

LIGHT AND ELECTRON MICROSCOPIC ASPECTS OF PEMPHIGUS HERPETIFORMIS (EOSINOPHILIC SPONGIOSIS) IN COMPARISON WITH OTHER ACANTHOLYTIC DISORDERS

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Abstract. Histopathological and electron microscopic analyses of pemphigus herpetiformis in a 64-year old woman with a previously operated atoxic goitre and suffering from a nephrotic syndrome and a chronic non-aggressive hepatitis revealed acantholysis preceded by eosinophilic spongiosis. Light microscopically the acantholysis is characterized by the occurrence of necrobiotic epidermal cells. No dyskeratotic cells are found. The mechanism of acantholysis depends on an exuberant elongation of desmosomes, leading to their disruption, *spongiotic acantholysis*. A conspicuous convolution of specific *tight intercellular spaces* is observed. Intracytoplasmatic occurrence of such tight intercellular spaces is seen in sections. Pseudomyeloid bodies of various types seem to be of significance. Particles closely similar to pox viruses are observed. The acantholytic process is compared with other acantholytic disorders in which the above-mentioned findings were not observed.

Key words: Acantholysis; Eosinophilic spongiosis; Pemphigus herpetiformis; Pseudomyeloid bodies; Virus-like bodies

The term pemphigus herpetiformis (PH) was first used by Jablonska et al. (12) to designate an acantholytic herpetiform disorder, a developmental stage of which was described as eosinophilic spongiosis by Emmerson & Wilson Jones in 1968 (9). In 1955 Flodén & Gentele (10) described a case of dermatitis herpetiformis with acantholysis. Winkelmann & Roth (18) reported two cases in which the patients had clinical features of both dermatitis herpetiformis and pemphigus. Doepfmer (8) described a similar case. Barranco (1) and later Knight et al. (13) have presented thorough studies on this subject, stressing the similarities to pemphigus. Several of these authors comment on the response of this disorder to sulphonamide derivatives. This therapeutical finding and the dynamic evolution of the disorder through an eosinophilic spongiotic stage to a clinical dermatitis herpetiformis-like and

a micro-morphologic pemphigus-like appearance justify the term pemphigus herpetiformis as suggested by Jablonska (12). It would therefore seem wrong to interpret eosinophilic spongiosis as heralding pemphigus foliaceus (5). Moreover, this is supported by the immunological findings of pemphigus and the therapeutical response as to dermatitis herpetiformis (6). Rather, it would appear more justifiable to recognize this disease as an independent entity and, consequently, use the term pemphigus herpetiformis.

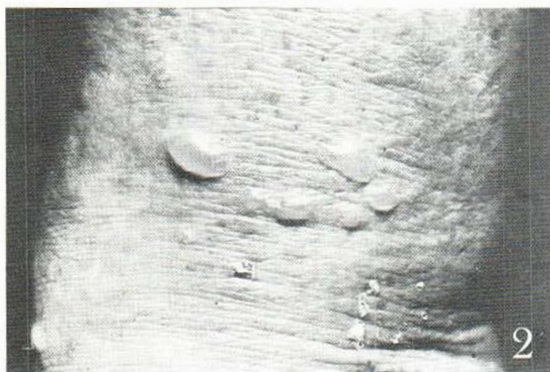
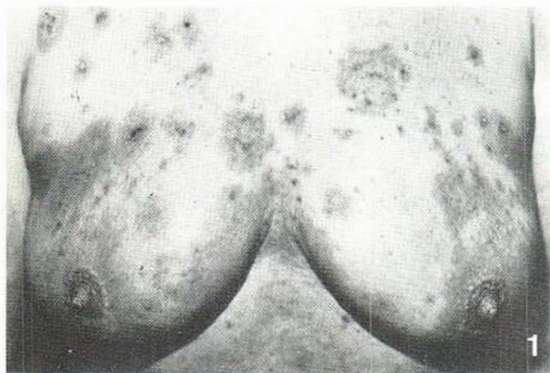
Light microscopical similarities to pemphigus vulgaris were first described by De Mento (7). This author also in the same paper presents two electron micrographs, mainly showing acantholytic separation of cells of the lower epidermis, though saying little about other details. Other light microscopical findings have also been reported (2). De Mento (7) seems to be the only author to perform electron microscopy, however summarily.

The present investigation was carried out to elucidate the mechanism of acantholysis of PH and to analyse other submicroscopic cellular changes in comparison with various acantholytic disorders.

MATERIAL AND METHODS

The specimens were obtained from a 64-year-old woman with no known heredity for autoimmune diseases. Six years ago she was operated on because of an atoxic goitre and has since then received substitution therapy with levothyroxine. The same year she developed a nephrotic syndrome on the basis of a membranous glomerulonephritis, though without positive serological findings, e.g. no

Abbreviations: D, desmosomes; IS, intercellular space; ME, melanosome; N, nucleus; TF, tonofilament bundles; T, tonofilament; TS, tight intercellular space; P, protuberance; PM, pseudo-myeloid body; VP, virus-like particles.



Figs. 1 and 2. Survey and detail pictures of the macromorphological lesions.

antibodies against glomeruli. Three years ago a diagnosis of chronic non-aggressive hepatitis was made. An autoimmune background of these manifestations cannot be rejected.

Skin lesions appeared about 2 months before biopsies were performed. The lesions were polymorphic and located over the shoulders, the back, arms and legs and were reminiscent of both erythema multiforme and urticaria as well as bullous pemphigoid and dermatitis herpetiformis. They consisted of annular and serpiginous infiltrated erythematous lesions, especially on the shoulders, erythematous maculae 3–15 cm large on the trunk and on the thighs, often with a raised indurated border and with central clearing and clear firm small vesicles on the arms and legs, both on healthy skin and on an erythematous base (Figs. 1 and 2).

Routine laboratory tests gave normal values but the differential count revealed a pronounced eosinophilia of 20–40%, and the total eosinophilic count was $1.8\text{--}3.5 \times 10^9$ cells/litre (normal range 0.04–0.44). Serum electrophoresis showed normal immunoglobulin values, including immunoglobulin E. The rheumatoid factor was missing. Results of immunological and serological examinations were in agreement with those in the pemphigus group.

Therapy and subsequent course. After completion of the investigations, a trial with Dapsone was made. On a

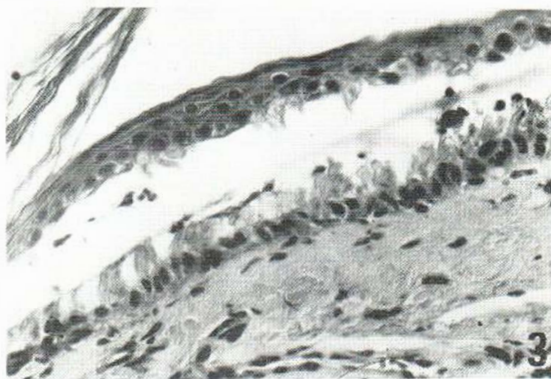


Fig. 3. Light microscopical picture presenting the micro-morphology. $\times 70$.

dose of 100 mg twice per day the patient's skin lesions improved; the infiltrated lesions became thinner and the vesicles and pruritus diminished. However, after a week she developed marked methemoglobulinaemia and increasing anginous symptoms. The dose was reduced to 100 mg per day and Prednisolone 40 mg was given. Due to the side effects, Dapsone was finally withdrawn the next week. The patient was then treated with immunosuppressive prednisolone and azathioprim. After one month on steroid therapy the patient was free of skin symptoms and after a further 3 months she was free from all symptoms on a maintenance dose of prednisolone 15 mg and azathioprim 50 mg.

Specimens for electronmicroscopy 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 Spurr and double-stained with uranyl acetate and lead citrate.

RESULTS

Light microscopy

The hematoxylin and eosin stained sections (Fig. 3) revealed a moderate subepidermally located inflammatory perivascular cell infiltrate rich in eosinophils and an evident spongiosis with a prominent exocytosis and an abundance of eosinophils. Later, intra-epidermal vesicles were revealed, located mainly suprabasally or with a basal delimitation at three or four cell layers. Especially in the suprabasal location, both the distal and proximal walls of the vesicles were partly populated by partly acantholytic necrobiotic cells. The necrobiotic cells

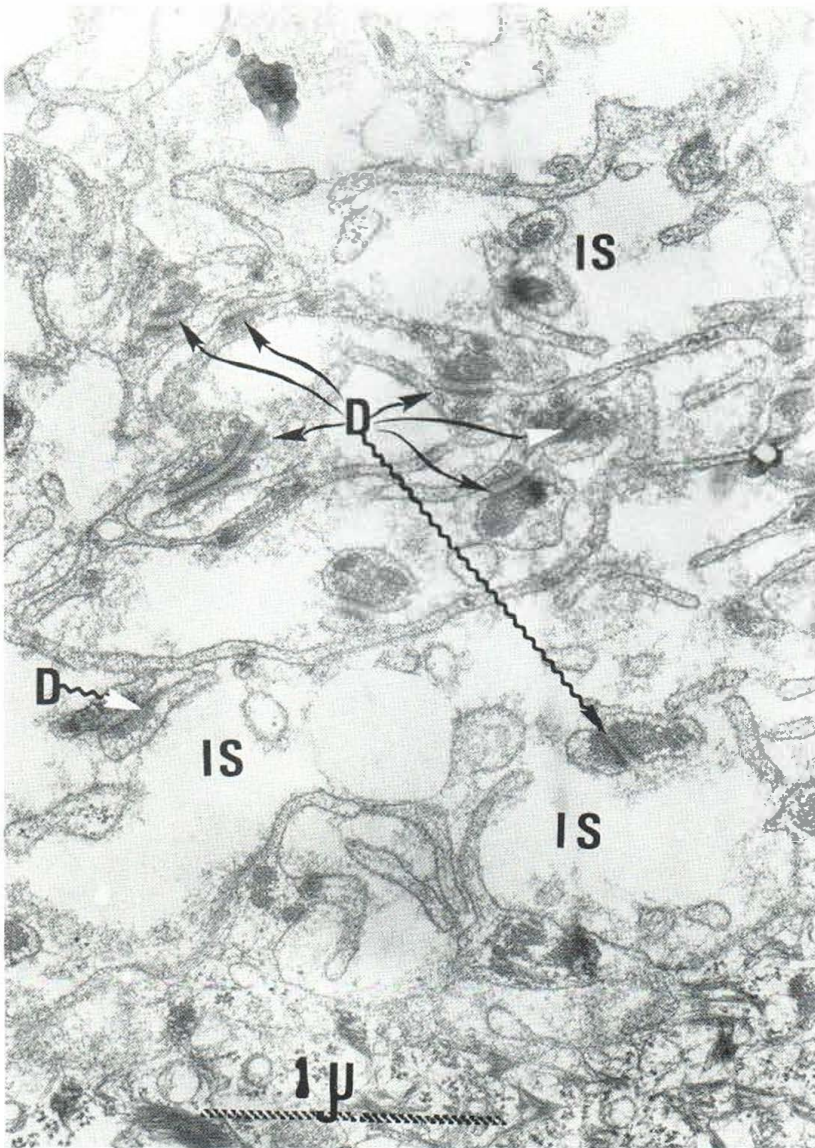


Fig. 4. Electron micrograph showing acantholysis. $\times 39\,780$.

of the proximal wall of the vesicles, seen light microscopically, have a preserved contact with the basal cell layer. In this acantholytic disorder such an abundance presumably reflects deficient nutrition. In the blister fluid, acantholytic—but not dyskeratotic—cells are observed to be intermingled with eosinophils. The corneal layer was orthokeratotic in correspondence to normogranulosis.

Electron microscopy

The dermo-epidermal border appeared highly folded, often forming comparatively extensive protrud-

ing dermal part. The anchoring fibrils seem to be well preserved. The lamina basalis, which was about 200–400 Å thick, followed the wavy contour of the dermo-epidermal border and showed comparatively rare but intermittent and clear discontinuities. Multifolding of the lamina basalis occurred. The electron-dense part of lamina basalis was separated from the epidermal basal cells by the less dense lamina lucida, varying moderately in thickness. Anchoring filaments were seen everywhere, traversing this space and connecting it with the basal cells. The half-desmosomes appeared to be

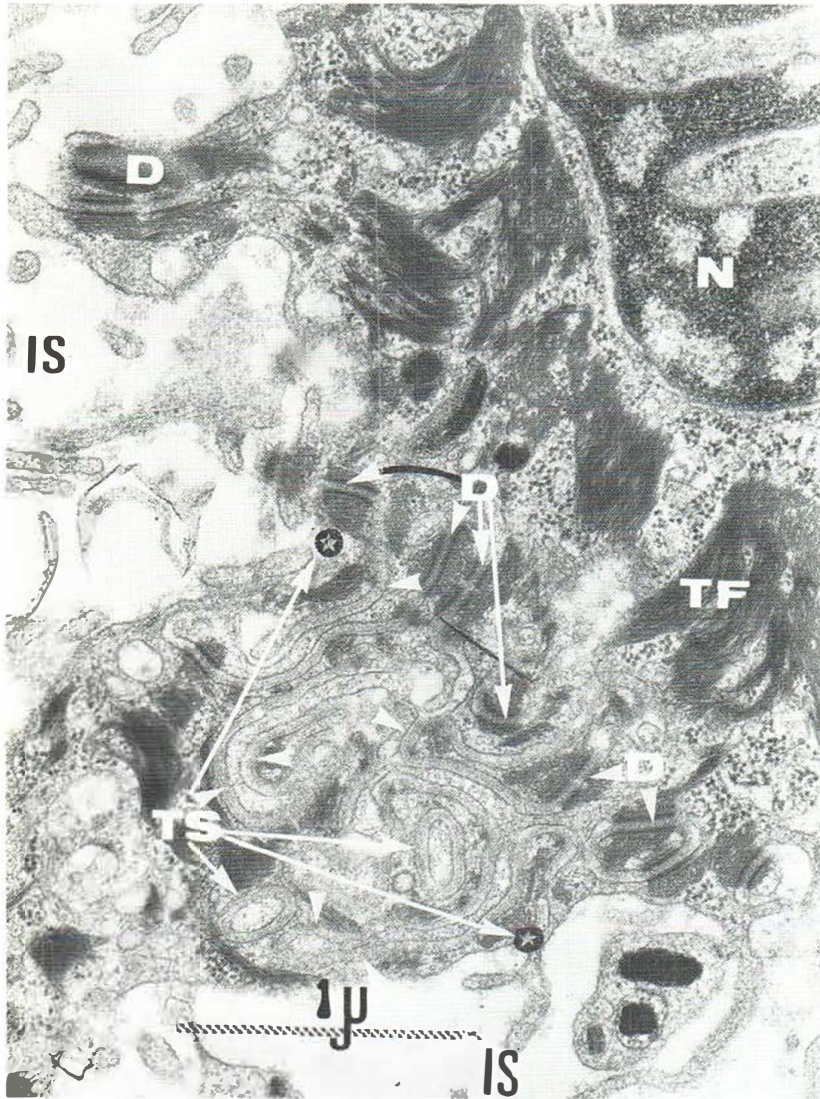


Fig. 5. Part of cells with the specific tight intercellular spaces. $\times 39\,780$.

submicroscopically normally organized with associated tonofilament bundles. The trimorphous unit membrane of the basal epidermal cells appeared normal. Thus the interfaces of the lamina basalis and the unit membrane limiting the basal parts of the basal epidermal cells with the normal half-desmosomes and the well preserved anchoring filaments showed no disintegration indicative of acantholysis or any similar process. However, in sections lying immediately above parts of the basal cells, portions of cells lacking the unit membrane but rich in ribosomes and granular material and having a tonofilamentous structure were observed.

Those cells undeniably correspond to the light microscopically observed necrobiotic cells. The neighbouring basal cells contained the usual number of cell organelles and a normal density of tonofilamentous material forming the tonofilament bundles. In some places there was a certain involvement of the desmosomes, commencing with considerable elongations of their cytoplasmic parts, heralding acantholysis. The spinous cells were abundantly observed in a dynamic process evolutionary of acantholysis (Fig. 4). This loss of coherence between the epidermal cells starts with an elongation of the cytoplasmic parts of the des-

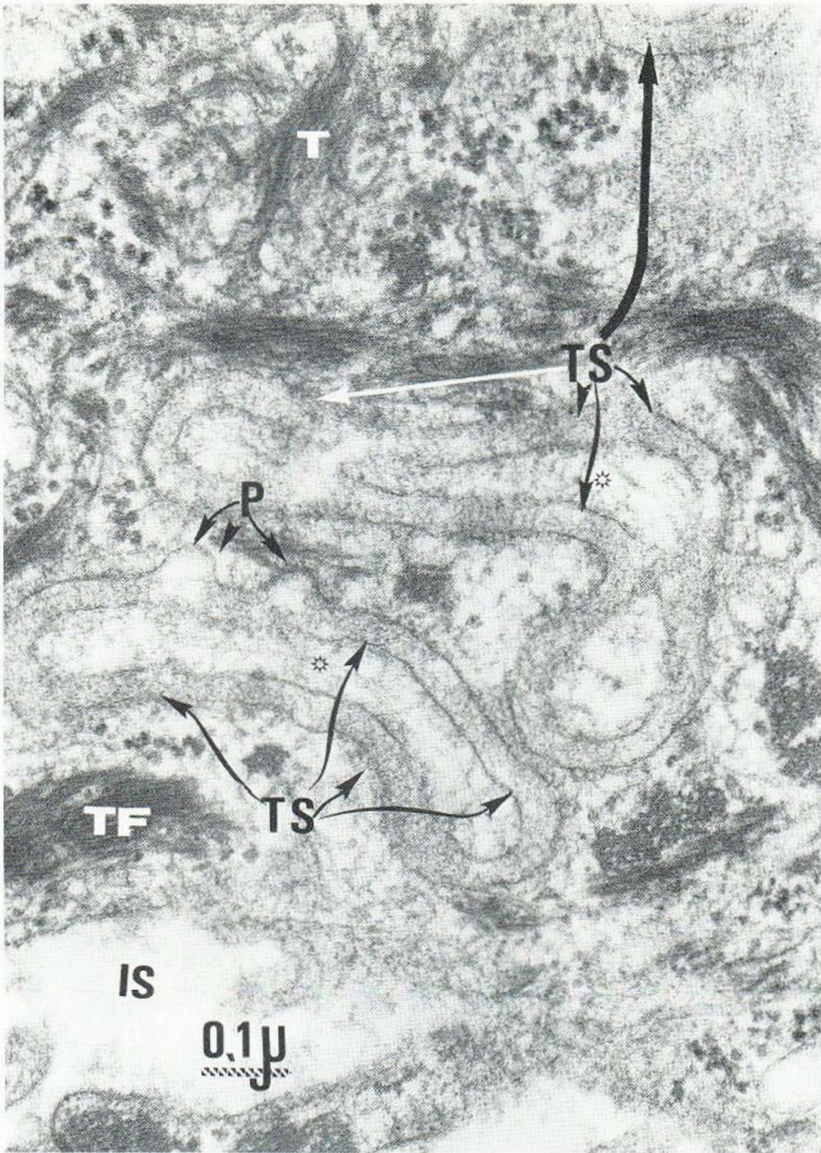


Fig. 6. Detail of an epidermal acantholytic cell showing a high magnification of annular tight intercellular spaces. $\times 112\,320$.

mosomes, in felicitous sections observable as serpentine configurations more or less occupying the intracellular spaces without any discernible damage of the desmosomal discs with their attachment plaques or the multilayered structure between. Often, however, the desmosome-tonofilament complexes are changed by a paucity or absence of tonofilaments associated with the attachment plaques. The final outcome of this ubiquitous protraction is a rupture of the cytoplasmic part of the desmosome, culminating in a significant form of

acantholysis. The abundance of this type of disintegration creates a pattern of microvillous projections in the intercellular spaces. These spaces are more or less widened and are to a small extent occupied by an amorphous material having the same electron-scattering property as intercellular cement-substance. The widened intercellular spaces are seen repeatedly, continuous with exceptionally tight spacial areas between adjacent cells in sections and always forming a pronounced convoluted appearance (Fig. 5). Those narrow, tight spaces are

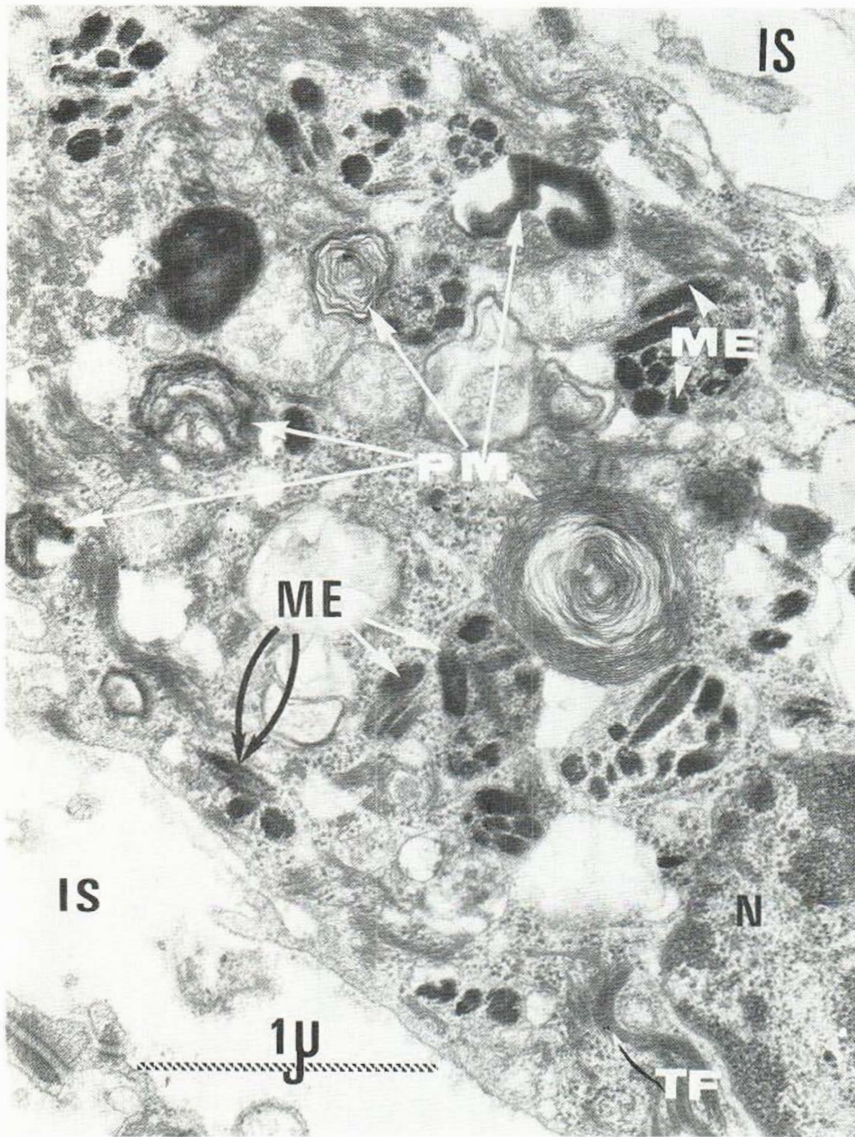


Fig. 7. Part of cytoplasm of an acantholytic cell with pseudomyeloid bodies. $\times 39\,320$.

constantly occupied by an amorphous material of the same electron density as the intercellular cement substance, presumably having the same character. The omnifarious convolute course of these specific narrow intercellular border zones is often complicated by breakage of the U-shaped loops of these tight border zones. Structures formed in this way are found incorporated within the cytoplasm, forming ovoid or circular or irregular ring-shaped configurations. Both the convoluted *tight intercellular spaces* and the intracytoplasmic incorporated parts are bordered by the unit mem-

brane. Furthermore, in continuous connection with those structures, desmosomes are often observed. The ubiquitous *tight intercellular spaces* form conspicuous protuberances (Fig. 6). These protuberances are always formed in the same manner, namely as centrifugally directed extrusions completely intracytoplasmically located, and bordered by the unit membrane. Desmosomes as well as ubiquitous tight junctions are—dependent on this special form of acantholysis—observed to be withdrawn into cytoplasm, often forming ring-shaped configurations.

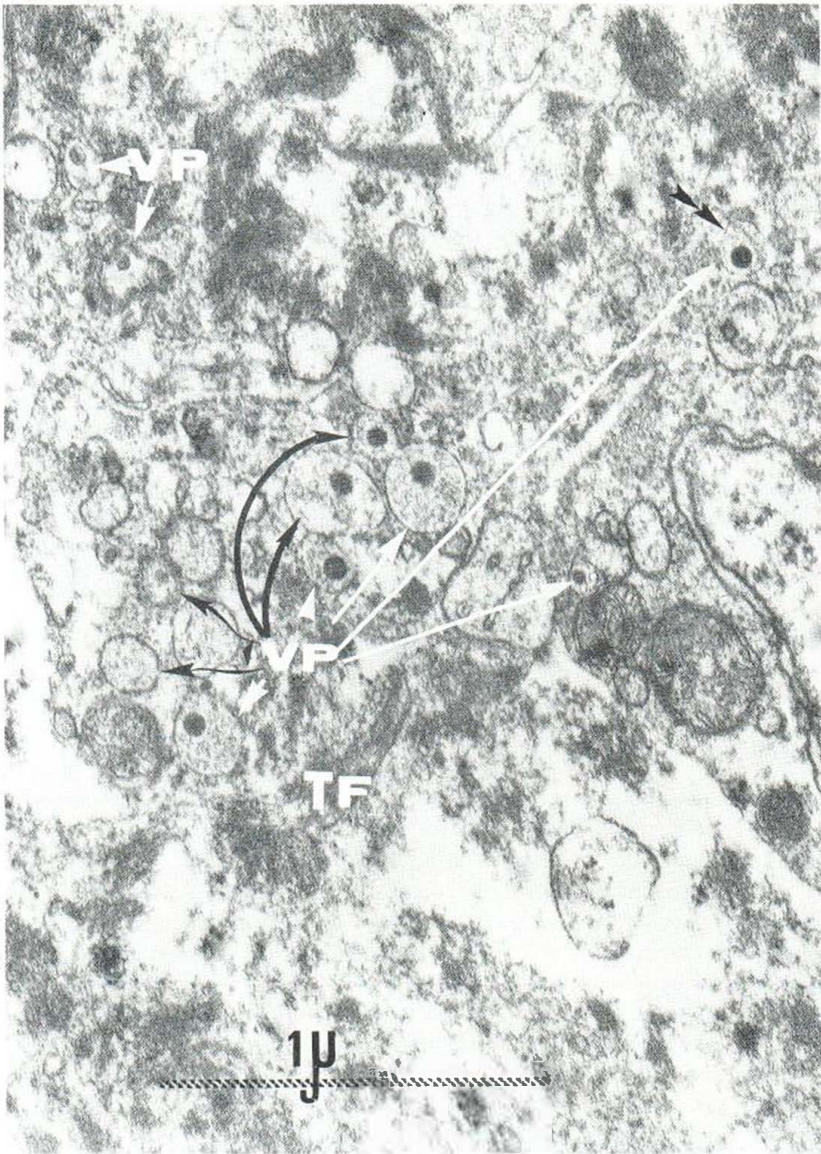


Fig. 8. Virus-like particles in cytoplasm. $\times 51\,480$.

The cytoplasm is rich in ribosomes, both single and in polysomal configurations and membrane-bounded. The endoplasmic reticulum is ubiquitous and quite hypertrophic, especially in cells in just perceptible vacuolation transformation. Mitochondria—and especially microtubules—are remarkably abundant. The cytoplasm of the acantholytic epidermal cells contain large quantities of the specific eosinophilic granules of eosinophilic leukocytes often observed in the acantholytic blisters and reflecting the extensive phagocytic property of the epidermal cells.

Pseudomyeloid bodies are observed in the cytoplasm of keratinocytes and very occasionally in macrophages of the dermis (Fig. 7). The substructure of these configurations is characterized by an outer zone of electron-dense concentric lamellae separated by lighter electron scattering spaces of uniform thickness diminishing successively in diameter and resembling the layers of myelin. The fifteen most peripheral lamellae are strictly concentric. The following twenty (approx.) lamellae are more loosely arranged and more irregularly shaped and have interposed light electron-dense

spaces of varying width. The very centre of the pseudomyeloid bodies seems amorphous even at extremely high resolution. Other types of pseudomyeloid bodies are observed which are rather irregularly outlined. This presumably indicates degeneration of the pseudomyeloid bodies with a gradual condensation of the lamellae arranged asymmetrically and culminating in strongly electron-scattering, irregularly shaped bodies. The cells containing pseudomyeloid bodies contain abundant melanosomes.

In every examined section one or two Langerhans' cells were observed, indicating an abundance of these cells or at least an increase in their numbers.

Several epidermal cells contained numerous particles haphazardly dispersed in the cytoplasm. The particles were round or slightly ovoid, measuring 270–330 nm × 190–220 nm. The particles were limited by a trimorphous membrane and contained an amorphous substance and a highly electron-scattering body, excentrically located.

DISCUSSION

The above-mentioned immunological and immunohistopathological findings in this case are in agreement with the results described earlier.

The light microscopical morphology consisting of the formation of intra-epidermally suprabasally located acantholytic vesicles reported by other authors seems not to have been completely characterized. The present findings reveal furthermore a characteristic occurrence of partly acantholytic cells presenting a necrobiotic appearance. These cells are also observed ultrastructurally as severely degenerated cells lacking a unit membrane, among other things. The abundance of these cells has not earlier been reported in any acantholytic disorders.

Regarding the mechanism of acantholysis in the pemphigus group, this does not yet seem to have been fully analysed or/and understood (15). Whatever the mechanism of acantholysis in this group may be, the formation of acantholysis in pemphigus herpetiformis appears to be of a quite particular character, separating this condition from other acantholytic disorders. In this acantholytic disorder the importance of spongiosis cannot be disavowed. Thus the term spongiotic acantholysis seems justifiable to designate the acantholytic mechanism of this disorder. A slightly similar phenomenon, acan-

tholytic spongiosis, is present in dyskeratosis follicularis (Darier).

In pemphigus herpetiformis there is not, however, the same submicroscopical intracellular organization of the withdrawn tonofilaments as in dyskeratosis follicularis or in the ordinary pemphigus group, in which diseases the tonofilaments are arranged perinuclearly. In the present acantholytic disorder there is no formation of keratohyalin as in pemphigus erythematosus, pemphigus brasiliensis (fogo selvagem) or dyskeratosis follicularis and very rarely in pemphigus chronicus benignus familiaris. The observed abundant microvillous formation of the surface of acantholytic cells in pemphigus herpetiformis is to a certain extent also described concerning pemphigus vulgaris erythematosus, pemphigus chronicus benignus familiaris, pemphigus brasiliensis and transient acantholytic disorder (Grover) (2, 3, 4, 15, 16, 18).

There is a paucity of Odland bodies in the acantholytic cells of the present disease, in contrast to pemphigus foliaceus, dyskeratosis follicularis and pemphigus brasiliensis. The acantholysis of pemphigus herpetiformis depends on an excessive elongation of the desmosomes, mostly combined with a withdrawal of the associated tonofilaments and culminating in the disruption of the elongated cytoplasmic part of one of two adjacent cells having a smaller part of the elongated cytoplasmic constituent continuous with the remaining desmosome remnants still in continuity with the other adjacent cell. These remnants are partly observed as microvillous projections into the intercellular spaces and also often seen to withdraw into the cytoplasm of this adjacent cell. In consequence of the dynamic evolution—and precursory to acantholysis—the intercellular spaces are considerably widened and contain patchy amorphous material mostly in contact with an epidermal cell. The character of this material is in concordance with the properties of the intercellular cement-substance. Presumably as a sign of pertinacity of preserving intercellular contact an abundance of *tight intercellular spaces* not earlier recognized are formed. These spaces are everywhere of the same width and completely filled with the amorphous cement-substance. They constantly present an excessively convoluted course. They are often observed in continuity with desmosomes. Through the highly convoluted course of these structures deep U-shaped loops are formed and those are often observed to be disconnected

from the other part of the tight spaces forming more or less irregular circular or ovoid intracytoplasmatic configurations. In the peripheral walls of such intracytoplasmatic circular or ovoid structures, small protuberances are formed, bordered by the unit membrane of the phagocytosing epidermal cell. Whether this formation reflects an active expansion of the circular or ovoid structures, or an intruding property of cytoplasm, is not clear. The diameter of the tight spaces is $360 \pm 17 \text{ \AA}$. The abundance of *tight intercellular spaces* reflects a tendentious endeavour to preserve the intercellular contact. The formation of the *tight intercellular spaces* of this peculiarity have not been observed in other acantholytic disorders. The *annular tight intercellular spaces* observed as loops detached from the tight intercellular spaces and apparently enclosed by cytoplasm may also be a transverse section through a loop in a plane perpendicular to the section. Other ring-shaped structures such as the annular nexus are much less frequently observed in the cytoplasm of acantholytic cells. The formation of an annular nexus is thought to be correlated with villous transformation of the cell surface and with an activated metabolic state. In this case, however, the villous formation has another explanation than metabolic activation, no less impeding the function of the nexus, i.e. indicating cellular degeneration. The ubiquitous vacuolization of cytoplasm of the acantholytic cells is also a sign of cellular deterioration. Abundantly lysosomes are found in cytoplasm, possibly cellular eradicators. The microtubules seem to be considerably increased in number, presumably reflecting an attempt to influence or normalize the intra-epidermal movement, or reflecting an association of cellular change of configuration by stabilizing cyto-skeletal elements that consolidate a shape alteration caused by cessation of intercellular contact.

Most of the acantholytic epidermal cells contain numerous vacuole-like structures, possibly only reflecting a cellular degeneration. At least some of them may, however, be pinosomes, in some places indicatively interacting with lysosomes and suggesting a saltatory movement.

Several of the acantholytic cells, Langerhans' cells and occasionally subepidermally located cells of unknown nature contain amphibological pseudomyeloid bodies more or less onion-shaped lamellar structures when transversely sectioned. The formation of myeloid figured structures is re-

garded as an expression of degenerative process in cytoplasm but they have never been found in acantholytic disorders. The origin of these bodies has not been elucidated but they might be generated from the endoplasmic reticulum or the Golgi apparatuses as well as through a paracrystallization of phospholipids. The occurrence of the pseudomyeloid structures has often been associated with various bacterial or viral infections. During an ultrastructural study on the skin of a patient with scleroderma, numerous myeloid bodies were found in the keratinocytes (17).

In this case, numerous particles have been found within epidermal cells. The appearance of those particles is very similar to the submicromorphology of certain pox viruses. In the present material these findings indicate the presence of virus-like structures whose significance one cannot elaborate on at the present stage, though a viral etiology cannot be dismissed.

The increased incidence of Langerhans' cells with a comparative abundance of specific granules seems to be of significance, but their biological function has not been elucidated by this ultrastructural analysis.

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