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TECHNICAL MANUSCRIPT 507

ELECTRON MICROSCOPY OF RIO BRAVO VIRUS IN AFRICAN GREEN MONKEY KIDNEY CELLS

John D. White
Theodore Tzianabos

MARCH 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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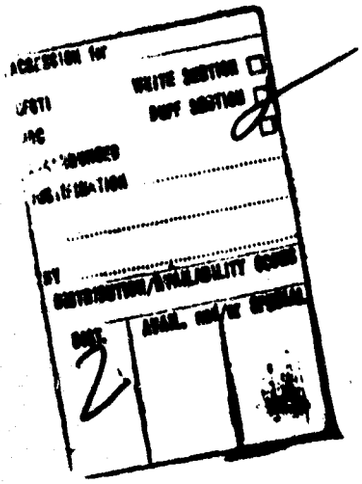

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John D. White

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Pathology Division
MEDICAL SCIENCES LABORATORIES

Project 1B061102B71A

March 1969

In conducting the research described in this report, the investigators 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

Rio Bravo virus, a Group B arbovirus, examined for the first time by electron microscopy and immunofluorescence, was found to be similar in appearance and size to other members of this group. It is a small virus, approximately 40 mμ in diameter, and consists of a dense core surrounded by a membrane. Inoculation of cultures of African green monkey kidney cells produced marked morphologic changes in the nucleus and cytoplasm. There was a striking loss of granular material in the nucleus. Electron-dense particles, 25 to 40 mμ in diameter, were seen in the nuclei of some cells, but the significance of these particles is unknown. The normal granular appearance of the cytoplasm disappeared, and there was a gradual and progressive increase in size of the mitochondria. Concurrently, the cell vacuoles increased in size, and large numbers of electron-dense particles were concentrated on the membranes of these structures as well as on the nuclear membranes. The particles are believed to be precursors of the mature virus because they were identical in size to the core of the mature virus and were frequently and consistently seen in cultures shown to be infected by immunofluorescence and viable assay.

ELECTRON MICROSCOPY OF RIO BRAVO VIRUS IN AFRICAN GREEN MONKEY KIDNEY CELLS*

Rio Bravo virus is a Group B arbovirus that was originally isolated from the salivary glands of Mexican free-tailed bats by Burns and Farinacci** in Texas. There have been no recognized natural human infections with this virus, but five laboratory-acquired infections have been reported by Sulkin et al.*** The disease was described as mild in two patients. The other patients required hospitalization with a moderately severe disease complicated by encephalitis, orchitis, or oophoritis.

There is a paucity of information other than serological data pertaining to this virus. Sulkin et al.*** described the experimental infection of monkeys. We examined cultures of African green monkey kidney cells with the electron microscope and immunofluorescence to determine what cellular changes are produced during infection.

Cultures of African green monkey kidney (Vero) were prepared in flasks and Leighton tubes and inoculated with the HA 119 strain of Rio Bravo virus. Samples were taken at 24, 36, and 48 hours after adsorption for virus assay, electron microscopy, and immunofluorescence.

The cultures grown in T-30 flasks were inoculated with 0.5 ml of heart infusion broth (HIB) containing $10^{7.35}$ $MICLD_{50}$ of virus. At the end of the 1-hour adsorption period at 37 C, the inoculum was washed from the cells. The concentrations of virus in the tissue culture medium at 24, 36, and 48 hours were $10^{3.9}$, $10^{5.25}$, and $10^{6.63}$ $MICLD_{50}$ per ml, respectively (Fig. 1). The gradual increase in virus concentration was also reflected in the cover slips stained with fluorescein-labeled antibody. By 24 hours postinfection, individual fluorescent cells could be identified (Fig. 2). Fluorescence was cytoplasmic, and discrete polar areas of intense fluorescence were seen. By 36 hours, the number of fluorescent cells, as well as the amount of fluorescent material in each cell, had increased (Fig. 3). By 48 hours, it appeared that most of the cells, as judged by immunofluorescence, were infected (Fig. 4).

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** Burns, K.F.; Farinacci, C.J. 1956. Virus of bats antigenically related to St. Louis encephalitis. Science 123:227-228.

*** Sulkin, S.E.; Burns, K.F.; Shelton, D.F.; Wallis, C. 1962. Bat salivary gland virus: Infections of man and monkey. Texas Rep. Biol. Med. 20:113-127.

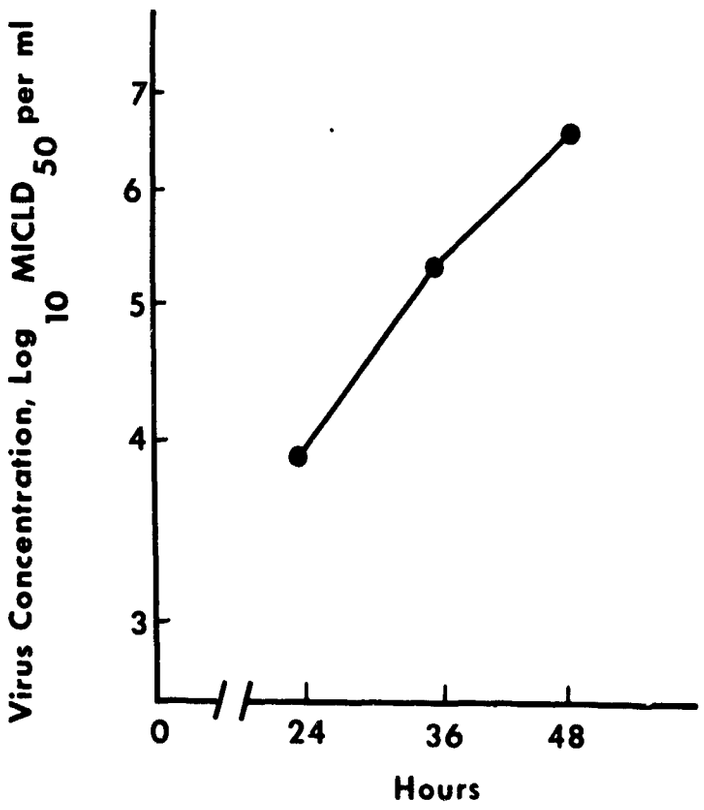

FIGURE 1. Concentration of Rio Bravo Virus in Cultures of African Green Monkey Kidney Cells.

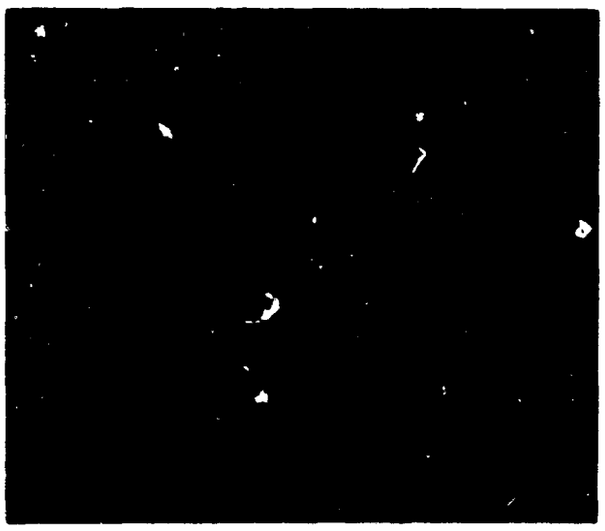

FIGURE 2. Immunofluorescent Stain of Culture at 24 Hours. Individual fluorescent cells were seen. 250X.

FIGURE 3. Immunofluorescent Stain of Culture at 36 Hours. The number of fluorescent cells has increased but non-infected cells can still be recognized. 250X.

FIGURE 4. Immunofluorescent Stain of Culture at 48 Hours. All cells appear to be fluorescing. 250X.

The appearance of a normal cell in the electron microscope is shown in Figure 5. There is one prominent nucleolus, the nucleoplasm has a uniform granular appearance, and the nucleus occupies a greater volume of the cell than the cytoplasm, which surrounds it as a narrow band. The cytoplasm also has a dense granular character. Vacuoles, mitochondria, rough and smooth endoplasmic reticula, and free ribosomes are easily recognized. The border of these cells has a fimbriate appearance created by numerous cytoplasmic protuberances.

There were striking changes in the subcellular morphology by 24 hours postinfection (Fig. 6). The uniform nuclear granularity disappeared, and the nucleoprotein material was aggregated in clumps with some margination. A nucleolus was not seen. Similarly, the granular appearance of the cytoplasm disappeared, and the cytoplasmic membrane was smooth with no projections. There was a noticeable increase in the size of the mitochondria and a concomitant loss in electron density. This is shown at a larger magnification in Figure 7. The vacuole in the middle of this picture is part of the rough endoplasmic reticulum.

At 36 hours, the superficial appearance was not changed appreciably (Fig. 8) except that the size of the mitochondria had increased (Fig. 9) and the christae appeared tubular and dilated. Various sized electron-dense particles, 25 to 40 mμ in diameter, were seen in the nucleus of some cells (Fig. 10). In addition, smaller electron-dense particles were seen along the nuclear membranes of cells. In Figure 11, numerous particles are seen adjacent to the nuclear membrane and the membrane of the vacuole located above the nucleus. The spatial relationships are more clearly represented in Figures 12 and 13, which show an alternating arrangement of particles on either side of a unit membrane.

At the end of 48 hours the most striking feature was the size of the mitochondria and the increase in number of electron-dense particles surrounding the endoplasmic reticulum and vacuoles (Fig. 14-16). These particles are approximately 20 mμ in diameter and are shown at a very high magnification in Figure 17. The structure of these particles is simple, and they cannot be conclusively identified as a virus. They most likely represent an early morphological entity in the development of the virus or are uncoated viral cores, because their size is identical to that of the viral nucleoid. It is well documented that arboviruses are coated at the time they are extruded through a membrane of the cell in which they develop. In this study it was exceedingly difficult to find mature virus particles in spite of the extent of cellular damage. In the instances when the virus was seen, it was similar in appearance to other arboviruses that have been described. It consisted of a dense-core nucleoid enclosed within a well-defined membrane (Fig. 18). The diameter of this particle was approximately 40 mμ, the core was 20 mμ. A portion of the suckling mouse brain suspension used for the inoculum was partially purified by differential centrifugation. The final pellet was fixed, embedded in plastic, sectioned, and examined in the electron microscope. Virus particles were seen (Fig. 19) that were identical to those shown in Figure 18.

FIGURE 5. A Normal Cell. The nucleus, which occupies the larger volume of the cell, has one prominent nucleolus and granular nucleoplasm. The normal appearance of the cytoplasm and its organelles is shown. 12,500X.

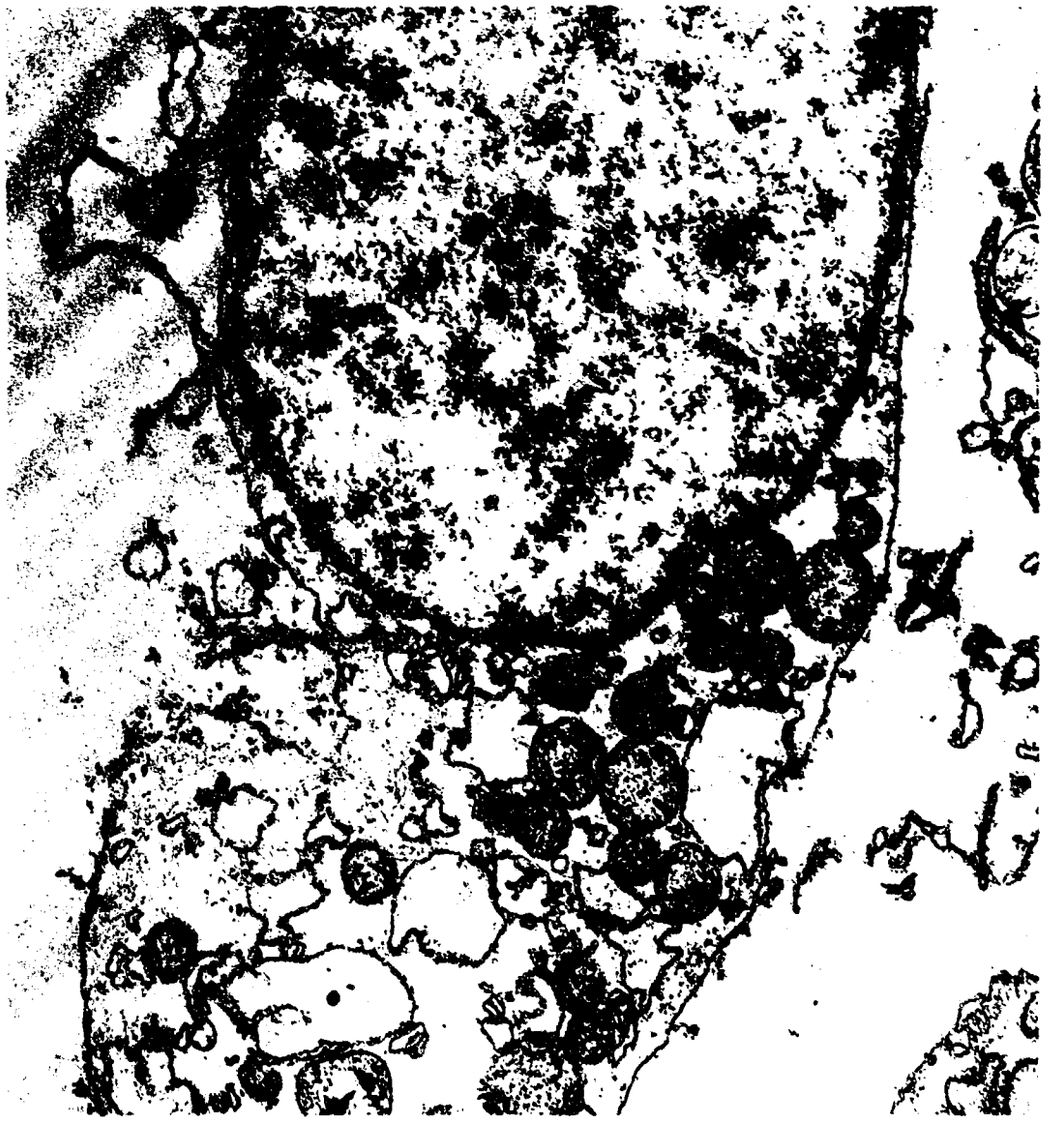

FIGURE 6. Infected Cell at 24 Hours. The nucleus has lost its dense appearance and marked changes in the cytoplasm are apparent. 17,500X.

FIGURE 7. Enlarged View of a Portion of Cytoplasm of the Cell in Figure 6. The endoplasmic reticulum is dilated and the mitochondria have increased in size. 70,000X.

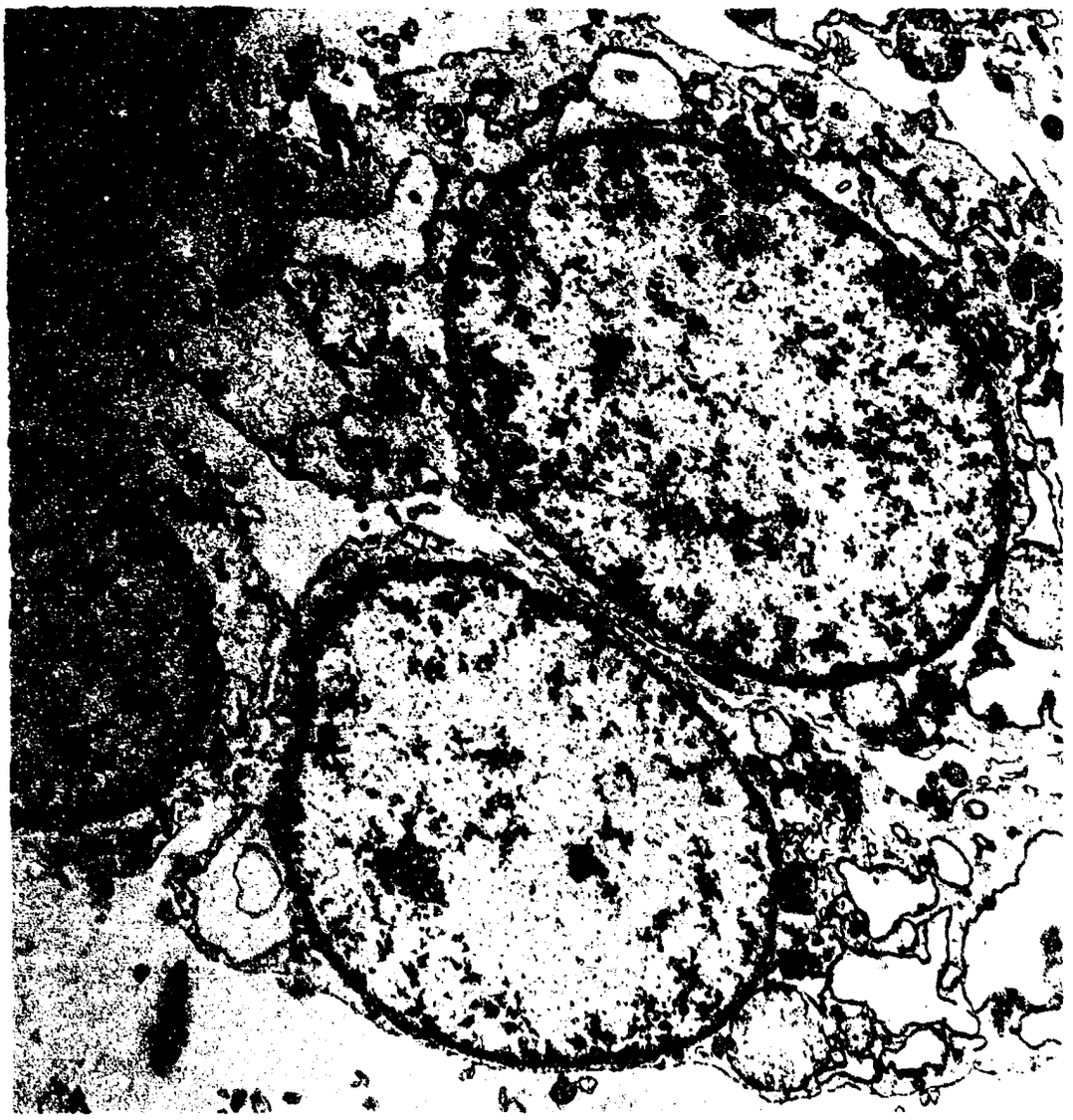

FIGURE 8. Low-Power View of Infected Cell at 36 Hours. 17,500X.

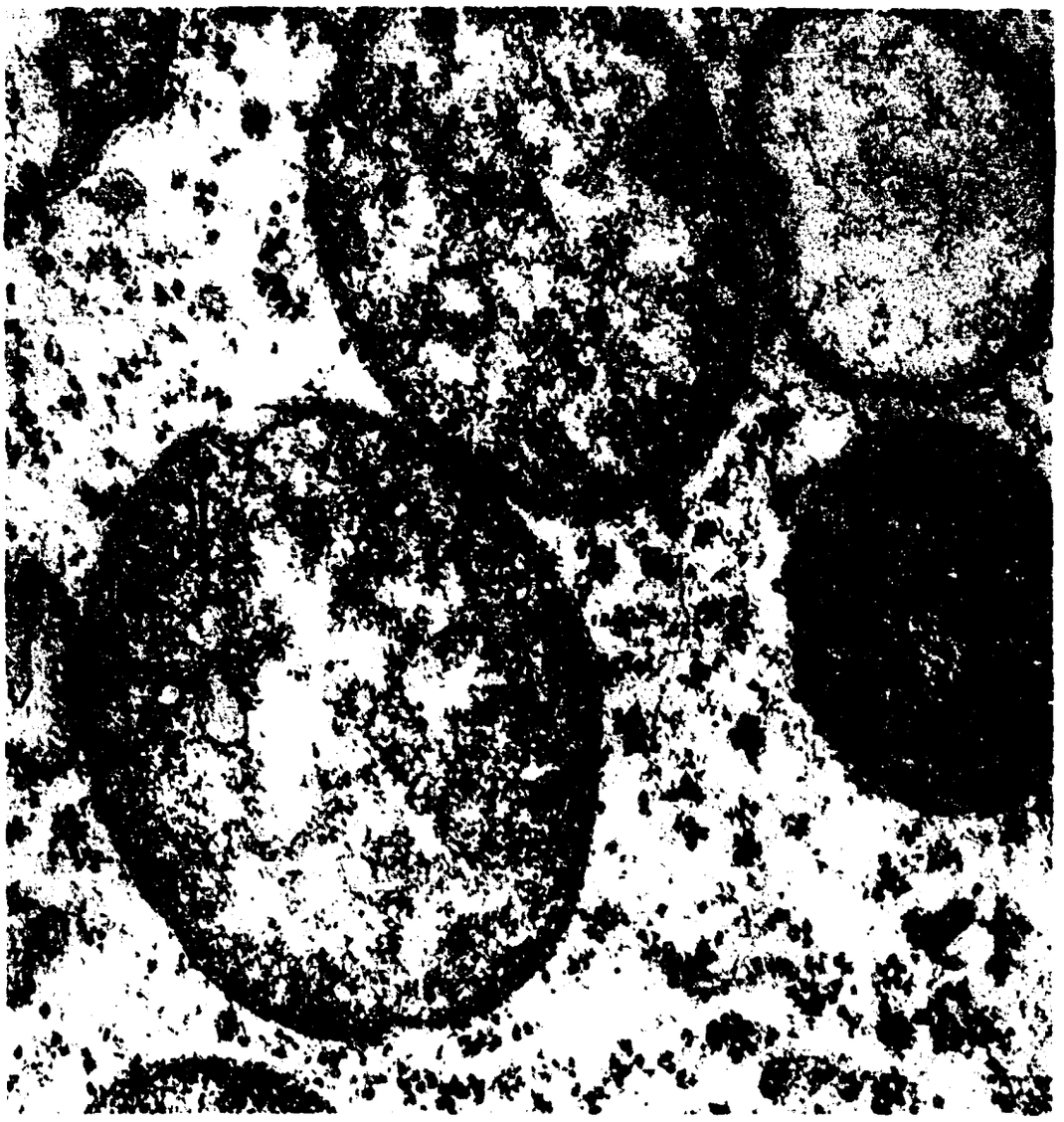

FIGURE 9. High Magnification of a Portion of the Cytoplasm of the Cell in Figure 8. The mitochondria are two to three times the normal size. 70,000X.

FIGURE 10. The Nucleus of the Cell in the Lower Half of the Picture Contains Several Particles Larger than the Nucleoprotein of the Nucleus. 70,000X.

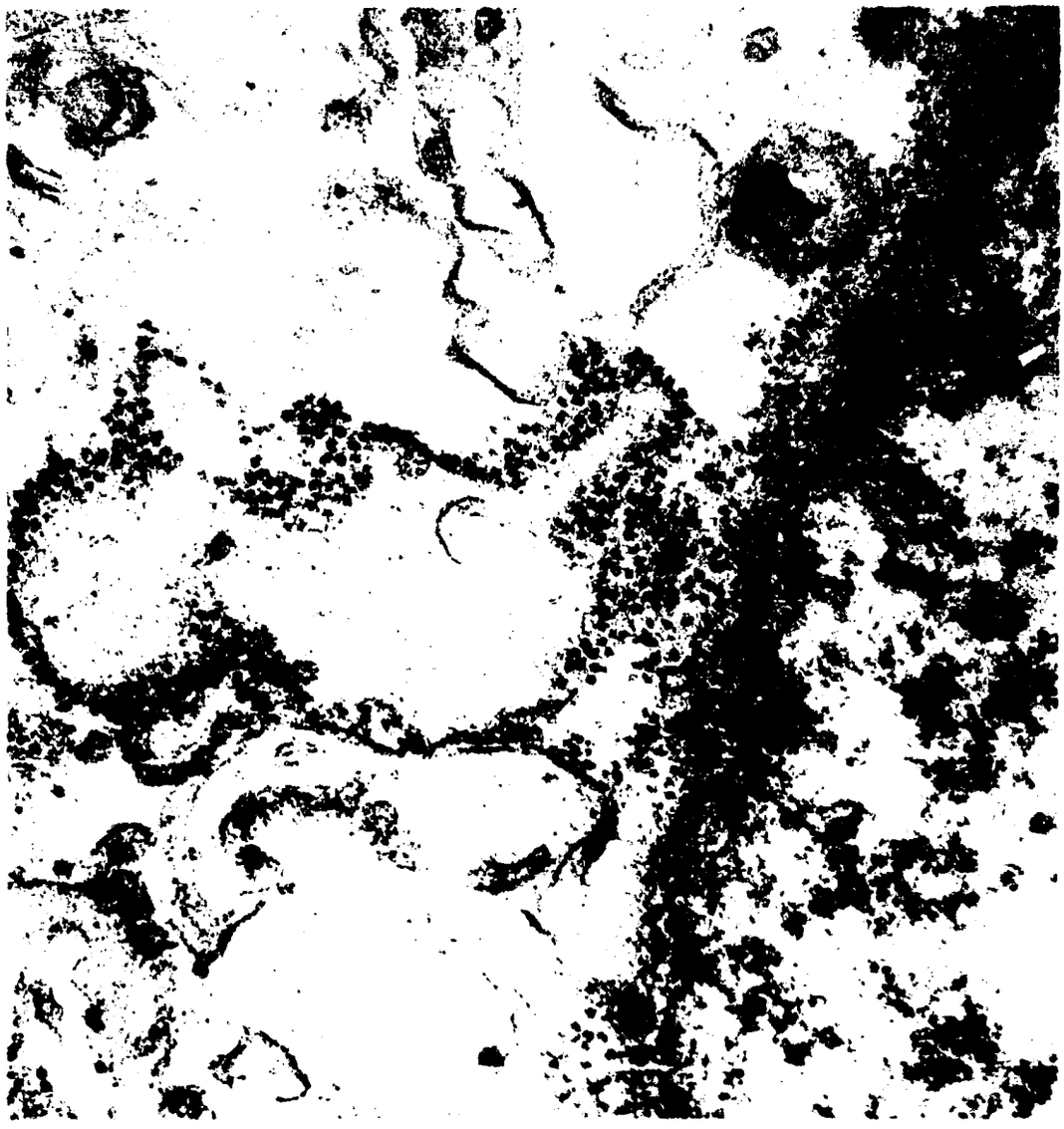

FIGURE 11. Dilated Endoplasmic Reticulum with Increased Number of Particles. The nucleus of the cell is to the right. 70,000X.

FIGURE 12. The Particles are in a more Orderly Arrangement on the Membranes in this Cell. Note the large particle in the nucleus. 70,000X.

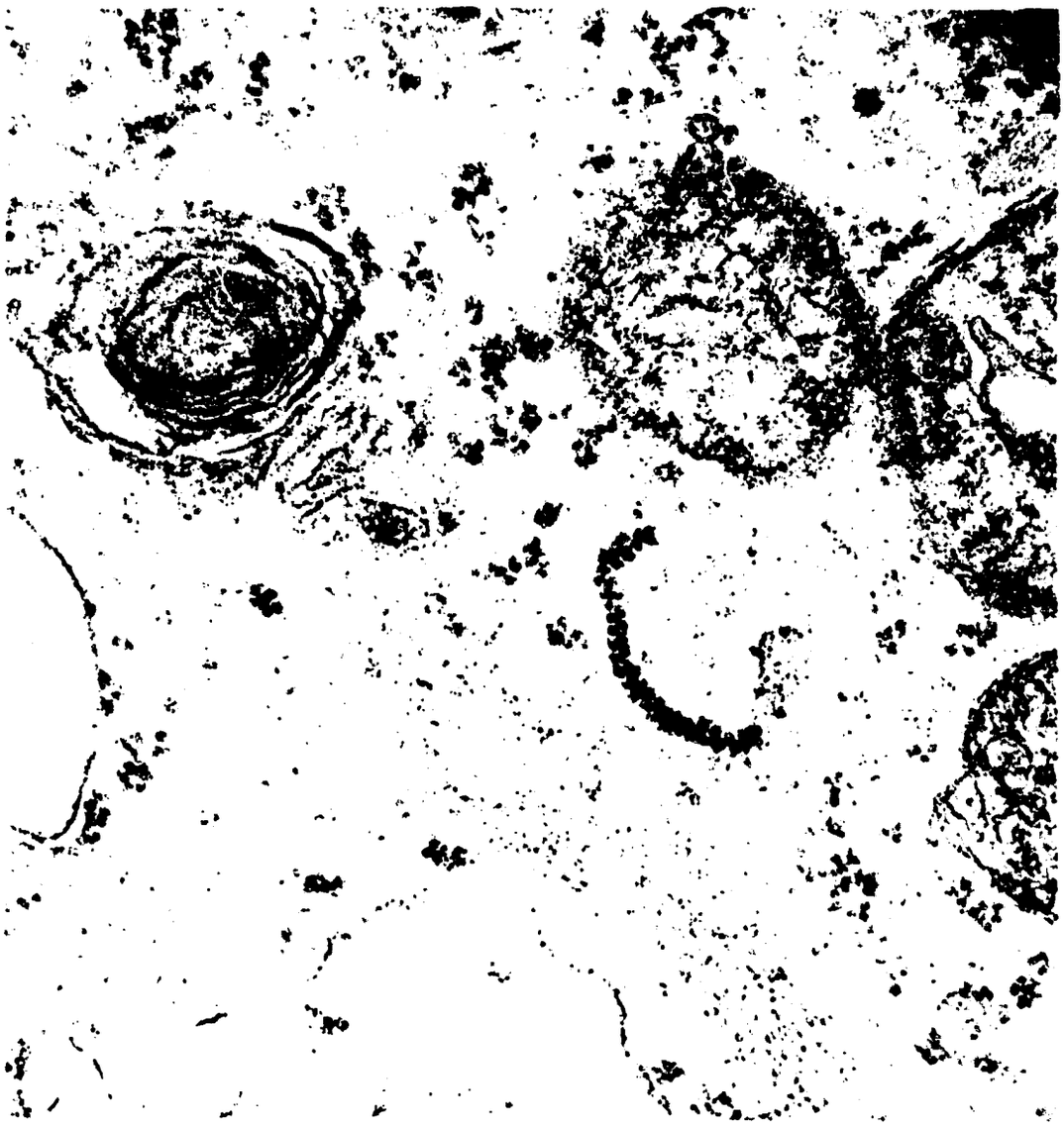

FIGURE 13. Cytoplasm of an Infected Cell Showing an Alternating Arrangement of Particles on a Membrane. 70,000X.

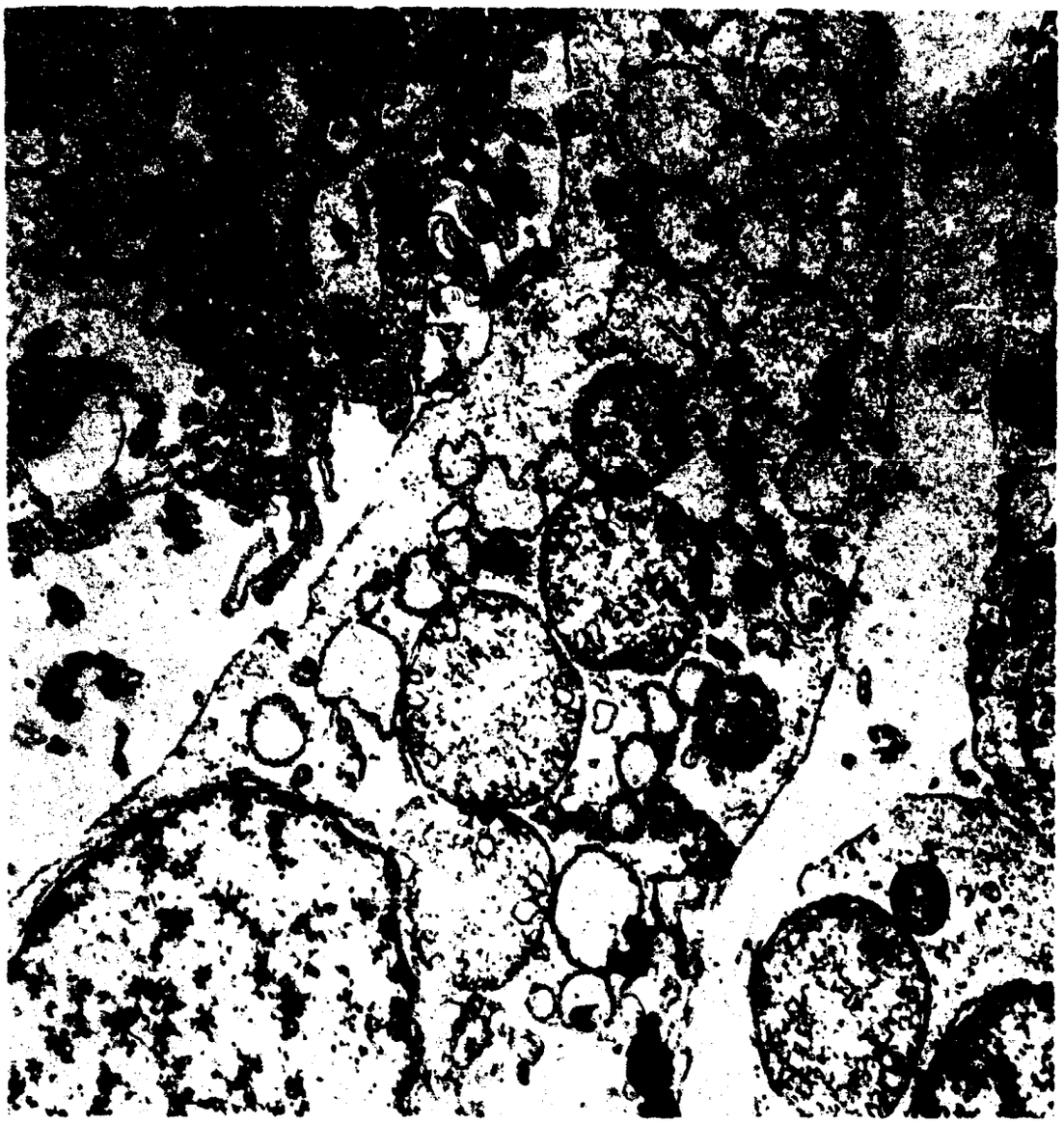

FIGURE 14. Low-Power View of Infected Cell at 48 Hours.
See Figure 8. 17,500X.

FIGURE 15. The Tremendous Size of the Mitochondria is Illustrated. The christae have a swollen tubular appearance. 70,000X.

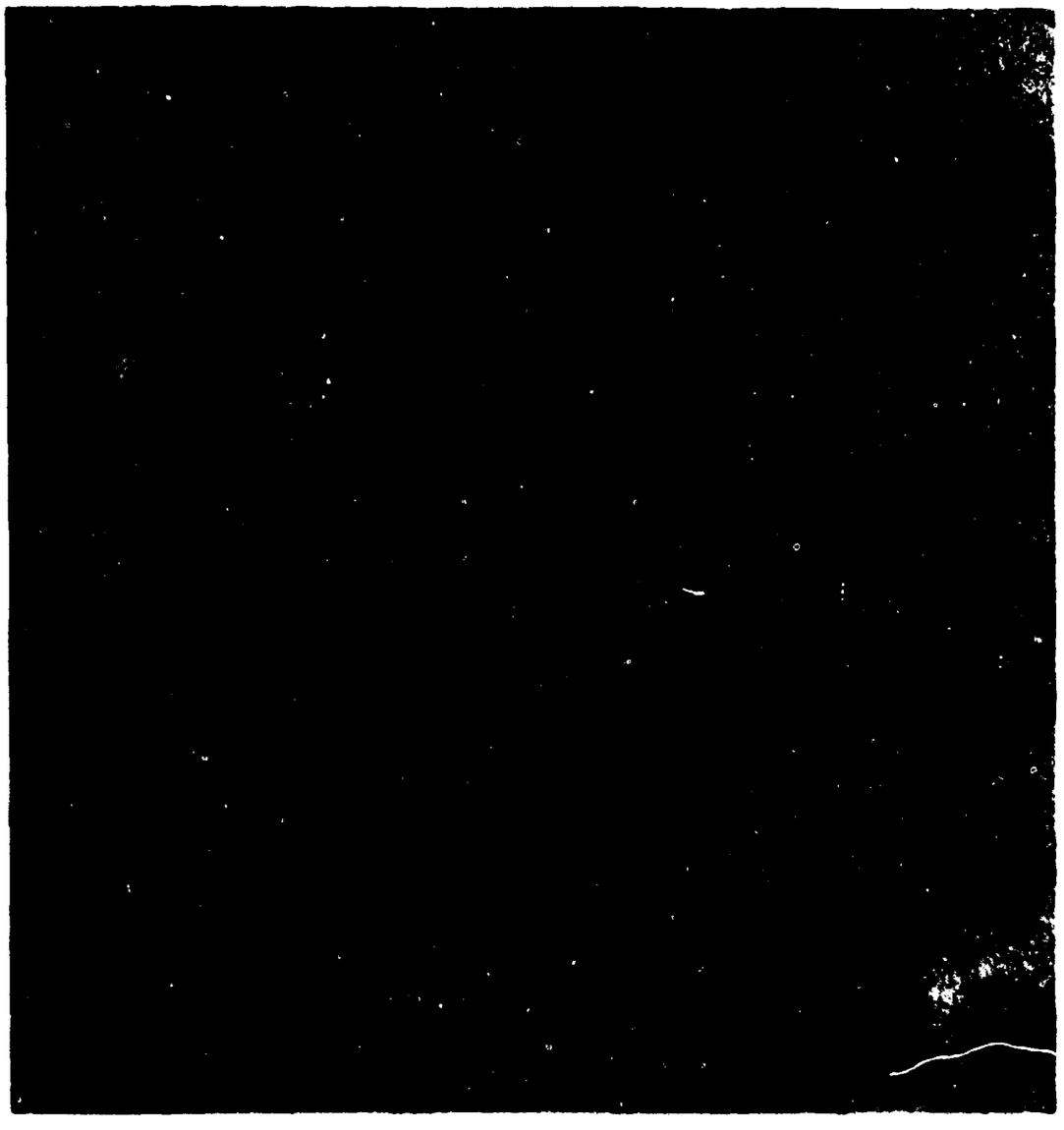

FIGURE 16. The Increase in Number of Particles is Shown. 70,000X.

FIGURE 17. Particles without Membrane. These most probably will become the core of the mature virus. 200,000X.

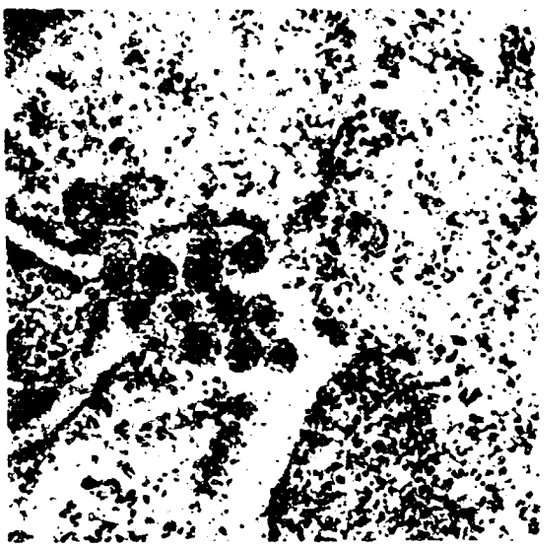

FIGURE 18. Mature Virus in Section of Tissue Culture. 150,000X.

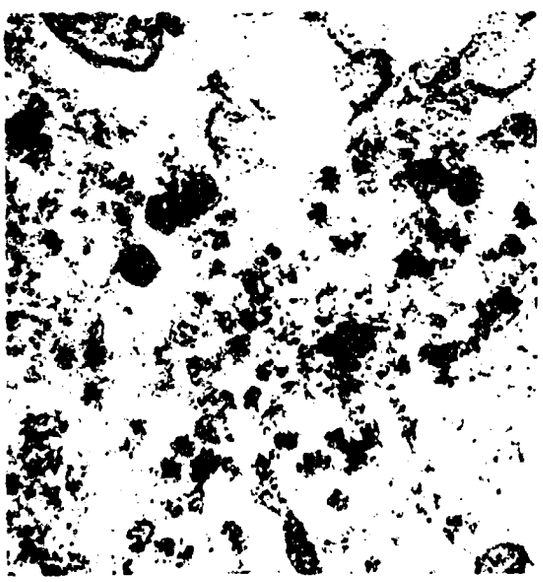

FIGURE 19. Mature Virus in Section of Partially Purified Suckling Mouse Brain. 150,000X.

The ultrastructural changes described were not seen in control cultures. The possibility does exist that the mitochondrial alteration was an osmotic artifact, particularly in view of the change in the cellular membrane. It is felt that these changes were caused by the virus infection because, at 24 hours, normal-appearing cells were seen in the same preparation as the affected cells and the mitochondrial damage was progressive. It is assumed that the numerous particles are viral precursor material because (i) the number of cells involved increased with time; (ii) the number of particles increased with time; (iii) the number of infected cells, judged by immunofluorescence, increased with time; (iv) the virus concentration in the tissue culture medium increased with time; and (v) the size of the particle was identical to that of the core of the mature virus.

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13. ABSTRACT

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14. Key Words

*Electron microscopes
*Rio Bravo virus
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