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SHORT COMMUNICATION

CRYSTALLINE AND LAMELLAR STRUCTURES IN LLCMK2 CELL CULTURES PERSISTENTLY INFECTED WITH DENGUE VIRUSES

SHIRO NII[1], TSUGUYA NAITO[2] AND PRAKORB TUCHINDA

Virus Research Institute, Department of Medical Sciences, Ministry of Public Health, Yod-se, Bangkok, Thailand

Electron microscopy of LLCMK2 cell cultures infected with rubella virus showed the presence of annulate lamellae and crystals in the cytoplasm of some cells and the possible involvement of these structures in the synthesis of virus material was suggested (Kim and Boatman, 1967). Crystalline structures were also found in RK13 cells infected with rubella (Holmes et al., 1968; Holmes et al., 1969) but not in BHK21 (Holmes et al., 1969; Tuchinda et al., 1969) and VERO cells (Tuchinda et al., 1969) infected with the same agent. Similar lamellar and crystalline structures were also seen in LLCMK2 cultures infected with Type 3 and 4 dengue viruses and this report describes observations on these structures.

The viruses used in the present investigation were strain H-87 of Type 3 dengue virus and strain H-241 of Type 4 dengue virus. The LLCMK2 strain of a continuous rhesus monkey kidney cell line was obtained from Dr. P. K. Russell (US ARMY Medical Component, SEATO, Bangkok) and serially propagated in 199 medium supplemented with 20% calf serum. Persistent infections with both the virus strains were readily established in this cell line (unpublished data). Samples for electron microscopy were prepared with these persistently infected cultures as follows: cells were washed once with 199 medium, scraped off and centrifuged gently to form a pellet. Cells were fixed for 1 hr in 1% glutaraldehyde, washed well and fixed for 30 min in 1% osmium tetroxide. Then they were dehydrated and embedded in epoxy resin. A Hitachi HU 11-C electron microscope was used for observation.

Fig. 1–5 show electron micrographs of cells prepared from cultures persistently infected with the H-241 strain of dengue-4 virus. Several lattice-like crystals are shown in Fig. 1 and 2. The fine structure of one crystal in Fig. 3 looks a little different from that of the crystals in Fig. 1 and 2 but this difference may have been caused merely by sectioning the crystals at a different angle, since all the crystals are thought to have essentially the same fine structure. Crystals were only found in the cytoplasm and not in the nucleus. Virus particles of 45–50 mμ diameter were observed in extracellular spaces as well as in the cytoplasm. Fig. 4 shows an aggregate of virus particles just outside a cell and some of these particles appear to consist of one outer envelope and an inner dense core. In the center of Fig. 5 an aggregate of virus particles is seen in the extracellular space and in the left, lower corner of the figure one crystal is seen in the cyto-

1 Present address: Department of Pathology, Research Institute for Microbial Disease, Osaka University, Yamada-kami, Suita, Osaka, Japan.

2 Present address: Department of Pathology, M. S. Hershey Medical Center, Hershey, Pennsylvania, U.S.A.

FIGURES 1. and 2. *Lattice crystals in an LLCMK2 cell infected with the H-241 strain of dengue-4 virus. Several crystals are located in the cytoplasm.*
Fig. 1 ×62,000 Fig. 2. ×56,000

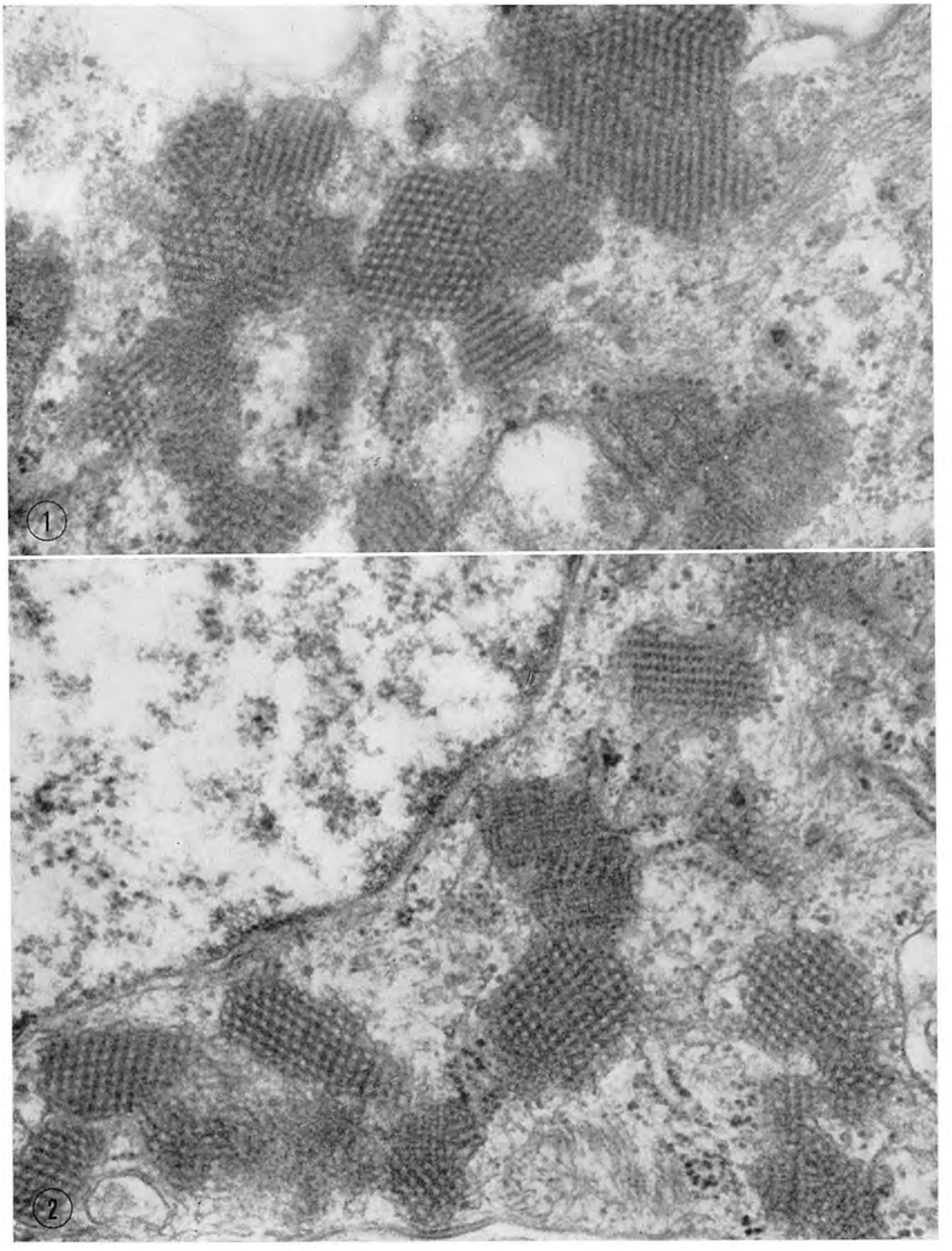
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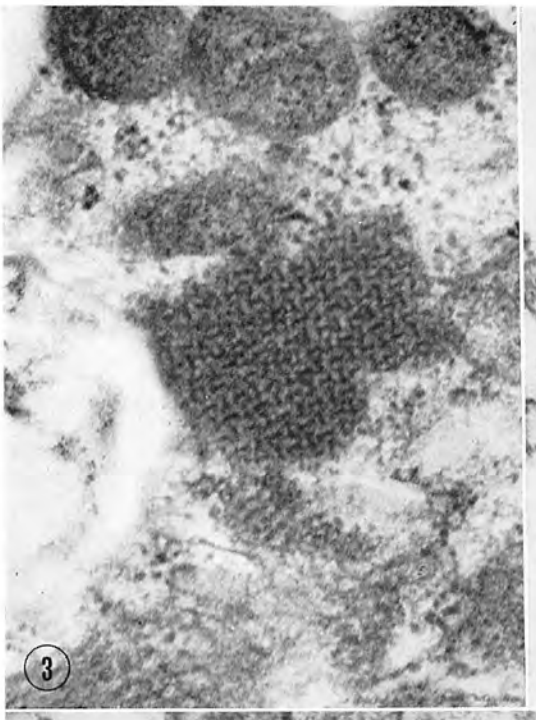
FIGURE 3. *A crystal structure in an LLCMK2 cell infected with the H-241 strain of dengue-4 virus.* $\times 59,000$

FIGURE 4. *An aggregate of virus particles is located just outside of the surface of an LLCMK2 cell infected with the H-241 strain of dengue-4 virus.* $\times 57,000$

FIGURE 5. *LLCMK2 cells infected with the H-241 strain of dengue-4 virus. One crystal in the cytoplasm is seen in the left, lower corner and an aggregate of virus particles in the extracellualr space is seen in the center of the picture.* $\times 38,500$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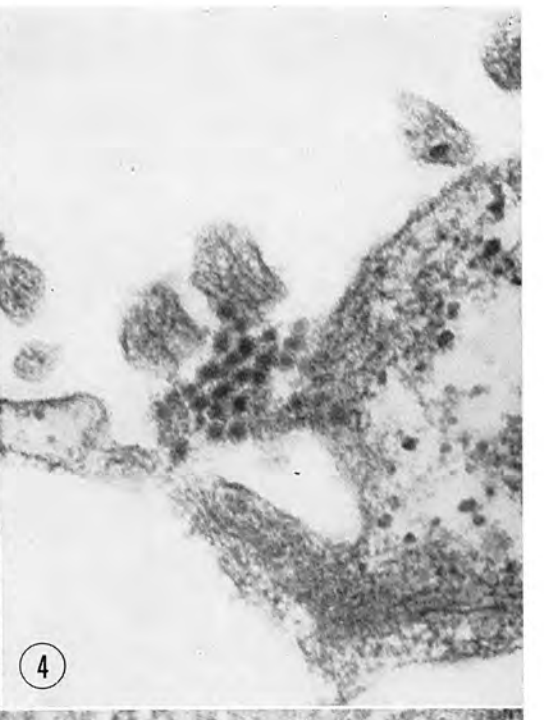
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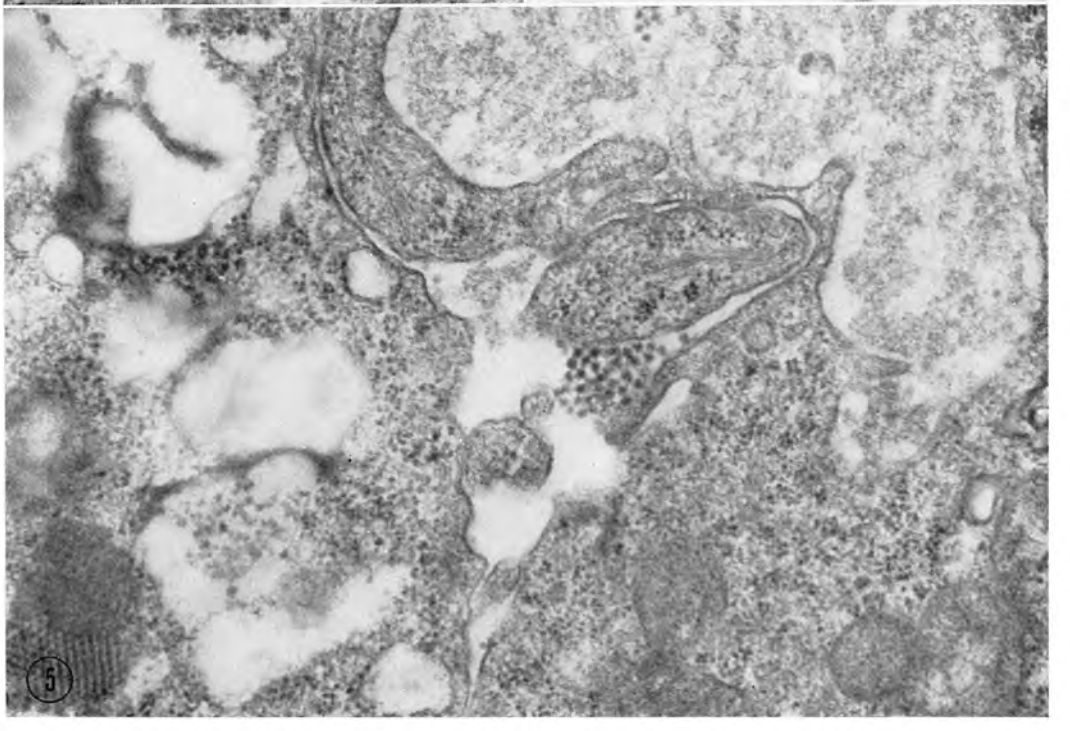
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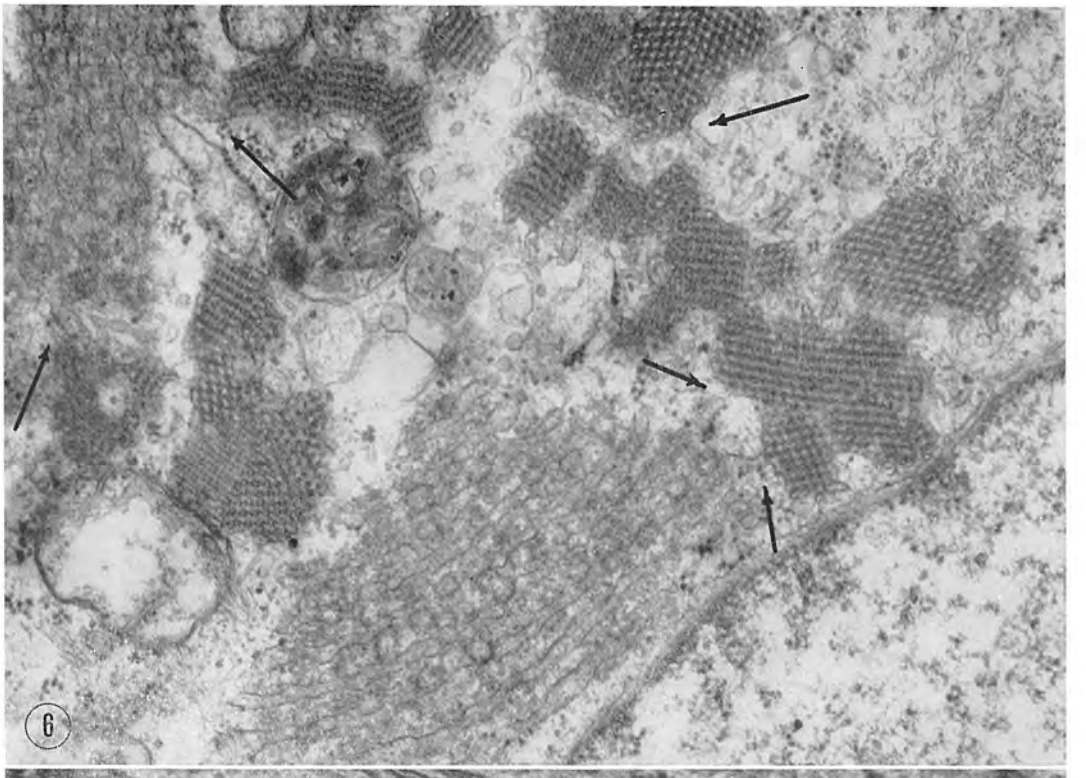
plasm.

Fig. 6 and 7 are electron micrographs of cells from cultures persistently infected with the H–87 strain of dengue-3 virus. Fig. 6 shows two lamellar structures, one in the left, upper part and the other in the lower, middle part of the figure. Several crystals are also observed. There are connections between lamellar or vesicular structures and crystals as indicated by the arrows, as found by Kim and Boatman (1967) in rubella-infected LLCMK2 cell cultures.

FIGURE 6. *Two lamellar structures and several lattice crystals are seen in the cytoplasm of an LLCMK2 cell infected with the H-87 strain of dengue-3 virus. Connections between lamellar or vesicular structures and crystals are indicated by arrows.* $\times 42{,}000$

FIGURE 7. *Virus particles in the cytoplasmic vacuoles of an LLCMK2 cell infected with the H-87 strain of dengue-3 virus.* $\times 46{,}500$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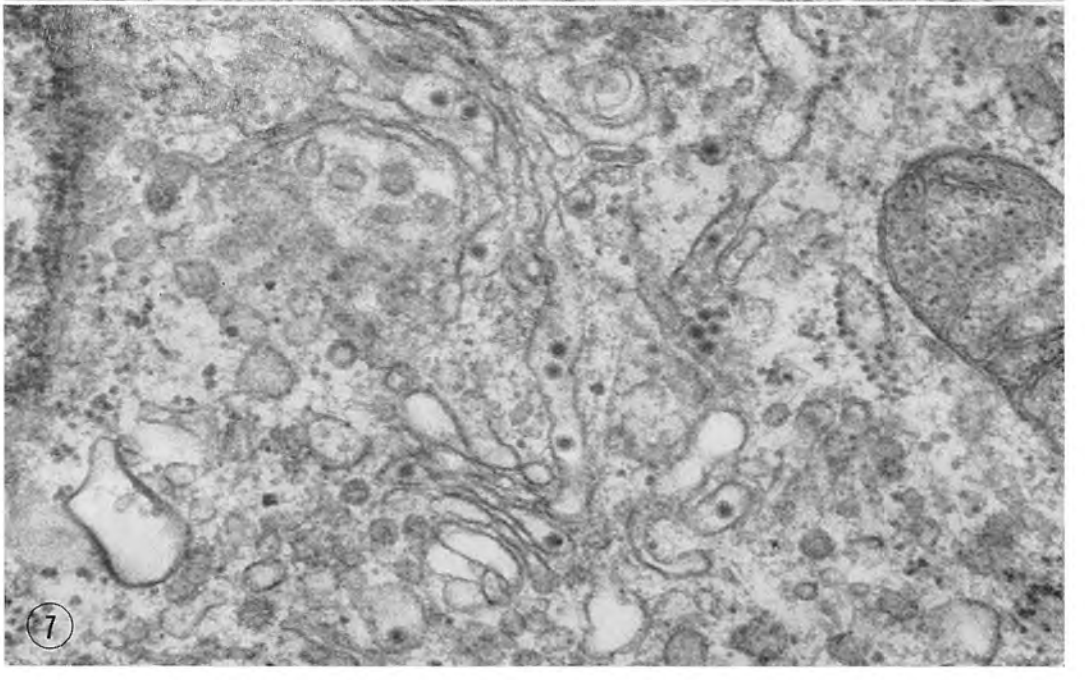
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The latter authors discussed the significance of annulate lamellae and crystals. They tentatively suggested that the structures observed were involved in the synthesis of virus material, since they were observed only in the cells from rubella-infected cultures. In our experiments with dengue-infected LLCMK2 cultures also the structures have so far only been detected in infected cultures and not in normal cultures. Moreover, it was found that the frequency with which cells containing cyrstals were detected in sectioned cells was approximately proportional to the percentage of cells containing antigen when parallel dengue-infected cultures were examined simultaneously by electron microscopy and fluorescent microscopy. However, no morphological evidence was obtained of a direct association between virus particles and either annulate lamellae and, or, crystals and this was also mentioned by Kim and Boatman (1967).

Kim and Boatman suggest an alternative interpretation, namely that these structures may reflect a reaction of the cells to viral infection rather than that these structures are actually involved in the synthesis of viral material and we are inclined to support this interpretation. Our present study showed that the appearance of crystalline and lamellar structures was not specific for rubella infection but that similar structures were also encountered in cultures of LLCMK2 strain cells infected with dengue virus. The kind of host cell infected with virus seems an important factor in their appearance. Thus Oyama et al. (1967) did not describe such structures in VERO cells infected with the same H-241 strain of dengue-4 virus as we used. Moreover, crystals were not found in BHK21 and VERO cell cultures infected with rubella while they were observed in infected LLCMK2 and RK 13 cells (Kim and Boatman, 1967; Holmes et al., 1969).

Lamellar structures like those observed in LLCMK2 cultures infected with rubella or dengue virus were also detected in cultures of the same strains infected with herpes simplex virus (unpublished data). They were distinctly different from the lamellae frequently observed in FL cells infected with this agent (Nii et al., 1968) and are thought to be virus specific (Nii et al., 1968). Annulate lamellae unrelated to virus infection have been reported in invertebrate and vertebrate germ cells, in neoplastic cells of various types and in some somatic cells (Swift, 1956; Gross, 1966). The structures described in these reports resemble those found in the present study.

Similar crystalline structures to those observed by Kim and Boatman (1967) and by us have also been found in cells of monkey tumors induced by Rous sarcoma virus (Munroe et al., 1964; Yamanouchi et al., 1967) and by Yaba monkey pox virus, a DNA type tumor virus (Tsuruhara, 1969). The crystals demonstrated in lamb kidney cells infected with Wesselbron virus (Parker and Stannard, 1967) and in HEp-2 cells infected with West Nile virus (Southam et al., 1964) may also be similar. Thus, the kind of virus does not seem to have any special relations with induction of these crystals in the cells.

The lamellae and crystals described above were observed in a variety of cells infected with various viruses. This suggests that these structures are not directly involved in the synthesis of viral material but appear as a response to virus in the host cells. Their significance and function, however, are still unknown.

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