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NOTES

Maturation of Rabies Virus by Budding from Neuronal Cell Membrane in Suckling Mouse Brain

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Two strains of tissue culture-grown rabies virus developed in suckling mouse brain predominantly by the process of virus budding from the neuronal cell membrane.

The intracytoplasmic development of virions and the lack of virus budding from the surface of the host cell have been considered characteristic of rabies virus replication in the central nervous system (2, 8, 10, 12, 14, 15) with one exception (17). The absence of virus budding was recently stressed in a report of rabies virus infection of organized cultures of mammalian neural tissues (13), although virus budding from host cell plasma membrane has been shown in various extraneural tissues of rabid animals (4, 16) and in dispersed cell cultures (1, 3, 5, 6). We here report the observation of rabies virus maturation by budding in the mouse central nervous system. To minimize the possible sampling error in an ultrastructural study of rabies virus replication in brain tissue, suckling mice were inoculated intracerebrally with large doses of virus and the infected brains were meticulously examined at various stages of infection. Yields of infectious virus in these brains were titrated.

Three-day-old ICR mice were inoculated i.c. with 0.01 ml of BHK-21 cell-adapted fixed rabies virus of strains CVS or ERA with titers of approximately 10^8 PFU/ml. Animals were sacrificed on days 1, 2, 3, 4, and 5 after infection. For the titration of virus yield, a portion (approximately 60 mg) of the cerebral hemisphere, opposite the site of inoculation, was homogenized and assayed by plaquing in agarose-suspended BHK-13S cells (18). Portions of cerebral cortex, diencephalon, cerebellar cortex, and pons were processed for electron microscope study as described elsewhere (7).

No clinical or ultrastructural differences were observed between the animals infected with ERA or CVS strain virus. The majority of the infected animals became inactive on day 4; the first death occurred early on day 5, and by the end of day 5 all animals were moribund. An increased virus titer was noted on day 2 and virus yield increased steadily thereafter (Fig. 1). Ultrastructural study revealed the presence of small aggregates of granulo-fibrillary material, identical to the structure known as matrix, in the perikaryon of numerous neurons on day 2 when virions were not detectable. On day 3, a few virions were seen in intercellular spaces and virus budding from neuronal cell processes was sometimes observed. On day 4, virions were commonly seen in the intercellular spaces. On day 5, virus budding from neuronal cell membranes (Fig. 2) and neuronal processes (Fig. 3) was frequently encountered. At this stage of

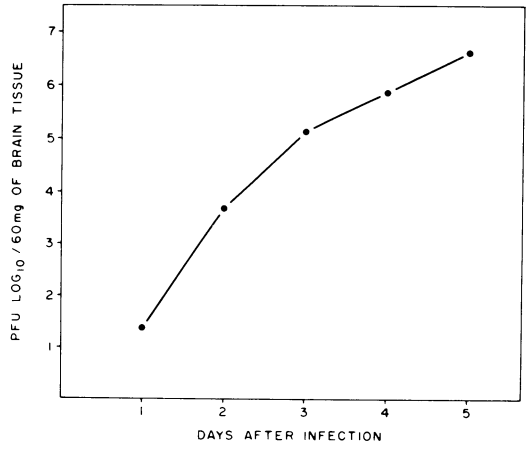

FIG. 1. *Growth curve of rabies virus (ERA strain) in mouse brain. A mean titer of two animals was recorded on each harvest.*

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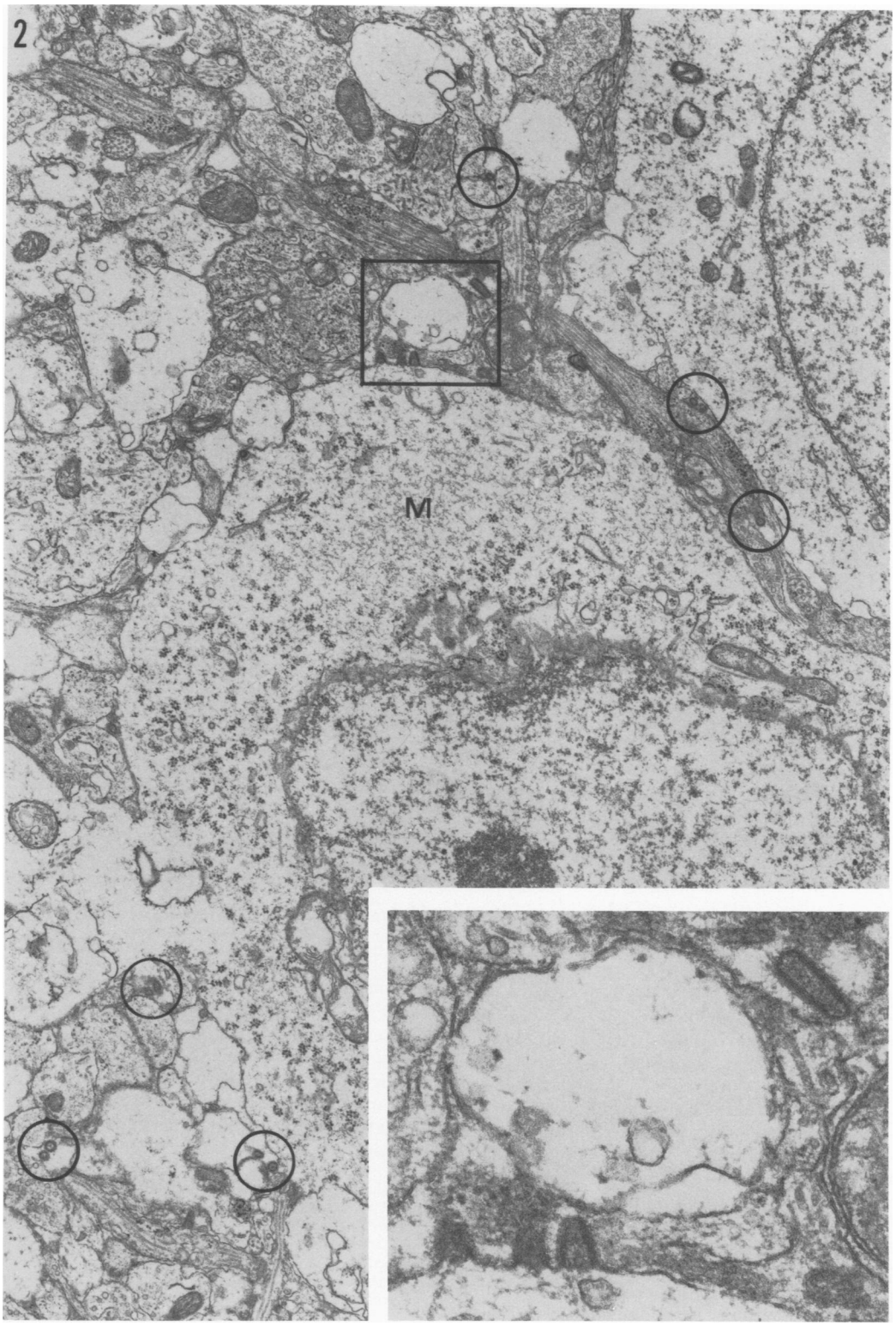

FIG. 2. *Portions of neuronal cells in hippocampus. Note virus budding (in rectangle) from the surface of a neuron bearing a matrix (M) in cytoplasm. Numerous virions (encircled) are seen in intercellular spaces. Inset: higher magnification of the area shown in rectangle. Three viruses are in the process of budding from cell surface (left lower corner) and a bullet-shaped virus particle is seen in right upper corner (5 days after infection). Magnifications: ×17,000; inset: ×66,000.*

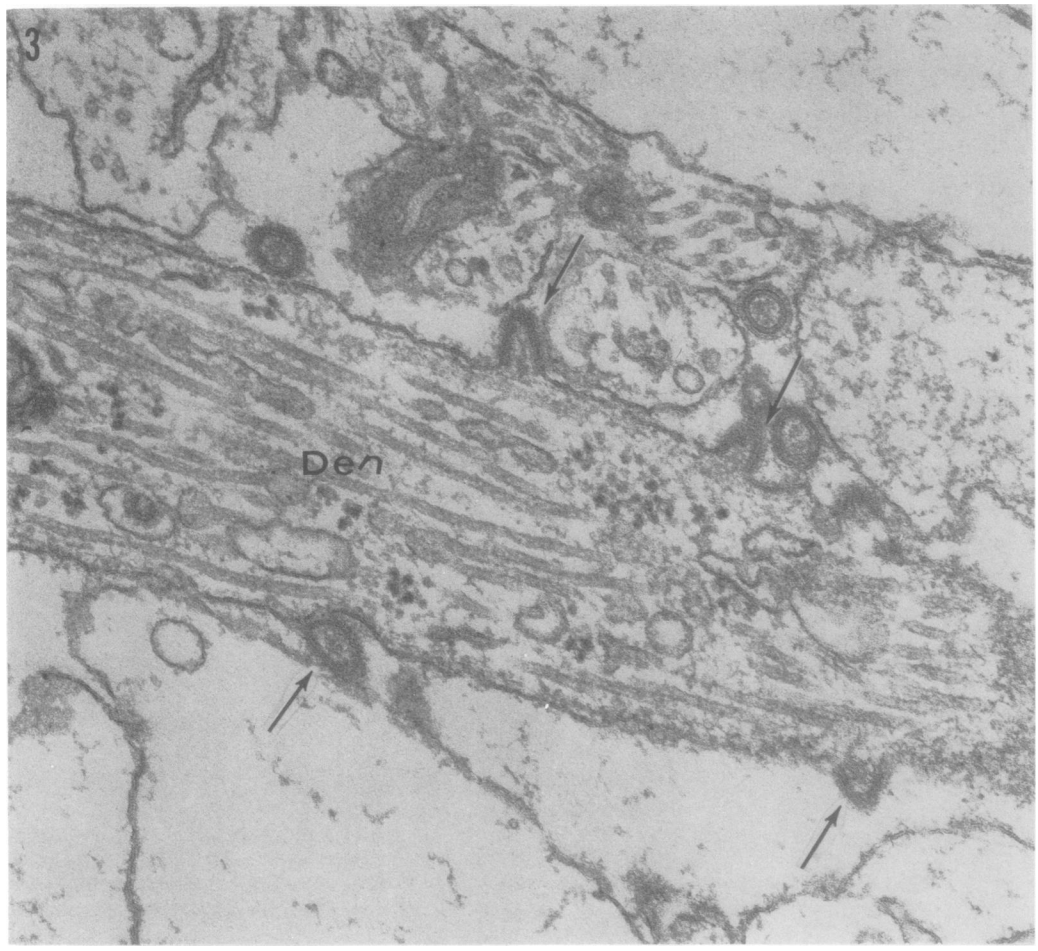

FIG. 3. *Virus budding from a dendrite (Den) in temporal lobe. Four particles (arrows) are budding from plasma membrane. Note the presence of virions in intercellular space (5 days after infection).* ×75,000.

infection, virions were also occasionally seen within the cytoplasm, but the ratio of the number of intracytoplasmic virions to that of intercellular virions remained less than 1:100.

The present study clearly demonstrated rabies virus budding from neuronal cell membranes in mouse brain, in agreement with recent observation of rabies-infected, young hamster brain (17). In the present study, virus budding from cell membrane apparently preceded the development of the virions within the cytoplasm as shown previously in an in vitro system (6). The increase in infectious virus titer in mouse brain appeared to parallel the increase in intercellular virions. Although the titer of virus used in this experiment was unnaturally high, the characteristic features of naturally occurring rabies, i.e., selective infection of neurons and a paucity of lytic changes in infected neurons, were well retained and probably represent the manner in which progeny virus is released from the host cell in nature. These findings may be of special significance in determining the manner of rabies virus dissemination in the central nervous system and in evaluating the immunopathogenic phenomena in rabies.

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