ULTRASTRUCTURAL AND AUTORADIOGRAPHIC INVESTIGATION OF THE SKIN IN ACANTHOLYTIC PEMPHIGUS

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The principal morphological manifestation of acantholytic (true) pemphigus is an acantholytic syndrome (acantholysis) with the subsequent formation of intraepidermal vesicles [2, 3, 6, 7]. The cause of the acantholysis is degeneration of epidermocytes with damage predominantly to intercellular junctions and with destruction of desmosomes. It has been suggested [12] that acantholysis in true pemphigus is due mainly to loss of intercellular cementing substance and not to destruction of desmosomes. These findings are in agreement with modern views on the autoimmune genesis of pemphigus, based on the action of antibodies against antigenic components of the intercellular substance of the epidermis [8, 11, 16]. Morphogenesis and clinical manifestations of true pemphigus are evidence of an extremely severe and diffuse lesion of the skin. However, under these circumstances plastic processes in the cells of the epidermis have not yet been studied (at the level of nucleic acid synthesis), although they determine their ability to regenerate.

This paper describes a comprehensive morphological investigation of biopsy specimens of skin in acantholytic pemphigus, using light-optical and electron microscopy and autoradiography.

EXPERIMENTAL METHOD

A histopathological investigation of biopsy specimens from the skin was conducted on 145 patients with true pemphigus aged from 25 to 83 years. Biopsy specimens of skin for electron microscopy and autoradiography were taken from 19 individuals (13 women and 6 men aged from 29 to 57 years). In nine cases the process was active and was accompanied by recent eruptions with numerous acantholytic cells in the contents of the vesicles, while seven cases were in the stage of remission: the cutaneous manifestations were absent or persisted in the form of healing erosions; three observations constituted the control group.

Tissue for light-optical investigation was fixed in 10% neutral formalin solution. Paraffin sections were stained with hematoxylin and eosin, by Van Gieson's method, and by the PAS reaction. Tissue samples for electron-microscopic investigation were fixed in a 4% solution of paraformaldehyde and postfixed in 1% OsO_4 solution. After standard treatment the tissue was embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on an LKB III Ultrotome. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined in the JEM 100B electron microscope.

The metabolic and proliferative activity of the skin cells was assessed by autoradiographic analysis [4]. Tissue samples immediately after the biopsy material was taken were introduced into flasks containing medium 199 and one of the labeled precursors. The intensity of RNA synthesis was studied by the use of ^{3}H-uridine in a concentration of 200 μCi/ml (specific radioactivity 29 Ci/mmole). DNA synthesis was studied by measuring incorporation of ^{3}H-thymidine, the concentration of which in the solution was 100 μCi/ml (specific radioactivity 48 Ci/mmole). Incubation was carried out at 37°C for 1.5 h. The tissue samples were then washed in three portions of Millonig's buffer (pH 7.2) and allowed to stand at 4°C for 12 h in a fresh portion of buffer in order to remove unincorporated labeled compounds. After fixation in 4% paraformaldehyde solution the tissue was treated by the standard method.

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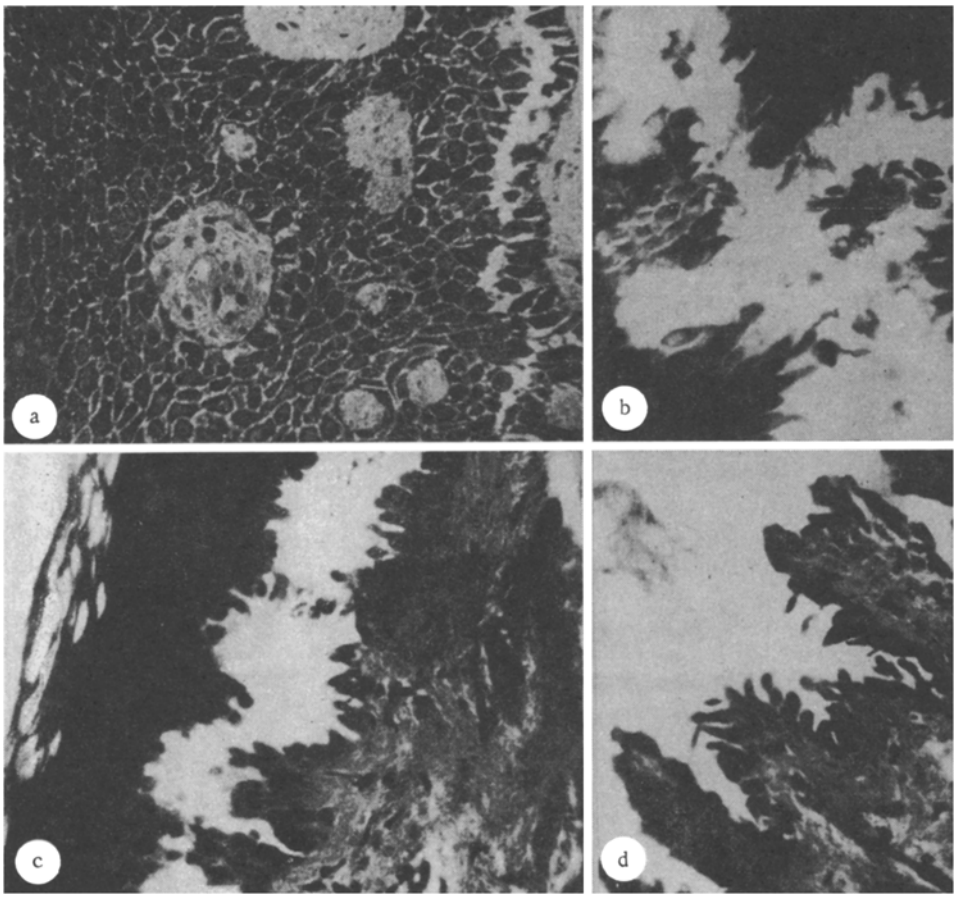

Fig. 1. Light-optical investigation of biopsy material from dorsal skin in acantholytic pemphigus; a) destruction of intercellular junctions in suprabasal layer with commencing acantholysis; b) marked acantholysis, preservation of chaotically arranged basal cells on basal membrane; c) intraepidermal vesicle containing acantholytic cells; d) complete desquamation of epidermis, skin papillae covered only by a layer of basal cells. a) Semithin section, stained with azure II. 700×; b, c, d) stained with hematoxylin and eosin. 280×.

The autoradiographic analysis was performed on semithin sections coated with type M emulsion. After exposure for 6 days at 4°C the autoradiographs were developed and the sections were stained with azure II. Quantitative analysis of the autoradiographs included calculation of the index of labeled cells and determination of the labeling density.

EXPERIMENTAL RESULTS

Light-optical investigation of biopsy specimens of affected areas of skin from patients with the active phase of true pemphigus revealed marked pathological changes in the epidermis and dermis. A typical feature was the formation of foci of multicentric acanthosis, accompanied by the formation of curiously branched papillary outgrowths into the dermis as a result of which the dermal papillae in some sections were visible in the depth of the epidermis. It must be pointed out that in paraffin sections, especially of the semithin kind, translucent or optically empty perinuclear zones, often extending over a considerable area of cytoplasm, and displacing the tonofibrils toward the periphery, could be seen in many epidermocytes of the stratum spinosum.

The most characteristic morphological picture of the biopsy material was destruction of the intercellular junctions, mainly in the zone of proliferating basal cells. In a wide area destruction of the intercellular junctions was observed in the suprabasal layer (Fig. 1a), with separation of the upper layers from the lower and the formation initially of slit-like defects, followed by vesicular. Total desquamation of the epidermis with erosion formation developed later (Fig. 1b, c). Some frequently chaotically arranged basal cells were still

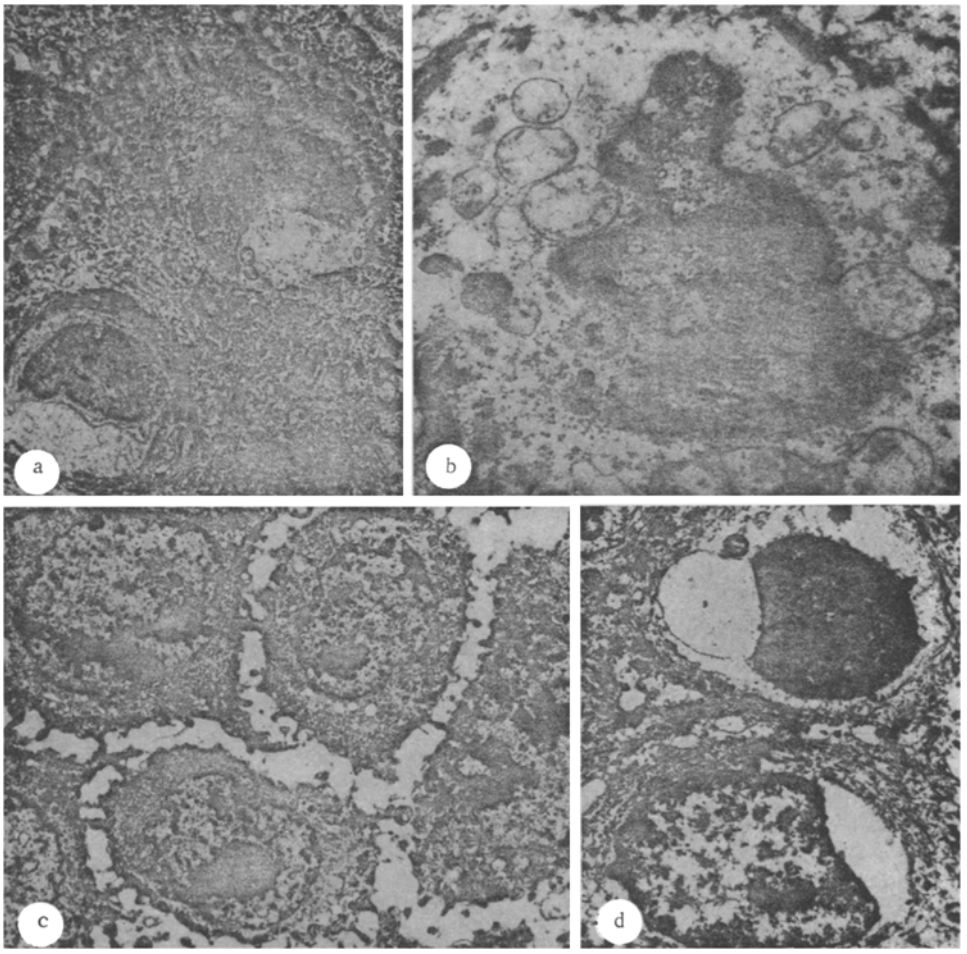

Fig. 2. Ultrastructural changes in epidermocytes in acantholytic pemphigus: a) coarsening and retraction of tonofilaments with formation of translucent perinuclear zone and partial necrosis of cytoplasm. 3300×; b) destruction of mitochondria in organelle-free perinuclear zone. 13,300×; c) marked changes in structure and localization of tonofibrils, reduction of intercellular junctions; increase in size of nucleoli and their frequent attachment to nuclear membrane. 2000×; d) collapse of nucleoli and pycnosis of nuclei in epidermocytes with signs of partial necrosis. 3300×.

present on the basal membrane (Fig. 1d). Their resistance was probably due to the fact that their attachment depended on anchoring filaments and not on the intercellular matrix [13]. Freely lying cells, highly heterogeneous in structure, could be seen in the newly formed defects of the epidermis. Some of them showed marked evidence of degeneration, whereas others had a well preserved nucleus, containing predominantly euchromatin, a clearly outlined nucleolus, and a well developed cytoplasmic reticulum, mitochondria, and keratohyalin granules. Heterogeneity of acantholytic cells also has been observed by scanning and transmission electron microscopy [9, 14],

Beneath the altered epidermis the stratum papillare of the dermis was edematous with signs of disorganization of connective tissue and disturbance of its staining properties, and sometimes with fibrinoid swelling and fibrinoid necrosis of the collagen fibers. The walls of the blood vessels of the superficial layer and the capillaries leaving it were infiltrated by lymphocytes, neutrophils, eosinophils, and macrophages, with spreading of the infiltration to the perivascular tissue and the formation of extensive and frequently confluent "cuffs." Inflammatory-cell infiltration in the stroma of the skin appendages was less marked. The reticular layer was moderately edematous and sometimes infiltrated by lymphocytes.

In the phase of remission the acantholytic syndrome was absent; the epidermis under the light microscope preserved its general structure plan with alternation of regions of acanthosis and thinning, although it still contained a large number of epidermocytes, in which the

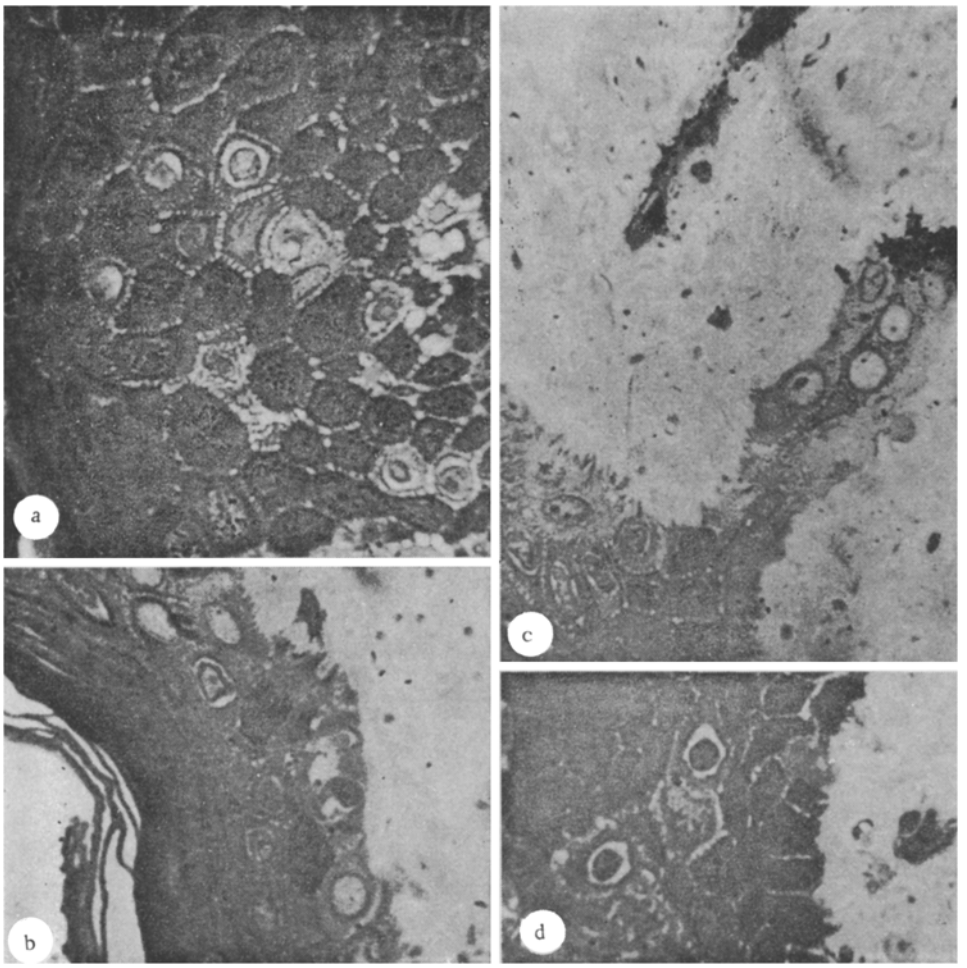

Fig. 3. RNA and DNA synthesis in skin cells in acantholytic pemphigus. a) Intensive RNA synthesis in cells of stratum basale and stratum spinosum of relatively unchanged skin; b) RNA synthesis in single epidermocytes; c) marked structural-metabolic heterogeneity of epidermocytes of stratum basale with respect to level of RNA synthesis, active incorporation of ^{3}H- uridine label by endothelial cells of blood capillaries; d) ^{3}H-thymidine label above nucleus of a cell in stratum basale. a, b, c) Biopsy material incubated with ^{3}H-thymidine; d) with ^{3}H-thymidine. Semithin sections, stained with azure II. 700×.

perinuclear zone was translucent, and sometimes optically empty. The cells themselves took up the stain poorly and often had the appearance of ghosts.

Electron-microscopic investigation of skin samples in true pemphigus, irrespective of the disease, regularly revealed uniform changes in the epidermocytes.

Epidermocytes of the stratum basale were mainly pale cells together with a few dark cells. The ultrastructure of the dark epidermocytes had features of biosynthetically active cells: many free ribosomes and glycogen granules were present in the cytoplasm, the small mitochondria were normal in structure, and tonofilaments were virtually indistinguishable.

The pale cells as a rule showed dystrophic changes with coarsening (fusion) and retraction of the tonofilaments, leading to the formation of a perinuclear zone with a low density of organelles. Mainly mitochondria with a lysed matrix and destroyed cristae were located in this zone, the number of free ribosomes was appreciably reduced, but outlines of the rough endoplasmic reticulum were seen. Parallel with these changes there was a decrease in the number of desmosomes between neighboring cells, whereas there was no significant change in the number of polydesmosomes, by means of which the basal cells were attached to the basal membrane.

Dystrophic changes in the epidermocytes in the stratum spinosum were the most marked of all. The most noticeable changes were found in the filamentous structures of the epidermocytes: as a rule their structural organization was grossly disturbed. Tonofilaments formed electron-dense tonofibrils, which were distributed haphazardly throughout the cytoplasm and often concentrated around the nucleus. However, the most typical change was retraction of the tonofibrils: the formation of a halo, without any fibrillary structures, around the nucleus (Fig. 2a). Destruction of cytoplasmic organelles was observed in the translucent perinuclear zone (Fig. 2b) and signs of partial necrosis appeared.

Progression of these changes led to the formation of clumps of tonofibrils which were more reminiscent of keratohyalin granules. Tonofibrils modified in this way were located exclusively at the periphery of the cells (Fig. 2c). Only solitary intercellular junctions between neighboring epidermocytes were observed in such cases. Some cells had lost all their junctions, but still preserved cytoplasmic outgrowths on their surface. Later, zones of depopulation were formed at the site of these cells.

Changes in the cytoplasmic organelles were accompanied by reorganization of the nuclear apparatus. The fraction of euchromatin was increased in the nuclei of epidermocytes of the stratum basale and stratum spinosum; hetero-chromatin was distributed marginally in the form of small clumps. The nucleoli were looped in appearance and enlarged. Considerable condensation of chromatin around the nucleolus was observed in epidermocytes with more marked dystrophic changes, and often such a complex was connected to their nuclear membrane (Fig. 2c); some collapsed nucleoli were seen (Fig. 2d).

The intercellular spaces in both layers were most frequently widened and electron-translucent; sometimes remnants of a finely granular ground substance were preserved in them.

The autoradiographic investigation revealed marked structural-metabolic heterogeneity of the epidermocytes. RNA synthesis took place only in single cells of the stratum basale (Fig. 3a, b); in one case RNA-synthesizing cells were recorded in the stratum spinosum. These cells were deeply stained with azure II and arranged in small groups or singly in the stratum basale. The labeling density varied considerably (from 5 to 30 grains of silver per cell) and the index of labeled cells was 4.5%. Intensive RNA synthesis was found in endotheliocytes of blood capillaries and in connective-tissue cells of the dermis, arranged around the capillary (Fig. 3c).

Proliferative activity of the epidermocytes in pemphigus was very low. Labeling with ^{3}H-thymidine was found very infrequently (Fig. 3d) and only in cells of the straum basale (index of labeled cells 0.67%). It is considered [15] that if the acantholytic cells were not labeled, this indicated their keratinization. In acantholytic pemphigus the endotheliocytes of the blood capillaries and the connective-tissue cells virtually did not synthesize DNA, unlike other pathological processes in the skin, when ^{3}H-thymidine was actively incorporated both by endotheliocytes of the proliferating capillaries and by perivascular fibroblast-like cells [1].

The principal ultrastructural changes in the epidermocytes in true acantholytic pemphigus are thus damage to the nucleolar apparatus, disorganization of the tonofilaments, destruction of desmosomes, and the almost total disappearance of the ground substance. Taken as a whole these ultrastructural changes in the epidermocytes [10] reflect disturbances of protein biosynthesis and they can be regarded as a manifestation of plastic insufficiency. The initial features of this process are to be found in cells of the stratum basale, but they are most marked in the stratum spinosum. At this stage of differentiation of the epidermocytes, metabolic processes decline sharply in the cells and structural protein synthesis is disturbed; as a result processes of physiological regeneration of tonofibrils and other cytoplasmic organelles are retarded.

On the whole, the data of light-optical, electron-microscopic, and autoradiographic studies of the skin show that the morphogenesis of acantholytic pemphigus can be regarded as a manifestation of a plastic deficiency syndrome. The picture of plastic insufficiency of epidermocytes is similar to the morphology of plastic insufficiency of cardiomyocytes when DNA-dependent RNA synthesis is disturbed [5].

However, disturbances of gene expression and the sudden reduction of protein synthesis in rapidly renewing cells such as epidermocytes and the epithelium of mucous membranes, lead to a marked process of desmolysis. Acantholytic pemphigus is thus a disease of systemic character [8] and it produces a diverse and serious clinical picture.

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ULTRASTRUCTURAL CHANGES IN THE MICROCIRCULATORY BED OF THE LUNGS IN ENDOTOXIN SHOCK

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Endotoxins are known to possess definite affinity for blood vessels and they damage capillaries through their direct toxic action on the endothelium [2, 3, 9]. The development of endotoxin shock (ES) is considered to be accompanied by disturbance of the systemic hemodynamics, by hypoperfusion of the internal organs, secretion of biologically active substances into the blood, and by disseminated intravascular clotting followed by fibrinolysis, and so on [4, 12, 13].

The reaction of the microcirculatory system to endotoxinemia is most marked in the lungs, whose vascular receptors are particularly sensitive to catecholamines and vasoactive substances [13].

Considering that the lungs are a target organ for introduced endotoxin, it was decided to undertake an electron-microscopic study of lesions of the microvessels in the course of ES.

EXPERIMENTAL METHOD

Experiments were carried out on rats, rabbits, and dogs. Rabbits and dogs were given an intravenous injection of 5 mg/kg of *Escherichia coli* lipopolysaccharide and rats were

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