:—

1. In the encephalomyelitis which may follow various infectious diseases, notably measles, varicella, variola, smallpox vaccination, or arise in the course of rabies immunization.
2. In acute disseminated encephalomyelitis of man and in multiple sclerosis.
3. In Schilder's disease.
4. In certain types of encephalomyelitis in dogs.
5. In monkeys as a spontaneous form of disseminated encephalomyelitis.

The demyelination which occurs in postvaccinal and other postinfectious conditions is predominantly perivascular in distribution; the blood vessels in the affected regions are sheathed in inflammatory cells of various types, but chiefly mononuclear. In comparison with the cerebrum, the spinal cord is relatively little affected.

This is true also of the demyelination in acute disseminated encephalomyelitis of man, and of course of the multiple sclerosis lesions which some authors are inclined to regard as a late or chronic form of disseminated encephalomyelitis. In Schilder's disease also, the demyelination, which may be very extensive, is largely restricted to the subcortical white matter of the brain.

The lesions in the nervous form of canine distemper have been studied by a number

of authors (7). Judging from the descriptions in the literature, they are accompanied by severe myelin destruction, both in brain and cord, and thus bear resemblance to JHM lesions. Many authors, however, have succeeded in demonstrating intranuclear and cytoplasmic inclusions identical with those present in other situations; similar inclusions have not been found in our mouse material. There are other differences in the pathology which make it improbable that the JHM virus should be closely related to that of canine distemper.

It is possible that dogs are subject to another form of demyelinating disease not directly associated with infection by the virus of canine distemper. Under the caption "Acute multiple sclerosis of dogs," Scherer (8) describes lesions which are essentially like those of acute disseminated sclerosis in man. Patches of demyelination occur in both grey and white matter; the neural elements are faultlessly preserved. On purely morphologic grounds, Scherer takes the position that this disease has nothing to do with the nervous form of canine distemper. The distemper etiology of demyelinating canine encephalomyelitis has recently been questioned also by MacIntyre, Trevan, and Montgomery (9) and by Innes (10). These authors describe a condition termed "foot-pad disease," characterized by hyperkeratosis of the foot-pads, and by extensive demyelinating and inflammatory lesions particularly in the cerebellar peduncles and folia of the cerebellum. This disease, unlike distemper, cannot be transmitted to ferrets, and does not produce typical distemper lesions when transmitted to dogs. The virus has not as yet been well characterized, but the disease offers a good example of a demyelinating infection in another species.

A disease which in its essential pathology resembles the JHM encephalomyelitis of mice occurs in monkeys kept in confinement. Scherer has assembled forty-nine instances of this "Leuko-encephalosis and myelosis," including twelve personally studied by him. It has been found in a variety of species—orang-utang, macaques, *Cercopitheci*, *Erythrocebus*, etc. The cord lesions consist of large but discontinuous patches of demyelination, irregularly effecting all tracts of the spinal cord. Inflammatory changes are not marked. Without going into details, it may be stated that the lesions are in many respects analogous to those of the mouse disease.

There have been a few attempts to demonstrate the infectious etiology of this monkey disease. Gärtner (11), starting from material of Schob's case in an orang-utang (12), inoculated a *Cercopithecus* and two *Papio hamadryas* monkeys, which developed similar symptoms, and proved to have confluent areas of demyelination in hemispheral white matter. Spinal fluid and blood from one of the *Papios* were injected intramuscularly into a *Macaca rhesus*. The animal given spinal fluid developed a very acute illness after several weeks, and histologically there were found areas of degeneration The description is incomplete. A third passage with brain suspension injected intramuscularly into a *Lasiopyga leucampyx* was positive, and spinal fluid and ultrafiltrate from brain material produced syptoms in a fourth passage. Since these experiments were never reported *in extenso* and no control animals maintained under similar conditions were examined, the results are not entirely conclusive.

Schaltenbrandt (13) has reported in great detail experiments which he regards as demonstrating the transmissibility of human disseminated encephalitis and multiple sclerosis to monkeys. Intracisternal injection of spinal fluid from human cases was regularly followed by pleocytosis in the spinal fluid of the monkeys, and in many cases

by the development, after widely varying periods, of demyelinating encephalomyelitis identical in its clinical and pathologic features with the spontaneous disease as described by Scherer and others. Indeed, one cannot exclude the possibility that Schaltenbrandt was dealing with the spontaneous "cage paralysis" of monkeys—particularly since monkeys in the same cage with the injected animal often became affected. It would be rash indeed to derive conclusions as to the virus etiology of the human disease on the basis of these experiments.

The experiments of Levaditi, Hornus, and Schoen (14) are also unconvincing. The infectious etiology of this monkey disease can therefore not be considered as proven.

The cause of these various demyelinating diseases continues to be the subject of lively discussion, and various ideas have been propounded:—

1. The lesions are a specific effect of the virus causing the primary disease.
2. They are due to the non-specific activation of latent viruses.
3. They are caused by accidental contaminating viruses. This has been discussed particularly in connection with cases of postvaccinal encephalitis.
4. They are due—and this applies especially to the encephalomyelitis arising during rabies immunization—to an allergic reaction following sensitization to heterologous brain tissues.

It is this last explanation and its possible bearing on the mouse disease that we wish particularly to discuss. During the past few years, beginning with the experiments of Rivers, Sprunt, and Berry there have appeared a number of important papers on the production of demyelinating encephalitis in monkeys, rabbits, and guinea pigs (15–27). In general, the most successful results have been obtained by repeated intramuscular injections of homologous or heterologous normal brain suspensions, the production of antibodies having been fortified by addition of killed tubercle bacilli and falba or mineral oil.[3] It would carry us afield to analyze these papers in detail. All the workers agree in attributing the lesions to an allergic response in sensitized tissue. Since the agent is not destroyed by heating or exposure to formalin (25) and since the brain disease is not transmissible, the intervention of a contaminant viral agent in such experiments can be excluded.

If one compares the lesions thus experimentally produced with those following infection with the JHM virus, certain differences are apparent. In the experimental disease, the demyelination and cellular infiltration are predominantly perivascular, although large areas may coalesce. The vessels are cuffed with inflammatory cells. The demyelination in the JHM-infected mice occurs in irregular patches which bear no obvious relation to vascular supply, nor is perivascular cell infiltration a conspicuous feature. Indeed, it may be absent in the early stages. The myelin destruction in our experimental animals is unaccompanied by the liberation of stainable fat in any quantity. In this it differs from

[3]In a recent paper (28), it has been shown that this allergic encephalitis may be produced in monkeys with suspensions of the animals' own brain tissue, obtained by lobectomy.

the usual demyelinating process. King (29), in his study of the pathology of dog "distemper" encephalomyelitis, has called attention to this form of myelin degradation. Morrison (30) has approached the problem experimentally and has shown that the lecithinase of *Clostridium welchii* toxin splits off the phosphorylcholine moiety of lecithin without the liberation of stainable fat, differing in its action from the cobra venom lecithinase, which acting *in vitro* upon the myelin, splits off the fatty acids with the appearance of stainable fat.

Giant cells are not a conspicuous feature in the "allergic" encephalitis. Rivers and Schwentker (16), it is true, refer to foreign body giant cells containing cell debris, and these are perhaps comparable to the large phagocytes filled with chromatin fragments which are commonly seen in the early JHM lesions. They also picture multinucleated elements occurring among the fat-laden macrophages, comparing these with the "globoid" bodies described by Greenfield in Schilder's disease. But we have seen no reference to large multinucleate cells arising from ependyma, glia, endothelium, liver cells, or the reticular cells of lymphatic tissue, which seem to be a characteristic feature of the JHM lesions. Kabat, Wolf, and Bezer (19), and Morgan (21), specifically noted the absence of giant cells in the monkey lesions.

More important is the broad question whether demyelination in the JHM mice could be the result, not of the direct action of the virus, but of non-specific sensitization to some component in mouse brain tissue, either in the inoculum or produced by the destructive action of the virus in the brain tissue of the infected mouse. Certain considerations speak strongly against either of these possibilities.

1. The time relations. Demyelination seems to begin very soon after the introduction of a single intracerebral injection and to increase progressively, reaching a peak at 7 days. There is little or no lag period after the injection, as one might expect if the lesions were dependent upon the development of antibodies following the introduction of an antigen. In experimental allergic encephalitis, the period between the first injection of antigen and the appearance of symptoms, as reported by various observers, ranges between 14 and 47 days.

2. The hypothetical antigen could not be something derived from normal brain tissue contained in the inoculum, since the demyelinating lesions are produced also by injection of suspensions of infected liver tissues.

3. Demyelination has not been observed in mice inoculated with normal brain tissue, or brain suspensions infected with a variety of other viral agents. It appears to be a specific reaction to this particular agent.

4. Attempts to produce allergic encephalitis in mice by injection of mouse brain suspensions have not been successful (25).[4]

[4]Since this paper was submitted for publication, Olitsky and Yager (*Proc. Soc. Exp. Biol. and Med.*, 1949, **70**, 600) have reported the successful production of allergic encephalitis in mice by repeated intramuscular injection of normal mouse brain reenforced with killed tubercle bacilli and mineral oil.

One is thus driven to the conclusion that the demyelinating lesions are brought about by the JHM virus. But how this is accomplished remains to be explained. Does the virus itself carry with it enzymes which destroy the myelin or the sustentacular tissues necessary for its integrity? Does the virus activate some preexisting enzyme system, or does it block some normal inhibitor? It will take prolonged and careful exploration to define the mechanisms involved, but one may hope that this viral disease will provide a useful implement for such studies.

SUMMARY

A description has been given of the pathologic changes produced experimentally in animals by the inoculation of a virus material obtained from a mouse with spontaneous encephalomyelitis. The most distinctive feature of the lesions in the central nervous system is the widespread destruction of myelin. Giant cells derived from a variety of tissue elements characterize the early lesions. The liver in the majority of cases is the seat of focal necrosis. In some mice, infected with large doses by the intravenous route, there is produced massive necrosis of the liver, with fat infiltration and calcification. Giant cells are occasionally found in lymphatic tissue, but no significant changes were noted in other organs. Inclusions or elementary bodies were not demonstrated in the lesions.

Similar lesions were produced by the inoculation of mouse virus into hamsters. In rats, the lesions were of a more chronic character.

The relation of this disease to other demyelinating diseases of man and animals is discussed.

We wish to express our thanks to Miss Eleanor Adams and Miss Sheila Richardson for technical assistance.

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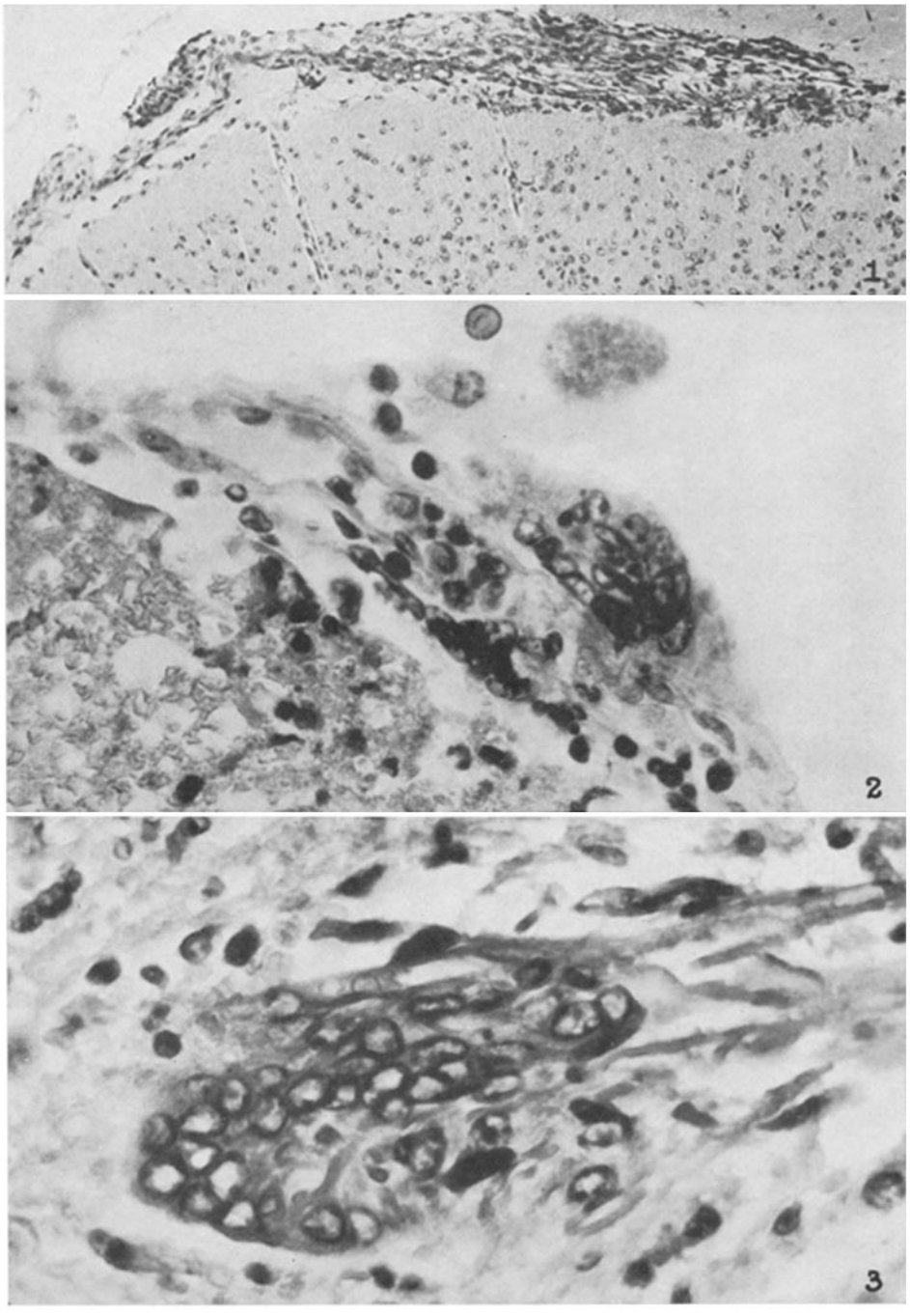
EXPLANATION OF PLATES

PLATE 9

FIG. 1. Fibrous plaque replacing meningeal exudate over cerebral cortex. Mouse E-48-137. 4th passage. 11 days after intracerebral inoculation. Hematoxylin and eosin. × 145.

FIG. 2. Giant cells in pia of cord. Mouse 2134. 45th passage. 2 days after intracerebral inoculation. Hematoxylin and eosin. × 860.

FIG. 3. Giant cell in capillary wall. Mouse 2314. Passage 4A. 4 days after intracerebral inoculation. Hematoxylin and eosin. × 860.



(Bailey *et al.*: Murine virus causing encephalomyelitis. II)

PLATE 10

FIG. 4. Lesions of perihippocampal region, anterior commissure, and alveus hippocampi. Necrosis of part of cornu Ammonis. Preservation of dentate gyrus. Mouse E-48-152. 4th passage. 1 month after intracerebral inoculation. Mahon's stain. × 36.

FIG. 5. Glia-lined cleft communicating with lateral ventricle. Mouse E-48-138. 4th passage. 11 days after intracerebral inoculation. Hematoxylin and eosin. × 117.

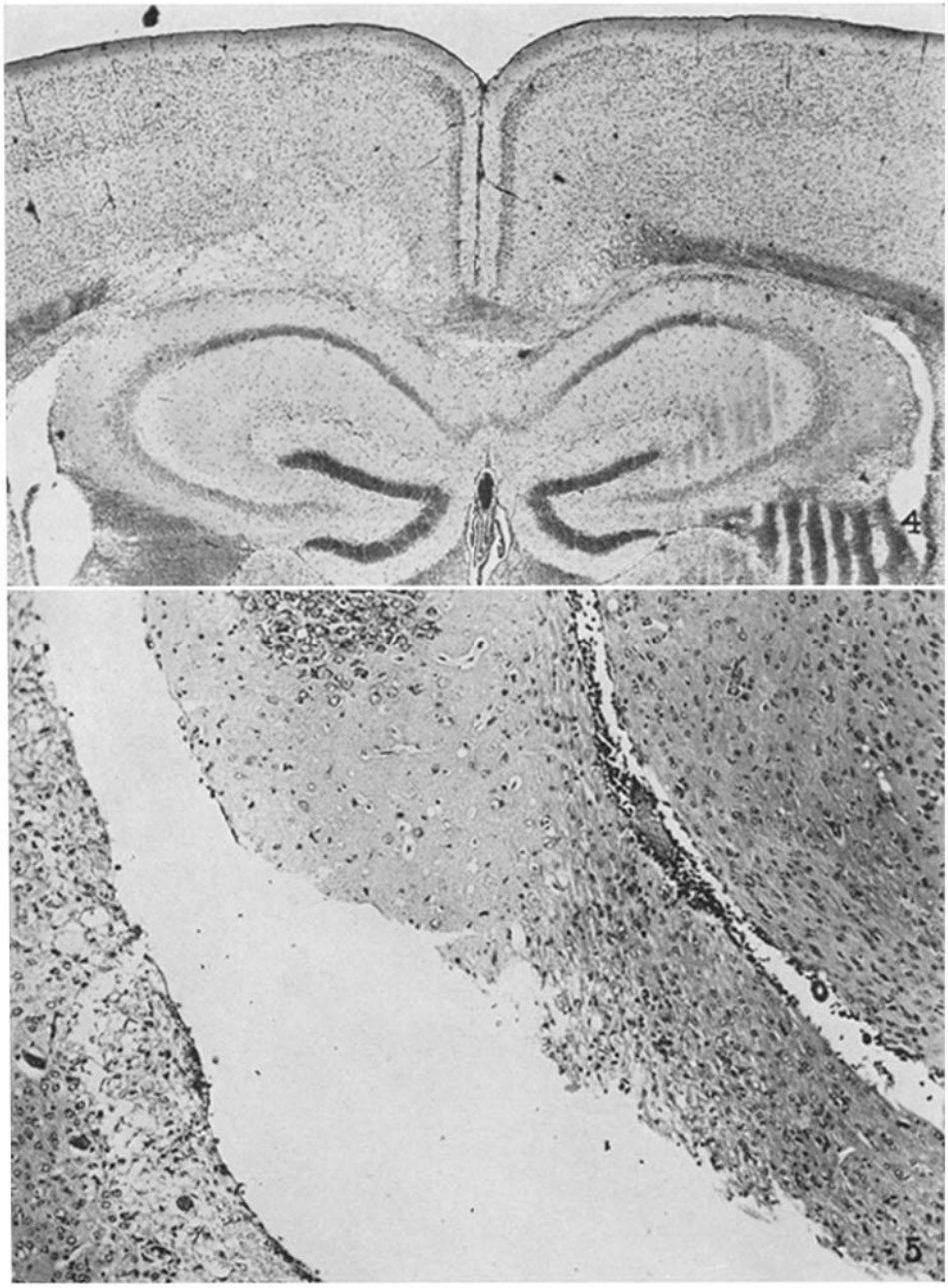


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PLATE 11

FIG. 6. "Slit lesion." Mouse E-48-120. 4th passage. 5 days after intracerebral inoculation. Hematoxylin and eosin. × 21.

FIG. 7. Acute ependymitis, with nuclear fragmentation and fusion of chromatin. Mouse 2322. 46th passage. 2 days after intracerebral inoculation. Hematoxylin and eosin. × 109.

FIG. 8. Large porencephalic cavity, extending from lateral ventricles. Mouse E-48-216. 3 months after intracerebral inoculation. Hematoxylin and eosin. × 16.

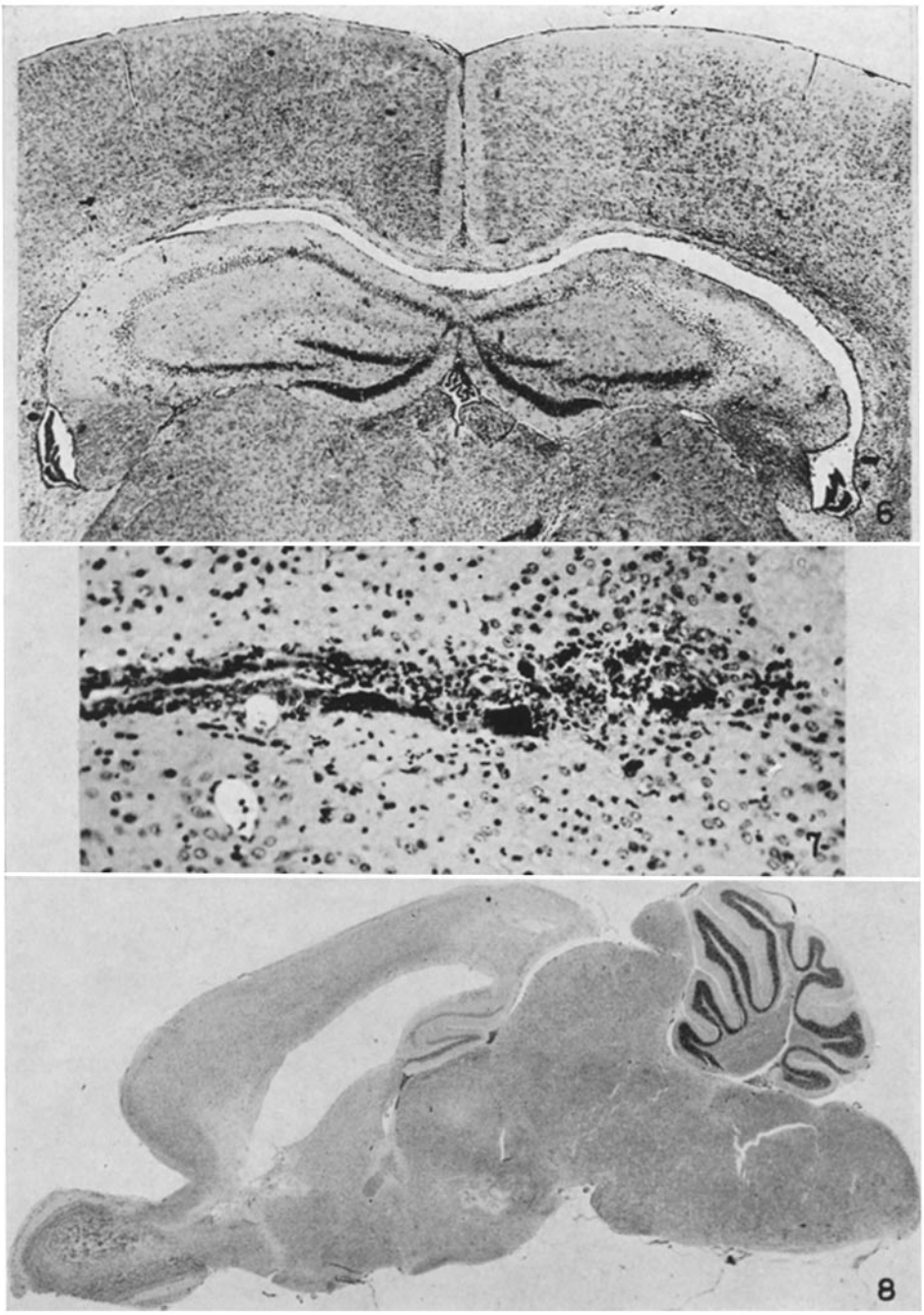

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PLATE 12

FIG. 9. Ependymal giant cell. Vascular cuffing. Mouse 2335. 46th passage. 4 days after intracerebral inoculation. Hematoxylin and eosin. × 105.

FIG. 10. Necrosis in olfactory lobe, with leucocytic infiltration and chromatorrhexis. Mouse E-48-117. 4th passage. 5 days after intracerebral inoculation. Hematoxylin and eosin. × 540.

FIG. 11. Area of rarefying necrosis in cerebral cortex. Mouse E-48-274. 6 days after intramuscular inoculation Hematoxylin and eosin. × 162.

FIG. 12. Demyelinating lesions of pons. Preservation of axis cylinders, and ganglion cells. Mouse E-48-249. 46th passage. 4 days after intracerebral inoculation. Bodian stain. × 97.

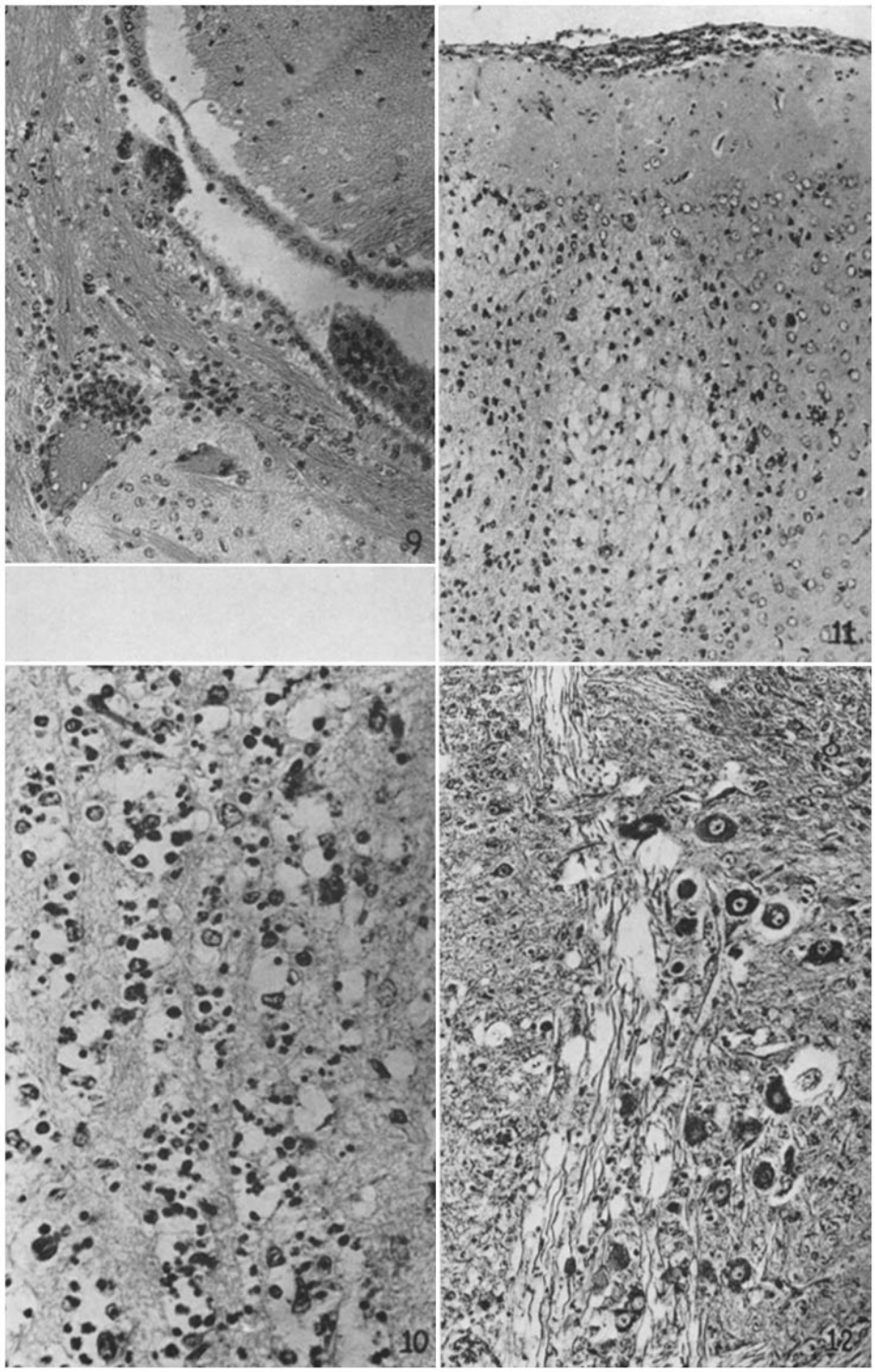

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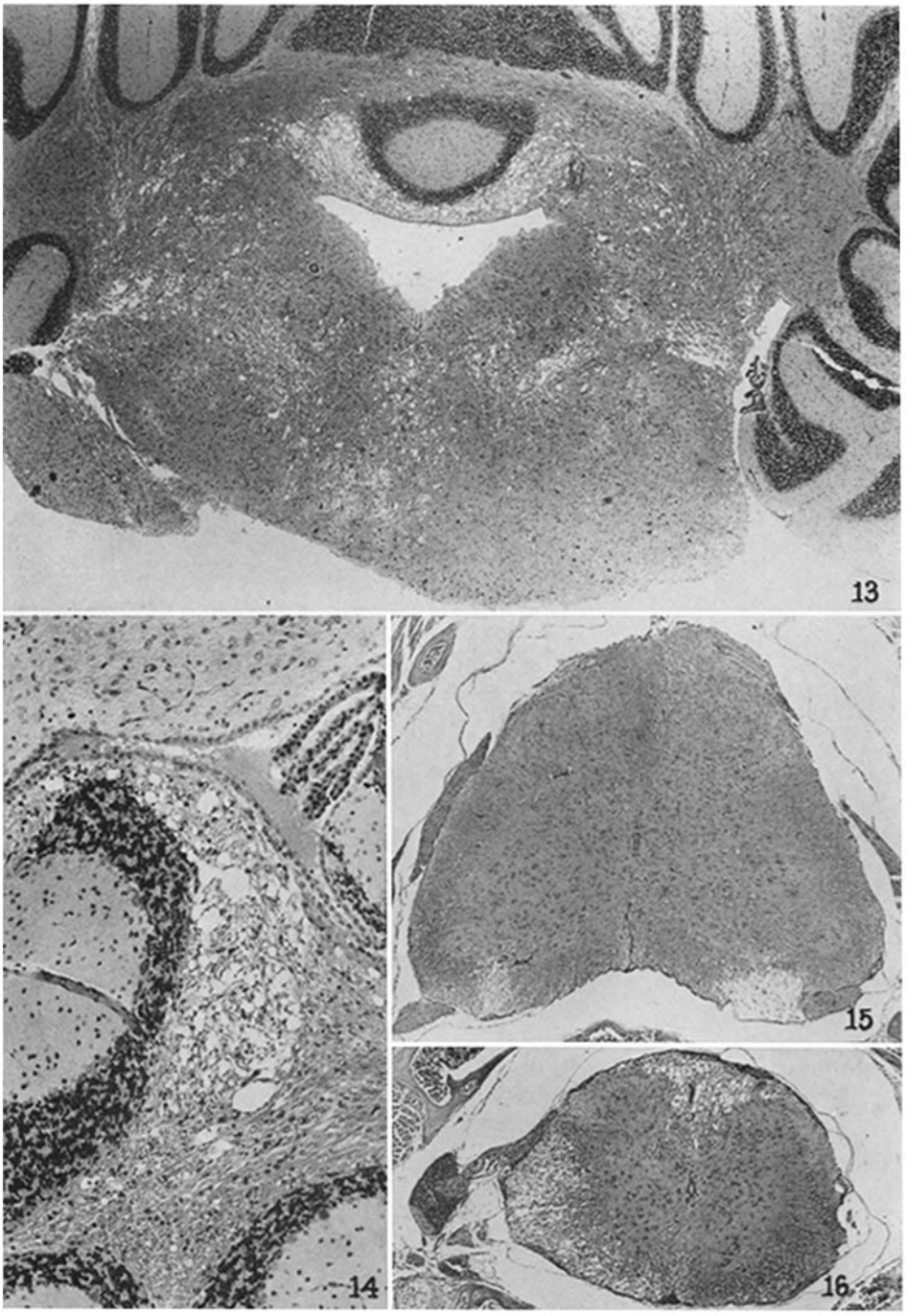
PLATE 13

FIG. 13. Extensive lesions of pons and cerebellar peduncles. Loss of ependyma in floor of 4th ventricle. Mouse E-48-138. 4th passage. 11 days after intracerebral inoculation. Hematoxylin and eosin. × 29.

FIG. 14. Demyelinating lesion in white matter of cerebellum. Mouse E-48-285. Passage 4A. 11 days after intracerebral inoculation. Hematoxylin and eosin. × 108.

FIG. 15. Early lesions of ventral tracts of spinal cord. Mouse E-48-120. 4th passage. 5 days after intracerebral inoculation. Hematoxylin and eosin. × 28.

FIG. 16. Extensive asymmetrical demyelination of white matter of spinal cord. Mouse E-48-138. 4th passage. 11 days after intracerebral inoculation. Hematoxylin and eosin. × 33.



(Bailey *et al.*: Murine virus causing encephalomyelitis. II)

PLATE 14

FIG. 17. Extensive patches of demyelination in dorsal, lateral, and ventral tracts. Note intact posterior nerve root and ganglion. No lesions of central grey matter. Mouse E-48-121. 4th passage. 6 days after intracerebral inoculation. Mahon's myelin stain. × 30.

FIG. 18. Very extensive demyelination. Mouse E-48-282. Passage 4A. 9 days after intracerebral inoculation. Hematoxylin and eosin. × 30.

FIG. 19. Microcystic degeneration in spinal cord. Mouse E-48-217. Passage 4A. 3 months after intracerebral inoculation. Laidlaw's reticulum stain. × 262.

FIG. 20. Mild lesions of white matter, which cease abruptly at transition to peripheral nerve structure. Mouse E-48-160. 4th passage. 1 months after intracerebral inoculation. Hematoxylin and eosin. × 33.

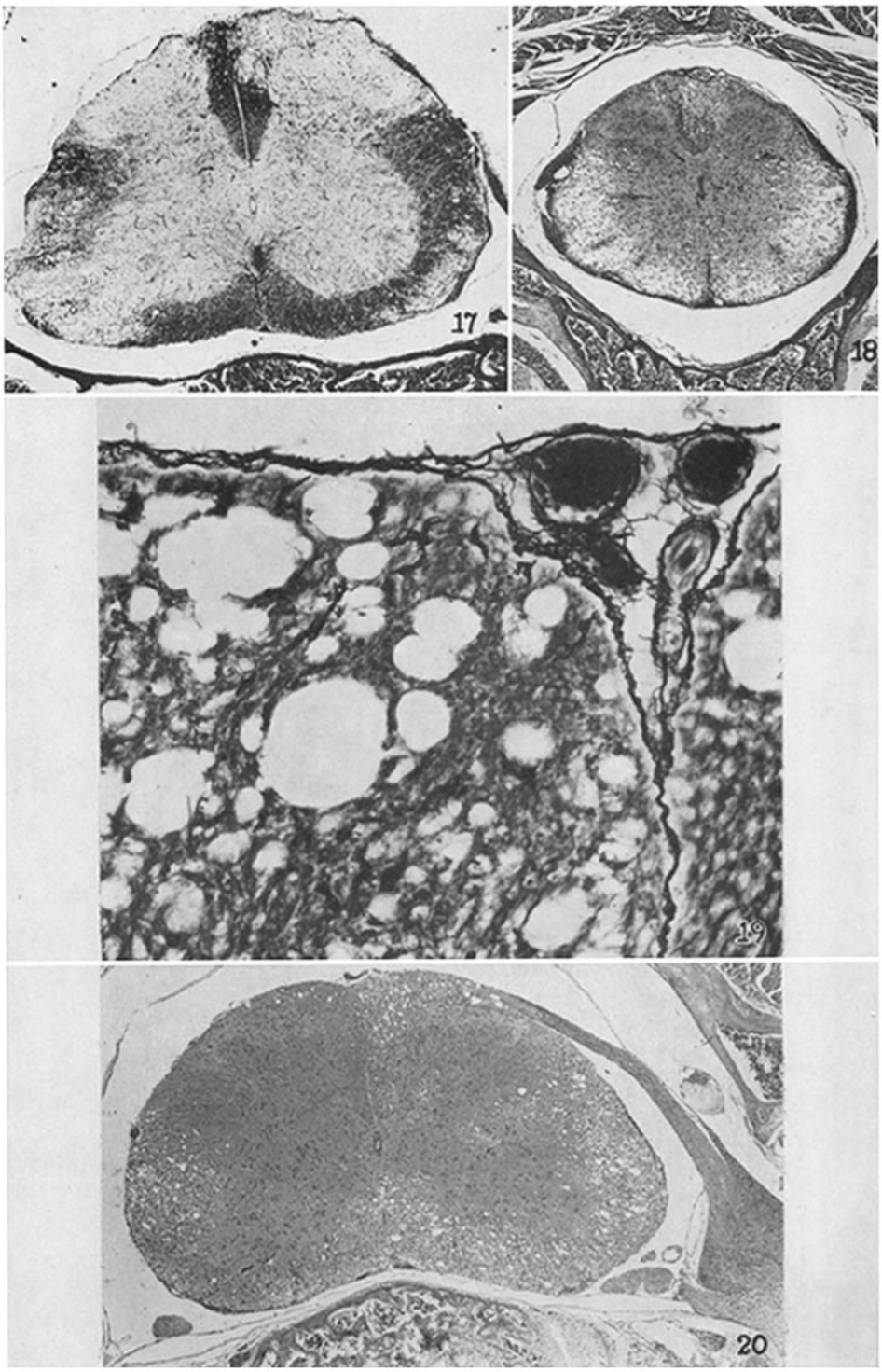


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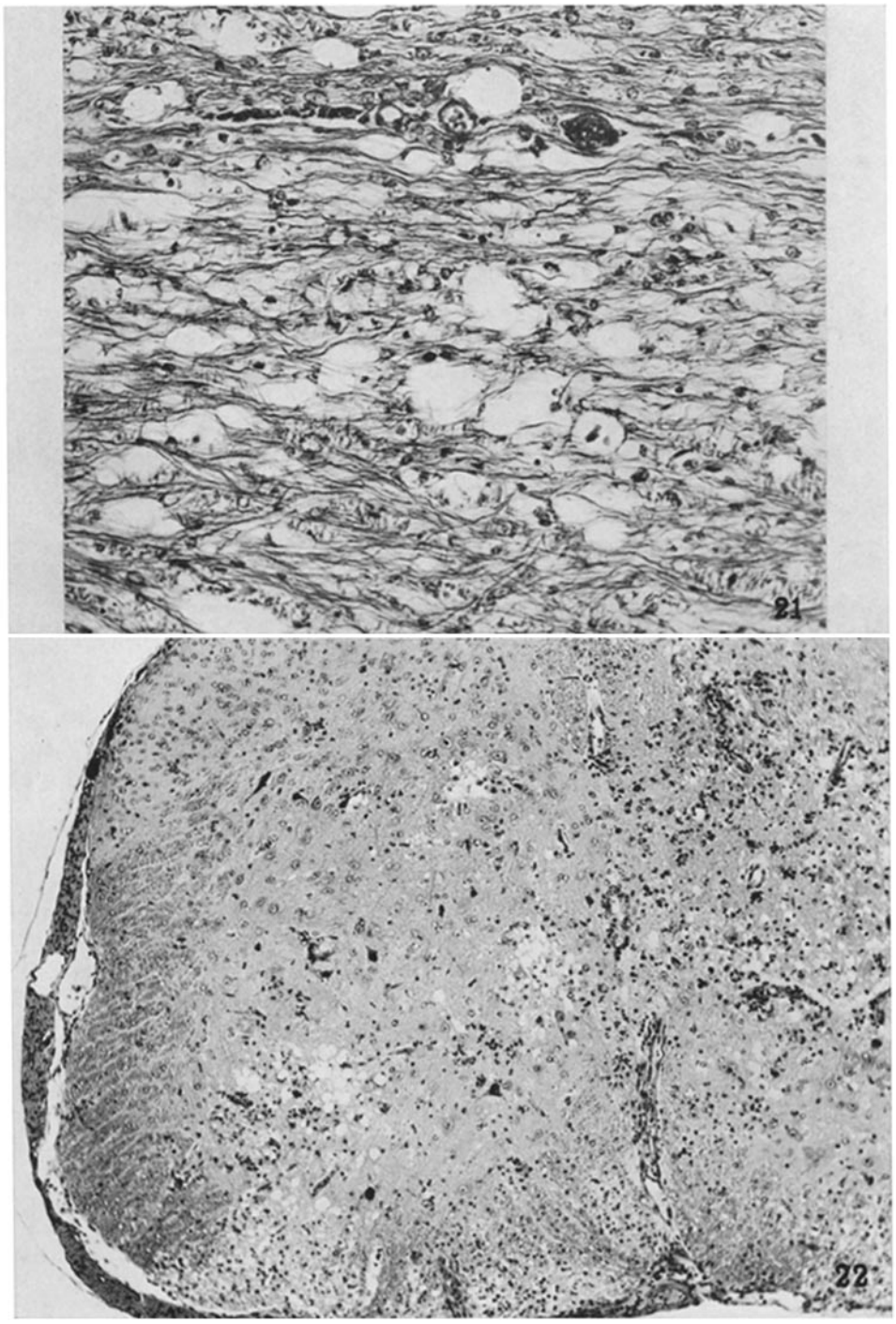
PLATE 15

FIG. 21. Longitudinal section through area of severe demyelination. The axis cylinders are pushed aside but not destroyed. Mouse E-48-161. 1 months after intracerebral inoculation. Bodian's stain. × 338.

FIG. 22. Extensive involvement of both white and grey matter of spinal cord. Areas of rarefying necrosis with leucocytic infiltration. Mouse 2140. 46th passage. 4 days after intramuscular inoculation. Hematoxylin and eosin. × 122.



(Bailey *et al.*: Murine virus causing encephalomyelitis. II)

PLATE 16

FIG. 23. Ingrowth of reticular fibers into demyelinated area of spinal cord. E-48-216. Passage 4A. 3 months after intracerebral inoculation. Laidlaw's reticulum stain. × 310.

FIG. 24. Focal necrosis of liver. Mouse 2469. 49th passage. Killed 4 days after intravenous injection of 49th passage material, 100,000 LD_{50}. Hematoxylin and eosin. × 354.

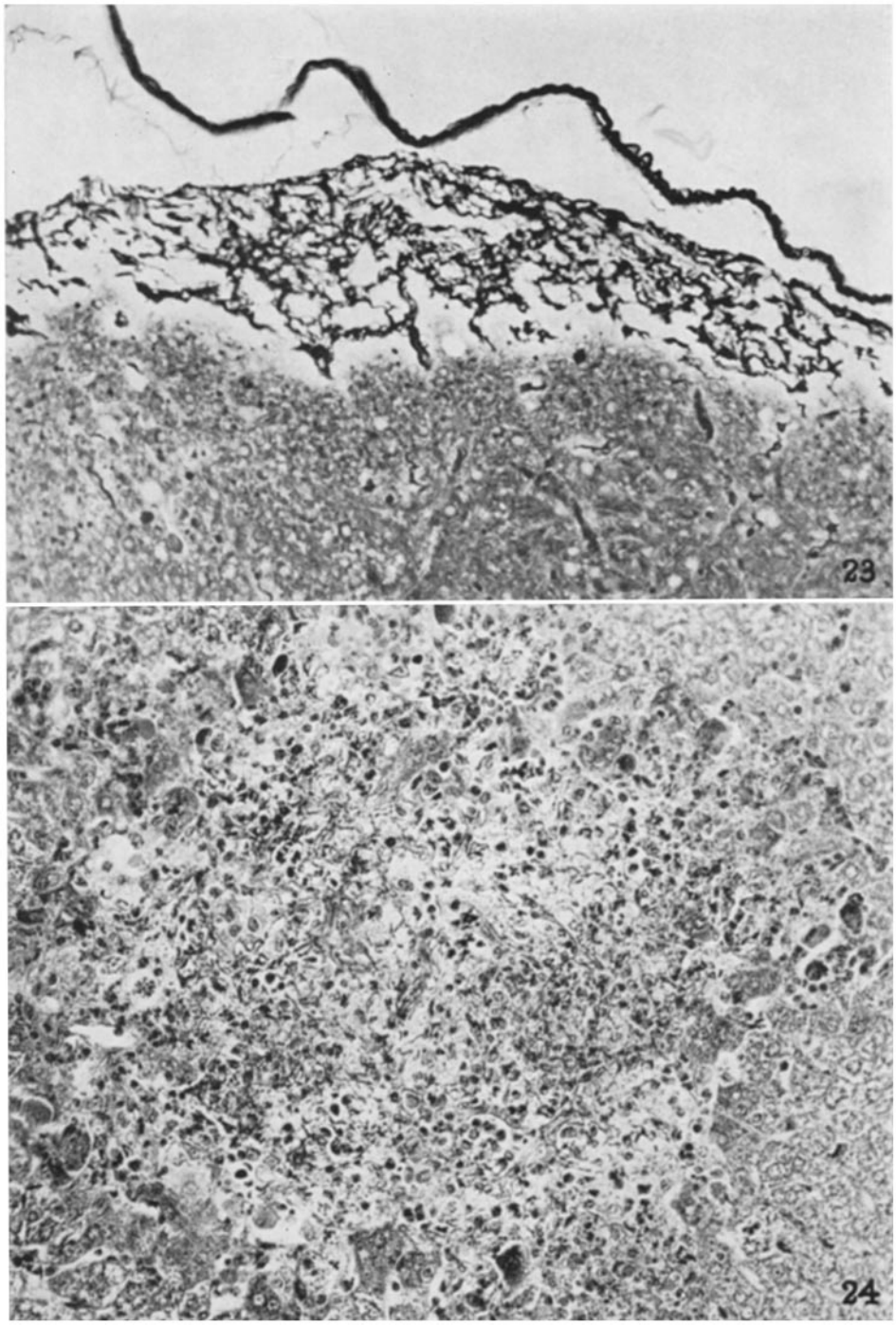


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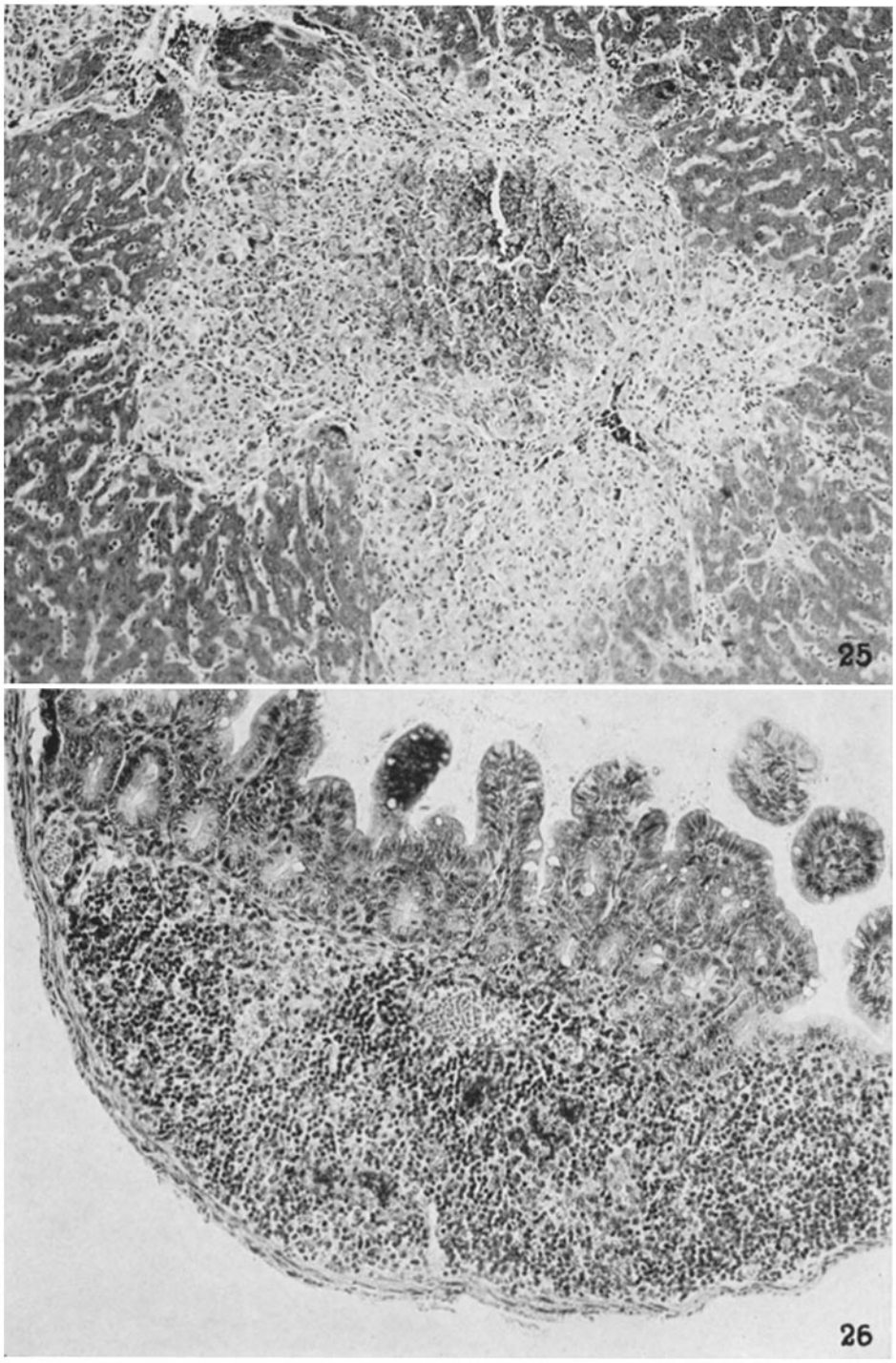
PLATE 17

FIG. 25. Focal necrosis of liver, with fibrous and central calcification. Mouse 2783. Killed 9 days after intravenous injection of 1000 LD_{50} 52nd passage material. Hematoxylin and eosin. × 169.

FIG. 26. Giant cells in Peyer's patch. Mouse 2353. Passage 6A. 3 days after intracerebral inoculation. Hematoxylin and eosin. × 110.



(Bailey *et al.*: Murine virus causing encephalomyelitis. II)

PLATE 18

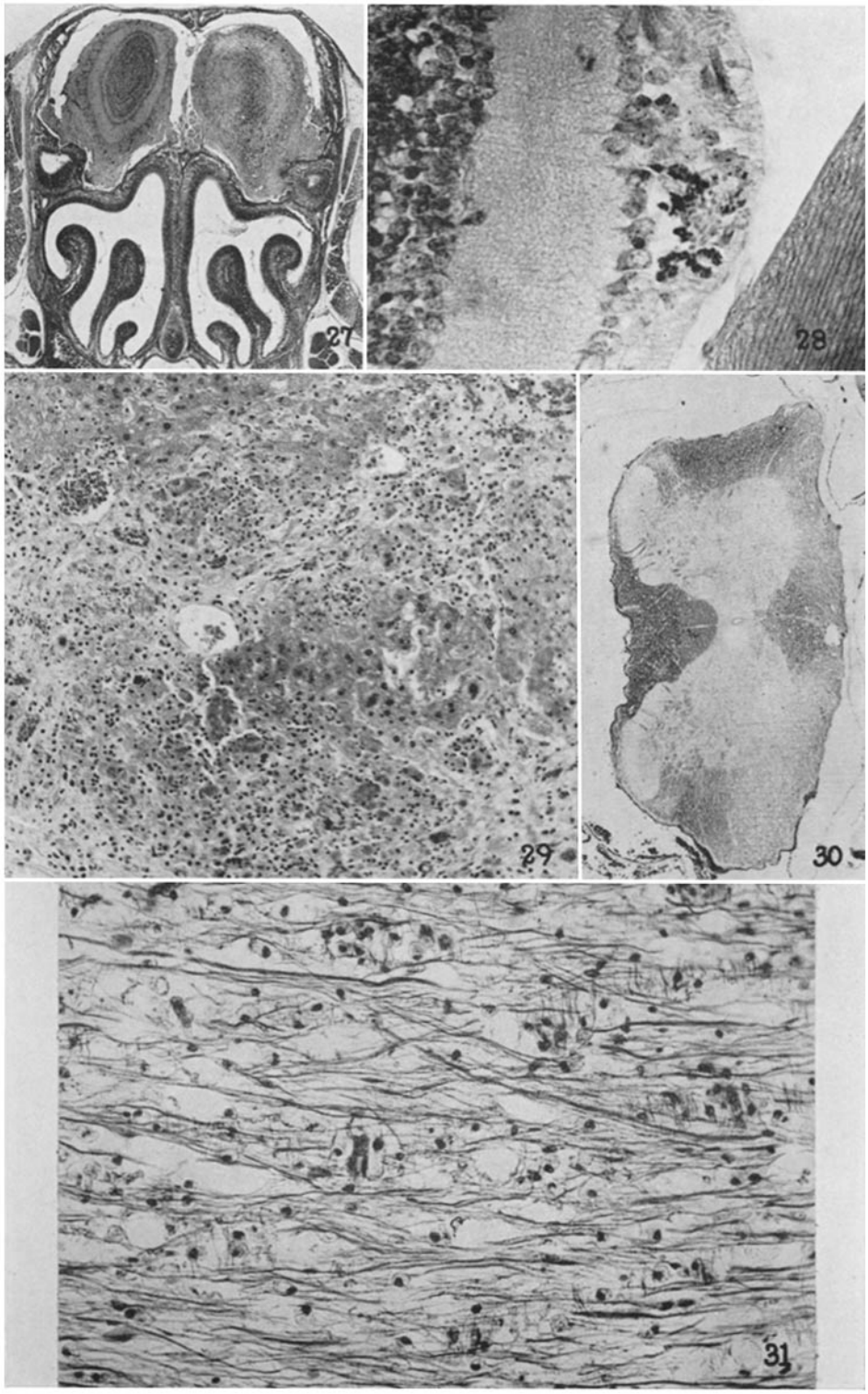
FIG. 27. Necrotizing lesions of right olfactory lobe. Note absence of inflammatory changes in nasal mucosa. Mouse 2246. 46th passage. 3 days after intranasal inoculation. Hematoxylin and eosin. × 16.

FIG. 28. Small inflammatory focus in fiber layer of retina. Mouse 2243. 46th passage. 4 days after intranasal inoculation. Giemsa stain. × 540.

FIG. 29. Massive necrosis of liver, involving all parts of lobule. Mouse 2776. 6 days after intravenous injection of 1000 LD_{50} 52nd passage material. Hematoxylin and eosin. × 162.

FIG. 30. Extensive demyelination in spinal cord of rat. Rat E-49-85. 16 days after intracerebral inoculation of 52nd passage mouse brain 10^{-1} Hematoxylin and eosin. × 48.

FIG. 31. Longitudinal section of spinal cord of rat. Preservation of axis cylinders in demyelinated area. Occasional fibers show swelling. Rat. E-49-85. 52nd passage. 16 days after intracerebral inoculation. Bodian's stain. × 325.



(Bailey *et al.*: Murine virus causing encephalomyelitis. II)