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REGENERATION OF THE EPIDERMIS IN LYELL'S SYNDROME

A Light- and Electron-Microscopic Study

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Abstract. One patient with advanced manifestations of Lyell's syndrome was followed with repeated biopsies, for electron-microscopic examinations, from the damaged area and the regenerated epidermis. At an early stage after treatment had begun, the epithelium was formed by a few layers of cells without cornification. The special epidermal organelles essential for normal keratinization appeared about 14 days after the first symptoms of the disease. This was followed by a period of "overproduction" of cell layers with keratohyalin. Specimens taken $1\,^1/_2$ months after the first signs of the disease, when the patient had recovered, showed a normalized epidermis. A close correlation was found between the fluid loss and the degree of maturity of the epithelium.

Lyell's syndrome, or toxic epidermal necrolysis, described by Lyell in 1956 (14), is a serious dermatologic disease which proves lethal in 30–40 % of cases (4, 23). The etiology of the disease is still obscure, but in several studies a toxicoallergic mechanism has been regarded as the probable cause. In many cases the disease has appeared in connection with sulfonamide medication. Clinically, the disease is characterized by red efflorescences of the skin which may become confluent. Within the reddened skin areas blisters are formed to a greater or lesser extent, together with necrosis and detachment of the epidermis. The skin damage frequently causes very large losses of fluid, which require fluid compensation.

From a histopathologic point of view the course of the disease may be divided into two stages (8): 1) The necrobiotic phase, and 2) The reactive-reparative phase.

Light-microscopic investigations show that the damage is concentrated in the epidermis, which displays necrosis. Either the entire epidermis is involved in the formation of blisters between the epidermis and the dermis, or is affected by a superficial intra-epidermal necrosis without visible blister formation (14). The necrosis is either of a coagulative (1) or of a colliquative type, and first appears focally in the superficial or central epidermis (8). The nuclear changes are characterized by karyorrhexis or karyopyknosis (21). Keratohyalin granules are decomposed (7) and hydrodropic degeneration of the basal cells causes the formation of subepidermal microvesicles.

Braun-Falco & Wolf (8) have described ultrastructurally the changes that affect the epidermis during the necrobiotic phase from the stratum basale up to the stratum granulosum, with decomposition of the mitochondria, endoplasmatic reticulum and pronounced nuclear changes. Finally a cell death of varying extent appears.

The reactive-reparative phase often begins with a strong infectious reaction in the dermis, clinically characteristic of erythema; most frequently it occurs a few days after onset of the necrotic phase, if regeneration gets properly started. The entire regenerative phase is completed within 10 days (4). In this phase hyperemia is also manifested with neogenesis of capillaries in the dermis (8). The reactive-reparative phase has not been studied in detail from an ultrastructural point of view. Consequently, it would be interesting to have this phase more closely analysed and compared with epidermal regeneration of another type, especially with regeneration in connection with second degree burns.

Recently one patient with Lyell's syndrome has been treated at the Burns Unit of Karolinska sjukhuset. The clinical course of the disease in

Fig. 1 a. Specimen from an extremely red area taken 9 days after first symptoms of disease. In the dermis (*der*) there is severe edema with sparse fibrils. The basement membrane (*BM*) is thin but continuous. Only sparse tonofilaments (*to*) are observed but more frequent in basal cells (*BC*) than in other cells. Between the cells are large intercellular spaces (*is*). *des:* desmosomes; *m:* mitochondria; *N:* cell nuclei. × 11 300.

this patient has been reported previously (5, 6). The aim of the present investigation is to demonstrate the ultrastructural changes in the skin of the patient mentioned above, especially in respect of the regenerative course during the healing phase.

MATERIAL AND METHODS

A woman aged 21 years, with a clinical diagnosis of Lyell's syndrome, was followed by means of repeated small biopsies from representative skin areas. The biopsies were fixed in 1% buffered osmium tetroxide (19). Fixation was carried out in an ice-bath for 3 hours, after which the specimens were rinsed with saline, dehydrated

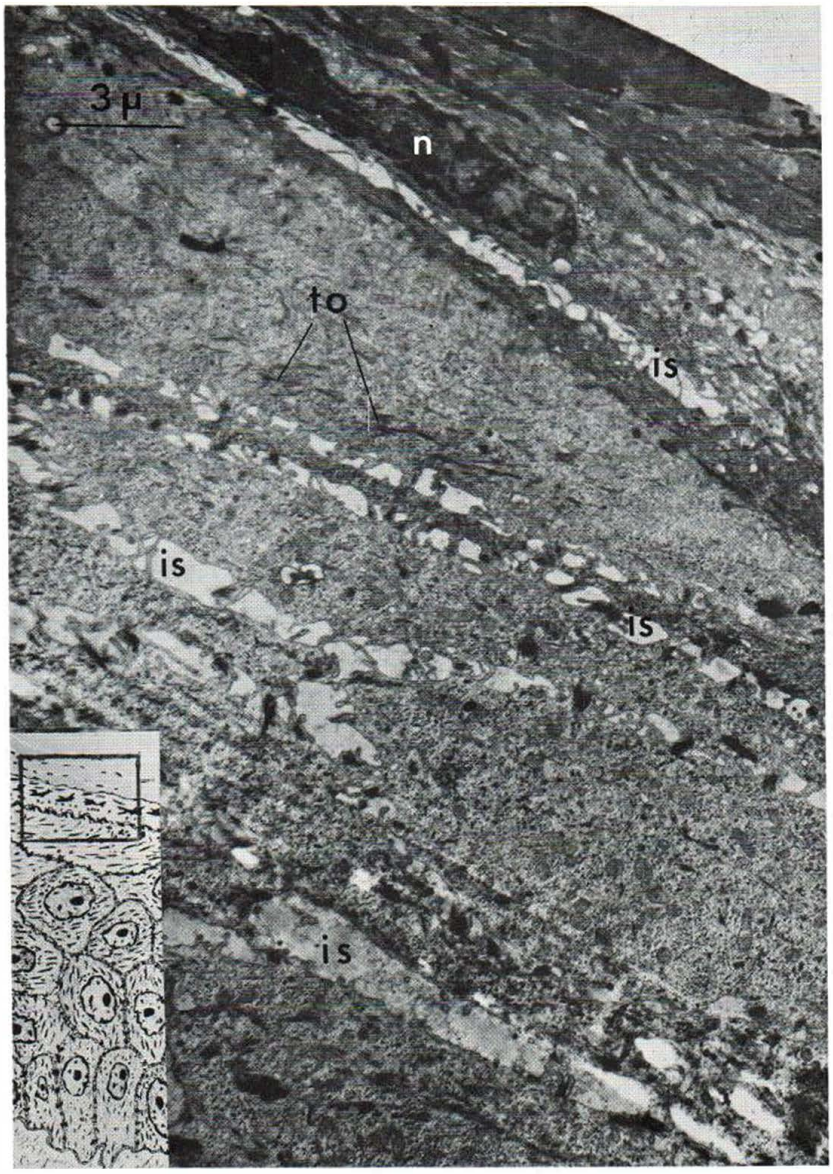

Fig. 1 b. Specimen from the same area as Fig. 1 *a*. The cells are flattened against the surface, with nuclei remnants (*n*). A few tonofilament bundles (*to*), but no keratohyalin or Odland bodies are observed. *is:* intercellular space. × 6 000.

in increasing concentrations of ethyl alcohol, and embedded in Epon (13). Sections, 1–3 µ in thickness, were stained with toluidine blue (21) and studied under a light microscope. Sections, about 300–500 Å in thickness, were stained with uranyl acetate (24) and lead citrate (18) and studied in Siemens Elmiskop I, a transmission electron microscope, at 80 kV with 2 000–40 000 primary magnification.

Case histories and macroscopic description

A previously healthy woman, who had been treated with sulfamethoxydiazinum for urinary tract infections, was admitted to the Burns Unit at Karolinska sjukhuset with widespread skin lesions covering about 80–100% of the body surface. The patient was a 21-year-old married woman in the fourth month of pregnancy who had been prescribed sulfa on account of urinary tract infection. After 10 days' treatment a rash developed on her arms and face with itching all over her body. Despite the withdrawal of sulfamethoxydiazinum and the administration of promethazin tablets, the skin changes increased. On admission to the Department of Dermatology her general condition was affected, with high temperature, redness and pea-sized papules over the entire body, and pronounced erruption of bullae on the face, chest and back. The process progressed until these bullae were present over a large part of the body. The patient was

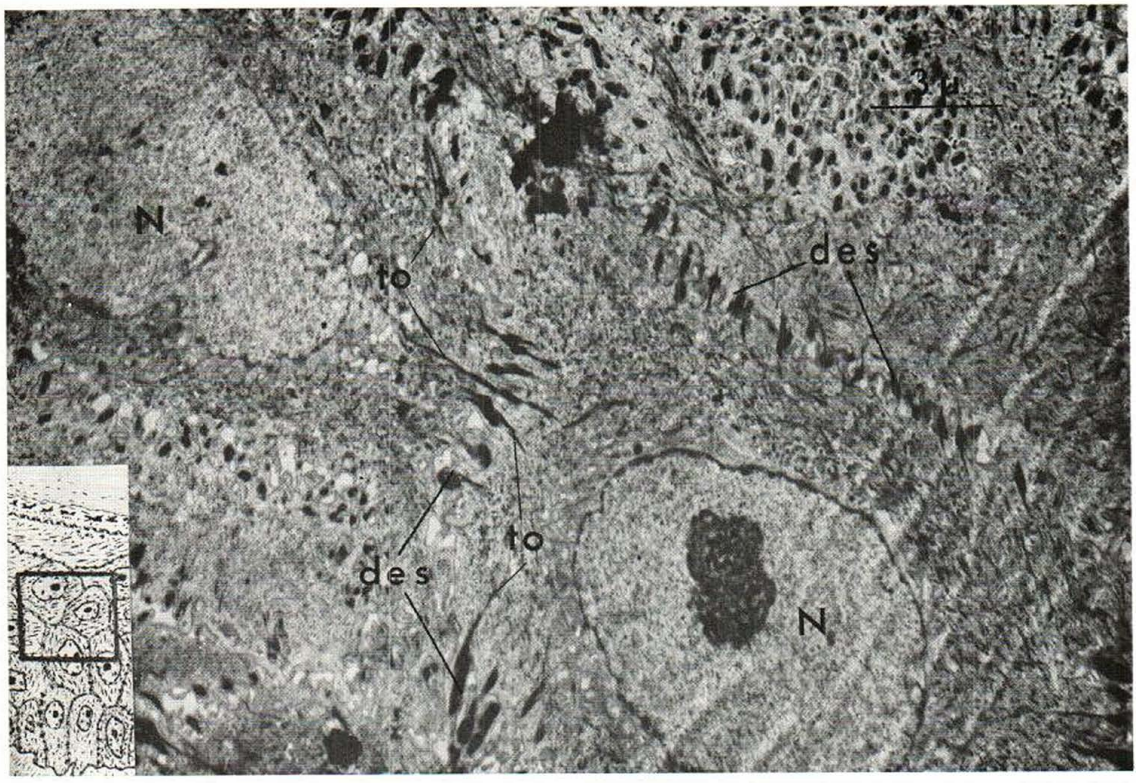

Fig. 2 a. Specimen from a dark red area at the same time as Fig. 1. Thin-walled nuclei (*N*) with diffuse chromatin are observed. Tonofilament bundles (*to*) are stretched out and connected to all the desmosomes (*des*). × 5 600.

treated with intravenous and peroral hydration, cortisone, antibiotics, and warm dry air (2, 3). After treatment for about 3 days there was distinct improvement with gradual normalization of the skin. Skin biopsies were taken 7, 12 and 45 days after admission to the Burns Unit. At the first biopsy the skin was characterized as consisting partly of normal skin areas, and partly of glaring red areas, especially some of the skin on the back, and also areas that had a brownish-red appearance. Specimens were taken from all these three areas. After a further 5 days the intensely red areas disappeared and were replaced by light red areas, and at the same time the dark brownish-red areas increased in size. The patient was discharged as healthy; and specimens were taken after another month, when, in part, entirely normal skin was observed, and in part, brownish-red pigmented areas from which specimens were taken.

ULTRASTRUCTURAL FINDINGS

Normal skin

Biopsies from the areas that seemed to be normal in colour and appearance did not show any ultrastructural changes. Normal cell layers and normal ultrastructure in the different cell layers could be demonstrated.

Specimens taken 9 days after first symptoms of disease

In specimens taken from an extremely red dorsal area, light microscopic surveys showed no signs of demarcation between the various layers of the epidermis. The epidermis was composed of a few layers without visible cornification.

Electron microscopic studies revealed a basal layer consisting of dense basal cells. The basal protrusions from the cells were few and short and the basement membrane was thin, but continuous. The area below the basement membrane was edematous, with very sparse fibrils. The nuclei were irregular and contained chromatin with rather small nucleoli. The cytoplasm was richly provided with ribosomes and mitochondria. Only sparse tonofilaments were observed, which formed thin bundles (Fig. 1 *a*).

The few layers of the stratum spinosum cells had intercellular protrusions with and without normal-looking desmosomes. The cells were separated by wide spaces which were bridged only

Fig. 2 b. Surface cells from the same specimen as Fig. 2 *a*. Towards the periphery, flattened cells with few cell organelles are identifiable. No Odland bodies or keratohyalin granules are observed. Tonofilaments (*to*), few and elongated, are spread throughout the cytoplasm. *is*: intercellular space. × 9 200.

by the intercellular protrusions. The nuclei contained fine granular chromatin and nucleoli. Like the basal cells, the stratum spinosum cells contained ribosomes partly attached to membranes which formed presumably cisterna and profiles of endoplasma. Rod-shaped mitochondria were spread throughout the cytoplasm. Bundles of short tonofilament were attached to the desmosomes, but practically no tonofilaments were found elsewhere in the cytoplasm. No signs of the formation of Odland bodies were observed anywhere in these stratum spinosum layers.

Some partly dehydrated cells with flat nuclei were found above the stratum spinosum layer. No keratohyalin was seen. Cell organelles were present even in these cells, and there was no stratum corneum (Fig. 1 *b*).

In specimens taken from a dark red area of the epigastric region *the light microscopic survey* showed that the epidermis was somewhat thicker than in the above-mentioned specimens. *The electron-microscopic study* revealed that the basal cells appeared to be somewhat denser than in the earlier stage. Short tonofilament bundles were attached to the desmosomes.

There were several layers of stratum spinosum cells with sparse tonofilaments in thin bundles between the organelles of the cytoplasm. The ribosomes formed clusters and groups. Tonofilament bundles were connected to all the desmosomes. Nuclear envelopes with diffuse chromatin were observed (Fig. 2 *a*).

No lamellated bodies, Odland bodies, or keratohyalin occurred. Towards the periphery, flattened cells were present where the intracellular protrusions were smaller, and the cell organelles were less identifiable. The flattened configuration of nuclei continued throughout the outermost dehydrated layer (Fig. 2 *b*).

Specimens taken 14 days after first symptoms of disease

Specimens taken from a light-red area of the left arm. *The light microscopic survey* demonstrated

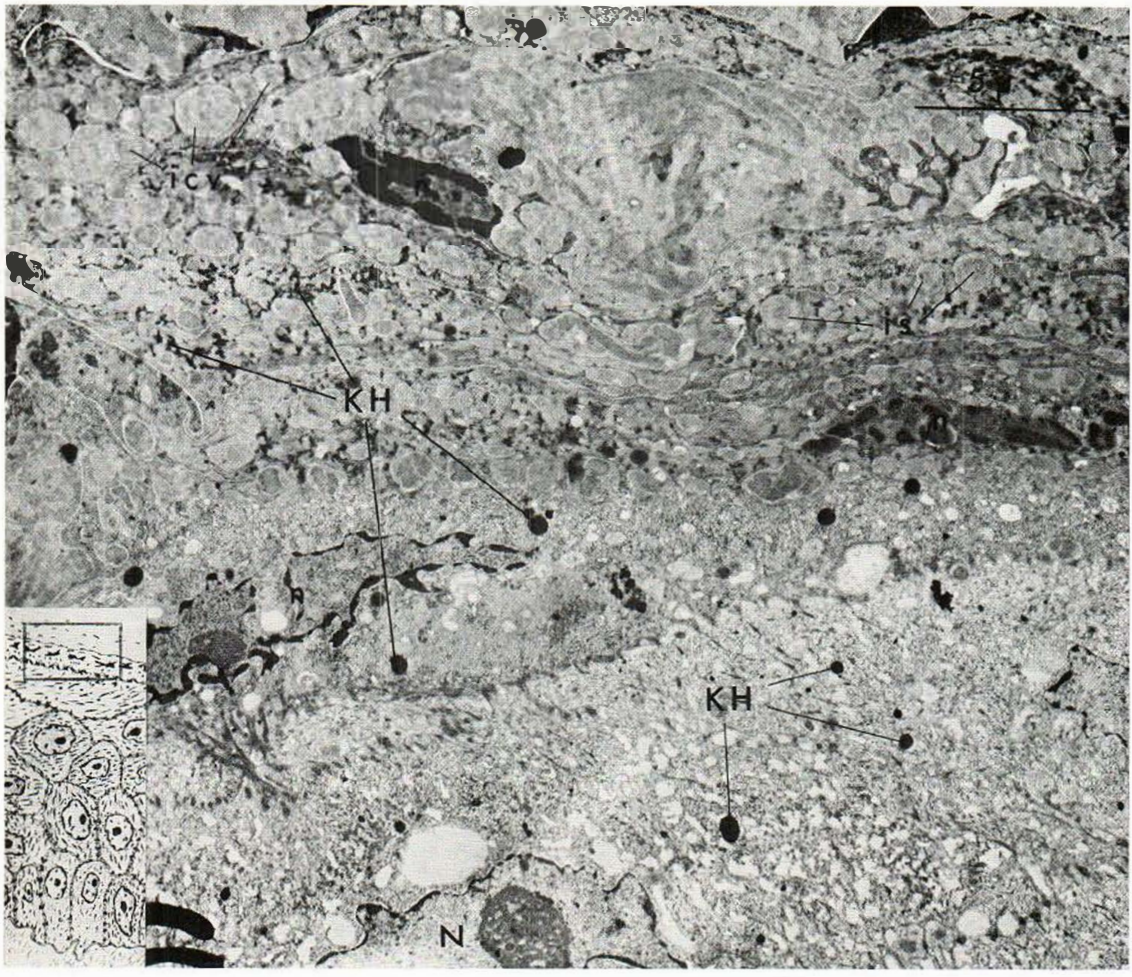

Fig. 3. Specimen from a light-red area taken 14 days after first symptoms of disease. The surface cells have flattened nuclei remnants (*n*) and some dense spots, possibly defective keratohyalin formation (*KH*), but few tonofilament bundles. Numerous intracellular vacuoles (*icv*) or globular formation up to 2 μ with hyalin material are observed. Intercellular space (*is*) with homogeneous material. × 4 700.

a thick epithelium with a dense covering layer, and no signs of cornification in the epithelium.

Electron microscopy showed that the basal cells and the stratum spinosum differed only slightly from the specimens taken after 9 days from the brown-red area. Sparse tonofilaments were observed in the basal cells and in the stratum spinosum cells. No Odland bodies were seen, but some dense particles, possibly keratohyalin granules, were observed. In this specimen, however, there was a comparatively thick layer on the surface of the epithelium of an irregularly layered substance containing nuclear and cytoplasmic remnants, and numerous globular formations with hyalin material (Fig. 3).

Specimens taken from the dark red area on the left thigh show, *in the light microscopic survey* of this material, a somewhat more normalized picture and a differentiation of basal cells, stratum spinosum cells, stratum granulosum and a stratum corneum.

The electron microscopic studies revealed the dense general appearance of the basal cells with a nucleus that had several invaginations and a dense chromatin network forming multiple dark bodies (Fig. 4 *a*). The basal protrusions were

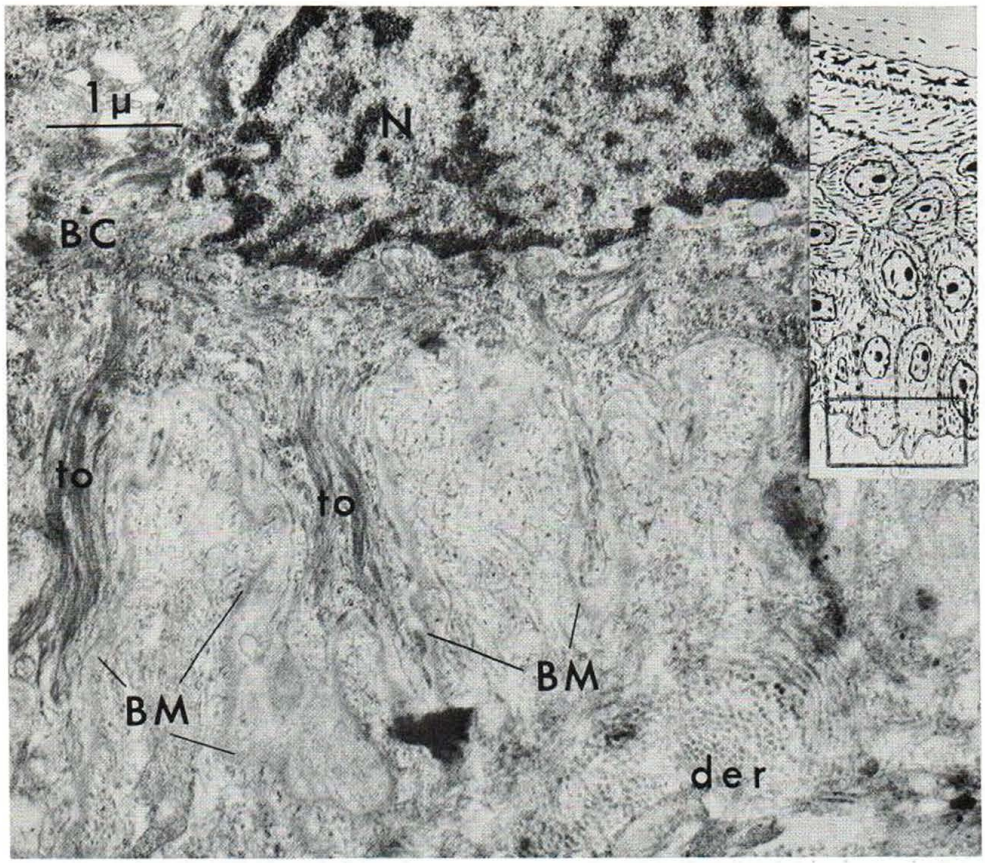

Fig. 4 a. Specimen from dark red area at the same time as Fig. 3. Basal cells (*BC*) show normalizing appearance, protrusions, tonofilament (*to*) and nuclei with dense chromatin network. The basement membrane (*BM*) is forming several processes into the dermis (*der*). × 18 000.

multiple and branched. The basement membrane was well developed, sometimes forming several processes, and provided with a rich system of aperiodic fibrils. The edema in the subepithelial tissue had been abated, and there were collagen filaments close to the basal cells whose cytoplasm contained a large number of clusters of ribosomes and a bundle of tonofilaments (Fig. 4 *a*).

Like the basal cells, the stratum spinosum cells contained large amounts of tonofilaments and, in the outermost layer of the stratum spinosum cells, Odland bodies were observed in close relation to tonofilaments and ribosome clusters (Fig. 4 *b*).

Several cell layers formed a stratum granulosum containing, besides the filaments, a large number of keratohyalin granules of varying size and shape (Fig. 4 *c*).

The stratum corneum was found to have normal properties.

Specimens taken 1 $^1/_2$ months after first symptoms of disease

Light microscopy showed that in specimens taken from a brownish area the epidermis had a normal appearance, with basal cell layers, stratum spinosum and stratum granulosum cells and a thin corneum at the surface.

The electron-microscopic pictures demonstrated that the epithelium had been normalized. The number of layers with keratohyalin granules had been reduced to about three, and there were no differences between the cytological appearance of the various cell layers in this epithelium and the epithelium taken from normal skin (Fig. 5).

DISCUSSION

Lyell's syndrome, toxic epidermal necrolysis, is a very serious disease forming more or less wide-

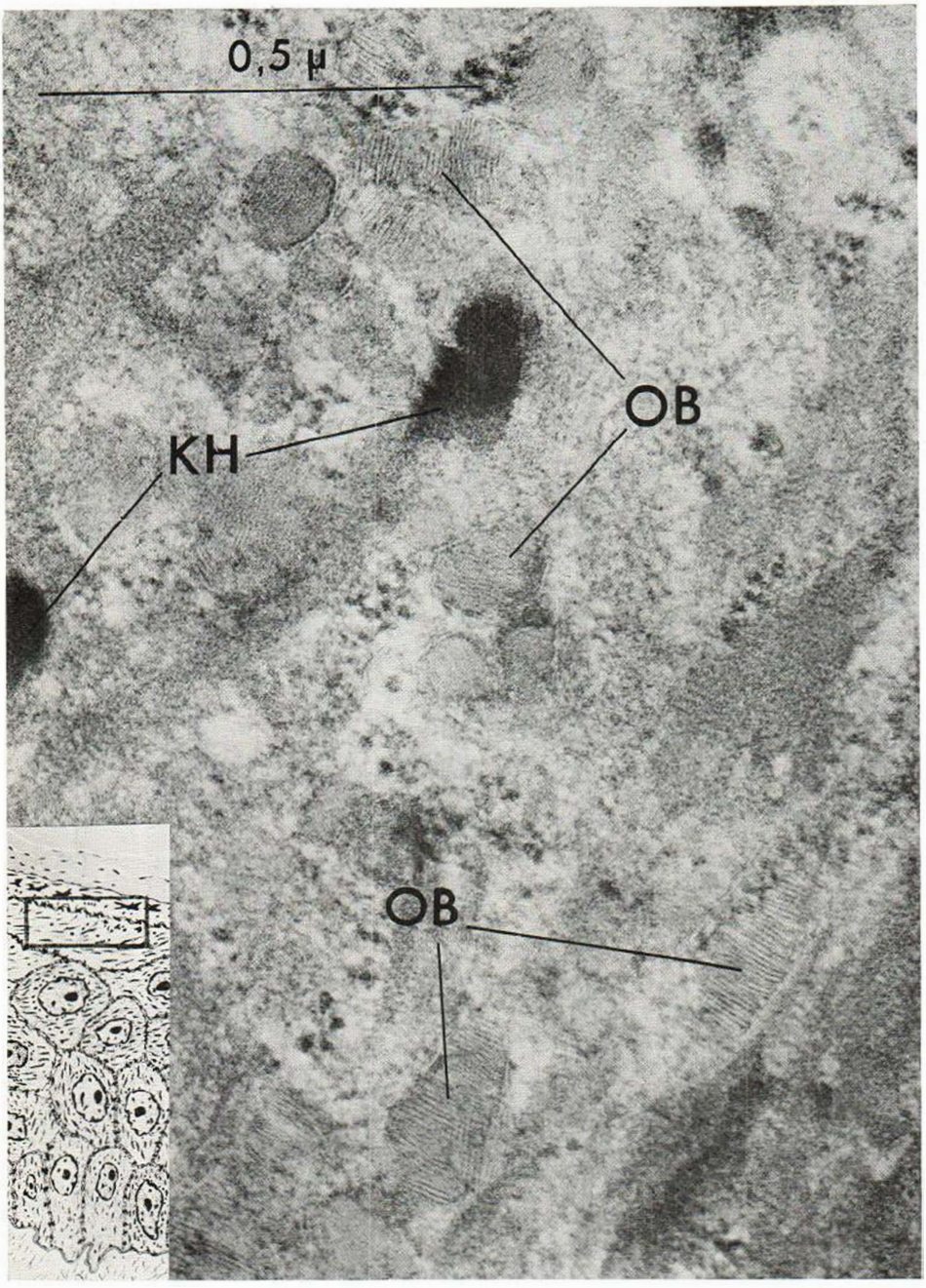

Fig. 4 b. Stratum granulosum from the same specimen as Fig. 4 *a*. Now several keratohyalin granulae (*KH*) and Odland bodies (*OB*) begin to appear. × 120 000.

spread areas of necrotizing epidermal epithelium, causing severe fluid loss and serious systemic deterioration. The disease is characterized by a stage of epidermolysis and reconvalescence (8). Epidermal manifestations develop from various lesions, with large blisters and more or less complete detachment of the epidermis. The disease develops for 3–5 days, after which, if the patient

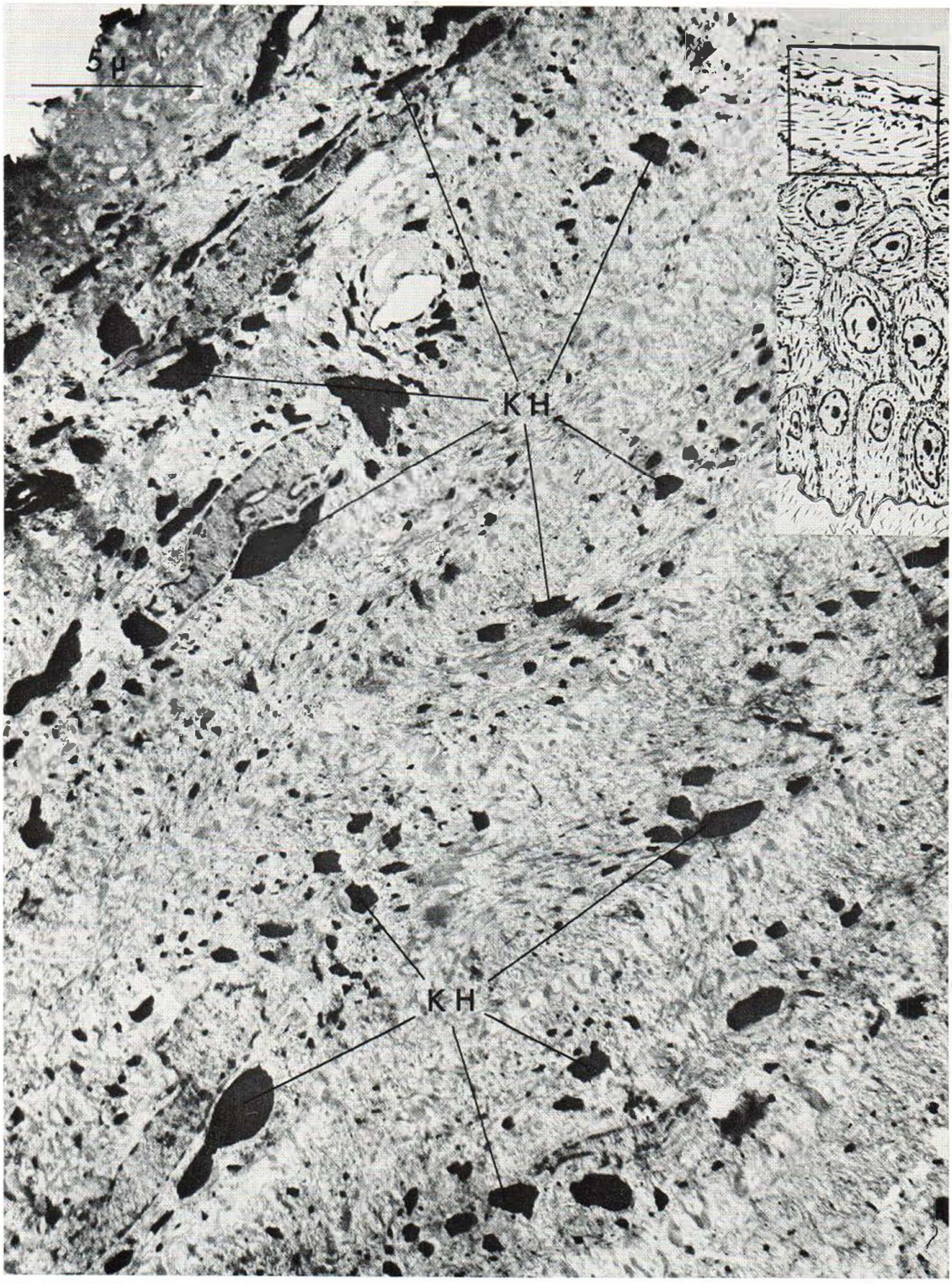

Fig. 4 c. Stratum corneum in the same specimen as 4 *a* and 4 *b* shows six layers with keratohyalin granules. × 5 300.

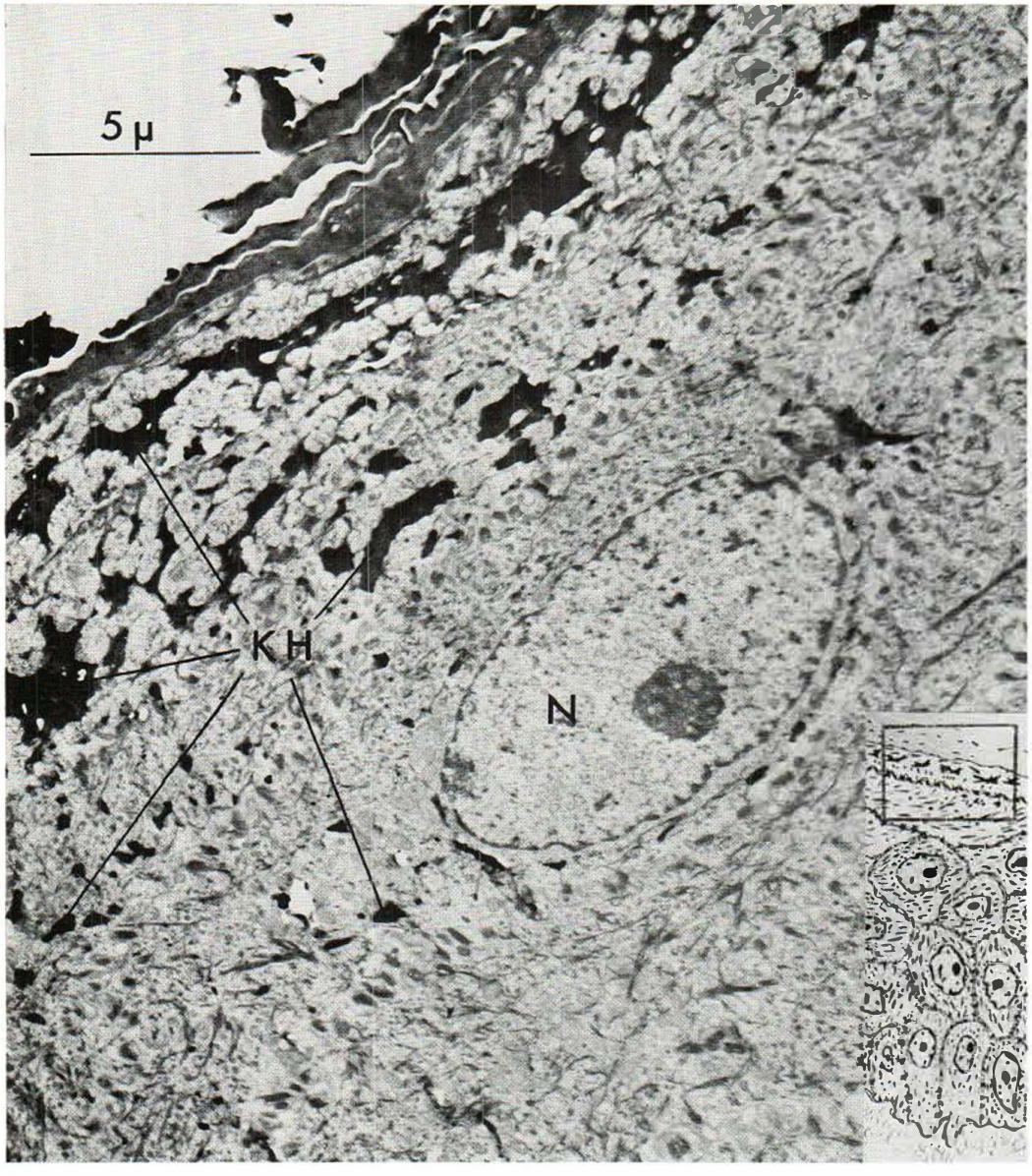

Fig. 5. Specimen from a brownish area 1 ½ months after first symptoms of disease. Stratum granulosum has now reduced to keratohyalin (*KH*) in three layers. × 6 000.

survives, recovery occurs. The specimens from the present patient were taken during the recovery phase. The epithelial structures observed at the various regenerative stages closely resembled these stages in a second degree burn (10) and in human split skin autografts during healing (11). The earliest stage was seen in a specimen taken from an extremely red erythematous area 9 days after the first symptoms of disease; there were then no signs of keratinization, only regeneration of the basal cells and the stratum spinosum cells of the epithelium. It seems likely that there generation of this epithelium proceeds from sweat-gland ducts and hair follicles as has been observed in regeneration after second degree burns. It is possible, however, that some of the

basal cells might remain and start to regenerate the epithelium, similar to the process observed in split skin autografts (11). Clinical and microscopical findings indicate that stages of both skin degeneration and regeneration occur simultaneously. In the present study two specimens taken on the ninth day show slightly different regenerative phases. The second specimen which was taken from a reddish-brown area had more epithelial layers and incipient tonofilament bundles as the first sign of keratinization. The same applies to specimens taken after 14 days, where the specimen from the reddish-brown area differed only slightly from the specimen taken on the ninth day of the disease, as the other specimens taken from the dark brown area showed advanced keratinization with the appearing of Odland bodies, keratohyalin granules and even a normal keratin layer.

From the present study it is apparent that the regeneration in the skin in Lyell's syndrome follows very closely the pattern observed in regeneration after second degree burns (10). Thus, in the early stages there is a high degree of activity in the regenerating cells and the appearance especially of a large number of free and membrane-bound ribosomes and mitochondria, indicating intense metabolic activity in the cells. The tonofilaments appear first in the periphery of the cells in close proximity to the desmosomes. At this early stage the cytoplasm contains only extremely thin filaments located in the periphery of the cell. On the 14th day of the disease, when the Odland bodies and the keratohyalin reappeared, the most serious period of the disease had already passed. Profuse fluid loss occurred even in areas where the first stages of regeneration were discernible, but was considerably reduced as soon as the first signs of keratinization became visible in the epithelium, with the tonofilament bundles, Odland bodies, and the first keratohyalin granules.

Intra- and intercellular spherical vacuoles with obvious hyaline material have been observed in the surface cells during the regeneration (Fig. 3). The diameters range from 0.3 μ to 2 μ and have light homogenous appearance. Intracellular spherical or oval lacunae about 0.5 μ have been observed in stratum granulosum and stratum corneum after ultraviolet irradiation (16) in psoriatic skin (12, 15, 9, 22) and epidermal regeneration after second degree burns (10). Nix (17) suggested that it can be a complex glycolipid or mucopolysaccharide, precursor of the interfilamentous cement. As in ultraviolet-induced epidermis this epidermis has accelerated renewal time and the increase in keratinization is associated with rapid cellular differentiation. This phenomenon might be a sign of defective incipient keratinization and more injured here than in second degree burns.

It is evident that during the regenerative stage there is a certain "overproduction" of layers containing keratohyalin granules which, however, become normalized within a few weeks. It is also evident that in areas where the cells have survived the toxiconecrotic stage, it is possible for skin regeneration to occur, with the formation of a normal epidermis. A similar "overproduction" of keratohyalin granular layers has been observed in epidermal regeneration after second degree burns (10) and in human split skin autografts (11). This is probably due to hyperemia in the dermal layer.

It is important to point out that at the same time as real cornification appeared with keratohyalin and Odland bodies (Fig. 4 *b, c*) and other cell organelles, the evaporative loss decreased (5).

It is likely that the prognosis is dependent not only on the possibility of applying treatment for severe fluid loss, infection and a general reaction during the first days, but also, to a greater extent, on whether sufficient remnants of epithelial cells are available in areas of the body from which regeneration can be obtained. Perhaps one of the reasons why some patients survive this disease even when very large areas of the body are involved is that various parts of the skin are affected at somewhat different times. Thus, some parts of the skin may already be regenerating while other parts are at a severely necrotic stage.

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