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Herpes oesophagitis

II. Electron microscopical findings

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Summary. Ultra-thin sections obtained from routine biopsy specimens and cytological smears of 3 cases, together with one autopsy case suggestive of herpes oesophagitis, clearly demonstrate herpes viruses. The infected epithelial cells reveal different stages of virus replication and propagation. Cowdry A type inclusion bodies, however, representing early alterations in the course of infection are less frequent. Ground-glass looking nuclei of light microscopical balloon cells and infected multinuclear giant cells of epithelial origin are characteristic changes of the late ulcerative stage of herpes oesophagitis usually seen at the time of detection. These typical virus induced cell changes are mostly to be found at the ulcers edge.

Key words: Oesophagitis – Herpetic cell change – Ultrastructure

Introduction

The ultrastructural cell changes due to herpes virus infection have been demonstrated in a variety of herpetic lesions in man after death (Swanson et al. 1966; Patrizi et al. 1968; Nash and Foley 1970; Viloria and Garcia 1976; Schäfer et al. 1981). However, to our knowledge, only a single case report deals with the post mortem electron microscopic findings of concomitant herpes-monilial oesophagitis (Mirra et al. 1982). In the present study the ultrastructural findings of herpes oesophagitis are reported based on biopsy specimens and routine cytological smears of 3 cases as well as one autopsy case.

Material and methods

The material consist of two forceps biopsies of oesophageal tissue featuring typical viral alterations of the epithelium and one post mortem case with an oesophageus showing characteristic herpetic punched-out ulcerations. For electron microscopy, small tissue blocks of

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formalin-fixed or deparaffinized material were thoroughly washed in cacodylate buffer before postfixation in buffered osmium tetroxide (McDowell 1978), processed routinely and embedded in Epon 812. Ultra-thin sections were contrasted with uranyl acetate and lead citrate.

In the third ante mortem case herpes oesophagitis was diagnosed by endoscopy and brush cytology, which could not be confirmed by histology, because no epithelial cells were present in the specimen. Therefore, cells showing cytological changes suggestive of virus infection by light microscopy were removed from stained smears for electron microscopy according to the technique of Smith and Coleman (1983)

Clinical data and immunhistochemical findings of the present ante mortem cases are given in part I of our study (Feiden et al., 1984; case No. 1, 2 and 4).

Results

The semi-thin sections of the oesophageal lesions demonstrate sqamous epithelial cells with ballooning degeneration and a dense inflammatory infiltrate consisting mainly of neutrophils (Fig. 1). Most of the balloon cells have enlarged ground-glass nuclei. Occasionally, the nuclei contain a fine granular material suggestive of viral inclusions (Fig. 1). Multinuclear giant cells are frequently encountered. Their nuclei appear either ground-glass like or granular (Fig. 1).

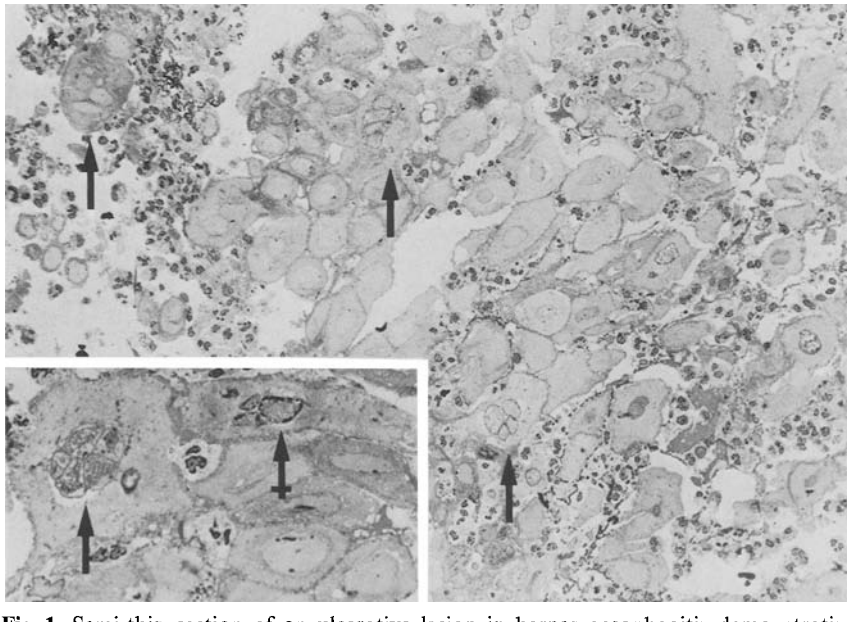

Fig. 1. Semi-thin section of an ulcerative lesion in herpes oesophagitis demonstrating many balloon cells with ground-glass nuclei and several multinucleated giant cells (*arrows*). Methylenblue-azur II, ×300. *Inset*: Multinucleated giant cells with intranuclear granular material suggestive of virus inclusions (*arrow*) or marginated chromatin (*crossed arrow*). Methylenblue-azur II, ×630

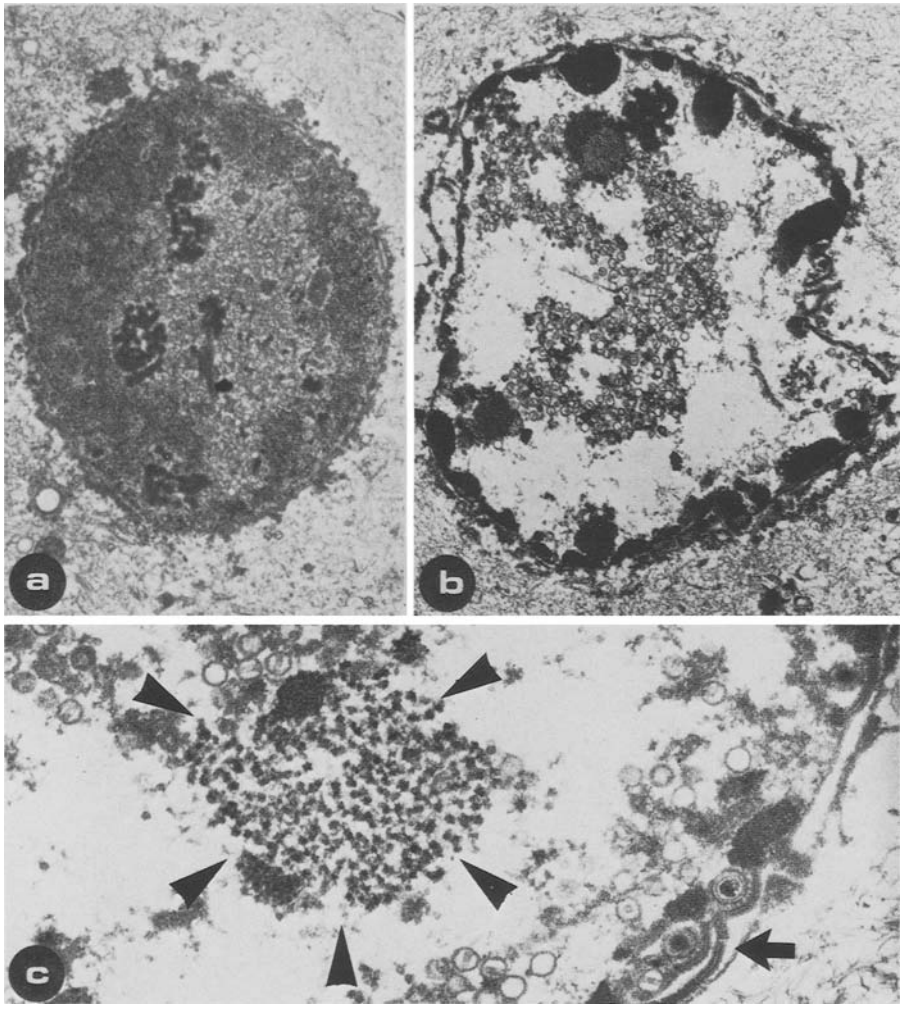

Fig. 2. Ultrastructure of intranuclear viral inclusions in herpes oesophagitis: **a.** Intranuclear crystalline formation of virus particles enclosed by coarsened, sometimes markedly clumped chromatin. ×12000. **b.** Intranuclear viral inclusions surrounded by a clear halo representing the ultrastructural equivalent of a Cowdry A type inclusion body. The chromatin is beaded at the nuclear membrane. ×12000. **c.** Intranuclear so-called vermicellar body (*arrowheads*); virus particles adjacent to the nuclear membrane and envelopment of virions by the nuclear membrane (*arrow*). ×36000

Electron microscopy

The nuclei of many epithelial cells contain virus particles consisting of a central electron-dense core and a capsid of similar electron density measuring about 95 nm in mean diameter. The central core appears as either solid or hollow or bar-shaped and is separated from the capsid by an electron-lucent

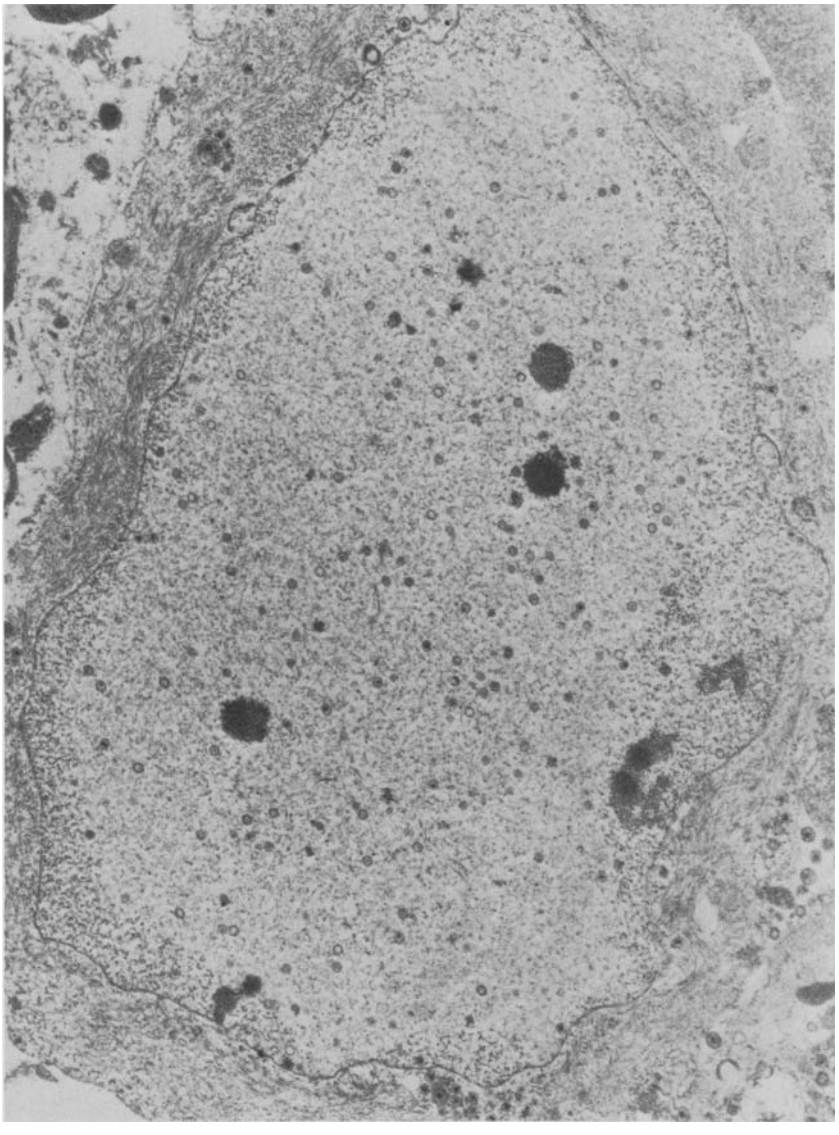

Fig. 3. Ultrastructure of a ground-glass nucleus of a balloon cell in herpes oesophagitis. The nucleus has a barren appearance with largely disintegrated chromatin. Virus particles are scattered throughout the nucleus and cytoplasm. × 13 500

halo. The cores have an average diameter of 38 nm. However, intranuclear particles often appear as empty capsids.

Some of the nuclei of the affected epithelial cells are filled with densely packed aggregates of virus particles enclosed by coarsened clumped chromatin (Fig. 2a). These viral aggregates are composed of empty and cored particles partly associated with small filaments (Fig. 5b) and vary in size and shape.

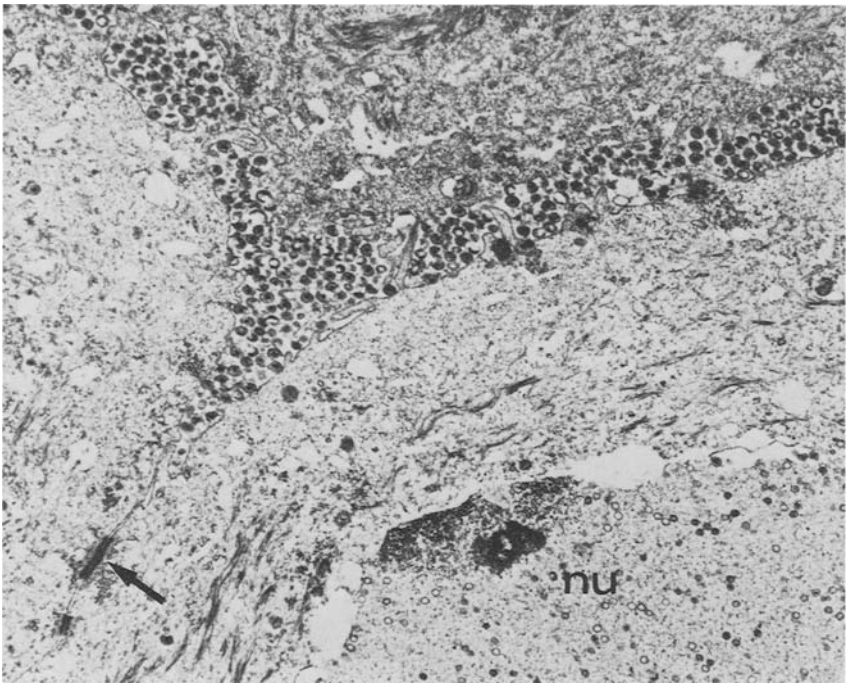

Fig. 4. Advanced stage of virus propagation in herpes oesophagitis. Viruses are accumulated in the intercellular space of a preserved epithelial sheet (*nu*: nucleus; *arrow*: desmosome). × 13000

Nuclear inclusions usually regarded as Cowdry A type inclusion bodies consist of paracrystalline viral arrays surrounded by a halo, while the coarse chromatin is beaded at the nuclear membrane (Fig. 2b). In addition, so-called vermicellar bodies (Ghadially 1982) are present in some nuclei (Fig. 2c).

Alignment of capsids and virions along the inner nuclear membrane and envelopment of virions by the nuclear membrane is frequently observed (Fig. 2c). The cytoplasm features only a few enveloped viruses measuring 130 nm in their mean diameter. Sometimes intracytoplasmic desmosomes occur as a result of acantholysis.

The ultrastructural equivalent of the ground-glass looking nuclei are swollen nuclei with dissolved chromatin, which is slightly condensed at the nuclear membrane (Fig. 3). Virus particles are scattered throughout the karyo- and cytoplasm of these cells.

Dying cells containing only a few virus particles within their desintegrated cytoplasm and lytic nuclei are occasionally found. They correspond to the "shadow cells" in light microscopy.

Accumulation of enveloped viruses is noticed in the intercellular spaces of preserved sheets of infected cells having the characteristic swollen nuclei as described above (Fig. 4). Furthermore, virus particles can be found, sometimes within the cytoplasm of largely degranulated neutrophils or ad-

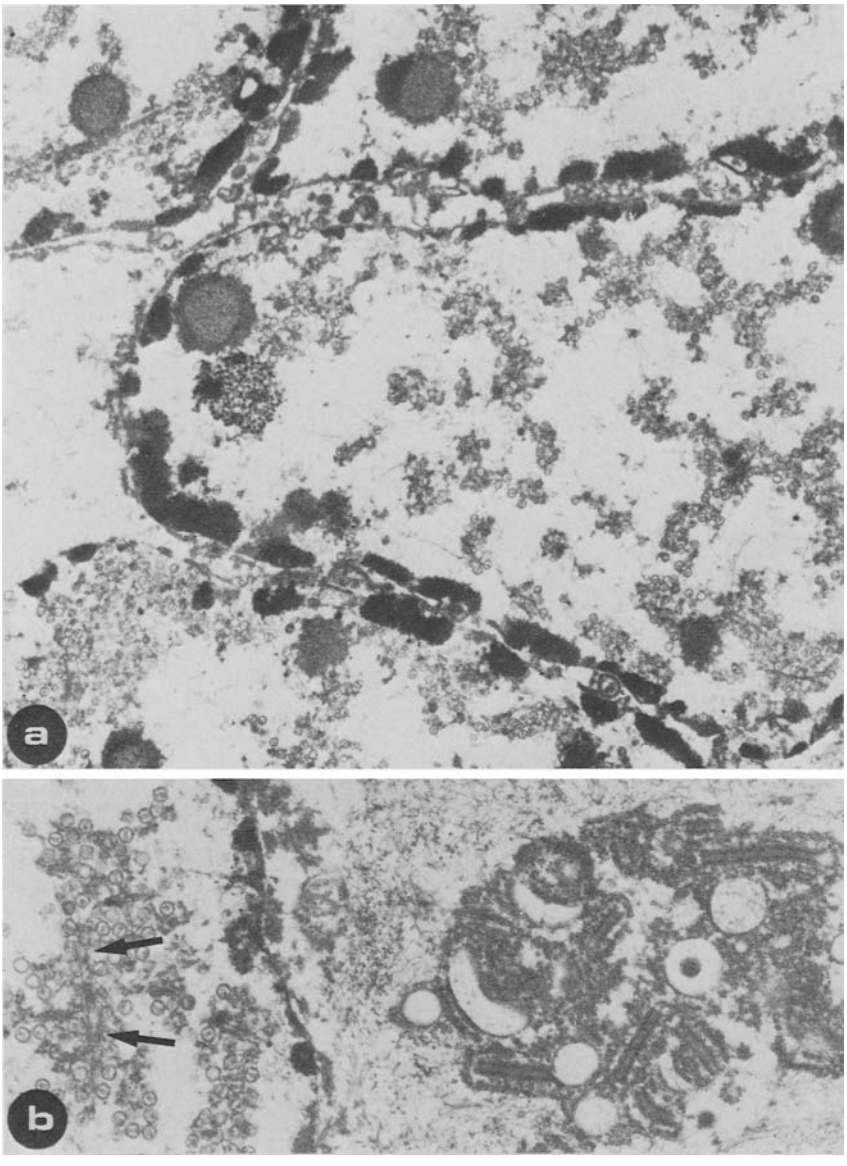

Fig. 5a. Multinucleated giant cell showing intranuclear viral inclusions. ×12500. **b.** Virus particles within one nucleus associated with small filaments (*arrows*). Cluster of intracytoplasmic desmosomes (*right*). ×20500

mixed with fibrin. The latter observation was the only hint of virus induced oesophagitis obtainable from the specimen lacking epithelial cells, while ultra-thin sections prepared from smears clearly demonstrate infected epithelial cells.

The multinuclear giant cells are also infected (Fig. 5a). Their nuclei show

both a swollen appearance with dissolved chromatin or marginated chromatin, and virus "crystals". A prominent finding is the presence of many tonofilaments and clusters of intracytoplasmic desmosomes within these polykaryocytes (Fig. 5b).

Discussion

Electron microscopy may be useful in confirming the diagnosis of virus induced oesophagitis. For this purpose many kinds of fixation are appropiate and paraffin-embedded material as well as cytological smears permit the identification of virus particles (Morecki and Becker 1968; Hübner 1981; Smith and Coleman 1983). In the present cases the structure of these virus particles is indicative of the herpes group. Further classification as herpes simplex has been done by immunhistochemical methods as shown in part I of our study (Feiden et al. 1984).

Sequential ultrastructural examination of herpes simplex infected cells in vitro has revealed that many different stages of virus replication and propagation are to be found in different cells until late in the course of infection (Nii et al. 1968). Margination, coarsening and clumping of nuclear chromatin are prominent findings of early stages of virus assembly, but are only seen in few of the infected epithelial cells in herpes oesophagitis. As a rule these nuclei contain numerous viral particles sometimes presenting as "crystals". Once outside the nucleus the virus particles are spread over the cytoplasm and subsequently extruded into the extracellular space. At this stage of virus propagation the nuclei are markedly swollen and have a barren appearance. Such nuclei are the striking feature of the affected cells known as balloon cells in herpes infection. Cell injury ultimately leads to cell death as indicated by nuclear lysis in the so-called shadow cells. These ultrastructural observations are in essential agreement with the results obtained by immunohistochemical methods demonstrating herpes simplex antigen in the presented cases (Feiden et al. 1984).

Intranuclear viral inclusions surrounded by a well defined halo are known as Cowdry A type inclusion bodies. They have been considered to be a specific material related in some way to the replication and maturation of viruses (Itabashi et al. 1966; Chou and Cherry 1967; Roy and Wolman 1969). However, the presence of Cowdry A inclusions and the surrounding halo represent an artifact largely depend on fixative of choice (Strano 1976). Since such inclusion bodies are most frequently found early in the course of infection (Strano 1976), they are usually less prominent in the late ulcerative stages of herpes oesophagitis, when biopsy is performed or brushings are commonly taken. Finally it should be noted that in a variety of reactions the enlarged nucleoli of oesophageal epithelial cells simulate viral inclusions. In summary, Cowdry A type inclusion bodies are less specific in herpes oesophagitis.

Virus induced alterations in herpes oesophagitis also include multinuclear

giant cells which are of epithelial origin as evidenced by the presence of numerous tonofilaments and the immunohistochemical demonstration of keratin (Feiden et al. 1984). These multinucleated cells are considered to be derived from the fusion of several infected cells (Chambers 1978) rather than derived by nuclear division (Leestma et al. 1968).

Dual infection by Candida albicans and herpes virus within individual epithelial cells may occur (Mirra et al. 1982), this was not observed in the present cases.

At the time of detection herpes oesophagitis usually presents as an ulcerative lesion. Typical virus induced cell changes are to be found preferentially at the ulcers edge or sometimes in shed cells of the ulcer bed. As in herpes genitalis (Ng et al. 1970) ground-glass looking nuclei and multinuclear giant cells are the characteristic alterations of these lesions.

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References

Chambers TJ (1978) Multinucleate giant cells. J Pathol 126:125–148

Chou SM, Cherry JD (1967) Ultrastructure of Cowdry type A inclusions. I. In human herpes simplex encephalitis. Neurology 17:575–586

Feiden W, Borchard F, Bürrig KF, Pfitzer P (1984) Herpes oesophagitis. I. Light microscopical and immunohistochemical investigations. Virchows Archiv [Pathol Anat] 404:167–176

Ghadially FN (1982) Ultrastructural pathology of the cell and matrix. 2nd ed. Butterworth London, Boston, Sydney, Wellington, Durban, Toronto. p 104

Hübner G (1981) Möglichkeiten und Grenzen der Erkennung einer viralen Pathogenese in Diagnostik und Forschung. Eletronenmikroskopie (Referat). Verh Dtsch Ges Pathol 65:29–47

Itabashi HH, Bass DM, McCulloch JR (1966) Inclusion body of acute inclusion encephalitis. An electron-microscopic study in a case of suspected herpes simplex encephalitis. Arch Neurol 14:493–505

Leestma JE, Bornstein MB, Sheppard RD, Feldman LA (1969) Ultrastructural aspects of herpes simplex virus infection in organized cultures of mammalian nervous tissue. Lab Invest 20:70–78

McDowell EM (1978) Fixation and processing. In: Trump BF, Jones RT (eds.) Diagnostic electron microscopy, vol 1. John Wiley & Sons New York, Chichester, Brisbane, Toronto. pp 113–139

Mirra SS, Bryan JA, Butz WC, Miles ML (1982) Concomitant herpes-monilial esophagitis: case report with ultrastructural study. Hum Pathol 13:760–763

Morecki R, Becker NH (1968) Human herpesvirus infection. Its fine structure identification in paraffin-embedded tissue. Arch Pathol 86:292–296

Nash G, Foley FD (1970) Herpetic infection of the middle and lower respiratory tract. Am J Clin Pathol 54:857–863

Ng ABP, Reagan JW, Lindner E (1970) The cellular manifestations of primary and recurrent herpes genitalis. Acta Cytol 14:124–129

Nii S, Morgan C, Rose HM (1968) Electron microscopy of herpes simplex virus. II. Sequence of development. J Virol 2:517–536

Patrizi G, Middelkamp JN, Reed CA (1968) Fine structure of herpes simplex virus hepato-adrenal necrosis in the newborn. Am J Clin Pathol 49:325–341

Roy S, Wolman L (1969) Electron microscopic observation on the virus particles in herpes simplex encephalitis. J Clin Pathol 22:51–59

Schäfer R, Ormanns W, Pfeifer U (1981) Zur autoptischen Diagnostik von Herpes simplex Infektionen. Verh Dtsch Ges Pathol 65:230–234
Smith J, Coleman DV (1983) Electron microscopy of cells showing viral cytopathic effects in Papanicolaou smears. Acta Cytol 27:605–613
Strano AJ (1976) Light microscopy of selected viral diseases (morphology of viral inclusion bodies). Pathol Annu 11:53–75
Swanson JL, Craighead JE, Reynolds ES (1966) Electron microscopic observations on herpesvirus hominis (herpes simplex virus) encephalitis in man. Lab Invest 15:1966–1981
Viloria JE, Garcia JH (1976) Human herpes simplex encephalitis (cellular events). Beitr Pathol 157:14–22

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