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DENSE BODIES IN DUCK EMBRYO CELLS INFECTED WITH TURKEY HERPES

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SUMMARY Electron-dense material appeared in duck embryo fibroblasts (DEF) after infection with a herpesvirus of turkeys (HVT). The substance was frequently enclosed in a limiting membrane and formed electron-dense bodies. This membrane was morphologically indistinguishable from the envelope of virions detected in the cytoplasmic area. Most of these virions contained electron-dense material between their envelopes and capsids. However, this material could not be seen in virions found in the nuclear vacuoles or the perinuclear cisterna. Thus virions of two different shapes were observed in HVT-infected DEF cells. Observations strongly suggested that electron-dense bodies were produced through the same budding process as most virions in the cytoplasm, the nucleocapsids of which egress from the nucleus and are enveloped in a cytoplasmic membrane.

INTRODUCTION

A herpesvirus of turkeys (HVT) was first isolated by Witter et al. (1970) from apparently normal turkeys. Comparative ultrastructural studies of this virus and Marek's disease virus (MDV) showed that a high percentage of naked particles of HVT were morphologically distinct; i.e., they lacked a single dense core and had an inner structure resembling an electron lucent cross (Witter et al., 1970; Nazerian et al., 1971).

Six strains of herpesvirus of turkeys were also isolated in our laboratory (Ono et al., 1973) and comparative electron microscopic studies on one of these and a strain of MDV isolated and kept in our laboratory confirmed the findings of the above authors. We also observed accumulation of masses of electron-dense material in the cytoplasm of cells infected with turkey herpes and their envelopmental process, resulting in formation of dense bodies. This report is on the latter findings.

MATERIALS AND METHODS

1. *Virus and cells*

The Biken T-3 strain of HVT was used in this study (Ono et al., 1973). Duck embryo fibroblasts (DEF) grown in Eagle's minimum essential medium (MEM) supplemented with 5% calf serum were used as host cells for the virus.

2. *Infection of cells*

Primary monolayer cell cultures of DEF were prepared in 50 ml Miharu plaque bottles. Cells were infected in one of the following two ways. (1)

Monolayers were seeded with approximately 1×10^5 cells, derived from a DEF cell monolayer previously infected with HVT, and one day later the medium was replaced by fresh medium. (2) Monolayers were inoculated with approximately 10^3 PFU of the virus. After adsorption for 2 hr at 37 C the inoculum was replaced by fresh medium.

3. *Electron microscopic technique*

Three days after infection, when cytopathic effects (CPE) in the monolayers had become marked, the cultures were used to make preparations for electron microscopy. The medium was discarded and 5–6 ml of fresh MEM were introduced. The cells were scraped off the bottles into the medium and centrifuged at low speed. The resulting pellets were fixed for 1 hr in 1% glutaraldehyde, washed well, fixed for 30 min in 1% osmium tetroxide, dehydrated and embedded in epoxy resin. Sections were made with a glass knife in an LKB microtome and stained

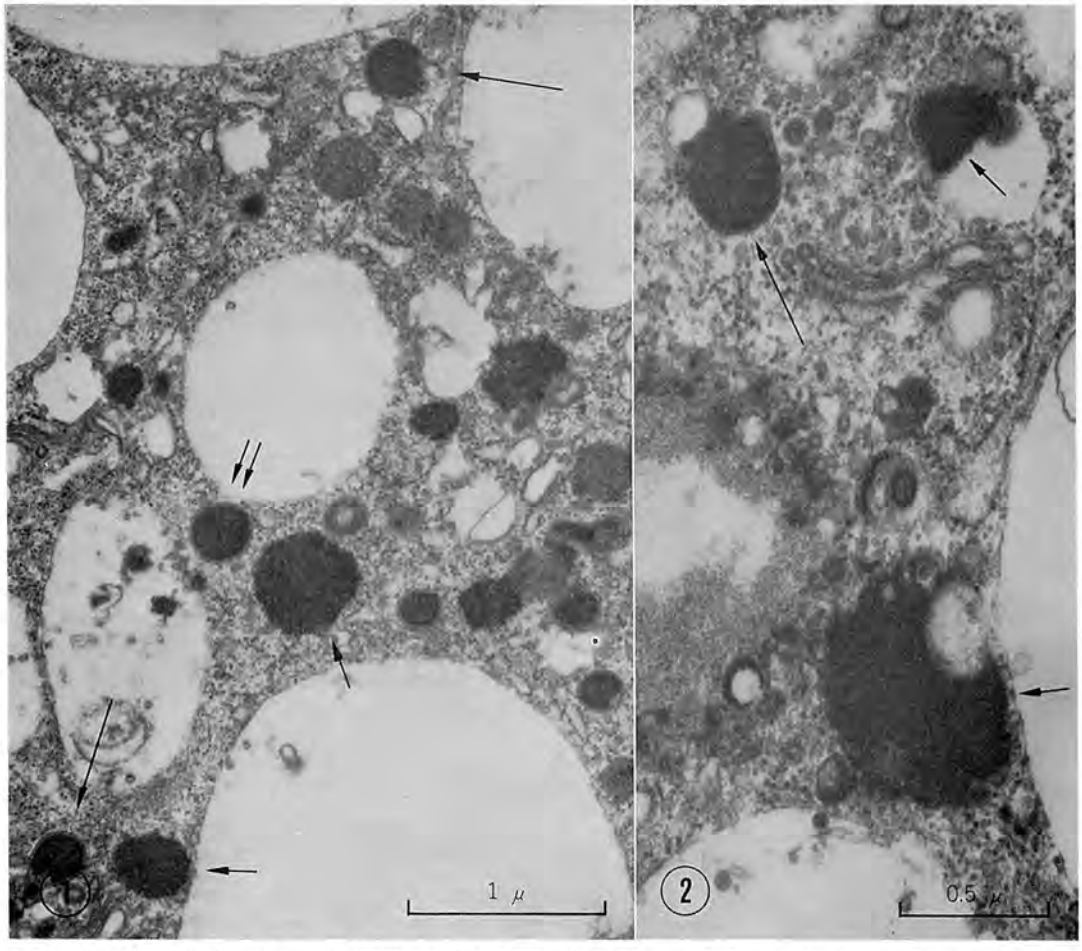

FIGURE 1. *Syncytial cytoplasm of HVT-infected DEF cells. Masses of electron-dense material are seen in the cytoplasm. Some have irregular outlines and do not seem to be associated with any membrane structures (short arrows). Others are half or almost completely enclosed in a limiting membrane (long arrows). The rest is completely enclosed in a membrane (double arrow) and are termed "dense bodies."*

FIGURE 2. *Three electron-dense masses in the cytoplasm. Two of them (short arrows) do not seem to be associated with a limiting membrane and have irregular outlines and the bigger one in particular looks amorphous. One electron-dense mass (long arrow) is half enclosed in a limiting membrane.*

with uranyl acetate and lead citrate. A Hitachi 11B electron microscope was used.

RESULTS

Syncytial formation in DEF cultures infected with HVT was observed by electron microscope as well as light microscope, as already described by Nazerian et al. (1971). In the cytoplasm of the syncytial cells many electron-dense masses were irregularly distributed, giving a patchy appearance (Fig. 1). Some dense masses were not associated with any membrane structures and seemed to be free in the cytoplasm (Figs. 1 and 2). These dense masses had more or less irregular outlines and occasionally looked amorphous. Others were partially covered with a limiting membrane and

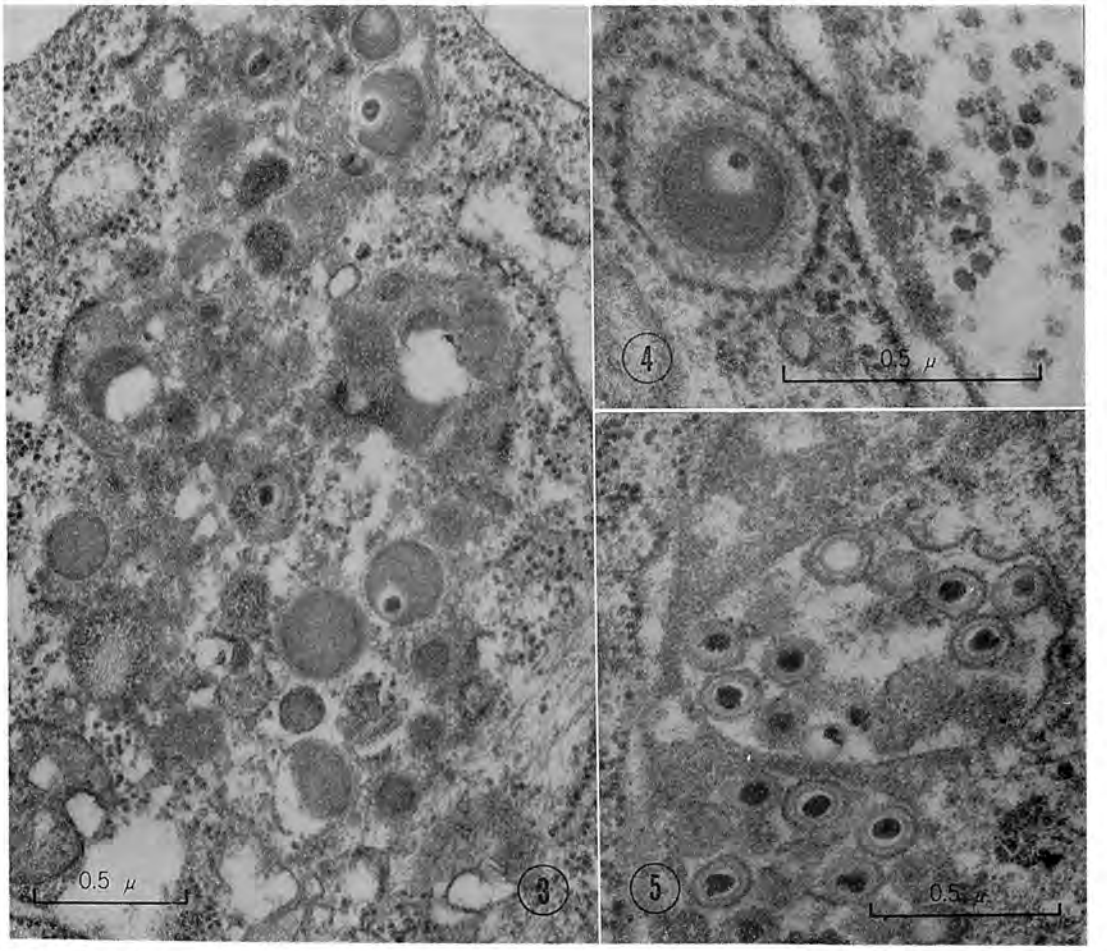

FIGURE 3. *Several electron-dense bodies which do not seem to enclose nucleocapsids are seen in groups in the cytoplasm concomitantly with enveloped particles. The dense bodies are similar in size to virions or smaller.*

FIGURE 4. *An enveloped particle in a tubule of rough surfaced endoplasmic reticulum. This particle contains electron-dense material between its envelope and nucleocapsid. In the nucleus many granules of 30–35 mμ in diameter are also seen.*

FIGURE 5. *Enveloped particles in nuclear vacuoles. Electron-dense material is not seen in the space between the envelopes and caspsids of these particles.*

so were partly circular and partly irregular in outline and occasionally had a cup-shape. Other dense masses were completely enclosed in a limiting membrane and looked spherical or ovoid. These membrane-limited particles of dense material were named dense bodies, following the terminology given to similar structures in cells infected with cytomegalovirus (Craighead, Kanich and Almeida, 1972).

Dense masses did not always appear in association with nucleocapsids and seemed to accumulate rather independently in the cytoplasm, while dense bodies were often found in groups concomitantly with enveloped particles. The dense bodies found in these groups were similar in size to virions or smaller (Fig. 3).

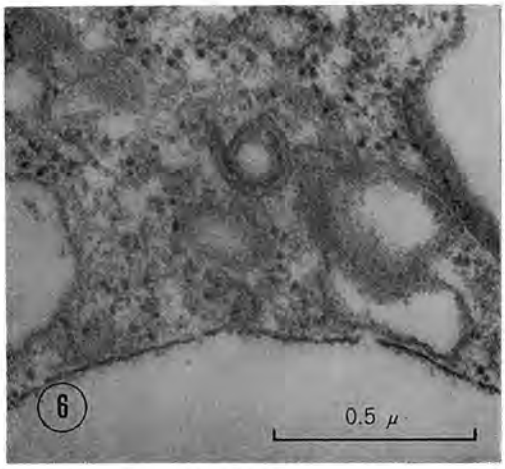

FIGURE 6. *A capsid, which seems to be empty, half covered with a cytoplasmic membrane.*

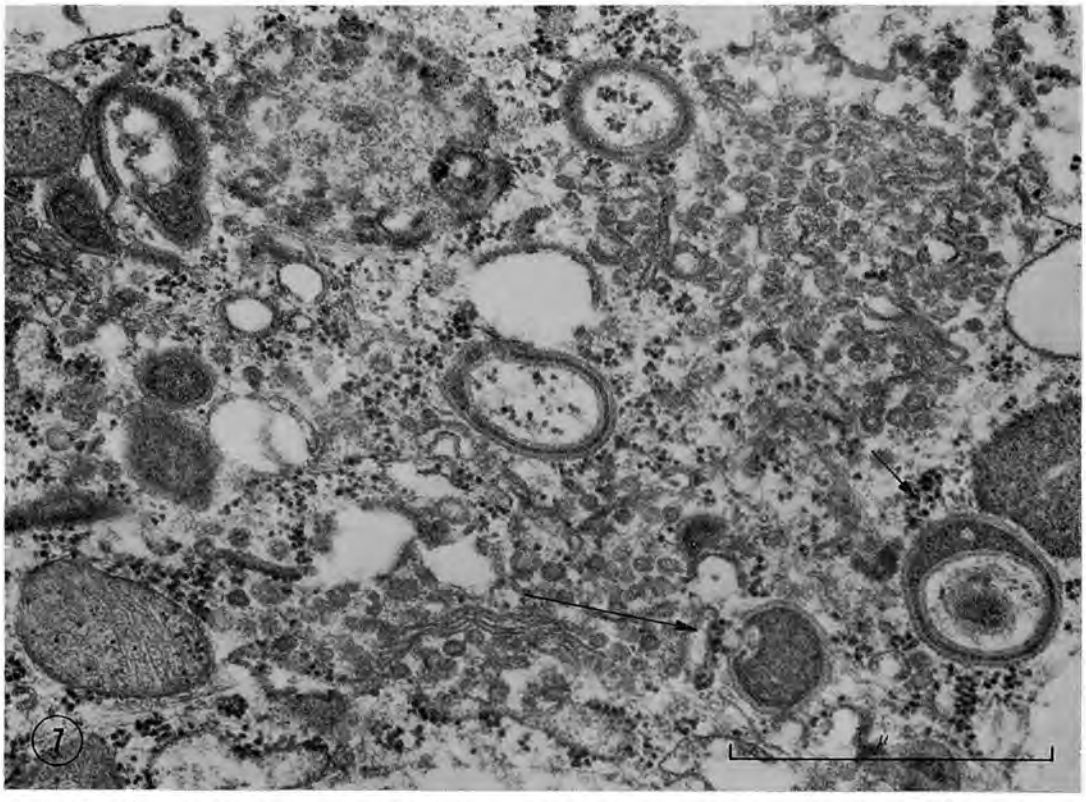

FIGURE 7. *Unusual development of membrane structures in the cytoplasm. One structure seems to be an aberrant form of a dense body (short arrow). A mass of electron-dense material almost completely enclosed in a limiting membrane (long arrow) is also seen.*

Most virions in the cytoplasm had larger envelopes than those found in the nucleus or perinuclear cisterna (Figs. 3, 4 and 5). The former particles contained electron-dense material between the envelopes and capsids. Dense material was not seen in the same position in the latter particles, which acquired their envelopes from the inner nuclear membrane. The process of envelopment of viral particles in the cytoglasm was also seen (Nazerian et al., 1971) (Fig. 6).

Unusual development of membrane structures and aberrant shapes of electron-dense bodies were also frequently observed in the cytoplasm (Fig. 7).

DISCUSSION

Electron-dense bodies or electron-dense material has only been found in cells infected with a few herpesviruses, i.e., human cytomegalovirus (Luse and Smith, 1958; McGavran and Smith, 1965; Becker, Melnick and Mayor, 1965; Patrizi et al., 1965), murine cytomegalovirus (Ruebner et al., 1964), varicella-zoster virus (Becker et al., 1965; Cook and Stevens, 1968; Nii, 1970) and herpes-type virus of frog renal adenocarcinoma (Stackpole, 1969). This material has received little attention and has been studied by only a few investigators. However, very recently Craighead et al. (1972) studied the morphogenesis of dense bodies produced in cells infected with cytomegalovirus and discussed their biological significance.

We found that HVT also produced electron-dense material in DEF cells and many homogenous electron-dense masses accumulated in the cytoplasm of infected cells. Uninfected DEF cells did not contain this material.

One of the most intriguing findings was that the dense masses were frequently enclosed by a limiting membrane forming structures termed "dense bodies" (Craighead et al., 1972). Envelopment of nucleocapsids was occasionally observed in the cytoplasm too. The ultrastructure of the limiting membranes of dense bodies could not be distinguished from that of the envelopes of nearby virions. Moreover the virions contained much electron-dense material between their envelopes and capsids. Therefore, some electron-dense bodies looked just like virions in the cytoplasm. These findings suggest that in the cytoplasm of DEF cells infected with HVT budding of nucleocapsids and of electron-dense masses occur by essentially the same mechanism.

Virions found in the nuclear vacuoles or perinuclear cisterna did not contain electron-dense material and looked much smaller than those in the cytoplasm. Thus two types of virions could be differentiated, as already reported by Nazerian et al. (1971).

It seems very likely that electron-dense material is a structural component of HVT for the following reasons: (1) electron-dense material appeared in HVT-infected cells but not in uninfected cells, (2) it was incorporated into virions and (3) it seemed to be able to initiate a budding process by itself. The great morphological difference between virions associated with the inner nuclear membrane and those in the cytoplasm might be explained as due to variation in the extent of accumulation of electron-dense material at sites where viral envelopment takes place.

One of the authors has already reported that amorphous particles of varicella-zoster virus were produced in the cytoplasm in just the same way as the dense bodies in HVT-infected cells (Nii, 1970). Further investigations may clarify the mechanism of formation of amorphous or aberrant-shaped particles in cells infected with herpesvirus and the cause of the cellular association of virus infectivity of subgroup B herpesvirus.

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