

## ELECTRON MICROSCOPE STUDY OF ECHO 19 VIRUS INFECTION IN MONKEY KIDNEY CELLS

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Virus particles in cells infected with adenovirus (1-6), poxvirus (7), herpes simplex virus (8-10), and one enterovirus Coxsackie B-5 (11), were recently reported. Such studies have not always revealed the presence of identifiable virus particles (12). The present brief note describes the presence of intracellular crystals in cells infected with ECHO 19 virus.

Kidney cells of *Macaca mulatta* Shaw, and *M. cynomolgus* Cuvier, were massively infected with ECHO 19 and harvested at different stages of infection. The samples were always taken within 48 hours and fixed in a solution of 1 per cent osmium tetroxide, for 60 minutes (7). The blocks were sectioned by means of a diamond knife in a Fernández-Morán ultramicrotome (13) and observed in a Siemens Elmiskop I electron microscope.

Fig. 1 shows two large crystals as well as two small groups in a portion of the cytoplasm of these infected cells. The whole cytoplasm appears to be vacuolated. Scattered dense masses can be seen which do not have a limiting structure. These might possibly be the source of material for the

formation of new virus crystals, some of which have a background density similar to that of the dense masses. Also some large, dense particles 40 to 50  $m\mu$  in size can be seen. They have never been encountered in normal cultivated cells (12) but, apparently, they should not be related to viruses because of their size and number.

Fig. 2 represents a markedly damaged cell, in which six different crystals varying in size and shape, can be seen in the cytoplasm. Virus crystal A contains regularly arranged particles side by side with other particles in an irregular arrangement. This might be either the initial stage of the crystal formation or a step during disintegration. The crystals observed in the center are bounded at certain points by a "membrane" but such "membranes" seem to be an artefact due to condensation of the cytoplasm because they are only observed in the damaged cells.

Figs. 3 and 4 show other crystals close to the cell membrane or attached to it. All the crystals observed were in the cytoplasm and appeared to be formed by rows of round or elongated particles, according to the plane of the section (cross-wise or oblique). The size of dense particles is  $14.5 \pm 2 m\mu$ . Particles in the vicinity of the crystals show

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approximately the same size, whereas less dense particles are usually smaller. No membrane formation was observed around these particles as noted in other virus groups, such as adenoviruses and poxviruses (6-10).

A variety of complex lesions were found in the infected cells, in both the cytoplasm and the nucleus, lesions that were never observed in normal cultivated cells. In addition, other alterations previously reported for normal cells were found (14). These findings will be the object of a detailed description at a later date.

In comparing these observations with those made on cells infected with another enterovirus, the Coxsackie B-5 (11), a great similarity was noted. Both kinds of particles were of about the same dimension, and were located in the cytoplasm with no membrane around them. The cells also showed identical alterations containing a number of small vacuoles (Fig. 1) and shrunken nuclei.

On the basis of the available evidence, the identity of the above described structures with ECHO 19 virus is not completely proved. However, the fact that they are found only in infected cells, their size, which agrees with that established by Barnes and Sabin for ECHO virus (15), and their similarity with another enterovirus Coxsackie B-5 (11), suggest that the particles here described are viral in nature.

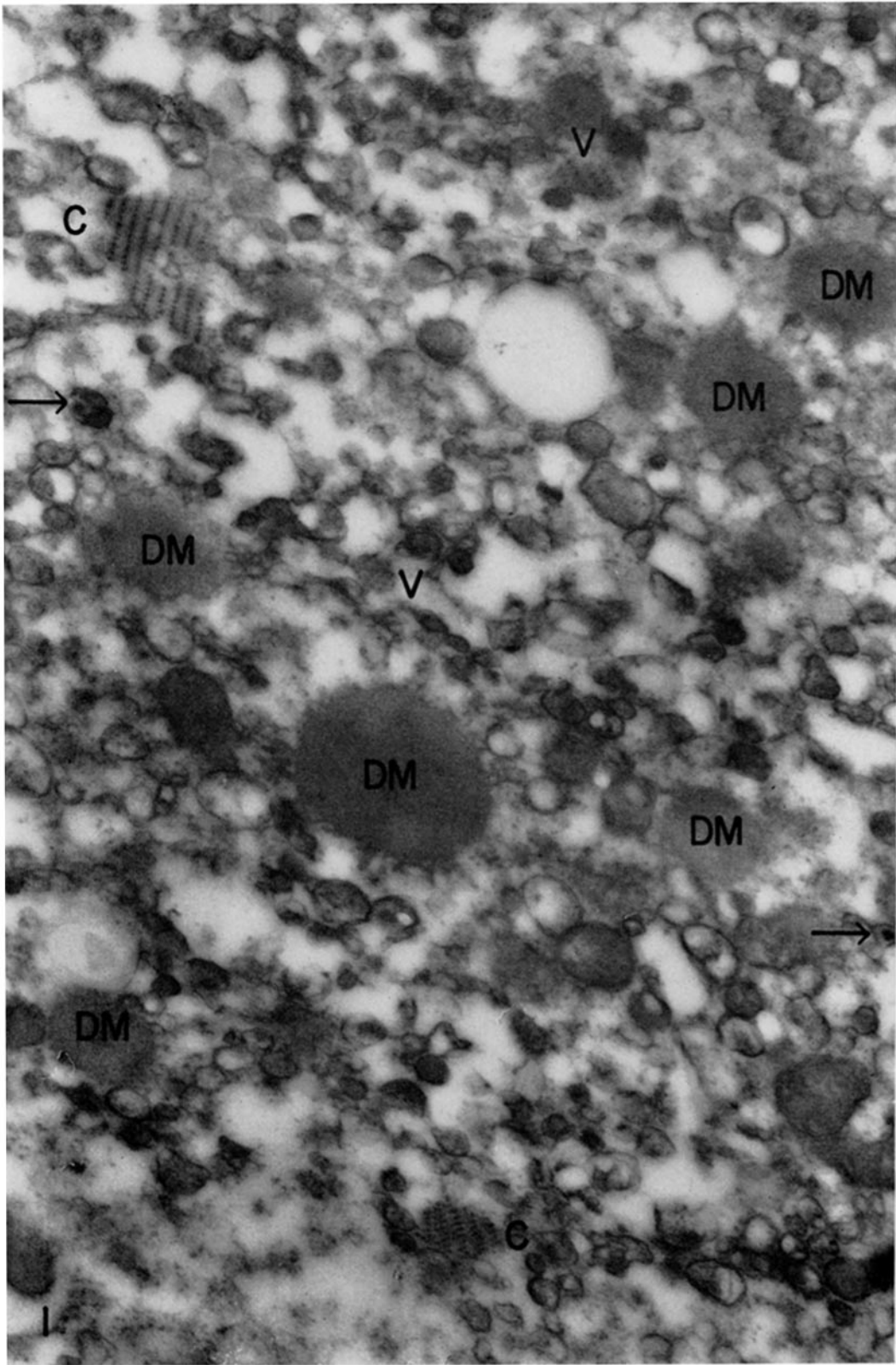
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## FIGURE 1

A portion of the cytoplasm of an infected cell which appears vacuolated, showing two large virus crystals (C) and two small groups of virus particles (V). Scattered dense masses (DM), plus a few large dense granular particles (arrows) are also in evidence.  $\times 45,000$ .



**FIGURE 2**

A greatly damaged or destroyed cell showing six virus crystals varying in shape and size. Crystal *A* is formed of regularly arranged particles side by side with other particles in an irregular arrangement. The crystals observed in the center are partially surrounded by membranes.  $\times 37,000$ .

**FIGURES 3 and 4**

Virus crystals located very close to the cell membrane. Fig. 3,  $\times 87,000$ . Fig. 4,  $\times 53,000$ .

