

SUBMICROSCOPIC ASPECTS OF THE KERATINIZATION, DYSKERATINIZATION AND ACANTHOLYSIS OF FOGO SELVAGEM

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Abstract. Histopathological, cytomorphological and electron microscopic analyses in a case of fogo selvagem are reported. Contradictory to the light microscopical findings, the acantholysis as seen with the electron microscope involves the basal layer but not the subcorneal layers—at least not the most superficial part of the granular layer. A conspicuous disintegration of the tonofilament-desmosome complexes give rise to the concomitant dyskeratosis. This aberrant process, including an association between retracting tonofilaments and defective Odland bodies, results in the terminal stage of monstrous defective and specific keratohyalin. In cytoplasm, target-like structures similar to virions of the herpes virus group were observed. The dynamics of the pathological process is discussed.

Key words: Acanthosis; Dyskeratosis; Fogo selvagem; Pemphigus

In 1940 J. P. Vieira (11) described a certain bullous eruption with a peculiar limitation to the states of Bahia, Graias, Mato Grosso and São Paulo in Brazil, namely fogo selvagem (=pemphigus brasiliensis). The cause of fogo selvagem (FS) remains obscure or unknown. The possibility of an infectious (viral) origin has been proposed (2). FS manifests itself in various morphologic forms classified by Vieira as acute, subacute, superacute and chronic types.

FS is characterized by transient bullous eruptions transforming into squamous or hyperkeratotic lesions with circinate, arabesque, bizarre configuration which is typical and characteristic. The disease is accompanied by a typical and diagnostic spiking fever curve in which the temperature is always elevated in the evening and normal or sub-normal in the morning, and sometimes by different systemic symptoms. For further detailed clinical characteristics, see the review by Brown (1).

Micromorphologically, FS is impossible to distinguish from pemphigus foliaceus, according to all authors (3, 7, 8, 9, 10). According to Brown (1) the histology is difficult to distinguish from certain stages of erythema multiforme and dermatitis herpetiformis.

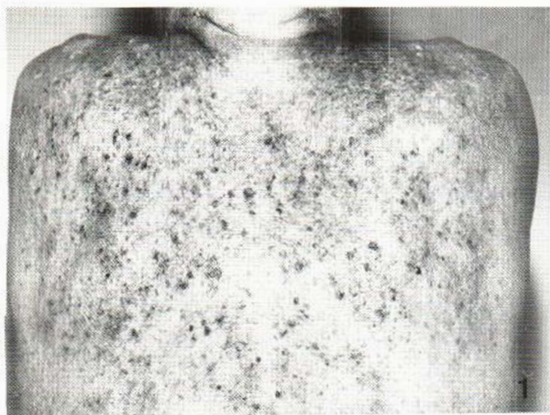
However, the most common concept seems to be that FS is a subcorneal acantholytic process, like pemphigus foliaceus. Brown (1) and Lever (7) emphasize that areas of acantholysis occur in the upper epidermis, leading to the formation of clefts and bullae in superficial, mostly subcorneal location. The dermis displays a certain vasodilatation and a more or less pronounced inflammatory infiltrate, among which eosinophils often are present. In contrast to other intra-epithelial disorders such as pemphigus vulgaris, pemphigus vegetans, pemphigus foliaceus, dyskeratosis follicularis and transient acantholytic disorder, FS has never been submicroscopically investigated with special analyses of the present peculiar dyskeratinization.

MATERIAL AND METHODS

The specimens were obtained from a 66-year-old Swedish man who had lived in São Paulo, Brazil, since 1932. Whilst temporarily visiting Sweden he was taken ill with a spiking fever curve, elevated in the evenings. The patient also developed polymorphous skin lesions consisting of macular erythema, bullous eruptions, crusty and scaling efflorescences, distributed mainly on the trunk but also on extremities (Figs. 1, 2). The patient complained of a burning sensation and discomfort of the skin.

Routine skin biopsy revealed a micromorphology equivalent to that of pemphigus foliaceus (Figs. 3, 4).

Laboratory investigations including several bacterial cultures and immunofluorescence studies on the serum, which proved negative. Cyto-diagnostic procedures according to Tzank were carried out, revealing numerous dyskeratotic cells (Fig. 5). Specimens for electron-



Figs. 1 and 2. Survey and detail pictures of the macro-morphological lesions.

microscopy were fixed in 2% glutaraldehyde buffered with a phosphate solution at pH 7 for 6 hours at 4°C. Post-fixation was performed in 2% osmium tetroxide buffered with the phosphate solution for 2 hours at 18°C. The specimens were rinsed in the phosphate solution and dehydrated in increasing concentrations of acetone. The specimens were embedded in Vestopal W and Spurr and double-stained with uranyl acetate and lead citrate.

RESULTS

Light microscopy

The hematoxylin and eosin stained sections revealed intra-epidermal bullae, located mostly sub-corneally, but also occurring in suprabasal sites and which contained several acantholytic cells presenting more or less advanced dyskeratosis, and also leukocytes, some of which were eosinophils. The walls of the intra-epidermal bullae showed an impressive formation of acantholysis. A slight, unspecific inflammatory, mainly perivascular, but also

disoriented, cellular infiltrate containing some eosinophils was present in the upper epidermis (Figs. 3, 4).

Cytologic examinations, introduced by Arnault Tzanck, revealed acantholytic and dyskeratotic cells, some of which had a peculiar appearance with a considerably increased diameter and irregularly shaped nucleus. They also had the typically dyskeratotic cytoplasm with its lighter perikaryon and denser peripheral zone and the grainy juxtanuclear material within part of the perikaryon (Fig. 5).

Electron microscopy

The dermo-epidermal border appeared highly folded, often forming comparatively extensive protruding dermal parts. Especially in these invaginated dermal areas, the collagen fibres seemed closely associated with the limiting lamina basalis, with a certain paucity of anchoring fibrils. The sub-structural organization of the collagen fibres appeared normal. The lamina basalis, which was about 200–400 Å thick, followed the wavy contour of the dermo-epidermal border, showed no discon-

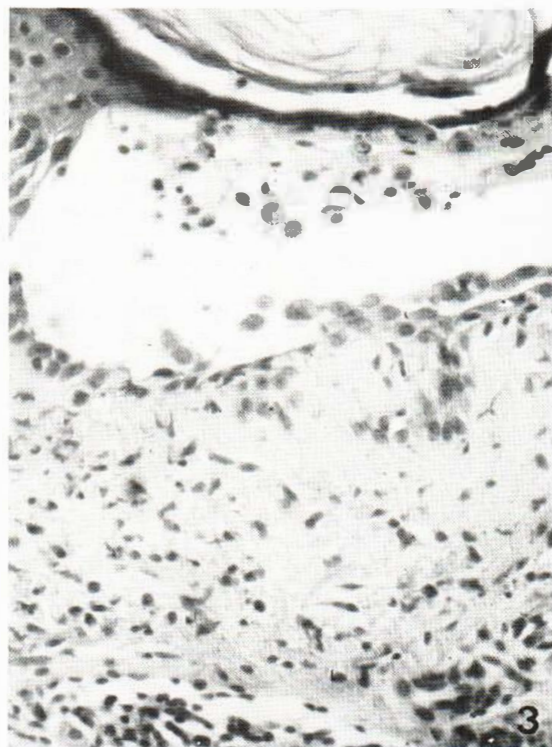


Fig. 3. Light microscopical picture presenting the micro-morphology.

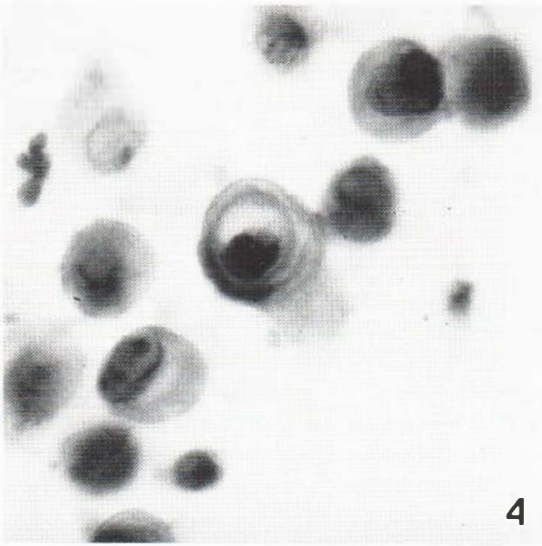


Fig. 4. The acantholytic cells in light microscopical section.

tinuities and was separated from the epidermal basal cells by a less dense space of greatly varying thickness. This space was frequently seen to be bridged by anchoring filaments connected with half-desmosomes (Fig. 6). The half-desmosomes appeared to be submicroscopically normally organized with associated tonofilament bundles. The trimorphous unit membrane of the basal epidermal cells appeared normal. The interfaces of the lamina basalis and the unit membrane limiting the basal parts of the basal epidermal cells with the normal half-desmosomes showed no disintegration indicative of acantholysis or any similar process.

The interfaces between neighbouring basal epidermal cells showed no conclusively manifest signs of acantholysis, although the cells in some places appeared loosely connected, presumably reflecting an illusory condition caused by the ultrathin sectioning. The basal cells contained a normal number of cell organelles and a normal density of tonofilamentous material forming the tonofilament bundles. Even in the border area between the basal layer and spinous layer there was a distinct and conspicuous disintegration of desmosome-tonofilament complexes, leading to the disappearance of desmosomes and a widening of the intercellular space. The spinous epidermal cells formed microvilli-like projections in the intercellular space. The tonofilaments retracted into the perikaryon.

The cell organelles were located mainly at the periphery of the cytoplasm, corresponding to the peripheral basophil cytoplasmic pattern as seen in the cytological imprints (Fig. 7). The Odland bodies were mainly of type II, intermingled with some of type I and not a few with signs of defective differentiation (Fig. 8). Ubiquitous crenations of the nuclei were found. In the intercellular space, free tonofilament bundles and degenerated cell organelles occurred. As shown in Fig. 10, part of the cytoplasm was rich in tonofilamentous material. The masses of tonofilaments were to a large extent grouped into loose bundles, often forming a network and randomly spaced whorls more frequent in the perikaryon. Caused by advanced cellular degradation, free tonofilament bundles were found in the intra-epidermal acantholytic vesicles. Microtubules were abundant and randomly dispersed in the cytoplasm, with peripheral tonofilament free spaces surrounding the tubules.

In the cytoplasm of both the acantholytic and the non-acantholytic cells, numerous membrane-bound areas were observed. The membrane appeared trimorphous, enclosing an amorphous—often stellate—outlined material. Possibly these structures were autophagosomes containing degraded cytoplasmic material.

The granular layer was very rarely involved in the acantholytic process. The keratohyalin in this layer was of normal appearance, with normally dif-



Fig. 5. Cytomorphology of an acantholytic cell of imprint.

ferentiated keratohyalin-tonofilament complexes. Orthokeratosis was superimposed on the granular layer (Fig. 9). In the cytoplasm of acantholytic and preacantholytic cells there was a prominent formation of keratohyalin masses, always juxtaposed with the nuclei. This keratohyalin had a conspicuous aberrant substructure (Figs. 10, 11). The keratohyalin had a marked variety of shapes. Within the keratohyalin there was a superabundance of circumscribed areas having greatly increased electron scattering properties. In the same cells, where this abnormal keratohyalin was found, similar circumscribed areas were observed within the mitochondria, though here with less electron scattering propensity (Fig. 11).

Cells with pronounced vacuolar cytoplasm and with more or less well preserved tonofilaments and bundles of tonofilaments, i.e. epidermal cells, were abundant. Numerous particles were haphazardly dispersed in the cytoplasm of those cells. The particles were round, or slightly ovoid. They were membrane-bound. The membranes were trimorphous. The centre of each particle was round and more or less electron-dense, or else annular. The ring-shaped centres always had larger diameters than the electron-dense homogeneous ones. The space between the trimorphous membrane and the electron-dense homogeneous centres, as well as between the annular centres, was fairly uniform, and was partly occupied by a fine granular material of a certain electron density (Figs. 12, 13).

DISCUSSION

The negative results of the immunofluorescence analyses of the patients' serum correspond to the regressive phase of this mild variety of FS. The light microscopic findings tally with earlier reports (7, 8, 10). No reports of more exhaustive light microscopical analyses of the acantholytic cells of FS appear to exist. In the sections described here, the acantholytic cells showed advanced dyskeratosis, with nuclear changes. The acantholysis involved both subcorneal and suprabasal layers.

The cytological observations on imprints of these cells also revealed advanced dyskeratosis, with prominent changes of an advanced pyknotic character. In juxtannuclear position, numerous basophilic granules were dispersed. This cytomorphology differs significantly from the various kinds of pem-

phigus and other acantholytic disorders. Multinucleation was not observed. The light microscopical and cytomorphological observations correspond to the submicroscopical ultrastructural organization.

The light microscopical observation that the acantholytic process involves not only subcorneal but also occasionally suprabasal layers is electron microscopically verified by this investigation. Certainly the acantholysis does not involve the half-desmosomes. Neither were any signs of disintegration of the half-desmosomes nor any dilatation of the intermembranous space seen. Disintegration of the desmosomes between the basal cells was conspicuous, however, as in the supraposed layers. This submicroscopical organization is characteristic of FS and contradicts the light microscopic conception. The ceasing of the tonofilament-desmosome relationship is fundamental to nearly all acantholysis and is also highly significant for this material. All tonofilament bundles were withdrawn from the periphery of the cytoplasm and were collected in the perikaryon. Under withdrawal, the tonofilaments were observed in different stages of association with the Odland bodies.

The outcome of this process is the synthesis of the monstrous and defective keratohyalin. Occurrence of keratohyalin of similar size is highly significant of certain acantholytic disorders such as pemphigus erythematosus and dyskeratosis follicularis (Darier's disease), though not of pemphigus foliaceus or pemphigus vulgaris. In FS, however, the substructure of the keratohyalin is highly characteristic, indicating a reduced synthesis of the monstrous keratohyalin, compared with that of pemphigus erythematosus and dyskeratosis follicularis. The monstrous keratohyalin of the latter two diseases does not obey the characteristic submicroscopical morphology of FS. The keratohyalin is totally absent in pemphigus vulgaris and

Abbreviations used:

A = A-cell	JG = half-desmosomes
B = B-cell	KH = keratohyalin
AF = anchoring filament	LB = lamina basalis
C = collagen	M = mitochondrion
DF = anchoring fibril	N = nucleus
D = desmosomes	O = Odland bodies
ER = endoplasmic reticulum	R = ribosomes
G = Golgi apparatus	TF = tonofilaments
IS = intercellular spaces	VP = virus-like particles

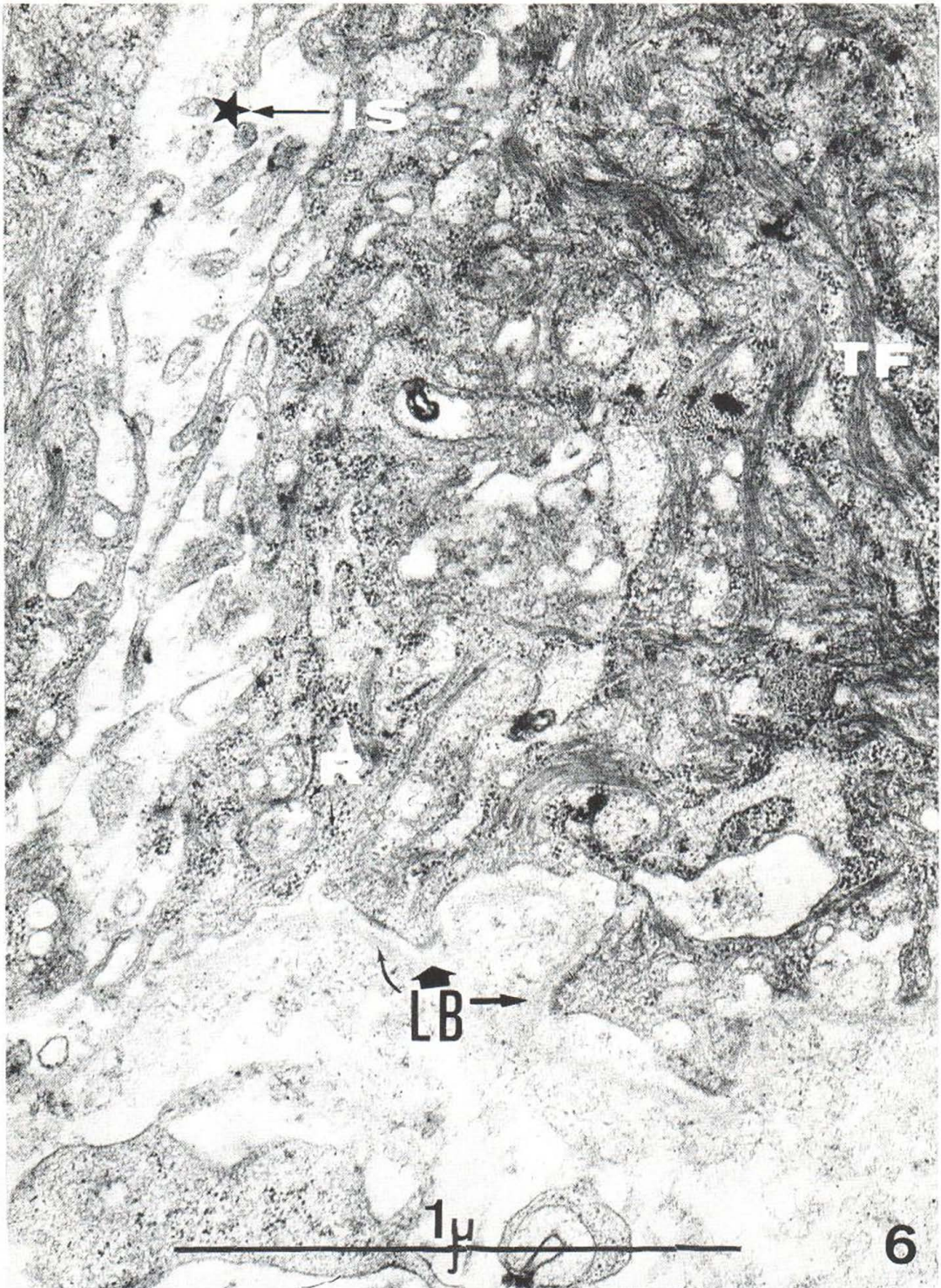


Fig. 6. Lamina basalis and parts of basal epidermal cells from a lesion as seen in the electron microscope. $\times 86\,000$.

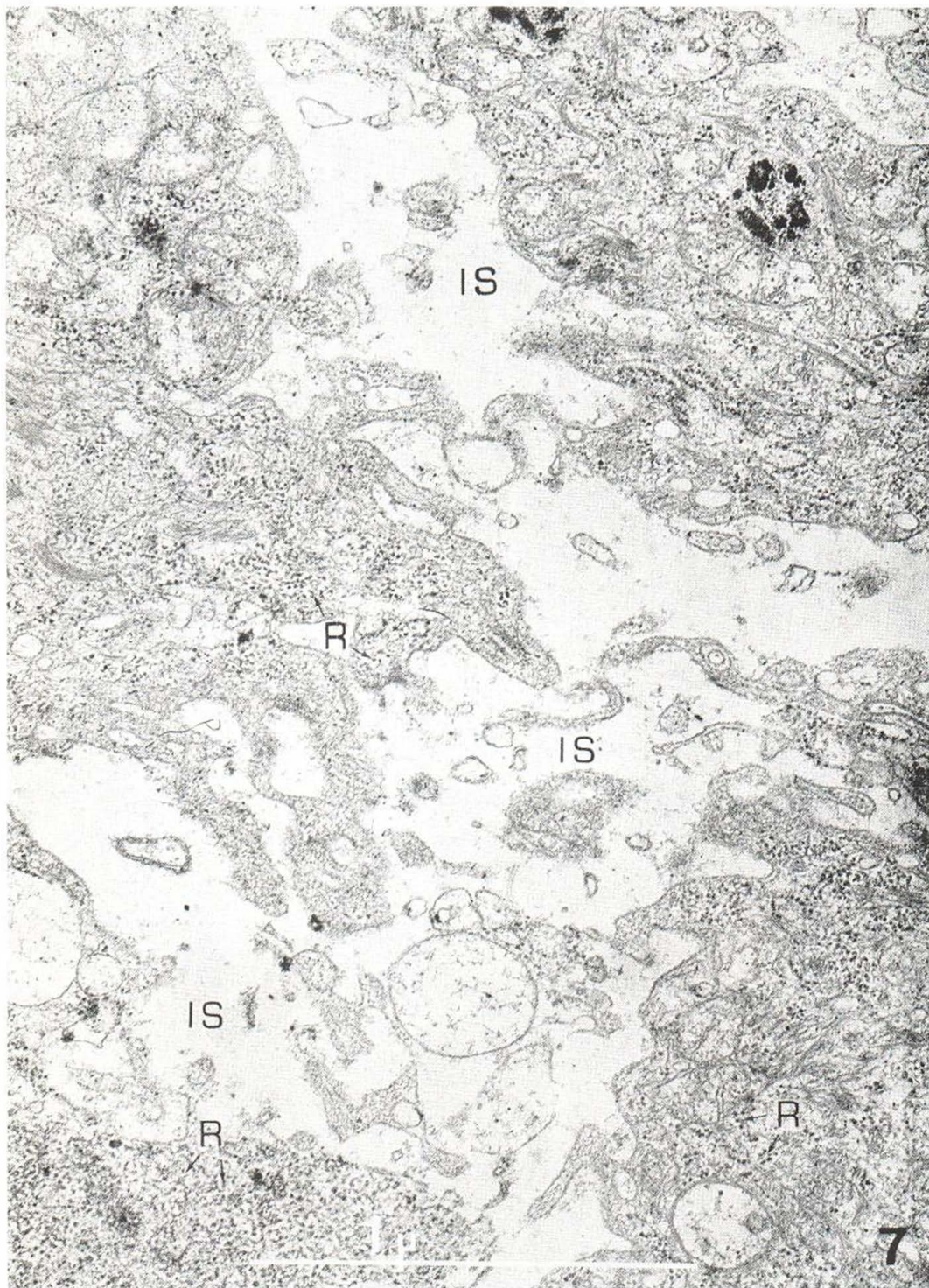


Fig. 7. Electron micrograph showing the interfaces between lower spinous cells. $\times 86\,000$.

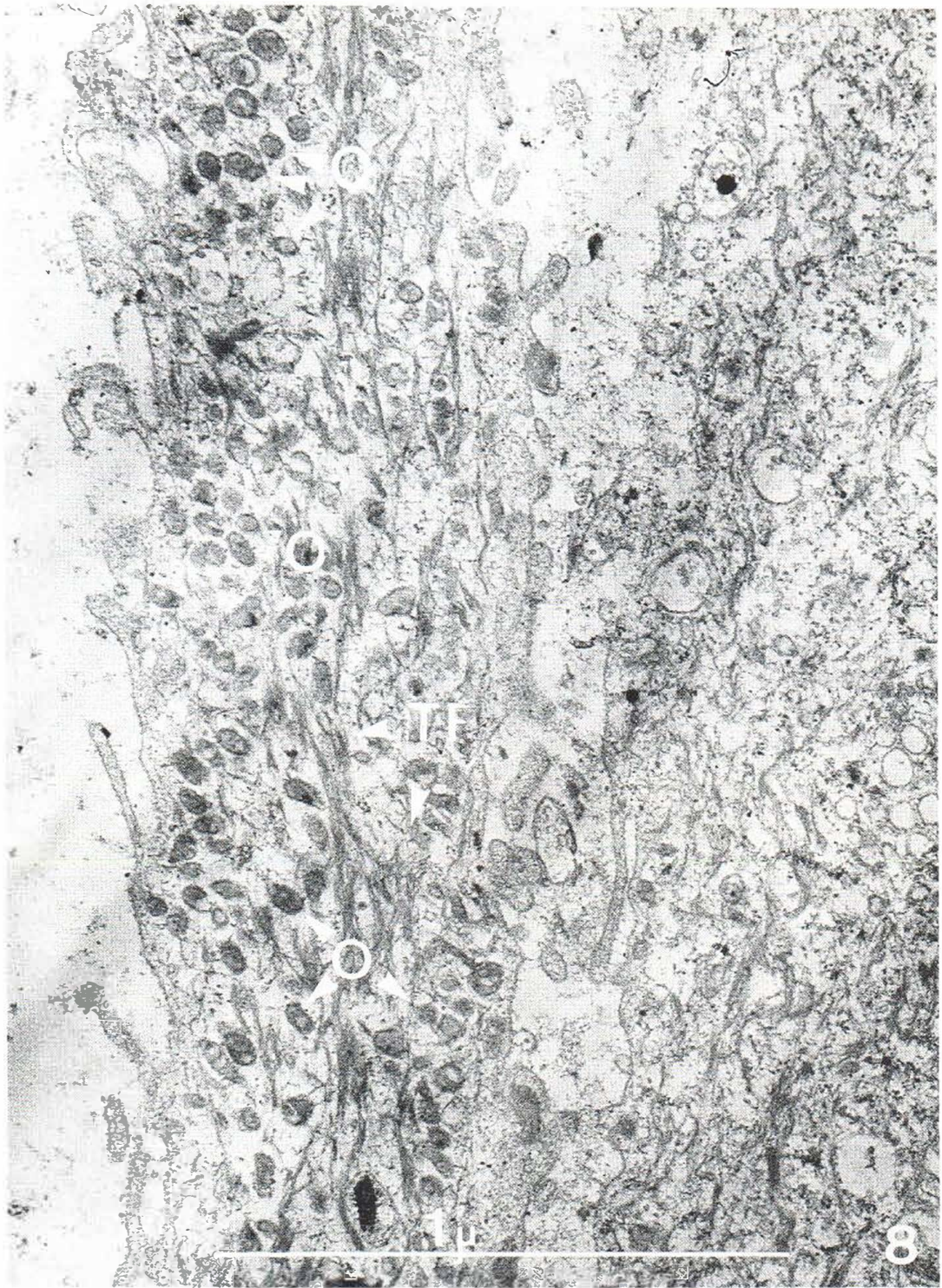


Fig. 8. Details of epidermal acantholytic cells showing Olland bodies. $\times 88\,000$.

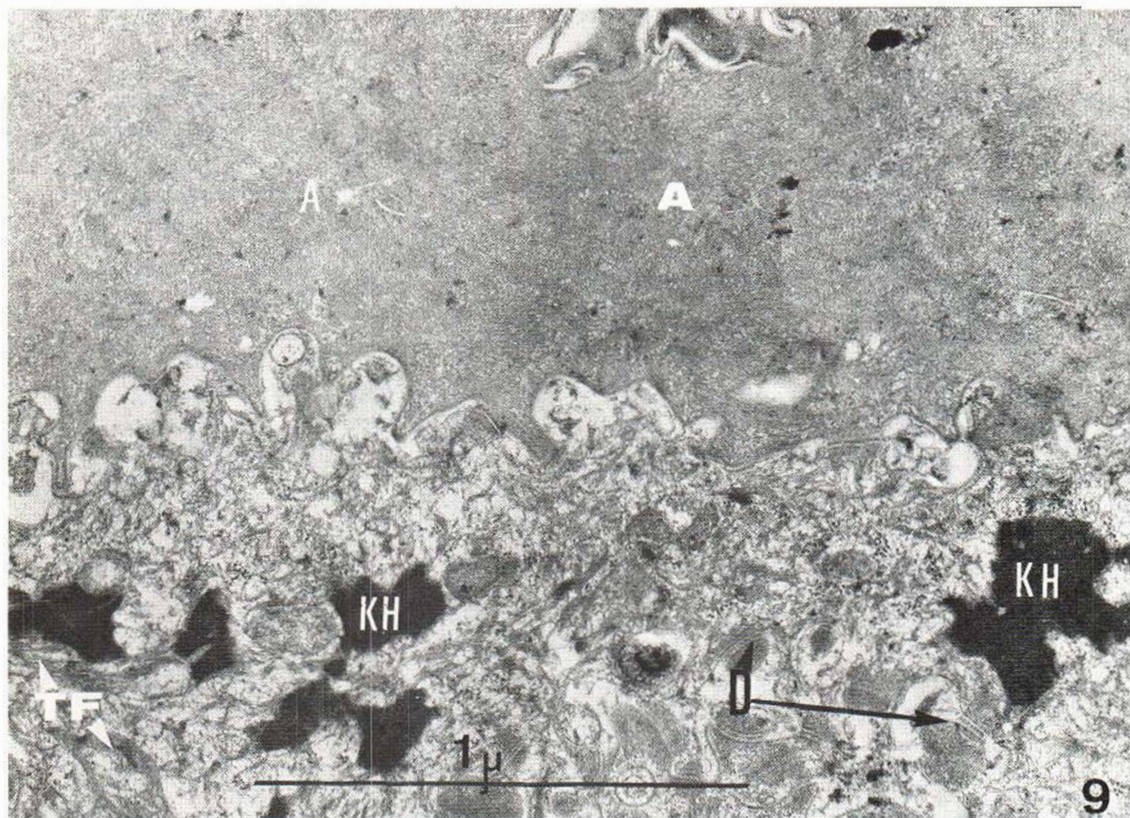


Fig. 9. Parts of granular and corneal cells. $\times 64\,500$.

pemphigus foliaceus. The dyskeratosis of the latter two diseases merely represents a conglomeration of tonofilament bundles having presumably no prior association with the Odland bodies and which reflects different degrees of damage to the tonofilament-desmosome complexes with a concomitant maturation disturbance. The dyskeratosis of FS, with the formation of monstrous keratohyalin, is preceded by a ceasing of the tonofilament-desmosome complexes and an association between tonofilaments and Odland bodies. The Odland bodies of FS are mainly of the described defective type and thus condition the synthesis of the defective monstrous keratohyalin with its peculiar substructure (4, 5, 9).

The etiology of the acantholytic diseases of the pemphigus group remains completely obscure, in spite of important findings. Virus as a cause of various types of pemphigus—and especially of

FS—has been suggested (1, 2, 6, 11), but no evidence has been adduced. Immunological analyses have, despite important results, been unable to provide an etiological explanation. In the present material, however, certain of the findings do, to some degree, support a viral etiology—or at least indicate the presence of virus-like structures. These are the particles in the cytoplasm of acantholytic cells which appear to consist of nucleoids and capsids, demonstrating a morphology similar to the viruses of the herpes group, which are known to cause acantholysis. These viruses, however, are karyotropic, but intracytoplasmic localization occurs, especially combined with extensive cytoplasmic vacuolization as found partly in the present material. Here, virion-like structures could only be found in cytoplasm, but are obviously of target structure. At the present stage, one cannot elaborate on the significance of these observations.

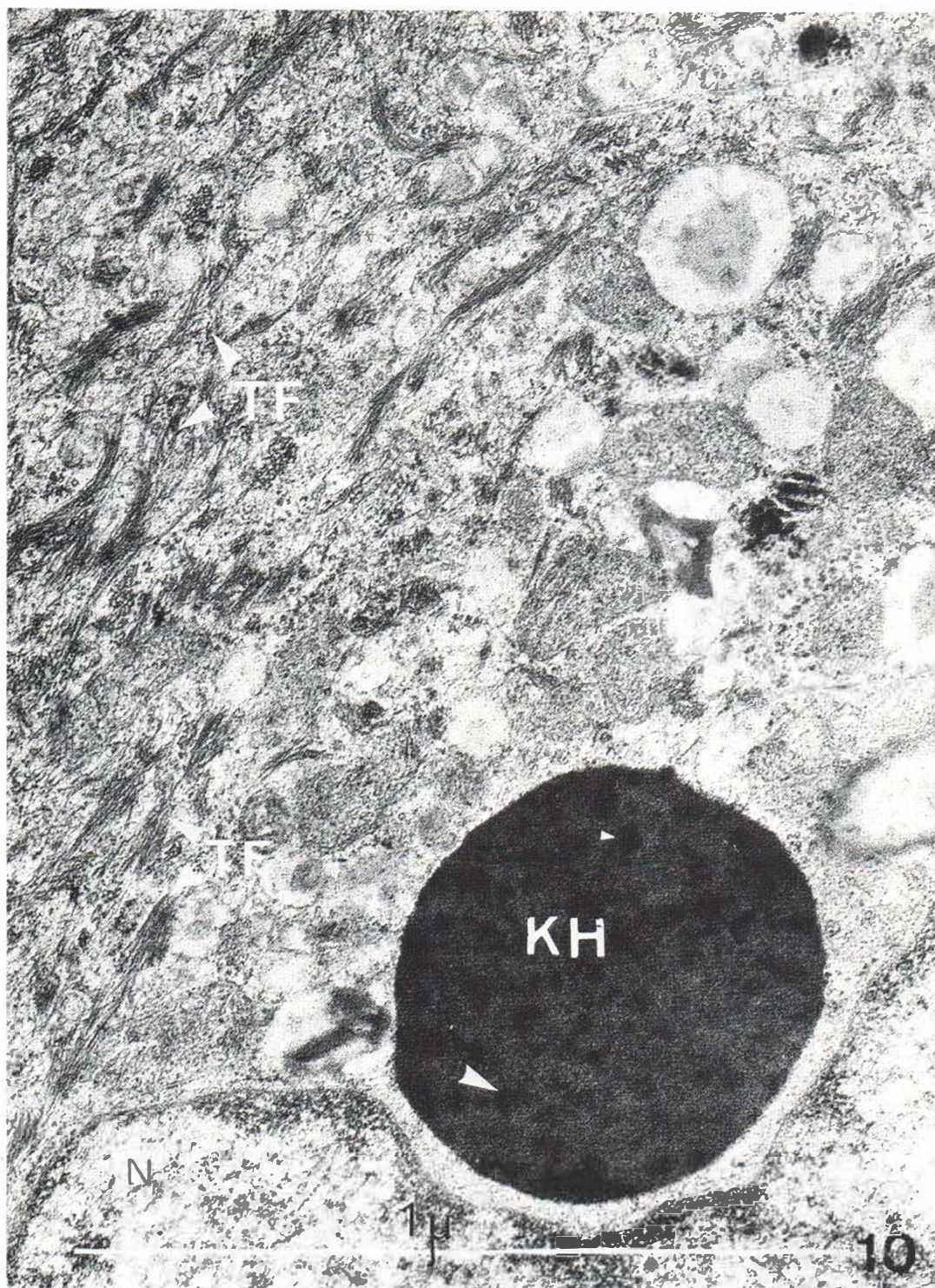


Fig. 10. Part of acantholytic cell with keratohyalin having an aberrant substructure. $\times 118\,000$.

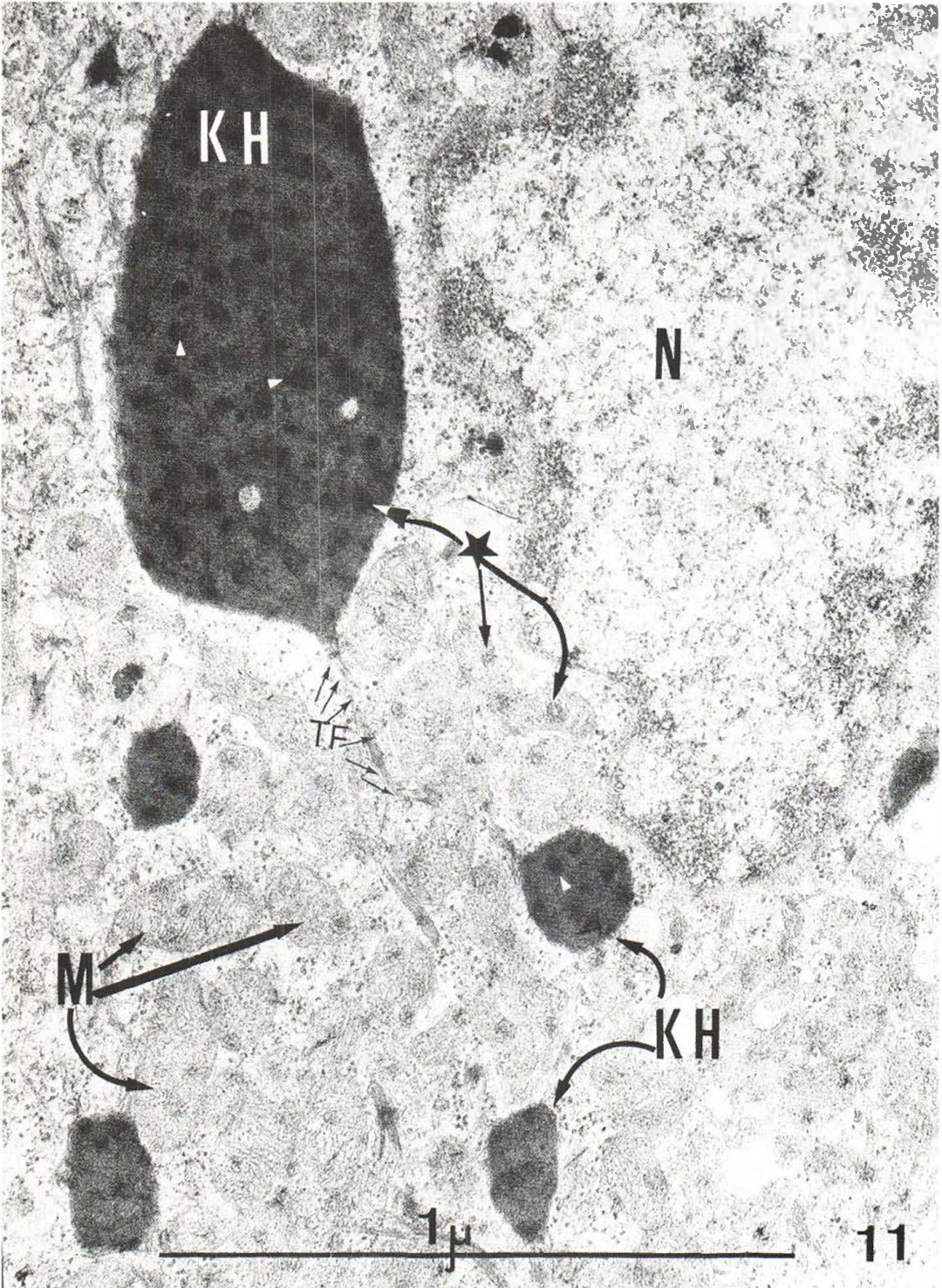


Fig. 11. Electron micrograph of part of an acantholytic epidermal cell showing monstrous keratohyalin and mitochondria having aberrant substructures. $\times 96\,000$.

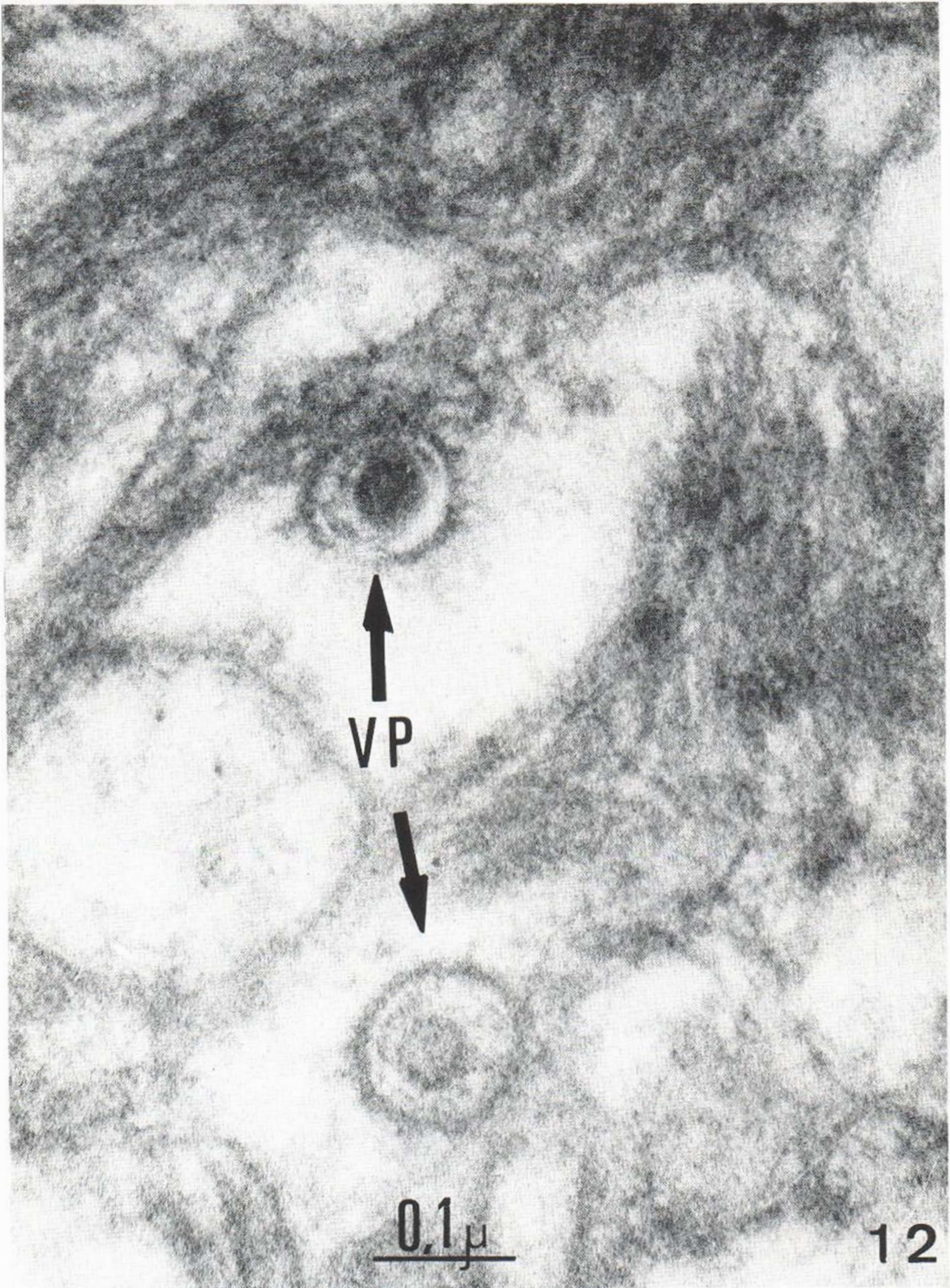


Fig. 12. High magnification of virus-like particles in the cytoplasm of acantholytic cells. $\times 215\,000$.

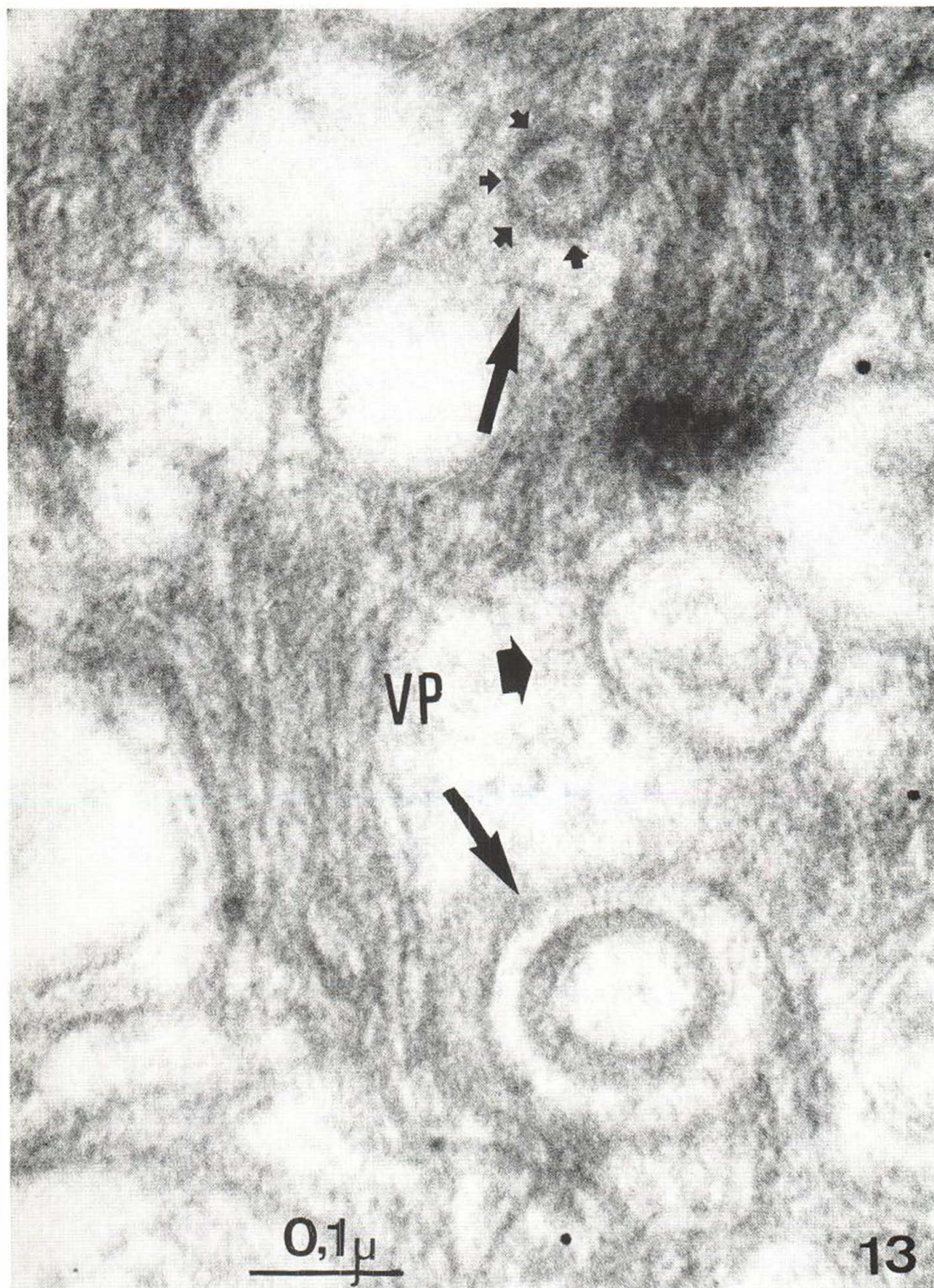


Fig. 13. Different plane of sectioning of virus-like particles. $\times 220\,000$.

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