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Ultrastructural study of human herpesvirus-7 replication in tissue culture

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Abstract Human herpesvirus 7 (HHV-7) was grown in a CD4+ lymphoblastic cell line (SupT1) and in cord blood mononuclear cells (CBMC). Virus infection was demonstrated by immunohistology with positive control sera, with monoclonal antibodies and by in situ hybridization for viral DNA. Cytopathic effects following HHV-7 infection generally resemble those after HHV-6 infection but are less pronounced. The ultrastructural appearance of HHV-7 and the replicative stages were similar to those described by Kramarsky and Sander for HHV-6. There were some minor discrepancies, including quite an extensive and space-filling tegument, a slightly different structure of the nucleoid, the frequent finding of nucleocapsids without any visible core and apparently scarce or delicate spikes on the envelope. These differences may suggest HHV-7 rather than HHV-6, but this finding needs confirmation. Mature HHV-7 particles measured 170 nm in diameter, with nucleocapsids of 90–95 nm and a tegument of about 30 nm.

Key words HHV-7 · Viral replication · Virus morphology · Ultrastructure · Herpesviruses

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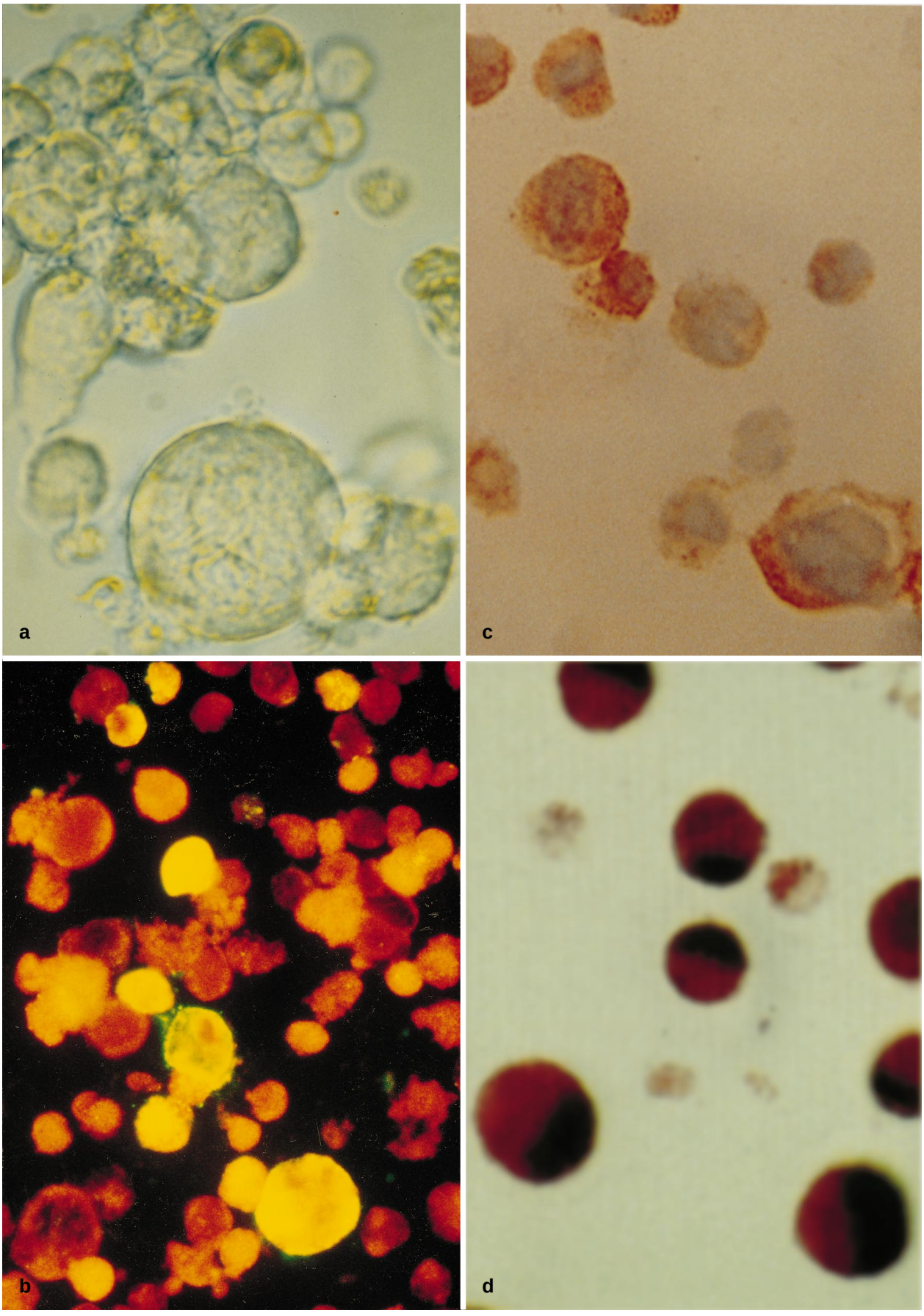
Introduction

Human herpesvirus 7 (HHV-7) was initially isolated in 1990 from CD4+ peripheral blood mononuclear cells (PBMC) of a healthy individual [16] and somewhat later from cells taken from a patient with chronic fatigue syndrome [4]. Antibodies against HHV-6 (another T-lymphotropic herpesvirus) from patients with AIDS and with lymphoproliferative disorders [13, 42, 50] show some cross-reactivity with HHV-7 and suggest antigenic similarities between the two viruses [1, 3, 52]. Although comparative DNA analyses have revealed limited homology between HHV-7 and human cytomegalovirus (HCMV) and shown that human herpesvirus 6 (HHV-6) and HHV-7 are very closely related [18, 32], HHV-7 is distinct from all previously characterized human herpesviruses [3, 52]. Monoclonal antibodies specific for HHV-7 have been described [1, 6, 14]. Like HHV-6, HHV-7 infects T-lymphocytes [3], but a significant and productive infection also occurs in cord blood mononuclear cells (CBMC). CD4, a membrane glycoprotein, was identified as a cellular receptor for HHV-7 [17, 31, 56]. HHV-6 and HHV-7, both β-herpesviruses, have been classified as members of the genus *Roseolovirus* [1, 3].

HHV-7 is frequently isolated from human saliva [11, 17, 31, 56], and seroepidemiological studies suggest widespread distribution of the virus [29]. Seroconversion usually occurs in early childhood, slightly later than for HHV-6 and earlier than in EBV infection [8, 52, 58]. Seropositivity for anti-HHV-7 in adults ranges from 75% to 95%, with some variation in different countries [8, 29, 41, 52–54, 58].

Although no specific diseases are currently known to be caused by HHV-7, virus isolation has been reported from patients with disorders mimicking chronic Epstein–Barr virus infection [22], upper respiratory tract infection, chronic fatigue syndrome (CFS), [4, 12, 43], and exanthem subitum [2, 49, 51, 55], and even from patients with neurological complications [51] and with nonspecific febrile syndromes [37]. HHV-7 DNA has also been detected by PCR in Kaposi sarcoma and normal skin tissue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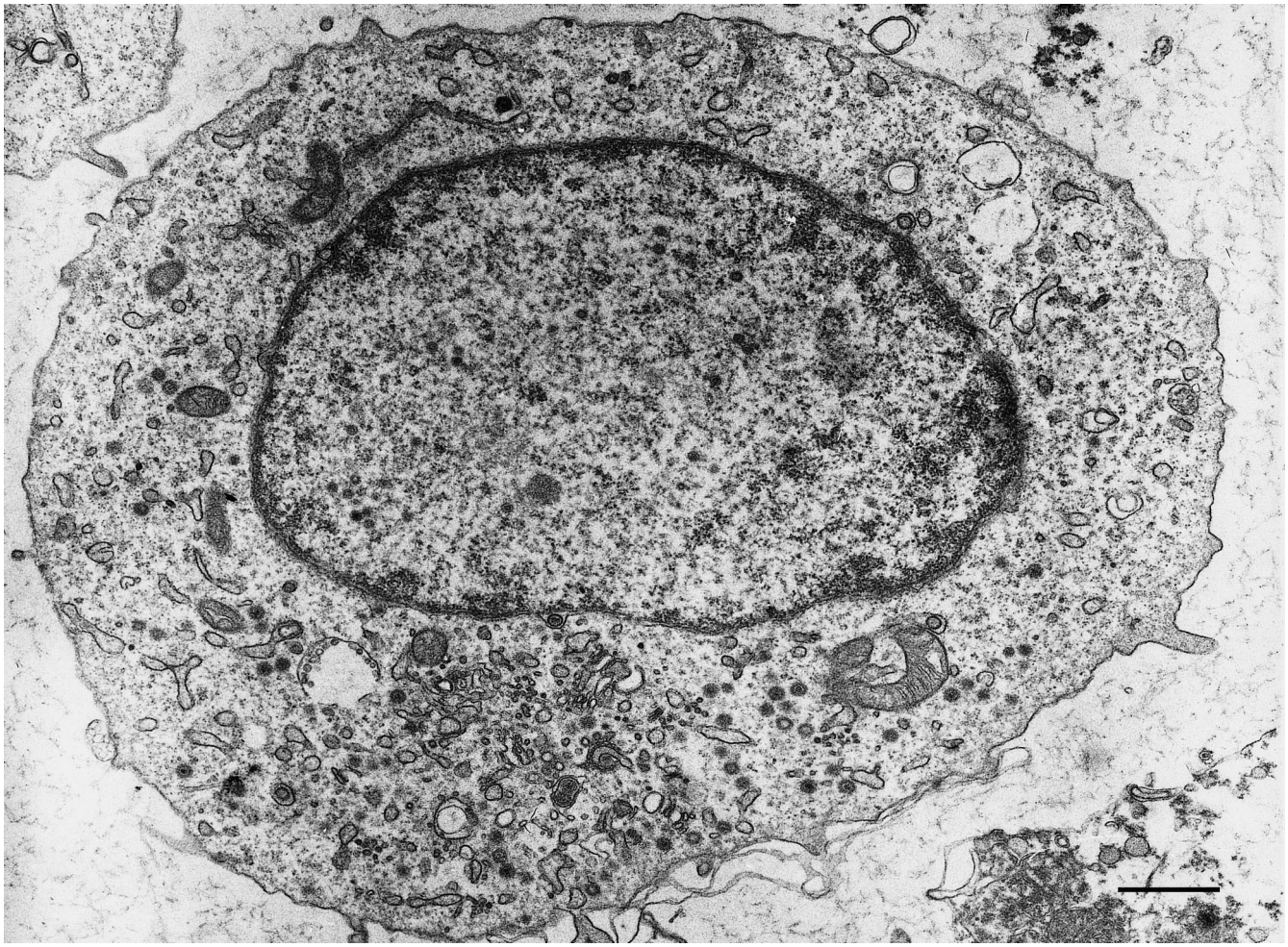

Fig. 2 HHV-7 infected cell showing nucleocapsids in the nucleus and tegument-coated virions free in the cytoplasm. Note the abundance of cytoplasmatic vesicles, while enlargement of the cell is not so pronounced. ×13,000, *calibration bar* 1 μm

of AIDS patients [23]. It should be noted that HHV-7 can apparently reactivate latent HHV-6 infections [15]. Moreover, HHV-7 may interfere with human immunodeficiency virus 1 (HIV-1), as both use the CD4 receptor [17, 31].

There are detailed descriptions of the ultrastructure of herpesviruses 1–6 [5, 7, 9, 10, 21, 26, 33–36, 38, 44, 45, 47, 48, 57] but few data on HHV-7 [1, 16, 43].

The objective of the present study was to describe the ultrastructural characteristics and replicative stages of HHV-7.

Materials and methods

HHV-7 (isolate JI) [4] was grown in PHA-stimulated SupT1 cells (DSM ACC 140, FRG) or in cord blood mononuclear cells (CBMC) in RPMI 1640 medium supplemented by 10% fetal calf serum and 10 units/ml interleukin 2 (Boehringer Mannheim, Germany). At 5–10 days after this, infection was accomplished by growing these cells in tissue culture supernatant containing an approximate infectious dose of 10^5 FFU/ml JI isolate as originally produced and described by Berneman et al. [4]. None of the cell lines contained other viruses before HHV-7 infection, as indicated by the absence of cytopathic effects and negative results of virus isolation attempts from their supernatants. SupT1 cells showed characteristic cytopathic giant cell formation upon HHV-7 infection. Infection was also demonstrated by indirect immunofluorescence assays using anti-HHV-7 positive patient sera reacting with HHV-7 infected cells but not with cells infected by HHV-6, CMV, EBV, or HSV; immunohistology (APAAP) using monoclonal antibodies against HHV-7 gp 110/56 viral envelope proteins (H-AR-7, kindly provided by J. Luka, Eastern Virginia Medical College, USA); and in situ hybridization with the pVL17A.1 probe of Berneman and the polymerase chain amplification reaction according to Klotman and his group [3, 4, 24, 25, 46] (Fig. 1) which was also effective. At lower concentrations, the H-AR-7 antibody cross-reacts with HHV-6, but cells were shown to be free of HHV-6 before and after infection with HHV-7 [27]. HHV-7 probes and primers are specific for HHV-7.

Cells were harvested at regular intervals after infection. Cell pellets were fixed for 90 min in 0.1 cacodylate buffer (pH 7.2) containing 3% glutaraldehyde and postfixed in 1% osmium tetroxide for 4 h. After staining with uranyl acetate, dehydration and propylene oxide treatment, samples were embedded in epoxy resin, and 50-nm sections were then cut with a Reichert-Jung ultramicrotome and stained with uranyl acetate and lead citrate. They were evaluated in a Philips 400 microscope operated at 60 kV.

◀ **Fig. 1** Demonstration of human herpesvirus 7 (HHV-7) infection in SupT1 cells: **a** cytopathic giant cell formation; **b** indirect immunofluorescence assay (IFA) with HHV-7 antibody positive patient's sera; **c** expression of HHV-7 gp 60/110 in infected cells; **d** in situ hybridization for HHV-7 DNA

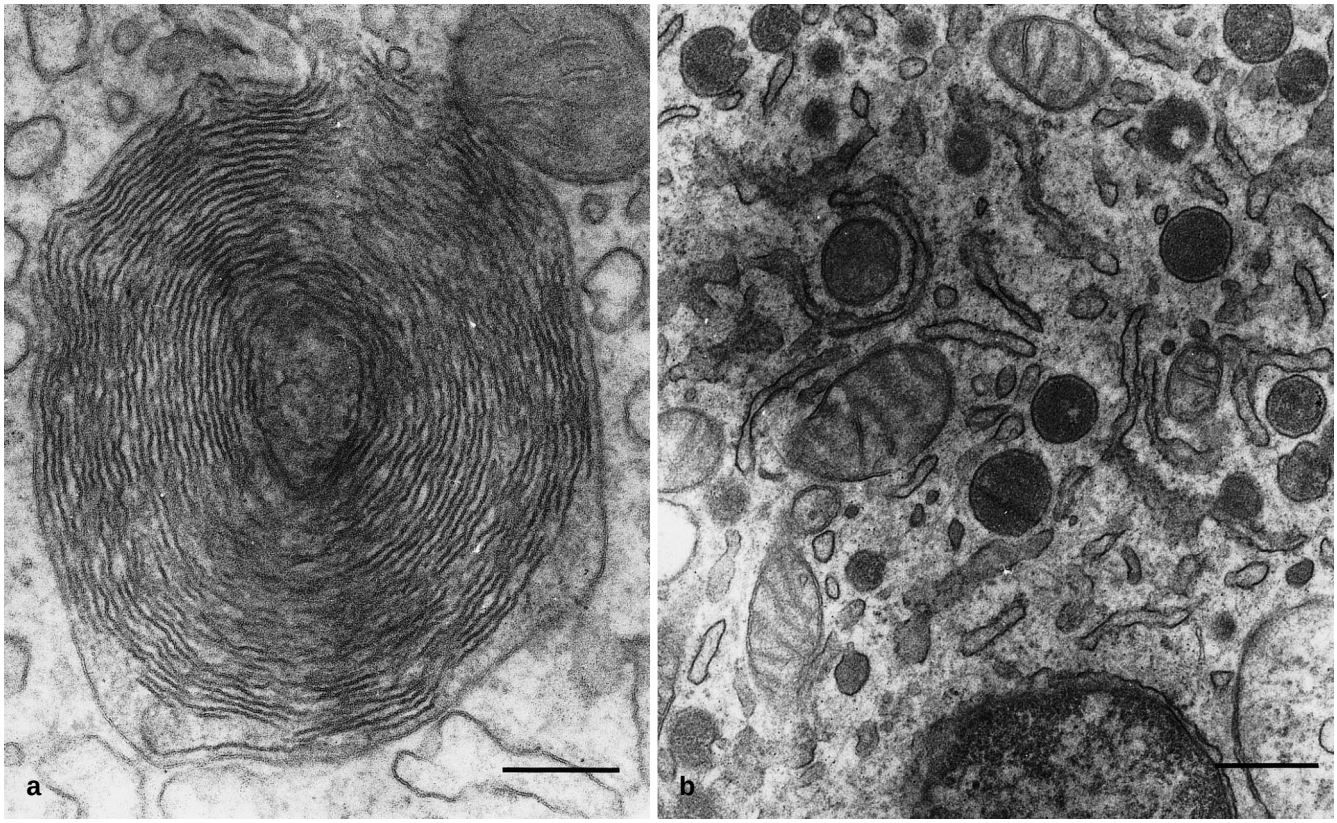

Fig. 3 **a** Proliferated and reduplicated cytoplasmatic membranes forming an concentric lamella structure. ×74,000, *calibration bar* 200 nm. **b** Cytoplasmatic electron-dense bodies. ×27,000, *calibration bar* 500 nm

Results

The characteristic features of HHV-7 infection in cell culture were demonstrated by: cytopathic giant cell formation; indirect immunofluorescence assay with HHV-7 antibody-positive patient sera, expression of HHV-7 gp 60/110 in infected cells, and in situ hybridization for HHV-7 DNA (Fig. 1).

Ultrastructural changes attributable to HHV-7 infection of CBMC or SupT1 cells can be classified as cytopathic and virological:

Cytopathic changes

The initial appearance of nonlytic HHV-7-infected cells was that of enlarged polymorphic blasts with lobulated nuclei (cytopathic giant cells; Fig. 2). In comparison with HHV-6-infected cells, the effects of infection were less pronounced [5, 7, 26]. The nuclei of HHV-7-infected cells exhibited some chromatin clumping and margination, which is typical for a herpesvirus [10]. The cytoplasm displayed an increased number of organelles, mainly smooth vesicles. Occasionally proliferation and reduplication of nuclear and cytoplasmatic membranes resulted in formation of concentric lamellar structures (Fig. 3a). This is also known for other herpesviruses [10, 35]. Cytoplasmatic dense bodies (300–400 nm in diameter; Fig. 3b) such as are seen in other herpesvirus infections were also observed [10, 21, 44]. These bodies are confined by a limiting membrane and completely or partially filled with electron-dense material, presumably products of excess synthesis of virus structural proteins. In later phases of infection, the nucleus became more and more electron dense and fragmented, a phenomenon described as apoptosis [24, 28]. Finally, there was cell lysis, but this degenerative process appeared more slowly and also less frequently than in HHV-6-infected cell cultures: infected cells can persist in culture for over 40 days [3].

Virological changes

The first recognizable step in virus assembly was the appearance of intranuclear compound granules. They were about 40 nm in diameter and surrounded by a clear “halo” separating them from indigenous nuclear chromatin (Fig. 4a). These particles, described elsewhere as “viral core-like subunits” [10, 34, 47] were found in both CBMC and SupT1 cells infected with HHV-7. At about 3 days after infection these structures were readily detectable. In SupT1 cells all subsequent steps of virus replication were much less apparent. Only aberrant forms were noted, although the cultures showed a characteristic cytopathic effect (CPE). In CBMC nucleocapsids (approxi-

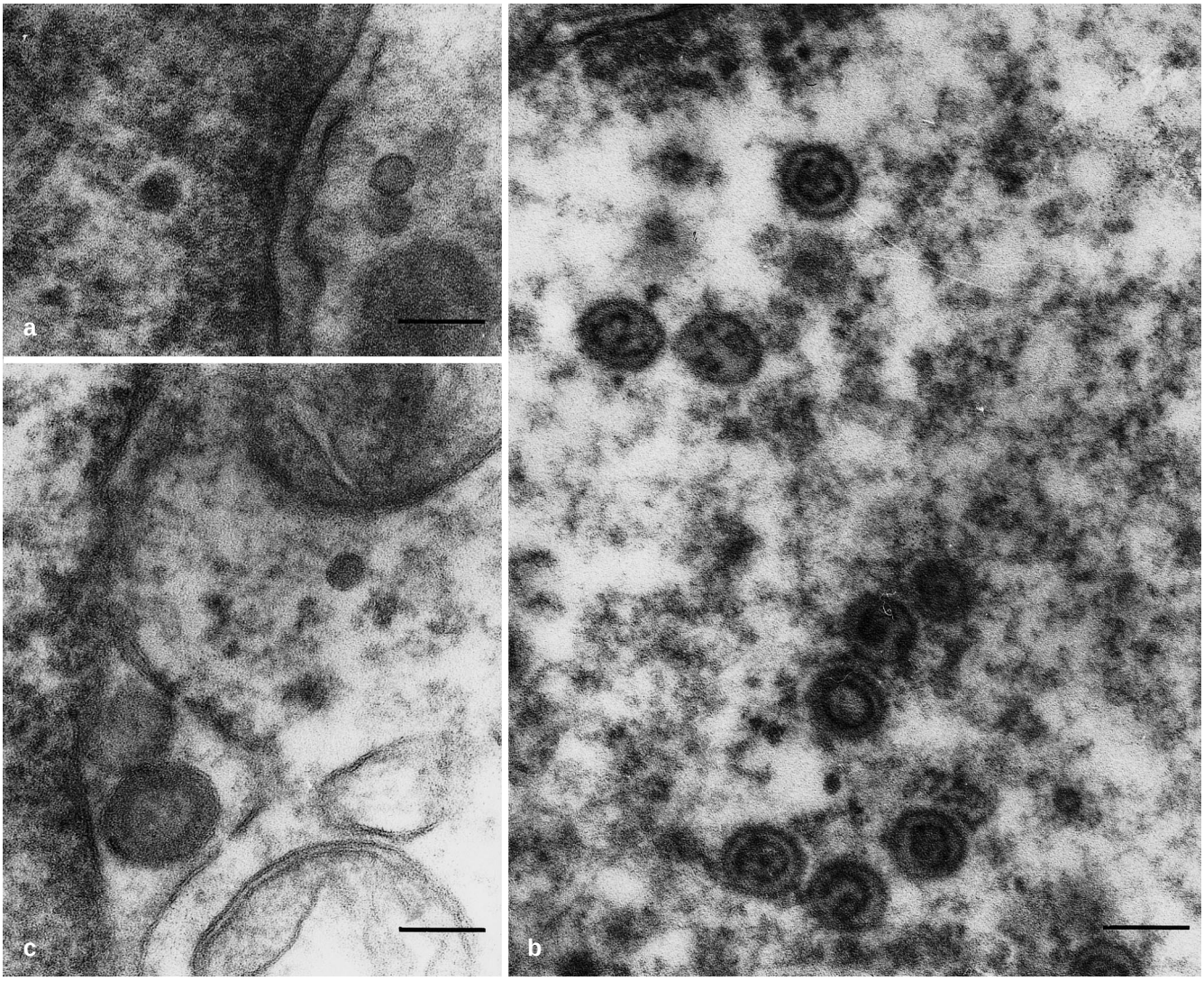

Fig. 4 **a** Intranuclear granules separated from chromatin by a "halo". ×120,000, *calibration bar* 100 nm. **b** Capsids in the nucleus with cores of different shapes. ×120,000, *calibration bar* 100 nm. **c** Enveloped virion in the perinuclear space with a thin tegument. ×120,000, *calibration bar* 100 nm

mately 90–95 nm in diameter) started to accumulate in the nucleus by day 4 after infection. Cores of nucleocapsids in the nucleus resembled the low density structures described for other herpesviruses [7, 35, 36, 47, 48, 57], and electron dense cores [34] were not detectable. Most cores were approximately ring-shaped, sometimes with irregular and zigzag appearances in detail; some had granules in the centre. Some cores had a beaded shape similar to those of HCMV [35, 47] (Fig. 4b). Capsids had a diameter of approximately 90–95 nm and capsids without a visible core [48] were not rare.

Few enveloped virions were located perinuclearly (Fig. 4c). These had already acquired a tegument (an electron-dense layer between capsid and envelope [40]). This tegument was obviously more frail than that of mature virions, supporting the observation that the acquisition of teguments may take place on the way from nucleus to cytoplasm [40]. This initial tegument supposedly originates from a patch of the inner nuclear membrane where the nucleocapsid buds [38]. Cytoplasmatic invaginations are easily found in nuclei of cells infected with HHV-6 (unpublished observations) [34] or other herpesviruses [10, 21, 33, 38, 45, 48]. This is not a common phenomenon in HHV-7 infection. In HHV-6 infection, cytoplasmic nuclear invaginations were described as putative site for tegument acquisition [39]. Unlike other herpesvirus infections [33, 44, 45] HHV-7 infection scarcely widened the perinuclear space.

The next step of HHV-7 development was the cytoplasmatic accumulation of unenveloped and tegument-coated virions (Fig. 5a). The tegument now regularly had the same width as mature enveloped virions in the extracellular space, namely about 30 nm. Usually, the core of these cytoplasmatic unenveloped virions was electron dense. However, quite a number of empty tegumental structures were seen (Fig. 5b). Unenveloped virions budded into smooth vesicular structures, probably

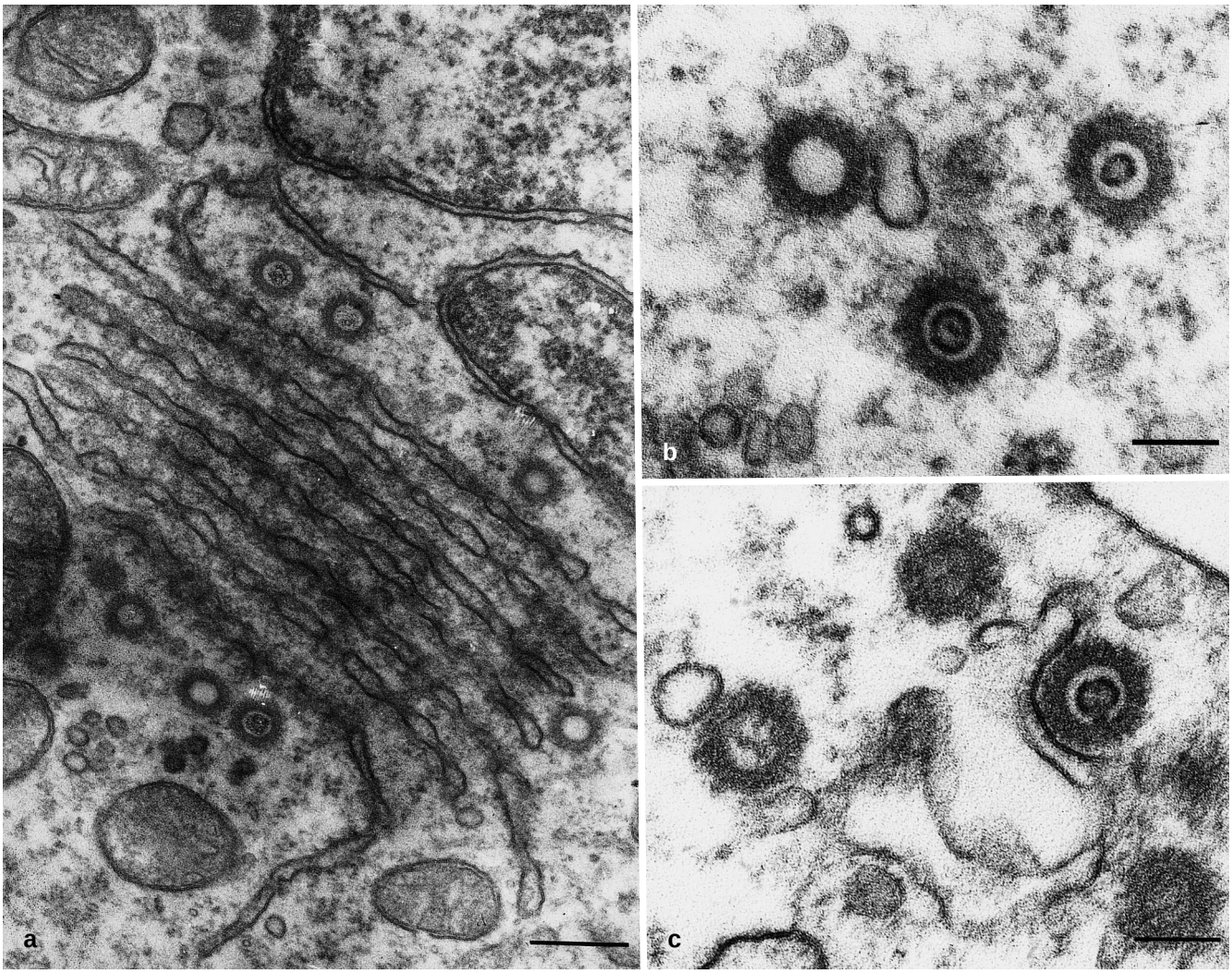

Fig. 5a Tegument-coated virions in cytoplasm near the endoplasmic reticulum. ×56,000, *calibration bar* 250 nm. **b** A tegument-coated virion with no visible core coming into contact with a cytoplasmatic vesicle. ×120,000, *calibration bar* 100 nm. **c** A tegument-coated virion budding in a cytoplasmatic vesicle. ×120,000, *calibration bar* 100 nm

of Golgi origin [20, 44] (Fig. 5c). There they became enveloped by cytoplasmatic membranes, as described for other herpesviruses [5, 26, 33, 45, 48]. Occasionally, we found two unenveloped tegument-coated virions budding into the same vesicle, forming a joint envelope for both virions (Fig. 6a). This appeared, however, to be a rather unusual phenomenon, and it was more common for only one virus particle to reside in one smooth vesicle (Fig. 6b, c). In the extracellular space many virions were arranged in heaps, while others were still attached to cellular membranes (Fig. 7a). Mature virions exhibited a distinct electron-dense core and a prominent fuzzy tegument occupying the entire space between nucleocapsid and envelope. Again, some particles consisted of tegument and envelope without a core (Fig. 7b). Complete and defective particles were approximately 170 nm in diameter, showing only delicate spikes on their envelopes.

Discussion

In semi-thin sections or conventional light microscopy, HHV-7-infected lymphoblasts showed less prominent cellular changes (CPE) than cells infected with HHV-6. Giant cells were smaller and less frequent. Nuclei of HHV-7-infected cells were lobulated, but undivided and cytoplasmatic organelles were not increased in the same way as in HHV-6 infection [26].

In contrast to HHV-6 infection of HSB2 cells, SupT1 cells contained very few mature virus particles intra- and extracellularly, although the CPE was obvious and viral DNA and antigen were found in the cells by both in situ hybridization and immunohistology. This may suggest certain defects in viral replication in these cells.

HHV-6 and HHV-7 differ in morphology and replicative activity [24]. Although stages of HHV-7 assembly in infected CBMC are comparable to those following HHV-6 infection, there are certain morphological differences.

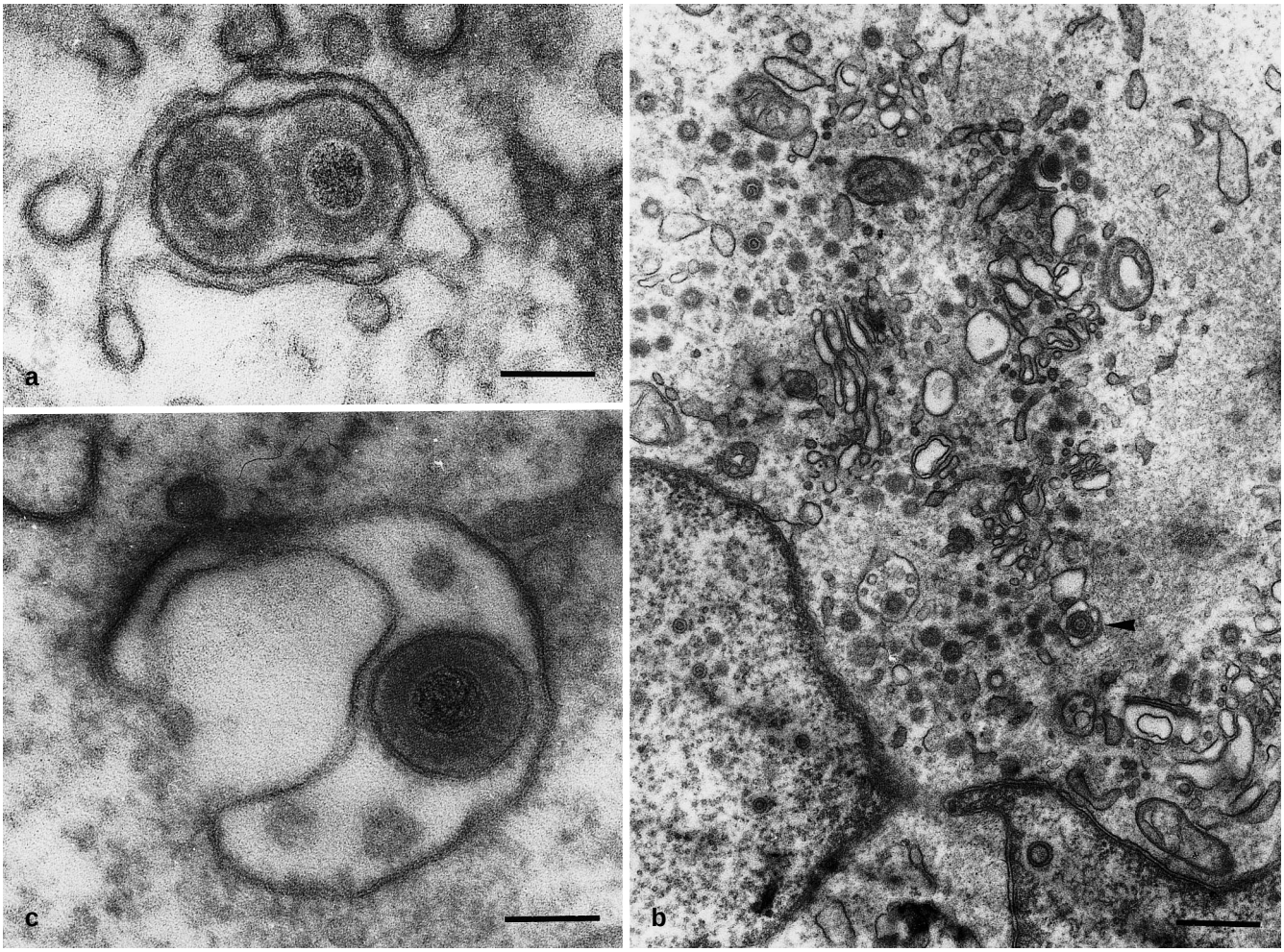

Fig. 6a Two tegument-coated virions have budded into one vesicle with a joint envelope. ×120,000, *calibration bar* 100 nm. **b** An overview: capsids in the nucleus, enveloped and unenveloped virions in cytoplasm. One virion appears to have just finished budding (*arrow*). ×21,000, *calibration bar* 500 nm). **c** Mature virion in a cytoplasmatic vesicle. ×120,000, *calibration bar* 100 nm

HHV-7 cores were more regular, mostly ring-shaped and in some cases with granules in the centre, as also reported for cores of VZV and HSV [9, 34, 35]. Only few HHV-7 particles had irregular or bead-shaped cores as described for HCMV [35, 47] Empty capsids were a frequent finding, which may reflect a discrepancy between the production of viral DNA and structural proteins. Because the morphological appearance of the cores varied and depended on the kind of fixatives [19] and dehydration agents used [35], we compared these observations with HHV-6 infected cells prepared in the same way.

The widening of the perinuclear space seen (without formation of prominent perinuclear cisternae) appears to be distinct from the cellular changes following infection with other herpesviruses. The rarity of virions in the perinuclear space may reflect either a rapid passage of virions from nucleus to cytoplasm or low-grade viral replication. The mechanism of viral passage through nuclear membranes is thought to include sequential envelopment and de-envelopment of immature virions, as reported for other herpesviruses [20, 26, 36, 45, 48]. It appears likely that virions acquire their teguments during this process, because all virions found in the cytoplasm were coated with mature teguments. The broad fuzzy tegument filling the entire space between envelope and nucleocapsid appeared to be more pronounced in HHV-7 than in HHV-6. Subsequent budding of tegument-coated virions into smooth vesicles is probably supported by vesicular reduplication, increasing the chance of tegumental attachment to the vesicular membranes. This would explain the perfect envelopment of coreless tegument-coated nucleocapsids (empty particles) and the occasional occurrence of two nucleocapsids in one envelope. Suggestively, vesicles serve as carriers for virions to the cell membrane, where (after fusion of vesicular and cytoplasmic membranes) mature virus particles are released [33, 44, 45].

Mature HHV-7 particles closely resemble HHV-6 virions [5, 7, 26], but they have a more uniformly structured core occupying the entire capsid and showing a more prominent fuzzy tegument. Figure 7 c and d gives an impression of mature HHV-6 and HHV-7 virions at the same magnification and treated with the same prepara-

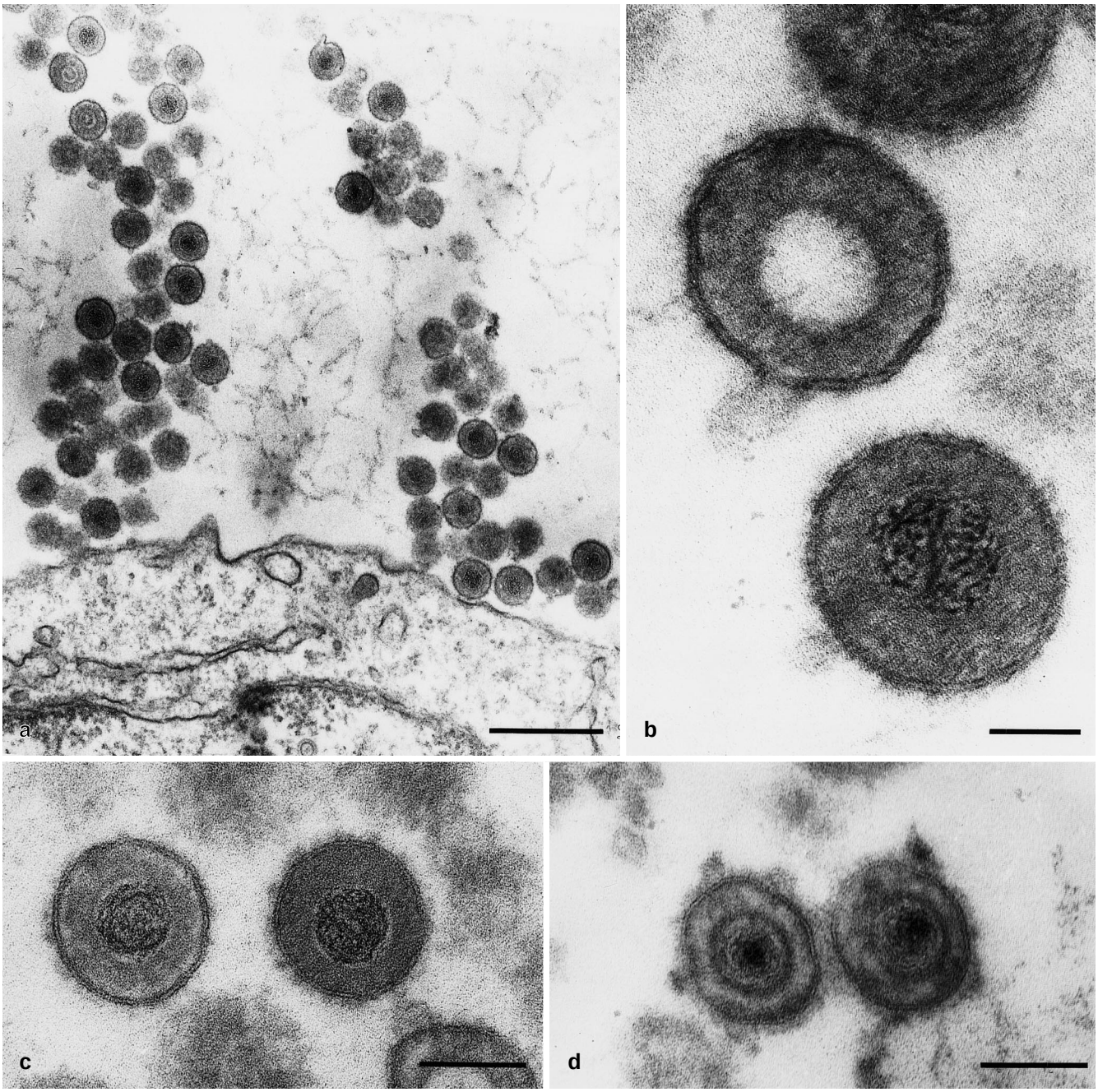

Fig. 7a Extracellular mature virions, some still attached to the cellular membrane. ×34,000, *calibration bar* 500 nm. **b** Details of mature virions: electron-dense core and no core visible, dense and bright tegument, spikes on the surface. ×270,000, *calibration bar* indicates 50 nm. **c** Mature HHV-7 and **d** HHV-6 at the same magnification. ×160,000, *calibration bar* 100 nm; note the uniformly structured core and the more prominent tegument of HHV-7

tion for electron microscopy. And although certain structural differences between HHV-7 and other herpesviruses can be seen, electron microscopy reveals structural similarities between HHV-6 and HHV-7, rather than lending itself for use as a tool for viral distinction.

So far, little is known about HHV-7, and future investigations will have to elucidate whether it is a dangerous organism [23, 30] or will be useful in future gene therapy concepts [31].

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