TABLE II. % Inhibitions of Cholinesterases of Mouse Brain, Bovine Red Cells and Horse Serum by Various Concentrations of Morphine and NAM. Each value represents the average of the 2 values obtained in each run.

Drug concentration	Mouse brain		Bovine RBC		Horse serum	
	Morphine	NAM	Morphine	NAM	Morphine	NAM
.00038 M	8	18	17	23	48	58
	12	21	19	23	49	60
.00075 M	33	38	24	32	50	66
	26	40	23	28	49	84
.0015 M	43	60	60	54	64	79
	44	45	35	42	54	78

and horse serum produced by N-allylnormorphine were measured and compared to the inhibitions produced by morphine. N-allylnormorphine is at least as potent an inhibitor as morphine. The implications of this finding for the theory of morphine action are discussed.

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The Electron Microscopy of Rabies Inclusion (Negri) Bodies. (18905)

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The great resolving power of the electron microscope together with our present ability to cut tissue sections thin enough for examination with this instrument has made it possible to visualize several viruses within their host cells. The present paper is a record of results of a preliminary attempt to recognize rabies virus in sections of diseased mouse brain. The Negri bodies that frequently are found in such tissue have long been studied under the optical microscope without, however, their developing any general agreement on the method of their development or their relation to the virus(1-7). First attempts to see Negri bodies with the electron microscope, using a modified smear technic, have given only very limited information about them(8).

For the present experiments, 3 to 4 weeks old Swiss mice were inoculated intracerebrally with strain 1449 of Street virus (obtained through the courtesy of Dr. Harry A. Rubin of the Communicable Disease Center Laboratory, Montgomery, Ala.) and sacrificed at 7, 9 and 12 days. After removal the brains were fixed in 10% formalin. Cross sections of the cerebral hemispheres at the level of the hippocampus were hardened for 3 days in 3%

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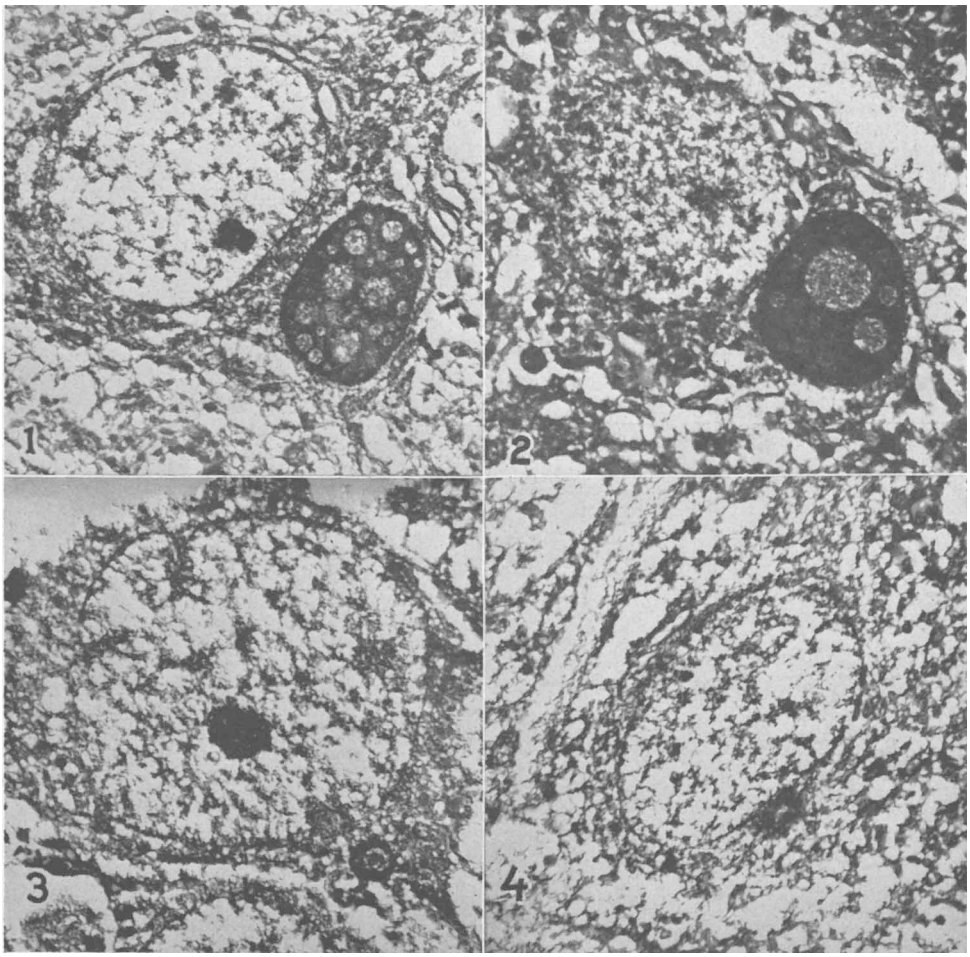

FIG. 1. Typical inclusion body containing vacuoles. Membrane of the nucleus, above to the left, is intact. In lower left corner of the figure are scattered granules suggestive of mitochondria by their size and density. Magnification = 3,800 ×.

FIG. 2. Inclusion body with 2 large vacuoles and characteristic, smaller, "daughter" vacuoles. Membrane of the nucleus in the upper part of the illustration has undergone dissolution. Magnification = 4,670 ×.

FIG. 3. Small inclusion with a single, central vacuole. Nuclear membrane is disrupted at several places. Magnification = 4,730 ×.

FIG. 4. Poorly demarcated, dense area with less dense central zone lying in the perinuclear cytoplasm. Nucleoli do not lie in the plane of section. Magnification = 5,000 ×.

aqueous solution of potassium bichromate, dehydrated in absolute alcohol, cleared in xylol and embedded in paraffin. Examination in the optical microscope of 5 μ thick sections cut from these blocks and stained with azure-eosin(9) accurately localized the large cell band of the hippocampus. Suitable tissue blocks selected in this way were immersed in xylol for 14 hours to remove the paraffin. From areas known to contain the large cells of the hippocampus small blocks, 1-2 mm in width, were then excised, embedded in methacrylate, thin-sectioned and prepared for the electron microscope by the methods outlined in previous papers. In addition 5 μ sections were cut from the methacrylate-embedded tissue, mounted on glass slides and the methacrylate removed by acetone. These thick sections were stained with azure-eosin and

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examined in the optical microscope. Control sections of the hippocampus from uninfected, 3-4 weeks old mice were prepared in the same way.

Comparisons with the optical microscope between tissue embedded in the original paraffin and those subsequently embedded in methacrylate do not reveal differences in the preservation of the cells. It should be mentioned, however, that using identical staining technics less eosin remains in sections of the methacrylate embedded tissue after dehydration and clearing.

In the hippocampus of moribund mice sacrificed 12 days after inoculation with rabies virus numerous large inclusion bodies can be identified in the optical microscope. In methacrylate embedded preparations examined by the electron microscope similar inclusions are readily recognizable. Fig. 1 shows a typical inclusion (Negri) body seen in the electron microscope. The body seems to contain small, irregular, poorly defined, dense granules and has multiple, oval vacuoles. Generally, as in this case, the vacuoles are filled by meshes of less dense material. Rabies virus as measured by filtration experiments (10,11) should be large enough to be clearly visible at this magnification. Distinct, dense, spherical granules are sometimes seen within vacuoles, but because they vary in size and are not consistently present it cannot be concluded that they are the elementary bodies of the virus. The larger forms probably are the "Innenkörper," the blue staining granules seen in the light microscope. The nuclear membrane of cells containing inclusions may be intact, as in Fig. 1, or partially or completely destroyed as shown in Fig. 2. The neurofibrillae in the cytoplasm of these cells, where they can be recognized, appear to lack an orderly reticular arrangement. This may be artifact, but sections through dendrites show a parallel arrangement; and this suggests that in the perinuclear region the neurofibrillae have variable courses, passing in and out of the plane of very thin sections.

In 7 and 9 days old lesions the electron microscope not only reveals the typical, multivacuolated inclusions just discussed but also more numerous, opaque objects too small to be recognized as inclusion bodies in the optical microscope. Fig. 3 shows one such body at the lower right, adjacent to the nucleus. The oval or spherical shape, density, presence of a central vacuole and perinuclear location suggest that these are small inclusions. Their number seems to preclude the possibility of their being the result of cutting through the periphery of large inclusions. Instead they may be early stages in development of the Negri bodies.

In 7 day old lesions similar, but poorly defined, dense bodies are found within the perinuclear cytoplasm (Fig. 4). Strands of cytoplasm can often be traced into them, suggesting a condensation or precipitation of opaque material in the cytoplasm. Such bodies are rarely encountered in 9 and 12 days old lesions. They are not present in control sections and perhaps are an especially early stage in the development of the inclusion body.

It has several times been suggested that the inclusions develop from nucleolar constituents extruded into the cytoplasm(1,5). The nucleoli of cells of both control animals and those containing inclusions vary greatly in size and shape and may lie against the nuclear membrane. Their position on the membrane is not, however, related to the position of the inclusion body, and there is no evidence from our electron micrographs that they contribute directly to the development of the inclusion body.

Summary. Electron micrographs of typical inclusion (Negri) bodies from the hippocampus of a mouse inoculated with rabies virus are shown together with smaller inclusions that may be early forms of the larger bodies. No particles have been recognized that could be identified with elementary bodies of this virus.

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