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The Replication of Rocio Virus in Brain Tissue of Suckling Mice. Study by Electron Microscopy

Brief Report

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With 4 Figures

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Summary

By electron microscopy studies, Rocio virus particles were about 43 nm and spherically shaped. They were found within the cisternae of the endoplasmic reticulum and Golgi complex of infected neurons. No precursor particles were detected nor virus budding was evident.

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Beginning in April 1975, several outbreaks of human encephalitis occurred in coastal areas of the State of São Paulo, Brasil. The etiologic agent was demonstrated to be a new flavivirus, subsequently named Rocio virus (3). The biological characteristics of this virus have been described as well as its epidemiological (4), clinical (10) and human pathological characteristics (8).

This report deals with ultrastructural aspects of Rocio virus replication in experimentally infected suckling mice. We have compared our observations with those describing other flavivirus which are the causative agents of human encephalitides.

Rocio virus (strain SPH 34675) was received from the "Seção de Vírus Transmitidos por Artrópodos" of the Instituto Adolfo Lutz, São Paulo, Brasil, in its sixth suckling mouse brain passage. Two-day-old Swiss mice from a colony maintained by the Instituto were inoculated intracerebrally (i.c.) with virus suspension at 10^{-1}, diluted in 0.15 M phosphate buffered saline with 0.75 per cent bovine albumin.

Brain tissue was harvested at 10, 16, 25, 40, 48, 59 and 73 hours after inoculation. These tissues were processed and included by classic techniques of electron microscopy. Ultrathin sections were stained with Reynolds lead stain (7) and examined at 60 kV in a Philips EM-200 and EM-400 electron microscope. In these sections, Rocio virus particles were always found in the lumen of membra-

nous cytoplasmic organelles, principally within Golgi membranes and endoplasmic reticulum, intracytoplasmic vesicles and the nuclear envelope. These particles were found in rows within the organelles, however individually scattered particles were also common.

In infected cells there was a hypertrophy of endoplasmic reticulum and Golgi complex. No particles were seen within the cytoplasmic matrix.

The diameter of Rocio virus particles was found to be around 43 nm in thin section studies. In order to measure the virus particles, we compared it with band repeating intervals of collagen fibril that was known to be of 64 nm in negative staining.

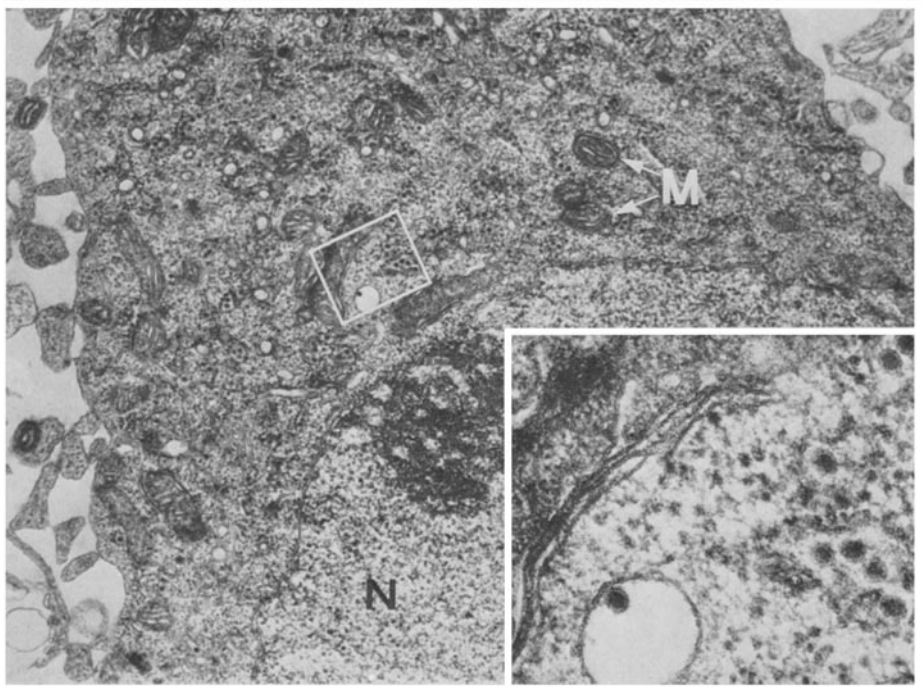

Fig. 1. Cytoplasm of mouse brain cell infected with Rocio virus, 73 hours post inoculation. Virus particles are present within cisternae of the endoplasmic reticulum. *M* mitochondrium 16,500×; *N* nucleous, Insert 90,000×

Rocio virus particles are spherical (Fig. 1); they have an electron dense core surrounded by a well delimited homogeneous transparent layer followed by a slight membrane. No virus particles could be detected in brain tissues collected before 40 hours after inoculation. From 40 hours onward the number of virus particles in infected cells increased rapidly, reaching its maximum when animals were sick and moribund, at 73 hours. However, no Rocio virus particles were found in the cytoplasmic matrix, no precursor particles could be detected, and there was no evidence of virus budding. Nuclei and mitochondria of infected cells appeared normal until very late in infection and there was not morphological evidence of nuclear participation in the virus replication. Within virus infected

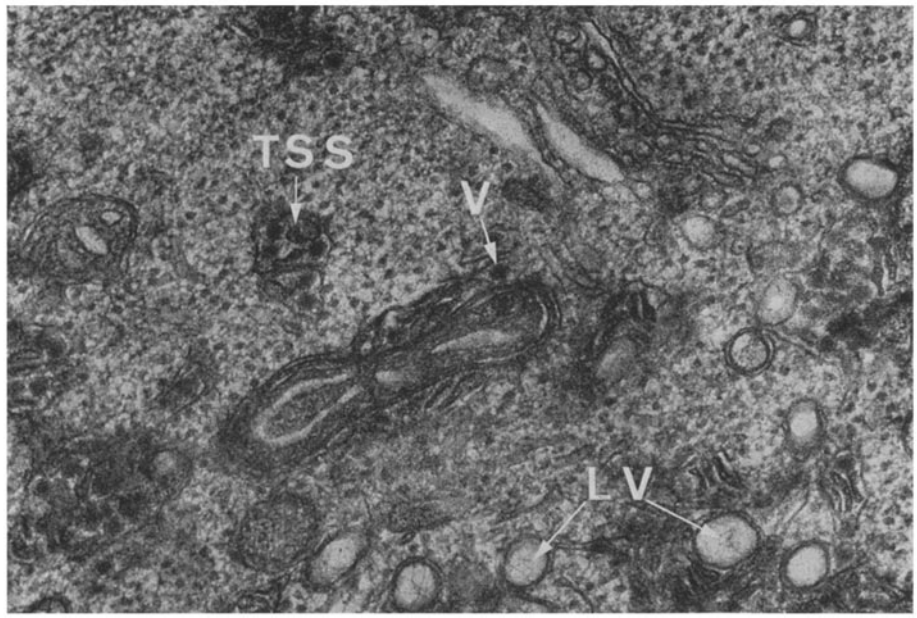

Fig. 2. Cytoplasm infected, 73 hours post inoculation. Lucent vesicles (*LV*), tadpole shaped structures (*TSS*) and virus particles (*V*) are present within cisternae of the endoplasmic reticulum. 70,000×

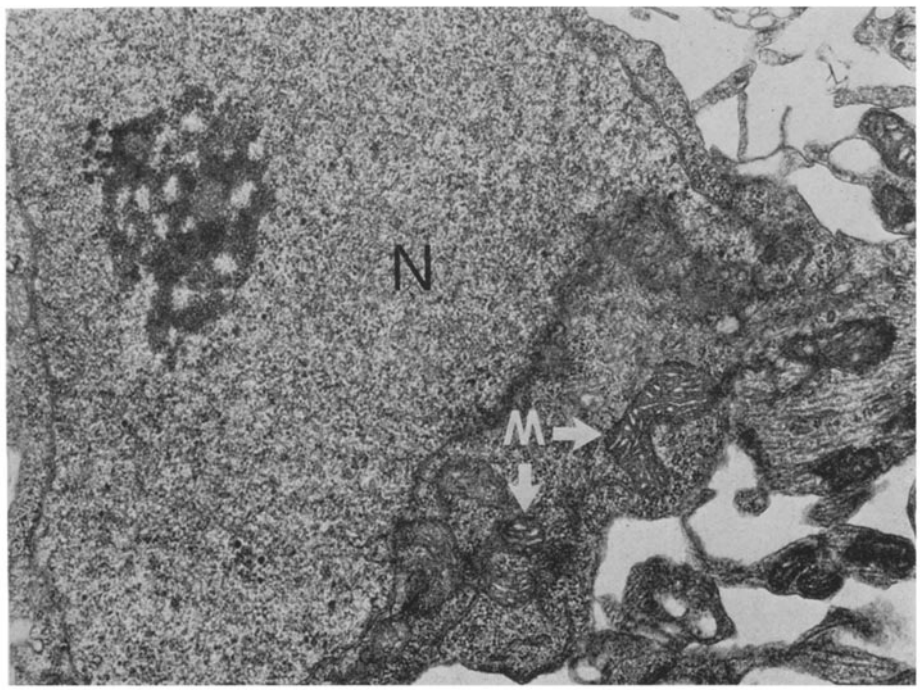

Fig. 3. Uninfected brain cell of suckling mice. *M* mitochondrium 17,500× ; *N* nucleous

cells there were abnormal structures (Fig. 2) which were associated with virus replication since they were never found in brain cells of uninoculated mice (Fig. 3). These were lucent vesicles, tadpole shaped structures, and inclusion bodies. The lucent vesicles had an almost spherical shape, were always associated with endoplasmic reticulum membranes and were first seen in those samples collected after 40 hours of infection (Fig. 2). Tadpole shaped structures were found within vacuoles, and never free in the cytoplasmic matrix (Fig. 2). Inclusion bodies consisting of electron dense masses were found in the cytoplasm of infected cells, and different from all other cytoplasmic organelles (Fig. 4).

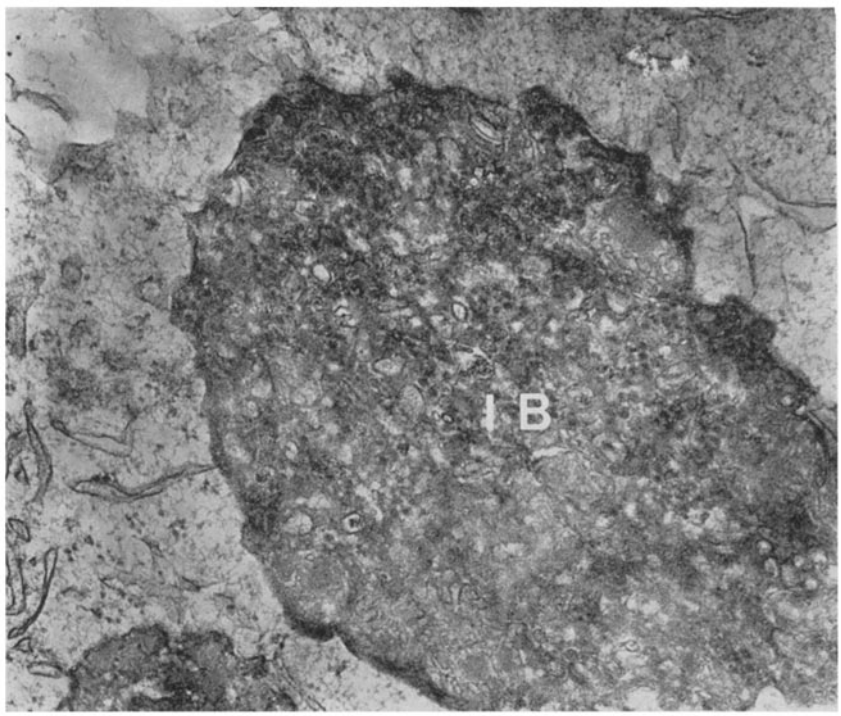

Fig. 4. Inclusion body within the cytoplasm of a mouse brain cell, 73 hours post inoculation. 46,000×

Several morphologic and morphogenetic studies have been done on flaviviruses which are the causes of sporadic and epidemic human encephalitis. All of these flaviviruses have been found to be spherical in shape, with a size ranging from 30 to 60 nm, and have an electron dense inner core when stained with uranium or lead citrate (1, 2, 5, 6). Similar morphologic details were found in Rocio virus (9). It was difficult to establish the real parameters of the virus replication, and some changes associated with infection, such as hypertrophy of cytoplasmic organelles, increase of perinuclear space and the appearance of cytoplasmic vacuoles, could not be ordered into a precise chronology. We suppose that the sequential events were obscured because the disease caused by Rocio virus in suckling mice developed very quickly, and the animals did not survive after 75 hours.

The appearance of precursor signs of cell infection and the involvement of Golgi complex and endoplasmic reticulum in Rocio virus infection were similar to that observed by FILSHIE (2), OYANAGI (6), MURPHY (5) and CALBERG-BACQ (1) in their studies on Murray Valley, Japanese Encephalitis, Saint Louis Encephalitis, and others flaviviruses.

Cytoplasmic matrix did not seem to be a place for virus maturation or accumulation. Mitochondria were preserved late in infection. Although no virus particles were visualized inside the nuclei of infected cells, nuclear participation in virus replication could not be excluded by the methods used.

Concerning the anomalous lucent vesicles observed (Fig. 2), it seemed that they must be associated with virus replication. According to MURPHY (5), they are always found associated with endoplasmic reticulum membranes and other cytoplasmic membranous organelles. Tadpole shaped structures, which functions are unknown, appeared more frequently in the final phase of the virus infection (Fig. 2) and seemed to be identifical to those found in Saint Louis Encephalitis virus infection in suckling mice (5).

Inclusions bodies (Fig. 4) were also found in final stages of infection, suggesting that they could be formed by an accumulation of electron dense material produced by cellular degeneration and metabolic changes.

From our observations, Rocio virus appears to be a typical flavivirus; it has morphologic and morphogenetic characteristics in brains of suckling mice which are very similar to other agents of this virus genus.

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