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Ultrastructure of Hamster Kidney Cell Culture Infected with Herpesvirus

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Hamster kidney cell culture was found susceptible to infection and replication of herpesvirus of turkey (HVT) as evidenced by cytopathic effect, induction of HVT-specific, complement-fixing antigen, and demonstration of virus particles by electron microscopy. Viral structures at different developmental stages were observed. Several infected cells showed a lamellar inclusion body possibly continuous with the endoplasmic reticulum. The function of these lamellar bodies is not known.

Strains of herpesvirus from turkeys (HVT) were isolated from kidney cell cultures of normal turkeys, and turkeys from flocks suffering from a lymphomatous leukosis (7, 24). The strain of herpesvirus used in this experiment, designated FC-126, was found to be antigenically related to the JM strain of type II leukosis, or Marek's disease (24). Nazerian et al. (14) carried out an ultrastructural study of HVT-infected avian cells. They found, in addition to different degrees of polykaryocytosis, a high concentration of 35-nm particles in infected nuclei, ribosome crystals, and virions that were similar to Marek's disease virus. The naked virus particles which were observed were morphologically characteristic of herpes-type virus, having an inner structure resembling an electron-lucent cross. Bedigian and Sevoian (1) reported on the susceptibility of hamster kidney cell culture to infection with HVT.

The purpose of this paper is to demonstrate the effects of HVT on the ultrastructure of hamster kidney cell culture.

MATERIALS AND METHODS

Virus. The FC-126 strain of HVT (24) was used to infect hamster kidney monolayers as described before (1).

Hematoxylin and eosin staining. HVT-infected hamster kidney cell cultures grown on cover slips were stained with hematoxylin and eosin (10) to determine the presence of intracellular inclusion bodies. Cell-free virus material was also prepared by the method described by Mikami and Bankowski (13) and was used to infect hamster kidney monolayer in other trials.

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CF test. Control and HVT-infected hamster kidney cell cultures were examined for complement fixation (CF) activity by a procedure similar to that for Marek's disease virus (4).

Electron microscopy. All cultures for electron microscopy were fixed in situ in 1% (wt/vol) glutaraldehyde in a 0.1 M sodium cacodylate buffer, pH 7.2, for 15 min at 4 C. After this initial fixation, the cells were gently scraped from the plastic surface with a rubber policeman and centrifuged at 800 $\times$ g for 10 min to form a soft pellet. This pellet was washed six times in cold 0.1 M cacodylate buffer for 1 h at 4 C. The cells were then washed in 0.1 M cacodylate buffer, dehydrated in a graded ethyl alcohol series, embedded in Epon 812 (11), and polymerized at 45 and 60 C for 12 and 24 h, respectively.

Thin sections were cut with a diamond knife on a Porter Blum MT-2 ultramicrotome (Sorvall, Inc.) and stained with uranyl acetate (6) and lead citrate (16). Sections were examined in a Philips EM 200 electron microscope equipped with a 50-μm objective aperture and liquid N_2 anti-contamination device.

RESULTS

Infection of hamster kidney cell culture with HVT. When monolayers of primary hamster kidney cell cultures were infected with HVT, cytopathic effects of the herpes type appeared at approximately 7 days postinoculation. Foci consisting of rounded refractile cells and syncytia were observed. These lesions progressed to clear plaques after a few days. Large syncytia having many vacuoles were also observed. Monolayers on cover slips which were stained with hematoxylin and eosin showed intranuclear inclusions similar to type A herpesvirus inclusions, reported by Cowdry (2). Electron microscopy of thin sections of infected hamster kidney monolayers showed polykaryo-

cytes which represented true multinucleated cells (Fig. 1). As can be seen in Fig. 1, the cell cytoplasm is not subdivided by cell membranes. Cultures which were infected with HVT-infected cellular material contained approximately six times more polykaryocytes than analogous cultures inoculated with cell-free virus material. In addition to the polykaryocytes observed in infection, single or uninucleate infected cells were also observed (Fig. 2). These infected cells showed characteristic margination of the nuclear chromatin along with viral nucleocapsids of approximately 100 nm. Most of the nucleocapsids were naked, lacking the outer envelope (Fig. 3). Some of these nucleocapsids lacked the central dense cores whereas others contained a variable number of internal dark areas (usually four) which gave the viral core the characteristic shape of an electron-lucent cross, the latter reported to be characteristic of HVT (14). The electron-dense particles were approximately 35 nm in diameter (Fig. 4) and are most probably the precursors of the central cores of the nucleocapsids. These structures were similar to those observed in cells infected with other herpesviruses (22, 23) and were similar in size and shape to the electron-dense cores of some nucleocapsids. Furthermore, these particles were observed in infected cell nuclei either associated with virus particles, or in their absence. Mature virus particles were present both within the cell nucleus juxtaposed to the nuclear membrane (Fig. 5) and free in the cytoplasm.

Virus-related structures in the cytoplasm. It was found that when hamster kidney cells were infected with HVT, there appeared in the cytoplasmic region lamellar membrane structures (Fig. 6). These membranes were composed of parallel units, each unit (membrane) being approximately 10 nm thick. When this electron-dense material was lost, there appeared to be a close association with the endoplasmic reticulum (Fig. 7). It is noted that viral-specific CF antigen could be demonstrated in these infected cells even before the demonstration of viral particles. Several of the virus particles appear to be in close association or proximity to a lamellar structure (Fig. 8). Thin sections from control hamster kidney cell cultures did not show any viral particles or these infection-related structures.

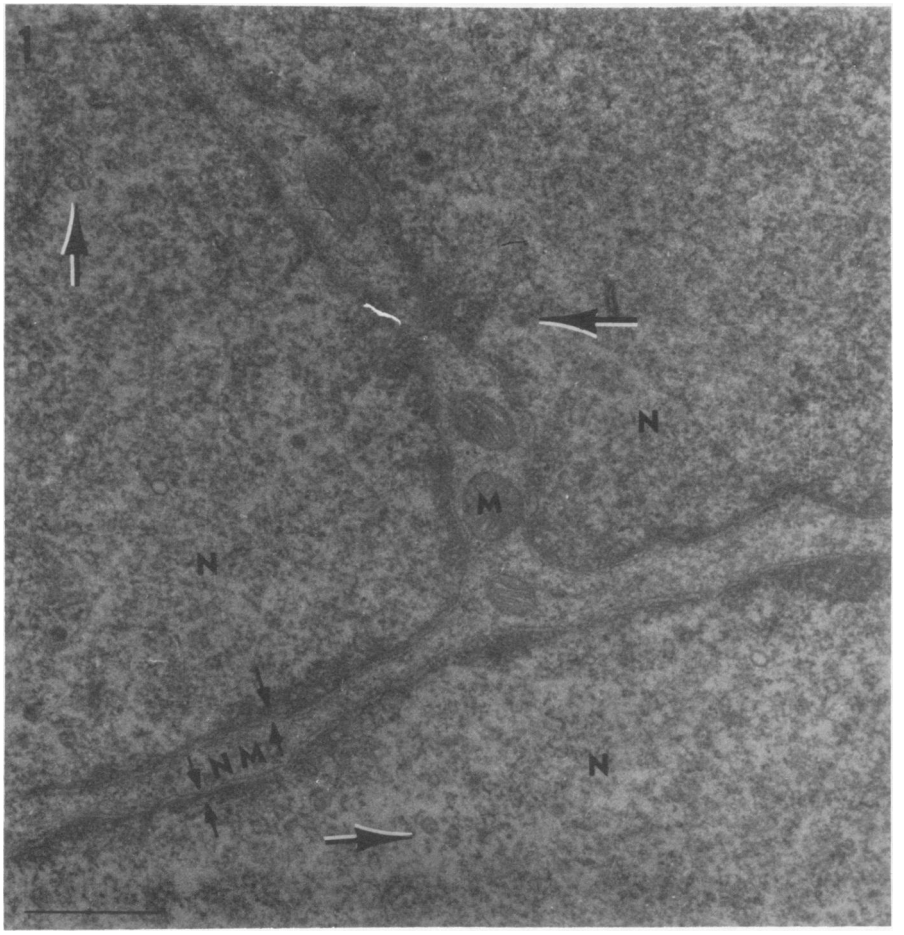
CF test. Both the HVT-infected hamster kidney and duck embryo fibroblast cell cultures showed specific CF activity to $\frac{1}{32}$ dilution. No CF was demonstrable with any of the HVT-infected hamster kidney or duck embryo fibroblast cell cultures with control negative rabbit serum. Control hamster kidney cell cultures did not show any specific CF activity.

DISCUSSION

Immunological and morphological data confirmed that hamster kidney cells could be infected with HVT with the production of characteristic cytopathic effects, induction of specific CF antigens, and the demonstration of HVT particles. While one must always be aware that viral infections of tissue culture cells may be due to latent viruses, it is highly improbable that the infection reported here was such. Uninoculated control cultures failed to show any cytopathic effects, specific CF antigens, or, for that matter, virus particles by electron microscopy.

It is most unlikely that the biological activities and the demonstration of virus particles were an expression of residual inoculated HVT-infected avian cells as the viral titer increased through six cell passages; the titer of CF antigen was comparable to that of HVT-infected duck embryo fibroblast cell cultures. In addition, virus particles were demonstrated in epithelial cells as well as in polykaryocytes formed by fusion of these cells. Nuclear inclusions were also detected in epithelial cells. Moreover, these results were duplicated using a cell-free HVT inoculum.

Polykaryocyte formation appears to be a general feature in herpesvirus-type infections (14). All the polykaryocytes observed in this study were true multinucleate cells in which the cytoplasm was not divided by membranes. We did not observe small membrane segments partially separating the nuclei. These membrane segments have been described in electron microscopy studies of virus-induced polykaryocytes (5, 9, 12). The formation of these polykaryocytes (Fig. 3) is consistent with the model for their formation proposed by Roizman (18). Roizman proposed that viral-induced polykaryocytes result from a fusion between infected and noninfected cells (17, 18). Antigenic changes of the cytoplasmic membranes are perhaps the decisive factor in polykaryocyte formation. Our observation agreed with Nazarian et al. (14) that polykaryocyte formation could occur as a result of the fusion between infected and uninfected cells, or of fusion of two infected cells, but it is not inconsistent with Roizman's suggestion because the apparent infected cells may not be infected until they integrate in the formed polykaryocytes. Moreover, it was significant that the polykaryocyte population was relatively higher in cultures



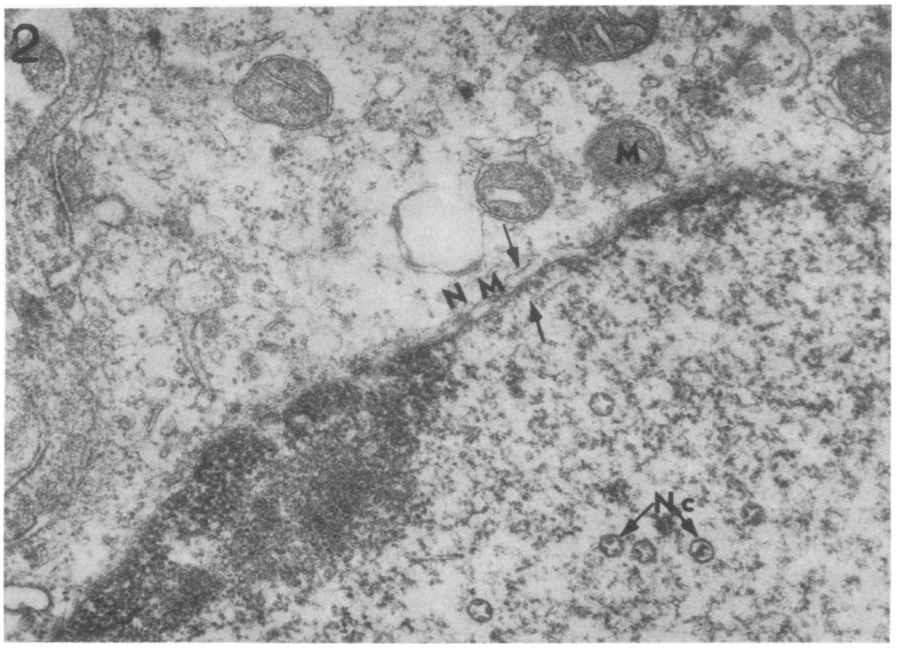
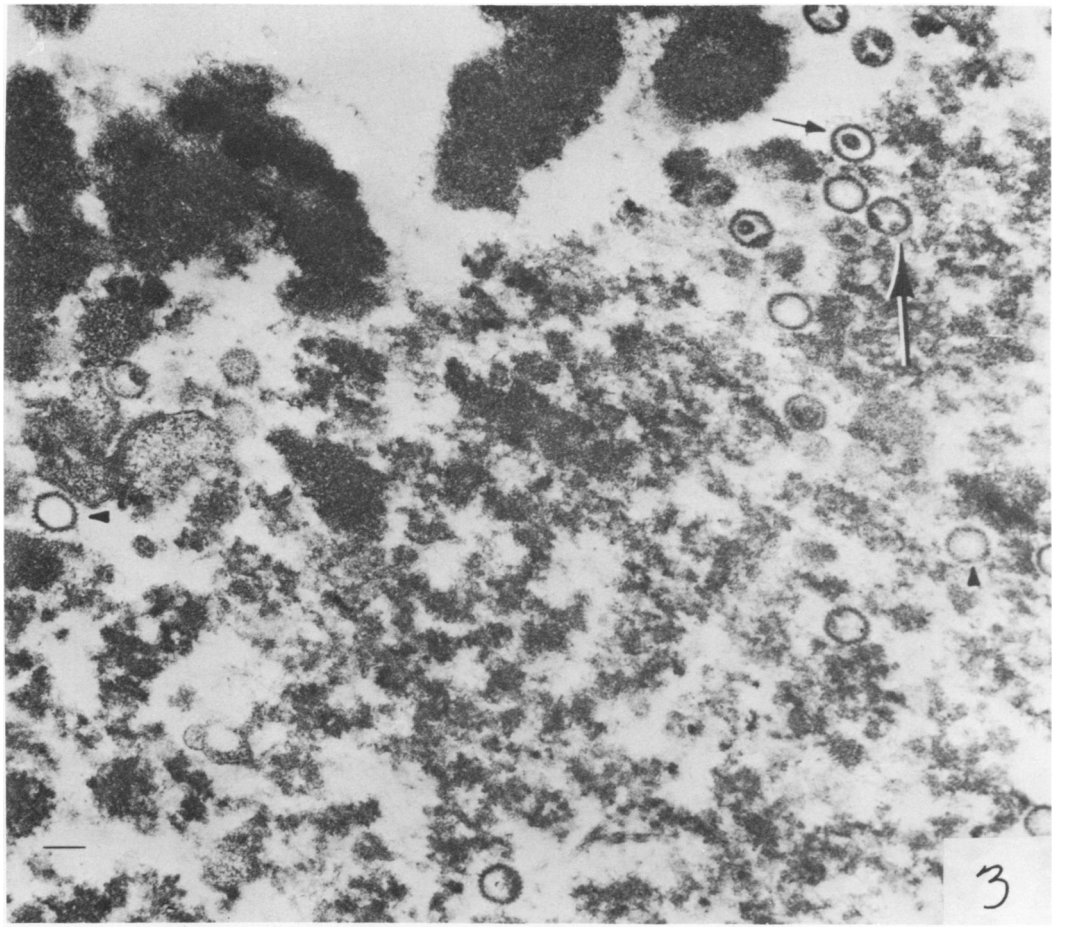
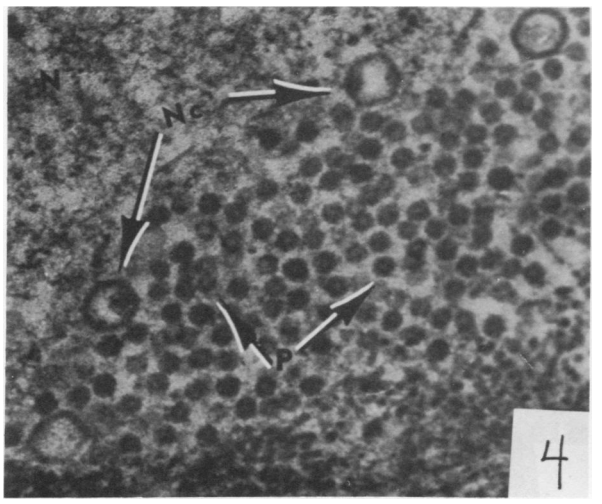


FIG. 1. *Electron micrograph of a thin section of hamster kidney cell culture infected with HVT. A portion of a polykaryocyte (three nuclei visible) showing a multinucleate cell in which the cytoplasm is not separated by membranes. Nucleocapsids (arrow), approximately 100 nm, are seen in the nuclei; NM, nuclear membrane; M, mitochondrion. Bar, 1 μm. All material for thin-sectioning and electron microscopy was fixed with glutaraldehyde-osmium and stained with uranyl acetate and lead citrate.*

FIG. 2. *Electron micrograph of thin section of HVT-infected hamster kidney cell culture. Nucleocapsids (NC) are observed. NM, Nuclear membrane; M, mitochondrion. Bar, 1 μm.*





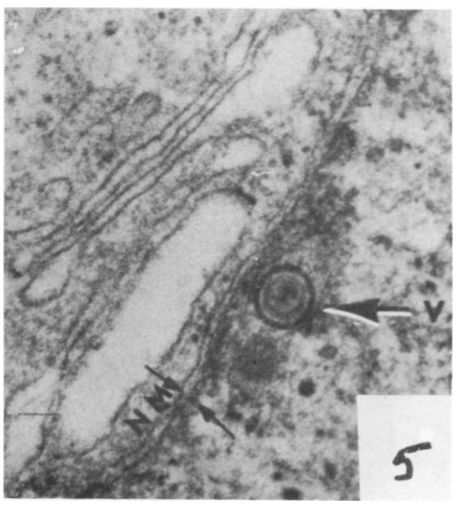
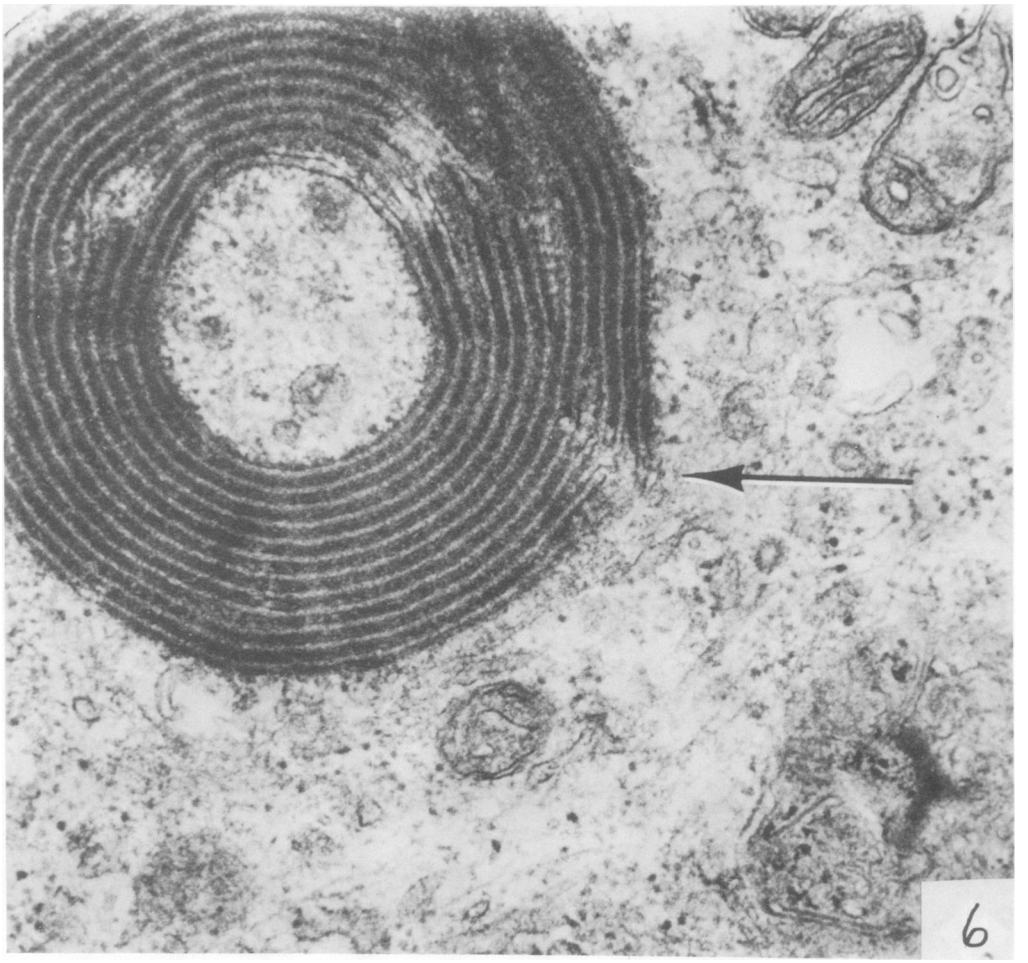
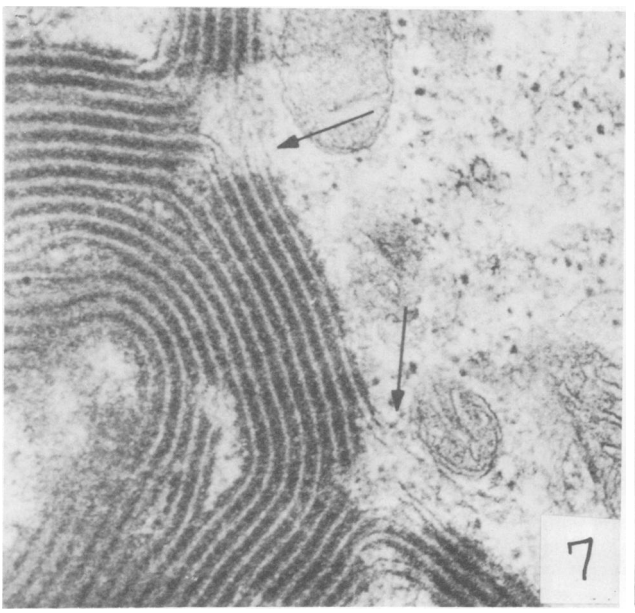


FIG. 3. *Electron micrograph of hamster kidney cell infected with HVT. Viral nucleocapsids (100 nm diameter) are observed in the nucleoplasm. Some are empty (arrow head), some contain dense central cores (small arrows), while others show the lucent cross shape (large arrow). The nucleoplasm shows extensive degenerative changes. Bar, 100 nm.*

FIG. 4. *Electron micrograph of HVT-infected hamster kidney cell culture. Particles (P) approximately 35 nm in diameter are in association with nucleocapsids (NC) in the nucleoplasm (N). Bar, 100 nm.*

FIG. 5. *Electron micrograph of HVT-infected hamster kidney cell culture. A virus particle (V) opposite the nuclear membrane (NM) was in the last stage of envelopment. Bar, 100 nm.*





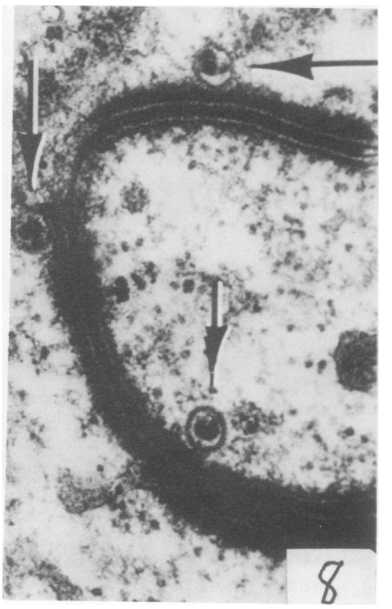


FIG. 6. *Electron micrograph of a thin section from HVT-infected hamster kidney cell culture. A large lamellar structure (arrow) is observed which apparently consists of multiple fused membranes of the endoplasmic reticulum. Bar, 100 nm.*

FIG. 7. *Electron micrograph of HVT-infected hamster kidney cell culture. The continuity of the lamellar structure with the endoplasmic reticulum (arrow) is observed. Bar, 100 nm.*

FIG. 8. *Electron micrograph of HVT-infected hamster kidney cell culture. Virus particles (arrows) are associated with the lamellar structure in proximity to the nuclear membrane (NM). Bar, 100 nm.*

inoculated with cellular material as compared to those inoculated with cell-free virus material. This observation also supports Roizman's model.

The lamellar material associated with the endoplasmic reticulum was found in several infected cells. Further study is needed to elucidate its chemistry and relationship to HVT infection. This structure does not seem to be related to ribosome crystallization as it appears in chicken embryo, cell cultures, normal chicken tissue, and chicken tissues affected with Marek's disease (21). No such structures could be detected in control hamster kidney cell cultures. In specimens where these structures were observed, HVT infection was confirmed by immunological and morphological data. Spear, Keller, and Roizman (20) indicated that the smooth endoplasmic reticulum became modified after infection of the cell with herpes simplex virus. In cells infected with OM-KI-68-69 herpesvirus of monkeys, King et al. (8) demonstrated similar structures and described them as multiple fused cytoplasmic membranes. Similar structures were demonstrated by Nii et al. (15) and were more prevalent in the nuclei as differentiated regions of the nuclear membrane. Nii et al. (15) pointed out that this fusion of the cytoplasmic membranes may be a result of changes in the antigenic determinants as a part of the infectious process.

Immunological light and electron microscope studies have made it clear that the nuclear and cytoplasmic membranes become altered after infection with herpes simplex virus (19), hypothetically as a result of binding with virus-specific products. It therefore seems reasonable to suggest that this lamellar structure arose from the endoplasmic reticulum of the cell modified by components associated with viral infection. In a recent work (P. A. Long, and L. F. Velicer, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, p. 204), soluble antigens in HVT-infected cells, similar to that of Marek's disease, were demonstrated. Analysis of these antigens by polyacrylamide gel electrophoresis showed that they were composed of five proteins. It is possible that the synthesis of these proteins and their binding to the cytoplasmic membranes contribute to fusion of the latter and the appearance of these lamellar structures.

In general, virion development of HVT in hamster kidney cells was analogous to that in avian cells, and virus maturation was similar to that in herpesvirus.

ACKNOWLEDGMENT

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