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ELECTRON MICROSCOPY OF CHIKUNGUNYA VIRUS INFECTION IN THE NERVOUS SYSTEM OF SUCKLING MICE

John D. White

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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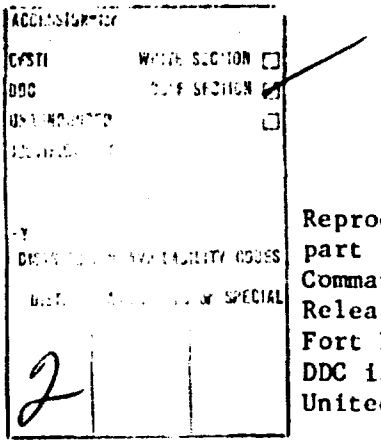

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In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

The African strain of chikungunya virus was used to infect newborn mice, and the central nervous system was examined in the electron microscope. Previously, it was shown that, with this virus strain, histological changes in suckling mice consisted primarily of necrosis of neurons in the cerebral cortex and spinal cord. In the present study, ultrastructural changes were found only in the cerebral cortex and spinal cord. Electron-dense particles were seen in the extracellular spaces of the neuropil and within axon fibers. A subtle change in the texture of the cytoplasmic substance and alterations of the endoplasmic reticulum were observed in neurons and glial cells. As with tissue culture cells, the endoplasmic reticulum appeared to be the main organelle involved in virus replication. The close association of electron-dense particles with this membranous structure and the presence of mature virus particles within the endoplasmic reticulum corroborated the findings in tissue culture. The viral core is apparently assembled at the endoplasmic reticulum, and the viral coat is formed from the cellular membrane, which is penetrated by the virus.

ELECTRON MICROSCOPY OF CHIKUNGUNYA VIRUS INFECTION IN THE NERVOUS SYSTEM OF SUCKLING MICE*

Chikungunya virus is an arbovirus characterized antigenically as belonging in group A. It is the etiologic agent of a dengue-like disease and has been implicated in epidemics of hemorrhagic fever.

Multiplication of the virus is possible in various tissue cultures. A lethal infection is produced in newborn mice but not in adult mice. Although a viremia develops after inoculation of other rodent species and subhuman primates, no signs of illness are seen. For this study, litters of newborn mice were injected intracutaneously with 1×10^3 SMICLD$_{50}$ of the African strain of chikungunya virus. Inoculated and control mice were sacrificed daily for 7 days after injection of the virus, and tissues were prepared in the usual manner for examination with both light and electron microscopes.

Multiple cross sections of the suckling mouse were examined with the light microscope. Morphologic changes that could be observed with the light microscope were limited to the central nervous system. The most prominent changes observed were necrosis of neurons (Fig. 1) and a mild vasculitis (Fig. 2). The vasculitis, which consisted of endothelial proliferation with a mixed inflammatory infiltrate, appeared to precede the neuronal changes. These lesions were seen in the cerebral cortex, basal ganglia, and spinal cord. In the cord, motor neurons were more frequently involved.

The earliest changes observed with the electron microscope were in sections of tissues obtained on the 4th day after injection, 1 day later than changes seen by light microscopy. Some sections of the cortex and spinal cord contained neurons in which the cytoplasmic structure was altered. At low magnifications, the cytoplasm was dense and the endoplasmic reticulum very prominent (Fig. 3). At a higher magnification, numerous spherical particles were seen surrounding portions of the endoplasmic reticulum. Some were scattered randomly, others were arranged in an orderly pattern throughout the cytoplasm (Fig. 4).

The perivascular changes noted in light microscopy were confirmed by electron microscopy (Fig. 5). Perivascular edema is evident and one mononuclear lymphocyte is located in this space. The cytoplasm of this glial cell, shown in Figure 6, is morphologically quite similar in appearance to that of the infected neuron. Note the arrangement of the particles, their distribution, and the virus-like appearance of the particles, which is quite evident in the three vesicles in the center of the figure. This

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is consistent with the morphology of arboviruses; namely, a dense core surrounded successively by a light area and a darker-appearing structure, the membrane. The size of these particles (60 nm) and the cytoplasmic changes are identical to those seen in tissue cultures infected with chikungunya virus (Fig. 7).

All cells were not as extensively involved as those shown previously. In some neurons, there were just a few clusters of particles (Fig. 8), and in others the only evidence of virus multiplication was the presence of mature virus in vesicles (Fig. 9). Occasionally, structures compatible with mature virus were seen in axon fibers (Fig. 10).

The nature of the neuronal alteration was the same in the cerebral cortex and the spinal cord. Myelinated fibers were found in sections of cord from mice sacrificed later than 5 days. In a few instances, lesions involving these myelinated fibers were seen. The nature of this type of lesion is seen in Figure 11. The continuity of the myelin sheath of this fiber has been destroyed, the cytoplasm of the Schwann cell contains various large inclusions, the cytoplasmic integrity of the surrounding glial cells is altered, an inflammatory infiltrate consisting of neutrophils and monocytes is present, and mature virus can be seen (Fig. 12).

These data show that the inoculation of newborn mice with chikungunya virus produces an encephalitis that primarily affects the cerebral cortex and spinal cord. The virus multiplies within the neurons and glial cells of these areas in a manner identical to that seen in L cells maintained in culture. Mature virus was seen within cells of the central nervous system as well as extracellularly.

FIGURE 1. Section of Cerebral Cortex Showing Neuronal Necrosis. Hematoxylin and eosin. ca. 875X.

FIGURE 2. Section of Cerebral Cortex Illustrating Vasculitis. Hematoxylin and eosin. ca. 875X.

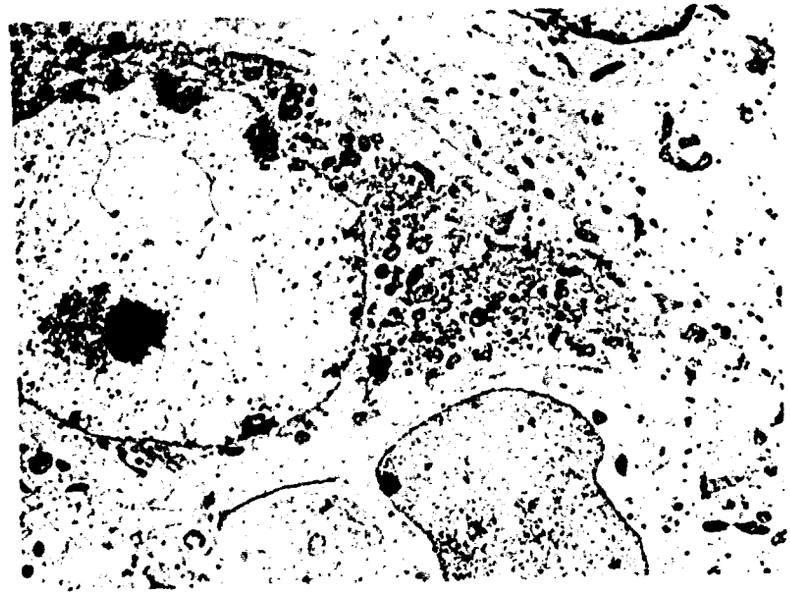

FIGURE 4. Section in Cerebral Cortex. Note granularity of the cytoplasm in the basal area. c.s. 5,800X.

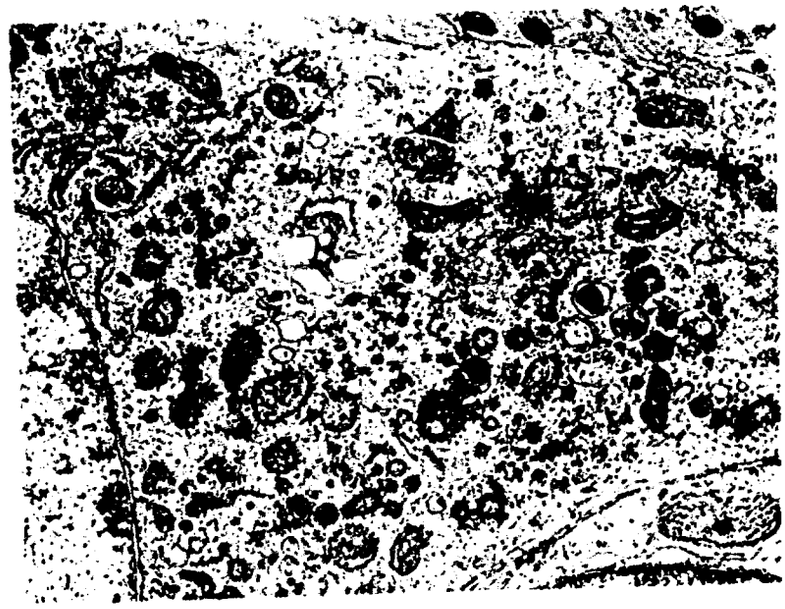

FIGURE 5. Higher Magnification of the Granular Portion of the Neuronal Cytoplasm. Numerous electron-dense particles surround the endoplasmic reticulum. Some mature virus is in the cytoplasm of the cell. c.s. 16,200X.

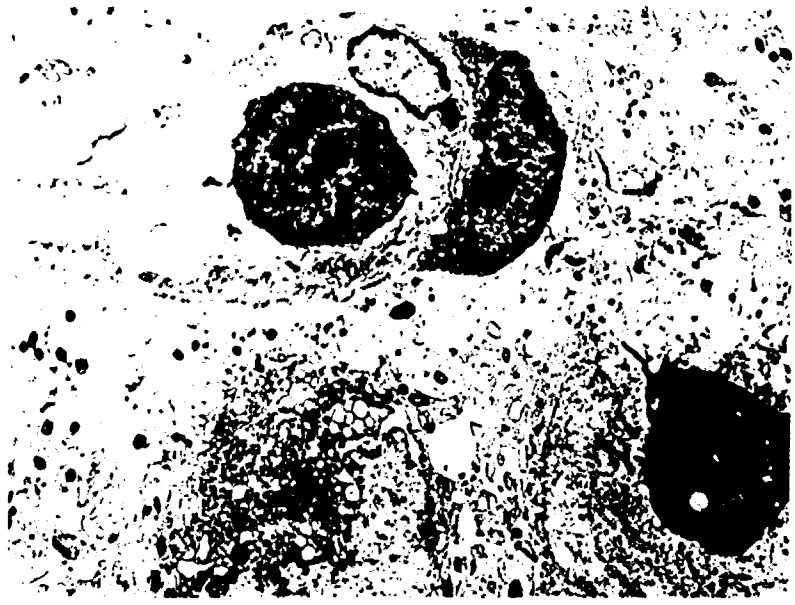

FIGURE 5. Cross Section of Small Blood Vessel in Cerebral Cortex. The perivascular space contains a histiocyte. Note granularity of the glial cell in lower center of the photomicrograph. ca. 5,800X.

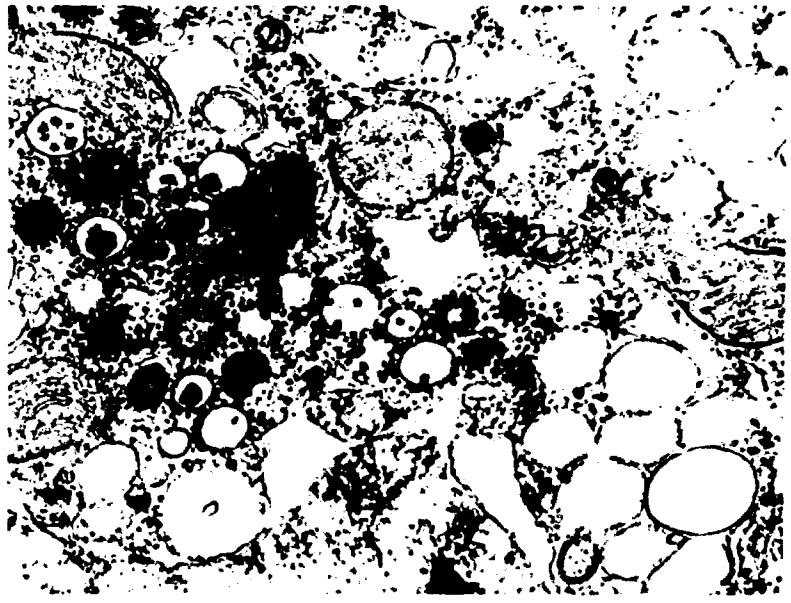

FIGURE 6. Higher Magnification of Glial Cell Shown in Previous Figure. ca. 18,500X.

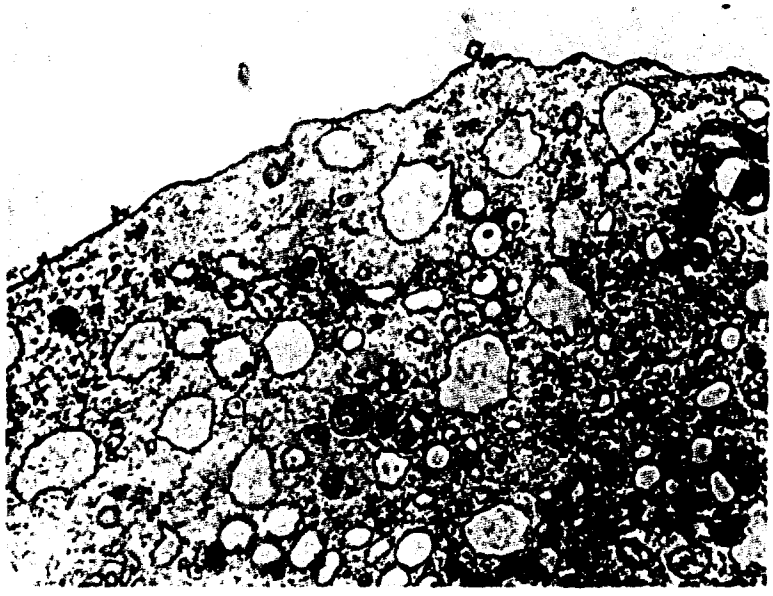

FIGURE 7. Tissue Culture (Mouse L Cell) Cell Infected with Chikungunya Virus. The appearance, size, and location of the virus are identical to those seen in glial cells and neurons. ca. In.200X.

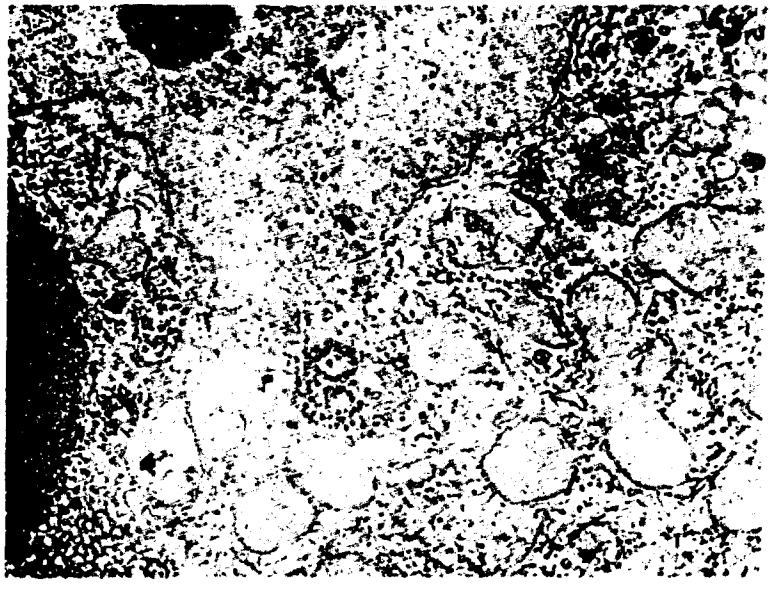

FIGURE [illegible] Spinal Cord. ca. 23,000X.

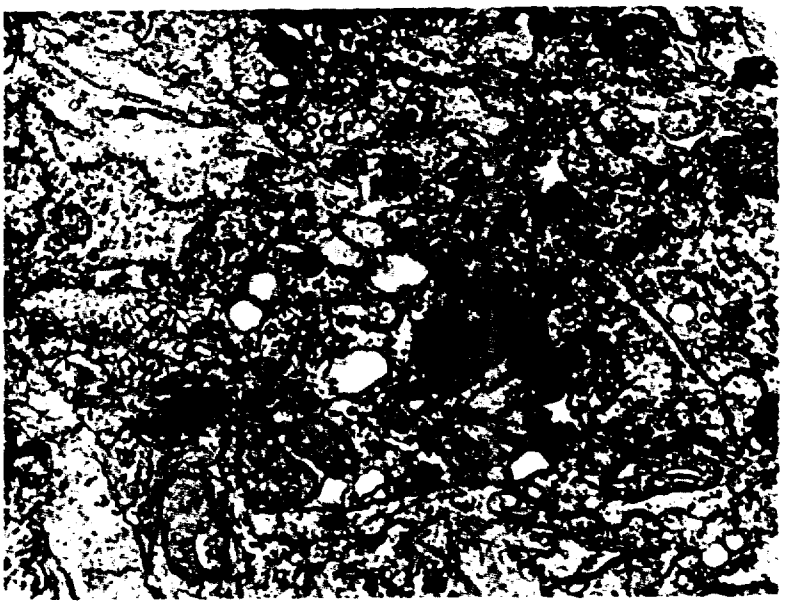

FIG. [illegible] Vacuoles Present in Extracellular Spaces of Spinal Cord [illegible]

[illegible] Nerve Fibers.

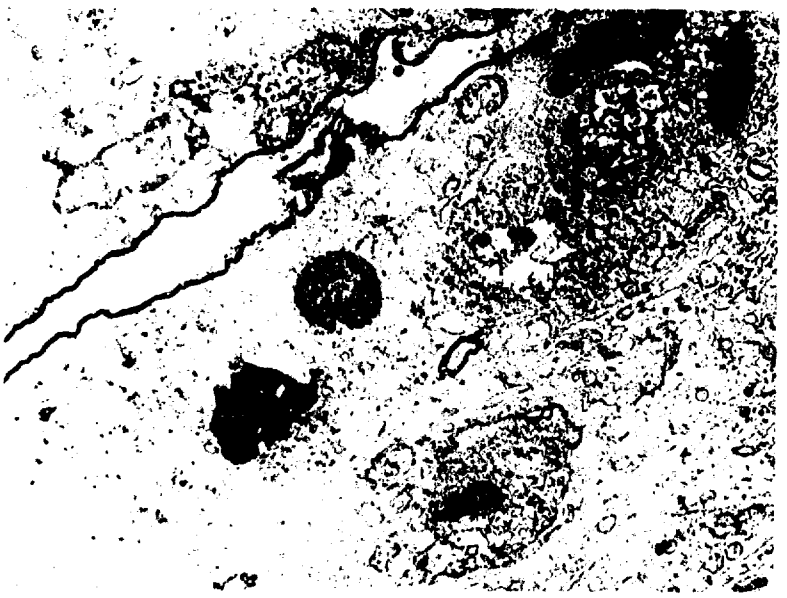

FIGURE 11. Myelinated Fiber in Spinal Cord. The continuity of the myelin has been destroyed. A neutrophil and some lymphocytes are shown. c.t. 5,800X.

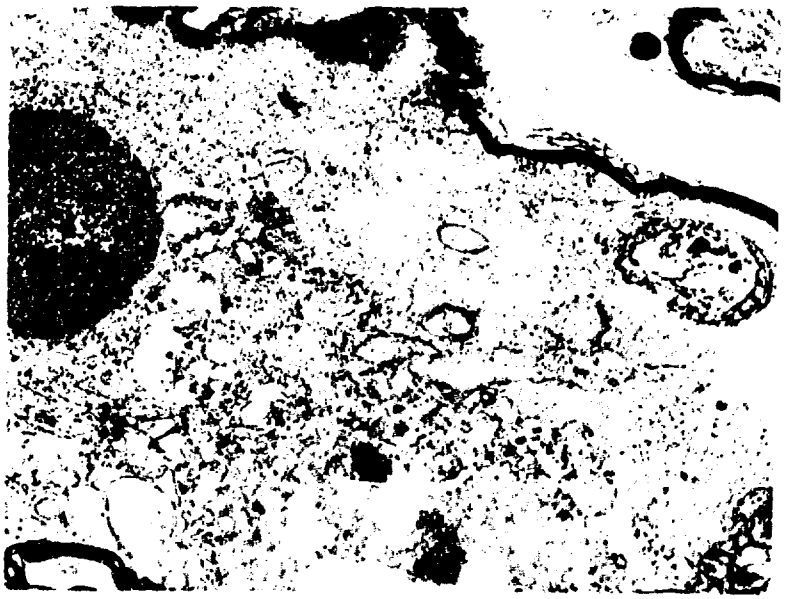

FIGURE 12. Myelin Debris are shown in the Cytoplasm of the Schwann Cell Above and the Neutrophil Below. c.t. 20,000X.

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14. Key Words

*Chikungunya virus
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*Nervous system
Arboviruses
Tissue culture
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*Electron microscopes

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