In a comparison of Fig. 1 with Fig. 2, it will be noted that with peptone broth 5 and 10 mg % were employed whereas 0.6 and 2.4 mg % were used in the synthetic medium. In peptone broth sulfathiazole serves to reduce bacterial proliferation by about 75% whereas a much smaller quantity of sulfathiazole in synthetic medium serves to reduce bacterial multiplication by more than 99.9%. From these figures it becomes evident that sulfathiazole is approximately ten times as effective in the synthetic medium as it is in meat-extract peptone broth.

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Histopathology of CNS of Mice Infected with Virus of Theiler's Disease (Spontaneous Encephalomyelitis.)*

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Since 1937, when Theiler[1] first described spontaneous encephalomyelitis of albino mice (Theiler's disease; "mouse poliomyelitis") and the virus causing it, two major developments have given this disease renewed importance because they confirm the impression that one is dealing here with an infection more closely related to human poliomyelitis than is any other known disease. In the first place, the finding by Olitsky[2] and by Theiler and Gard[3] of Theiler's virus in the intestinal contents and feces not only of mice showing the signs of the spontaneous or experimental malady but also of normal, young adult or mature animals, recalls similar results obtained in studies on human poliomyelitis.[4, 5] Again another epidemiological feature is shown commonly by the two diseases, *i. e.*, the analogous incidence of paralytic cases of one in more than 5000.

The second recent development under consideration concerns the successful transmission by Armstrong[6] of one strain (Lansing)

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1 Theiler, M., *J. Exp. Med.*, 1937, **65**, 705.

2 Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 434; *J. Exp. Med.*, 1940, **72**, 113.

3 Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49, 79.

4 For literature see Trask, J. D., and Paul, J. R., *Am. J. Public Health*, 1941, **31**, 239.

5 Sabin, A. B., and Ward, R., *J. Bact.*, 1941, **41**, 49.

6 Armstrong, C., *Public Health Rep.*, 1939, **54**, 1719.

of human poliomyelitic virus to the white mouse. This notable achievement enables one now to carry the comparison of the 2 diseases further than it was heretofore possible. The clinical resemblance of human and experimental (monkey) poliomyelitis on one side and mouse-encephalomyelitis on the other has been fully stressed before.[1-3] In the mouse, the two maladies are clinically indistinguishable.

In view of the striking similarities noted in many properties of the two diseases, it was thought desirable to study in detail the histopathology of spontaneous and experimental Theiler's disease (a) in the hope that clues might be yielded which in turn could be applied to problems in the pathogenesis of human poliomyelitis and (b) for the purpose, particularly, of comparing in the same host the pathology of the Lansing strain of poliomyelitis and of Theiler's disease.

Methods and Materials. The CNS of more than 30 mice sacrificed either at the height of spontaneous Theiler's disease, or at various stages of the experimental infection following intracerebral *(regio parietalis),* intranasal, intralingual, and intraäbdominal inoculation of the virus deriving from intestinal contents of mice were studied. Virus introduced by peripheral routes induced paralysis in approximately 10 to 15% of inoculated 14-day-old mice. In most instances the entire head from which the skin and lower jaw had been removed, and the entire spinal column were fixed in Zenker's solution plus 10% glacial acetic acid (for decalcification) for 30 hours before washing. The skull and contents were cut semiserially, each sagittal section being 6 microns thick; the first 6 sections of each ribbon of 30 were retained for mounting. The spinal column was cut only at the middle of the cervical, thoracic, and lumbar levels. Thus about 150 sections of the CNS were cut from each animal, and more than 5000 in all were studied. Eosin-methylene-blue or hematoxylin-eosin stains were used.

This method was first employed by Sabin and Olitsky[7] who showed thereby the definite and selective pathways taken in the mouse, from the site of inoculation through the CNS, by another neurotropic virus, vesicular stomatitis. The progression of poliomyelitic virus through the CNS of man and monkey has been delineated by Sabin,[8] Bodian and Howe,[9] and others.[8b, 9]

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7 Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.,* 1938, **67**, 201.

8 a. Sabin, A. B., and Olitsky, P. K., *J. Am. Med. Assn.,* 1937, **108**, 21; b. Sabin, A. B., *Am. J. Dis. Child.,* 1940, **60**, 1313.

9 Bodian, D., and Howe, H. A., *Brain,* 1940, **63**, 135.

Results of Microscopical Examination. We have not been able, however, to find such clear-cut patterns in the brain of mice having Theiler's disease. Instead, no significant differences in the types of lesion and their distribution were noted, regardless of the route of inoculation. Of particular interest is the fact that the olfactory bulbs exhibited no characteristic changes—either in those 8 mice receiving the virus intranasally and responding with paralysis, or in 22 others which were infected by other routes, or in the spontaneous disease. This is in agreement with what is found to be true in human poliomyelitis but is in sharp contrast to the bulb-lesions that characterize poliomyelitis in the monkey following intranasal instillation of virus.[8a] In this relation it should be pointed out that human poliomyelitis and murine encephalomyelitis are natural diseases of man and mouse but monkey-poliomyelitis is an experimental infection, artificially induced. Other pathways that may, possibly, be taken by intranasally instilled Theiler's virus are via the V nerve or the sphenopalatine (parasympathetic) or superior cervical (sympathetic) ganglia. It was noted, however, that the gasserian and superior cervical ganglia were affected not only after intranasal but also after intralingual and intracerebral inoculation of virus. In no instance have we been able to find lesions in the sphenopalatine ganglion.

Intracerebral injection of intestinal Theiler's virus in sufficient dosage was invariably followed by clinically apparent infection with an incubationary period extending from 7 to 37 (usually 10 to 20) days.[2] Animals so inoculated were sacrificed after 7 days, before clinical signs of infection (paralysis) were seen. In the brains, the most marked lesions were observed at the site of injection. The area immediately surrounding it showed endothelial swelling and proliferation chiefly of the smaller vessels and capillaries, and perivascular microglial and lymphocytic infiltration. Perivascular neuronal degeneration and, less often, necrosis also were present. The lesions extended from the site, rostrally to the septal area and caudally to the pontine level. They spread out mainly periventricularly.

In still later stages of the period of incubation after intracerebral inoculation (9th-11th day), the lesions had expanded over the adjacent structures but chiefly periventricularly. The vascular changes as mentioned were more in evidence, as was perivascular gliosis, the gliosis also occurring elsewhere, diffusely or focally. Neuronal degeneration and necrosis were more marked but neuronophagia was met with only infrequently and the cells active in this process were

neuroglial rather than polymorphonuclear. The pathological changes rostrally, at the septum or *corpus striatum* were not extensive and were predominantly of mesodermal-glial type; caudally, from the level of the thalamus to that of the *substantia nigra* or pons-medulla, they were more prominently neuronal (degeneration, necrosis; neuronophagia; loss of neurons with resulting vacuolization of the stroma), while the mesodermal-glial reactions became less conspicuous (Fig. 1).

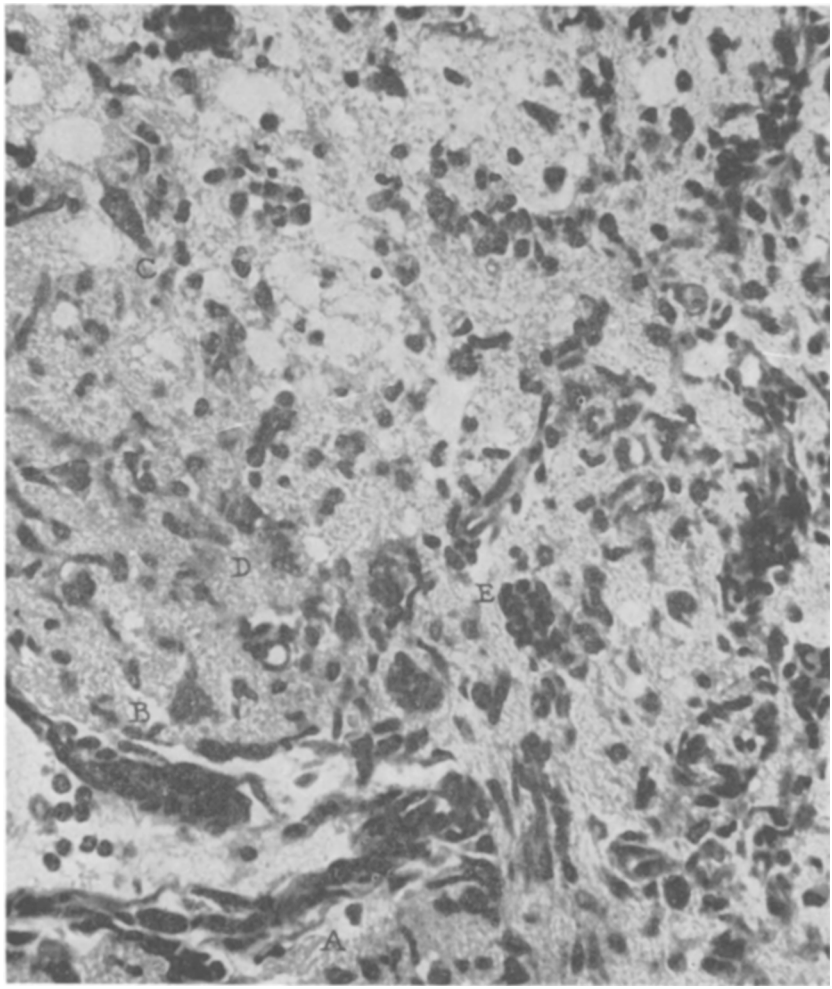

FIG. 1.
Substantia nigra in encephalomyelitis of the mouse. To be noted are the endothelial (A) and perivascular (B) reaction and neuronal degeneration (C), necrosis (D), neuronophagia (E) and vacuolization of the stroma. × 500.

Thus, at this stage, when clinical signs were still absent and the cord was still free from detectable changes, the characteristic picture of the fully developed disease was noted in the brain, except that in the latter stage the degree of reactions were more pronounced. The areas then most constantly affected were the *substantia nigra, tegmentum,* reticular formation, olivary nuclei, nuclei of V and VIII nerves, red and dentate nuclei. Cortical lesions were few in number, mainly vascular in type and sometimes consisted of neuroglial infiltration.

After onset of paralysis, in addition to the cerebral lesions, the anterior horns of the spinal cord were regularly involved: They revealed the vascular and perivascular reactions, neuronal degeneration, necrosis, and neuronophagia. The neuronal changes, however, were never as extensive as they are ordinarily in the monkey cords in experimental poliomyelitis—there was always a number of apparently normal neurons present and only a few polymorphonuclear cells were, as a rule, seen. It is of interest that certain neurons showed, usually at the onset of paralysis and chiefly in the anterior horns of the cord, the presence of Cowdry Type B, intranuclear inclusion-bodies, similar to those seen in human and monkey poliomyelitis.

The progression of pathological changes during the period of incubation following peripheral inoculation of Theiler's virus could not be studied in the same way, in view of the irregularity of production of clinically apparent infection by these means.

After peripheral inoculation when paralysis developed, however, the pathological picture differed in no significant manner from that seen after intracerebral injection (except for the more pronounced periventricular distribution in the latter), and was also similar to that shown by mice spontaneously attacked.

In conclusion, in the fully developed clinical disease the types of lesions and their distribution in the CNS were not essentially different when the malady was induced by various routes of inoculation, regardless of the length of incubationary period. As a rule, the rostral part of the brain showed mesodermal-glial reactions predominantly, while the caudal, including the cord, exhibited more marked neuronal degeneration and destruction.

The type of lesions and their distribution in Theiler's disease in albino mice closely resembled the picture in this animal at corresponding periods after infection with the Lansing strain of human poliomyelitis.[10]

[10] Lillie, R. D., and Armstrong, C., *Public Health Rep.*, 1940, **55**, 718.