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Herpes oesophagitis

I. Light microscopical and immunohistochemical investigations

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Summary. The diagnosis of herpes oesophagitis was established from routinely processed biopsy specimens and cytological brush preparations of six patients by immunoperoxidase staining of herpes simplex virus (HSV)-antigen. Macroscopically small round punched-out ulcers are the most frequent and characteristic feature of herpes oesophagitis, whether occurring in patients with serious debilitating illnesses, under immunosuppression, or without evidence of any significant disease.

Light microscopically the herpetic changes of squamous epithelium consist of ballooning degeneration, ground glass nuclei with margination of chromatin, eosinophilic inclusions and multinuclear giant cells. A specific positive reaction with anti-HSV is found only at the borders of the oesophageal ulcers. The immunostaining intensity of nuclei and cytoplasm varies from cell to cell according to the mode of HSV replication in productive infected cells. The latency of HSV in the vagus ganglion and centrifugal neural spread are discussed.

Key words: Oesophagitis – Herpetic cell changes – Herpes simplex virus – Immunoperoxidase

Introduction

Herpes simplex virus (HSV) often induces mucocutaneous lesions, such as stomatitis or gingivitis. Herpetic infection of the oesophagus may appear as an ulceration in autopsy studies (Pearce and Dagradi 1943; Fingerland et al. 1952; Berg 1955; Moses and Cheatham 1963; Rosen and Hajdu 1971; Nash and Ross 1974; Buss and Scharyj 1979).

Most of the autopsy cases had serious debilitating illnesses, such as cancer, Hodgkin's disease, haematological malignancies, or the patients were immunosuppressed. Herpes oesophagitis has been rarely established by

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biopsy or brush cytology. To date 34 cases have been reported in the literature (Weiden and Schuffler 1974; Shah et al. 1977; Lasser 1977; Lightdale 1977; Depew et al. 1977; Owensby and Stammer 1978; Fishbein et al 1979; Springer et al. 1979; Hemstreet et al. 1980; Clocuh and Hansen 1981; Giger et al. 1981; Bastian and Kaufmann 1982; Hoang et al. 1982). A few of these patients had no other significant disease prior to or during the infection, suffering only from acute odynophagia and retrosternal pain (Depew et al. 1977; Owensby and Stammer 1978; Springer et al. 1979; Bastian and Kaufmann 1982).

Most of the cases of herpes oesophagitis have been diagnosed by light microscopy alone. The histopathological characteristics are ballooning degeneration, ground glass nuclei with marginated chromatin and eosinophilic nuclear inclusions of squamous epithelial cells as well as multinuclear giant cells. These cell changes are the same as in other herpetic mucocutaneous lesions and were partly known to Unna (1894) and Lipschütz (1921). In single cases HSV-subtype 1 has been cultured from the oesophageal lesions (Lasser 1977; Owensby and Stammer 1978; Fishbein et al. 1979; Hemstreet et al. 1980; Giger et al. 1981; Bastian and Kaufmann 1982). To our knowledge, an immunomorphological demonstration of herpes oesophagitis has not been made. In the present report details are given concerning immunoperoxidase staining of HSV-antigens in routinely processed biopsy specimens and cytological brush preparations of six patients.

Materials and methods

Clinical data of the six patients are summarized in Table 1. In each patient diagnostic endoscopy of the upper gastrointestinal tract was undertaken and demonstrated characteristic alterations. In three cases small, punched-out ulcerations of the oesophageal mucosa were found (Fig. 1), in two of them the clinicians suspected that these alterations were caused by herpes infection. In each patient, forceps biopsies were taken, especially from the floor and border of the ulcer.

Table 1. Basic data of 5 patients with herpes oesophagitis

No	Sex	Age	Primary disease	Actual symptoms	Endoscopical diagnosis
1	F	14	Diabetes type I Ketoacidosis	Severe retrosternal burning pain	Herpes oesophagitis suspected
2	F	30	Collagenosis Cytostatic therapy	Retrosternal pain Herpes labialis	Herpes oesophagitis suspected
3	M	52	Alcoholism Fatty liver	Stomachalgia Singultation Nausea. Vomiting	Ulcerative oesophagitis
4	F	71	Arterial hypertension Coronary heart disease Heart failure	Stomachalgia Nausea	Reflux oesophagitis
5	M	55	Cerebral meningeoma Glucocorticoid therapy	Epigastric pain	Ulcerative oesophagitis
6	F	58	Adenocarcinoma	Nausea	Monilial oesophagitis suspected

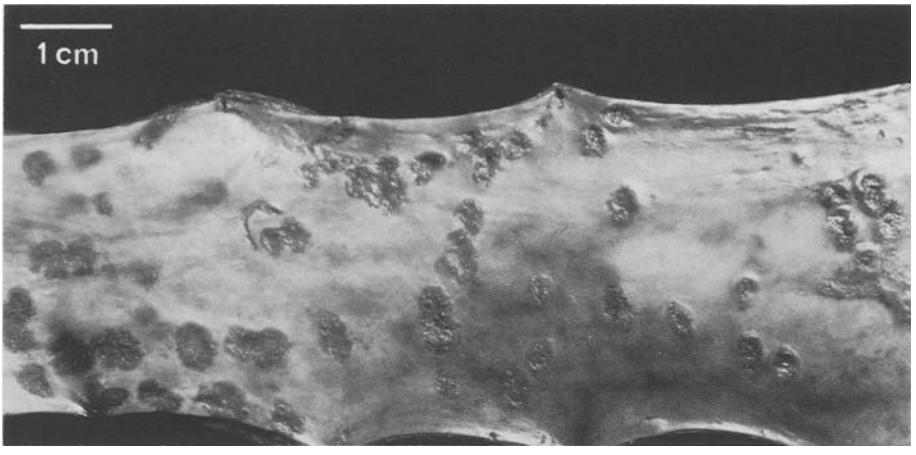

Fig. 1. Herpes oesophagitis with typical punched-out ulcers (autopsy case)

The biopsy specimens were fixed in 10% neutral formalin and processed routinely for light microscopy. For immunohistochemistry additional paraffin sections were stained by both the indirect immunoperoxidase method (IPM) using peroxidase conjugated secondary antiserum (DAKO-immunoglobulins, Copenhagen-Denmark) and by the avidin-biotin-peroxidase method (ABPM) using biotinylated secondary antiserum and preformed avidin-biotinylated horseradish peroxidase complex (Vector Laboratories, Burlingame-USA). The peroxidase was made visible by the diamino-benzidine-reaction. The primary rabbit antiserum against herpes simplex virus (DAKO-immunoglobulins) was polyspecific, containing precipitating antibodies against the major HSV-glycoprotein antigens and both viral subgroup antigens. According to the producer the specificity of the antiserum was tested by the neutralization technique, indirect immunofluorescence on HSV infected and non-infected cells, and counter and rocket immunoelectrophoresis. The specificity of the immunohistochemical methods used included positive controls of virologically proved cases of HSV-encephalitis (Feiden 1983). A further positive control consisted of an autopsy case of herpes oesophagitis with typical punched-out ulcers (Fig. 1). Specificity control of the immunostaining included substitution of normal rabbit serum and rabbit antisera of different specificity – keratin, lysozyme, complementfactor 3 and carcinoembryonic antigen (all antisera from DAKO-immunoglobulins) – instead of the primary anti-HSV-serum. As negative controls, three other oesophageal biopsies, two with ulcerative lesions and one with reflux oesophagitis, were stained immunohistologically. On light microscopy these biopsies contained some squamous cells with eosinophilic intranuclear inclusions while the other typical features of herpes oesophagitis were missing.

In two cases additional smears for cytological examination were obtained by oesophageal brushings. Smears were fixed in Merckofix® and stained with Papanicolaou's stain. Thereafter these slides were stained immunocytochemically by the IPM after removing the cover slip with xylol.

Results

Light microscopy

The HE-stained sections of the oesophageal forceps biopsies show squamous epithelium with ballooning degeneration and marked nuclear changes of cells. They are found only at the edge of the ulcers. In one biopsy an intact herpes blister is preserved in the oesophageal mucosa, and infected epithelial cells are located only adjacent to the blister (Fig. 2a). Most of the nuclei are

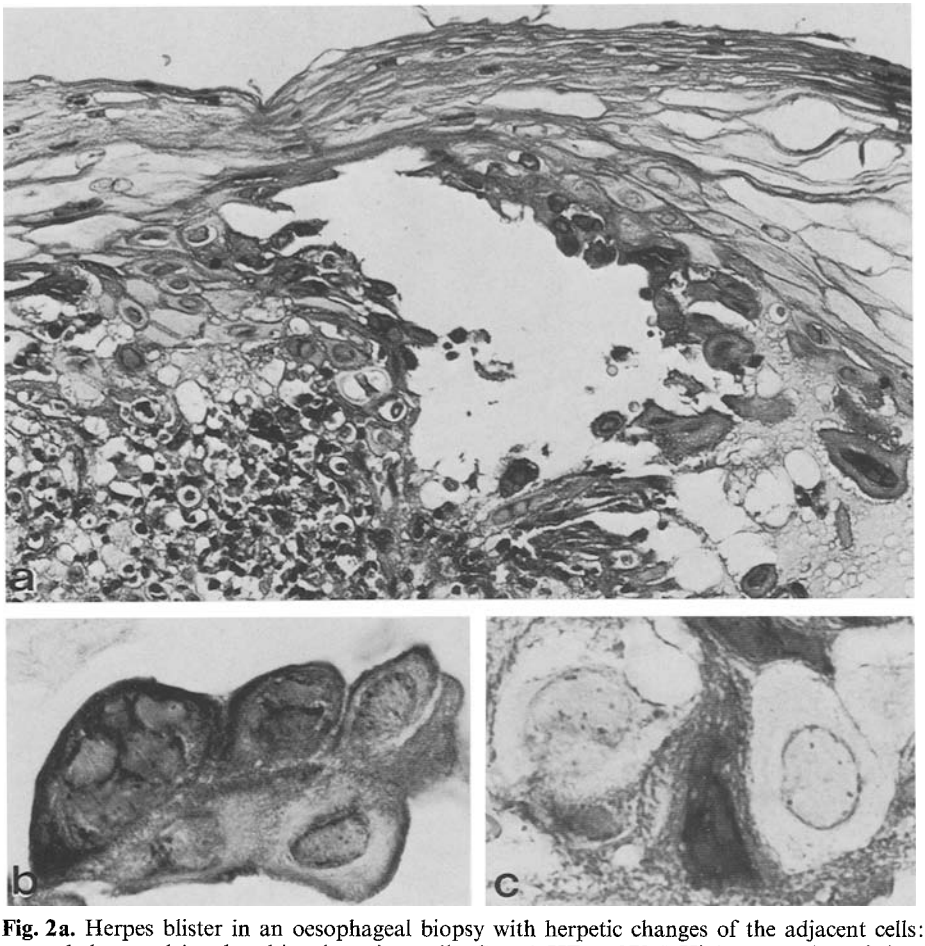

Fig. 2a. Herpes blister in an oesophageal biopsy with herpetic changes of the adjacent cells: ground glass nuclei and multinuclear giant cells. Case 4, HE, ×270. **b** High power view of giant cells and ground glass nuclei. Case 2, HE, ×800. **c** Shrinkage of a multinuclear cell with dark stained stick-like nuclei (*center*), balloon cells. Case 2, HE, ×1000

filled with a light staining basophilic-amphophilic material or a homogeneous to granular eosinophilic mass surrounded by marginated chromatin, so-called ground glass nuclei (Fig. 2b).

A halo is lacking in these cells. Eosinophilic intranuclear Cowdry type A inclusion bodies with a halo are very rarely seen. Some nuclei are only slightly enlarged with markedly decreased staining intensity and margination of the chromatin (Fig. 2b). Shrinkage of cells with dark stained stick-like nuclei is also observed (Fig. 2c). Many multinuclear giant cells showing typical molding are recognized (Fig. 2b). Giant cells and small complexes of squamous cells are seen over the ulcer bed obviously shed at the epithelial border of the ulcer (Fig. 3b). The floor of the ulcer contains acute and chronic inflammatory cells and varying amounts of fibrin and necrotic debris (Fig. 3a).

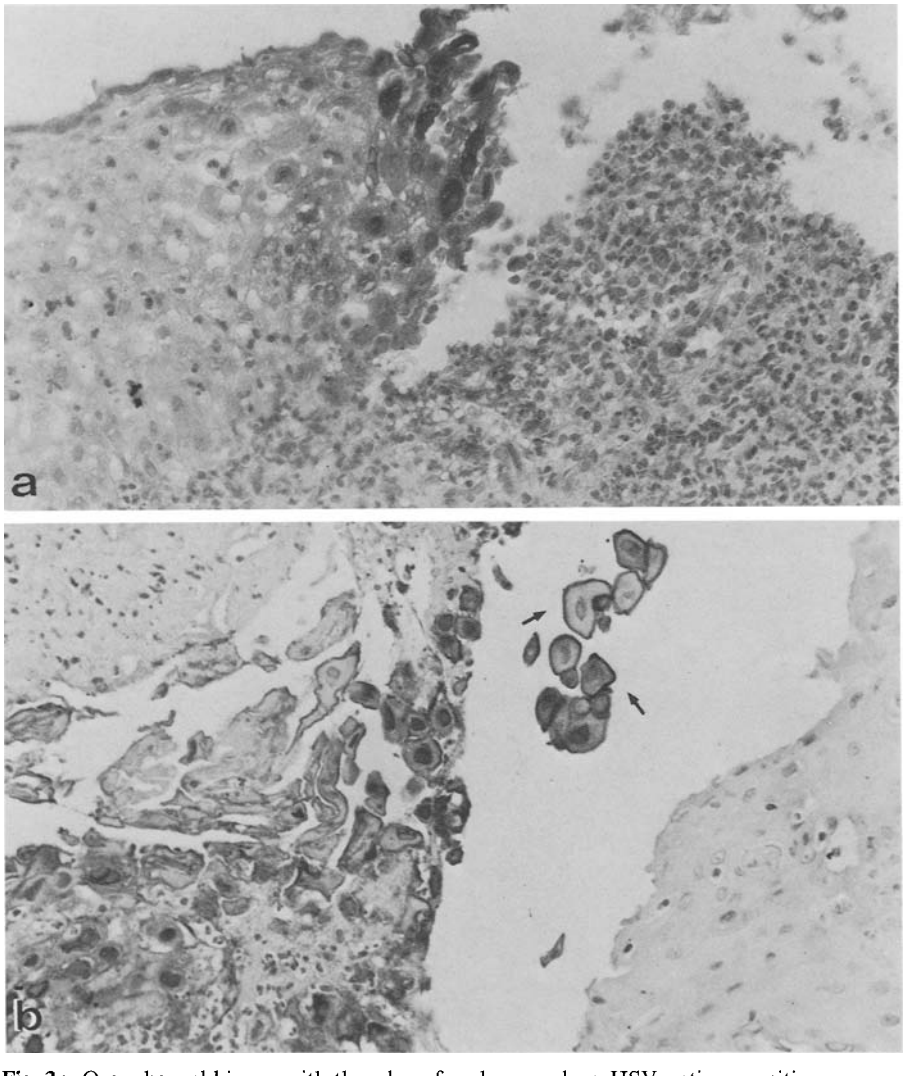

Fig. 3a. Oesophageal biopsy with the edge of an herpes ulcer, HSV-antigen positive squamous cells immediately at the edge of the ulcer, negative reaction of normal epithelium on the left, and of infiltrated cells on the right. Case 3, IPM with anti-HSV, counterstain with haematoxylin, ×310. **b** Shed squamous cells in the ulcer with focal intense positivity at the cytoplasmic membrane (↑), on the right negative staining of normal epithelium. Case 2, ABPM with anti-HSV, counterstain with haematoxylin, ×140

Cytology

Among the numerous squamous cells of the brush preparations there are some with ground glass nuclei and a few multinuclear giant cells displaying typical molding. The background is made up of numerous red and white blood cells.

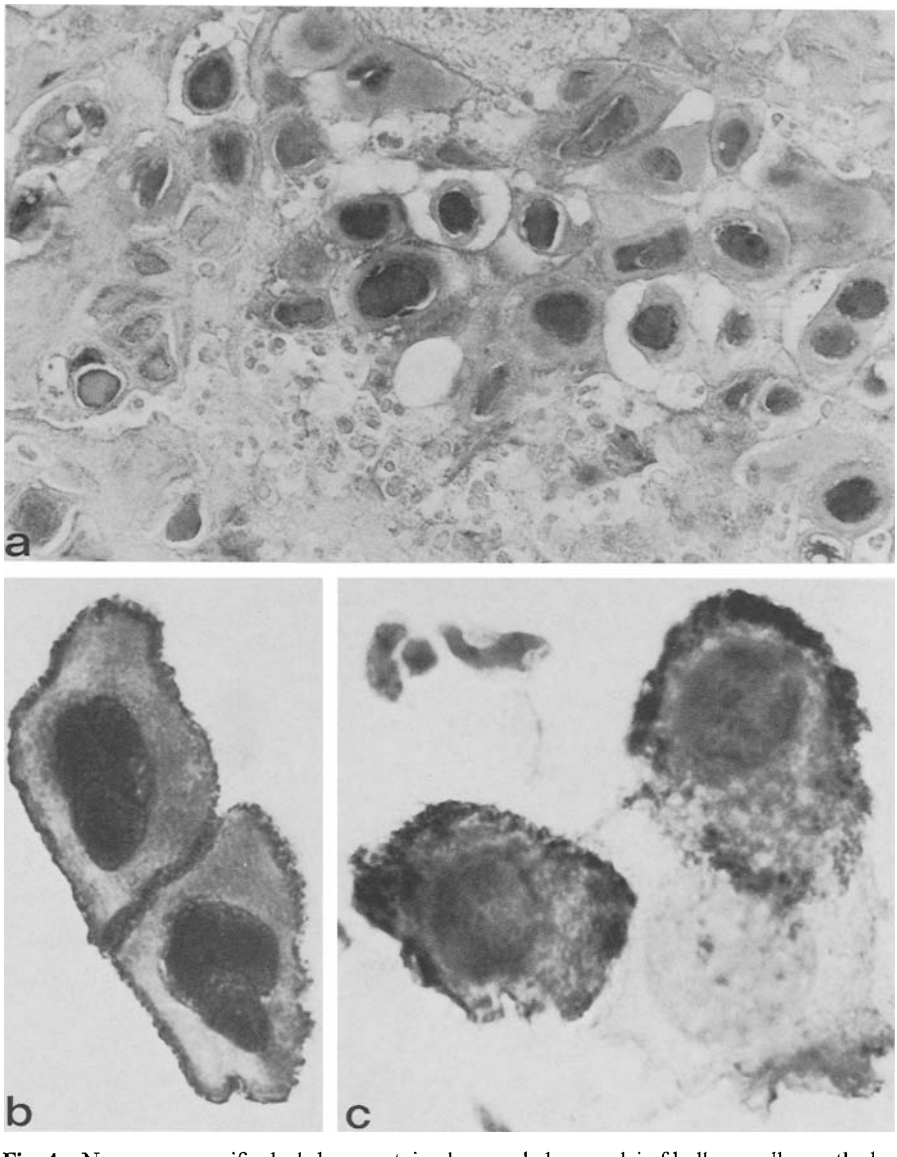

Fig. 4a. Numerous specific dark-brown stained ground glass nuclei of balloon cells; on the lower left very weak reaction or negative staining of necrobiotic 'shadow' cells, negative intranuclear inclusions. Case 2, IPM with anti-HSV, counterstain with haematoxylin, ×215. **b** Two shed still adherent multinuclear squamous cells with molding of HSV-positive nuclei, focal intense granular positivity of the cytoplasmic membrane and of the intercellular space. Case 2, ABPM with anti-HSV, counterstain with haematoxylin, ×850. **c** Cytological brush preparation, strong marginal reaction of the cytoplasm, negative squamous cell in the background. Papanicolaou stain, additional immunocytochemical staining, IPM with anti-HSV, ×850

Immunohistochemistry

The sections stained immunohistochemically with anti-HSV show the presence of a specific granular to homogeneous brown reaction product of different intensity within the nuclei and in the cytoplasm of squamous cells infected by the HSV (Figs. 3 and 4). All control sections lack specific staining. The normal oesophageal squamous epithelium at a distance from the ulcer and inflammatory and mesenchymal cells do not stain with anti-HSV. In addition, the cytoplasm of the multinuclear infected cells shows a positive staining with anti-keratin as do most of the normal and infected squamous cells. In the HSV-stained sections the intensity of specific immunostaining in the ground glass nuclei and in the cytoplasm varies, obviously reflecting different concentrations of virus antigens. Most squamous cells with strongly HSV-positive nuclei contain no or little virus antigen in their cytoplasm (Fig. 3b and 4a). On the other hand, some cells with a dark brown reaction product in the cytoplasm demonstrate only a weak diffuse reaction of the nucleus or a focal intense positivity of the nuclear membrane (Fig. 3a). The shed squamous cells usually reveal a strong marginal reaction of the plasmalemma (Fig. 3b) or of the adjacent marginal cytoplasm (Fig. 4c). In 2 cases a specific granular reaction product is also recognized in the intercellular space of still adherent squamous cells (Fig. 4b). In necrobiotic and 'shadow' cells only a few faintly positive granules are retained at the nuclear membrane or in the cytoplasm, or they show a total fading of specific staining (Fig. 4a, lower left).

Discussion

To establish the morphological diagnosis of herpes oesophagitis it is necessary to examine the borders of oesophageal ulcers because it is only there that squamous cells with herpetic changes can be found. Therefore, biopsy specimens or brushings have to be taken from the edges of the ulcerative lesions revealed at endoscopic examinations. Although the macroscopical lesions of herpes oesophagitis consist mostly of typical small round punched-out ulcers (Fig. 1), other authors have claimed that viral oesophagitis cannot be distinguished, on clinical or endoscopic grounds, from reflux oesophagitis (Giger et al. 1981).

If the biopsy specimen contains only a small portion of the ulcer, or if there are only a few squamous cells with some of the herpetic changes, it may be very difficult or even impossible to establish the diagnosis by HE-staining alone. In these cases we regard immunohistochemical staining of HSV-antigens as necessary. Eosinophilic intranuclear inclusions alone are not considered diagnostic of herpes infection and the eosinophilic intranuclear changes of our negative control cases did not stain with anti-HSV.

The different intensity of immunoperoxidase staining of HSV-antigens in the nucleus and cytoplasm of the affected cells is consistent with the mode of replication of HSV in experimentally infected cells (Lebrun 1956). The assembly of incomplete viral particles begins within the nucleus (Nahmias

and Roizman 1973), changing into a mass of light staining basophilic-amphophilic and later more eosinophilic material with marginated chromatin. These ground glass nuclei react strongly positive with anti-HSV (Figs. 3b and 4a). With the movement of viral particles from the nucleus into the cytoplasm this becomes immunohistochemically positive (Fig. 3a). The immunostaining of the nucleus decreases to a focal positivity at the nuclear membrane. Here, the envelopment of incomplete viral particles takes place, the virions accumulating between the inner and outer lamellae of the nuclear membrane (Nahmias and Roizman 1973).

If the infected cell does not undergo total lysis, herpes virions assemble beneath the plasma membrane, resulting in a marginal positivity. In this phase of the infectious cycle many nuclei may stain negatively (Fig. 3b). The negative reacting eosinophilic intranuclear inclusions of the late infectious cycle seem to be the burntout remnants of the 'viral factory'. After extrusion from the cell, virions can also accumulate in the intercellular space (Fig. 4b) as has been shown electron microscopically (Bürrig et al. 1984).

Typical eosinophilic intranuclear inclusion bodies of Cowdry type A surrounded by a halo were seen only very rarely in our cases. The reason for this observation could be that formalin is one of the poorest fixatives for the demonstration of these inclusions, the features of which are fixative-dependent artifacts in virus infected cells (Strano 1976). Zenker's acetic acid and Bouin's solution are considered to be the fixatives of choice for demonstrating such viral inclusions (Strano 1976). We think ground glass nuclei surrounded by marginated chromatin and multinuclear giant cells to be more constant herpetic alterations. However, ground glass nuclei and eosinophilic intranuclear inclusions do not differentiate between herpes simplex and varicella-zoster or cytomegalic virus infections. In the latter, oesophageal lesions may include nuclear changes in mesenchymal cells and also in the glandular epithelium.

As to the pathogenesis of herpes oesophagitis, the trigeminal and spinal ganglions are considered to be the source of recurrent herpes labialis and herpes genitalis infections since HSV has been found to be present in a latent form in these somatic ganglions (Bastian et al. 1972; Baringer and Swoveland 1973; Plummer 1973; Baringer 1974). Warren et al. (1978), isolating HSV-subtype 1 from the superior cervical and the vagus ganglions of humans, have discussed the possibility of virus latency in the jugular portion of the vagus ganglion, causing herpes oesophagitis and perhaps other diseases of the digestive tract innervated by the vagus nerve. It seems possible that after activation of latent HSV in the vagus ganglion, the virus migrates centrifugally along the vagus nerve and infects the susceptible squamous epithelium, displaying the cellular changes characteristic of productive HSV-infection. Since many infected cells undergo necrosis the typical blister develops and ruptures, leaving an ulcer whose margins show evidence of active herpetic infection. The occurrence of herpetic infection in other areas innervated by the vagus nerve, such as herpes tracheobronchitis and pneumonia (Herout et al. 1966; Nash and Foley 1970; Rosen and Hajdu 1971; Ramsey et al. 1982), supports this pathogenetic theory, although the swallowing of saliva

containing the virus can be considered in cases with previous oropharyngeal infection, as in our case 2. However, this is less probable because of the presence of antibodies and the observation that HSV characteristically infects contiguous cells by cell to cell spread (Nahmias and Roizman 1973).

Simultaneous monilial infection of the oesophageal ulcers was not found in our cases, but is well known, especially in debilitated patients with malignant disease (Rosen and Hajdu 1971; Buss and Scharyj 1979). Herpetic infection may precede secondary bacterial or fungal infection.

In our view, the establishment of the diagnosis of herpes oesophagitis may be particularly important in immunocompromised patients because of the availability of effective anti-viral chemotherapy (Mitchell et al. 1981).

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