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ULTRASTRUCTURE OF PALMAR AND PLANTAR PITS IN BASAL CELL NEVUS SYNDROME

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Abstract. Transmission and scanning electron microscopy were used for the study of palmar and plantar pits from two patients with the basal cell nevus syndrome. Under transmission electron microscopy the horny cells of the pits appeared normal, but severe pathological changes were observed in the remaining epidermal cell layers. These changes were different from those observed in basalioma. They consisted of lumpy tonofilament bundles and keratohyalin granules containing numerous white tiny spots. Large keratinosomes were present containing homogeneous lucent material. Cells with dark cytoplasm and a few tonofilament–desmosome complexes were observed. Scanning electron microscopy revealed a defect shaped like a somewhat irregular cone. The cornified cells of the wall were arranged in irregular tattered layers. The bottom of the pit was irregularly elevated over an annular furrow in the periphery.

The basal cell nevus syndrome (BCNS) is a genetic entity characterized by multiple cutaneous basal cell carcinomas, odontogenic keratocysts and skeletal abnormalities. Although primarily involving the skin and skeleton, several other organs may be affected including the nervous system and endocrine organs (4, 5). Pits of the palms and soles are hallmarks of the syndrome (3, 7, 8, 18). Development of basal cell carcinoma in palms and soles is rare, but development from pits in patients with BCNS has been observed (6, 17, 18).

The present paper is concerned with a study of the ultrastructure of pits in patients with basal cell nevus syndrome, and includes results obtained by transmission as well as scanning electron microscopy.

MATERIAL

Two patients with BCNS were studied.

Patient 1. A 26-year-old male with several manifestations of the syndrome including multiple jaw cysts, bifid spine and ribs, microsella turcica, calcification of falx cerebri, mental retardation, multiple basal cell carcinomas, fibropapillomas, and a dermoid cyst.

Patient 2. A 16-year-old male, previously published (16) as patient P. H., had jaw cysts, basal cell carcinomas, pes planus, and retention of the testes.

Both patients had several skin-coloured, pin-point sized pits in the palms and soles. Ham-coloured depressions or teleangiectatic macules, seen in some patients with BCNS by other authors (1, 5), were not observed.

METHODS

Punch biopsies (3 mm diameter) were obtained from palmar pits in patient 1 and from plantar pits in patient 2 using ethyl chloride for local freezing anesthesia.

The specimens for *transmission electron microscopy* were fixed in 6% glutaraldehyde solution in veronal acetate buffer (pH 7.2) with 7.5% sucrose at 4°C for 3 hours, and cut into little blocks. The tissue blocks were then washed overnight in ice-cold veronal acetate buffer (pH 7.2) with 7.5% sucrose and fixed again with 1% osmic acid in veronal acetate buffer (pH 7.2) at 4°C for 1 hour. After washing the tissue blocks in the same buffer, dehydration was carried out in a series of alcohol solutions of increasing concentration, whereafter the blocks were embedded in Epon 812. Ultrathin sections were cut by ultramicrotomes (Reichert OM2 and LKB) and stained with uranyl acetate and lead citrate. A Siemens electron microscope (Elmiskop 1A) was operated with double condensors at 80 kV.

For *scanning electron microscopy* the specimens were fixed in the same glutaraldehyde fixative as described above. After washing, the specimens were dehydrated in a series of alcohol solutions of increasing concentration up to 95% and further dried in vacuo. The surfaces of the dried specimens were coated with a 300 Å thick layer of carbon and gold. A JEOL scanning electron microscope (JSM-U3) was operated at 15 kV with a tilting angle of 45°.

RESULTS

The ultrastructure of the pits from the 2 patients was almost identical although the pathological changes were most pronounced in patient 1.

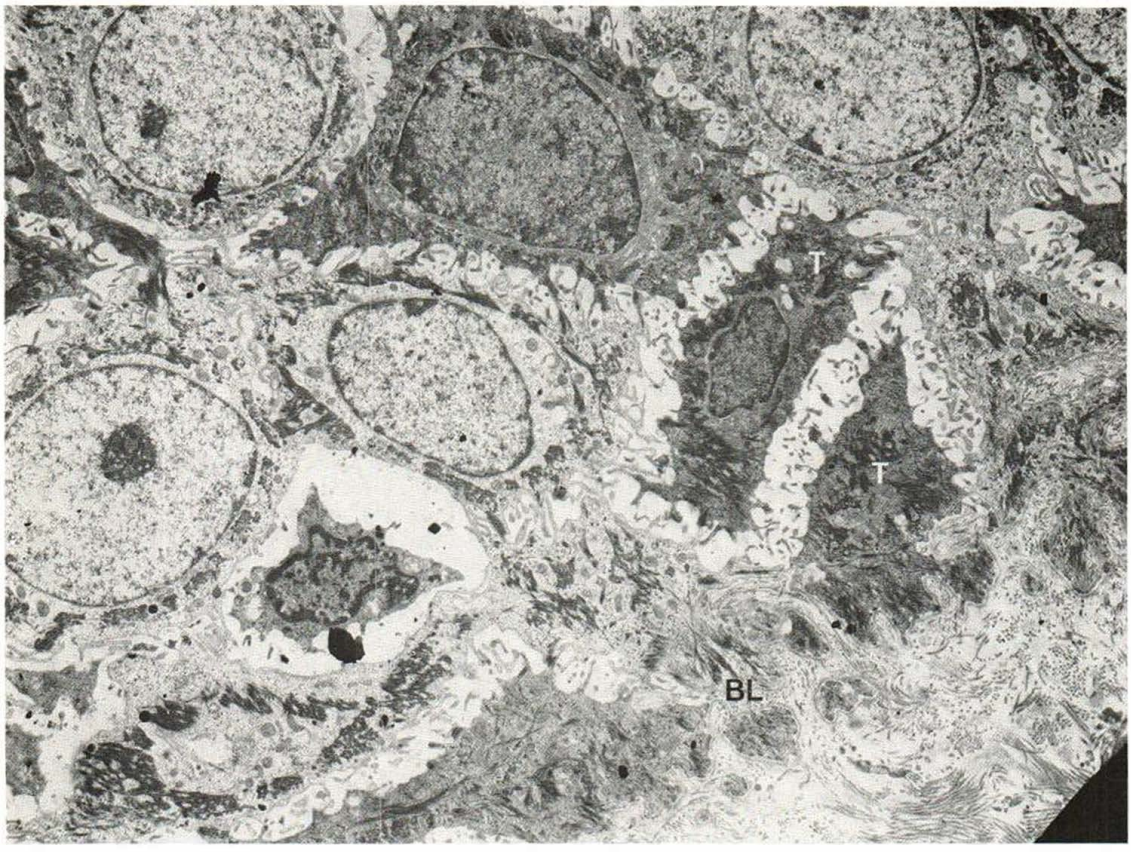

Fig. 1. In the wall of the pit, dark cells with dense cytoplasm and tonofilaments (*T*) aggregated in lumpy masses are seen. Light cells with large spherical nuclei are seen mixed with the dark cells near the basal lamina (*BL*) (× 4 600).

Under *transmission electron microscopy* the outer surface of both the bottom and the walls of the pits showed several layers of horny cells in parallel arrangement. The horny layer consisted of broad, flat cells containing dense, roughly packed keratin fibrils. The cells were closely connected by interdigitations and dense plates.

The cells of the Malpighian layer revealed either light or dark cytoplasm. In the walls of the pit both types of cells were seen (Fig. 1), whereas, in the bottom, dark cells were located in the lower part and light cells in the upper part of the epidermis (Fig. 2 *a*). Thick bundles of tonofilaments were seen in the cell periphery leaving a free perinuclear space in both types of epidermal cells through the entire epidermis (Figs. 1, 2 *a*, 2 *b*, 3). The bundles were thickest in upper Malpighian layer. Large keratohyalin granules containing numerous white, tiny speckles were found inside the bundles of tonofilaments in the upper Malpighian layer (Fig. 3). Keratinosomes were numerous in the upper Malpighian layer (Fig. 4). Most of them were spherical containing homogeneous, lucent material. Keratinosomes showing distinct, lamellar patterns were seldom found. Both types of keratinosomes discharged their contents into the intercellular spaces of the granular cell layer. Desmosomes were distinct (Figs. 2 *a*, 3, 4), and numerous cytoplasmic protrusions were also seen in the intercellular spaces (Figs. 1, 2 *a*). In the basal cell layer, thin tonofilament–desmosome complexes with narrow desmosomes were seen crossing the wide intercellular spaces (Figs. 1, 2 *a*). Around the dark epidermal cells, the intercellular space was wide, containing numerous finger-like protrusions of epidermal cytoplasm, while the complexes were few (Fig. 1, 2 *a*). In many places basal cells were separated from

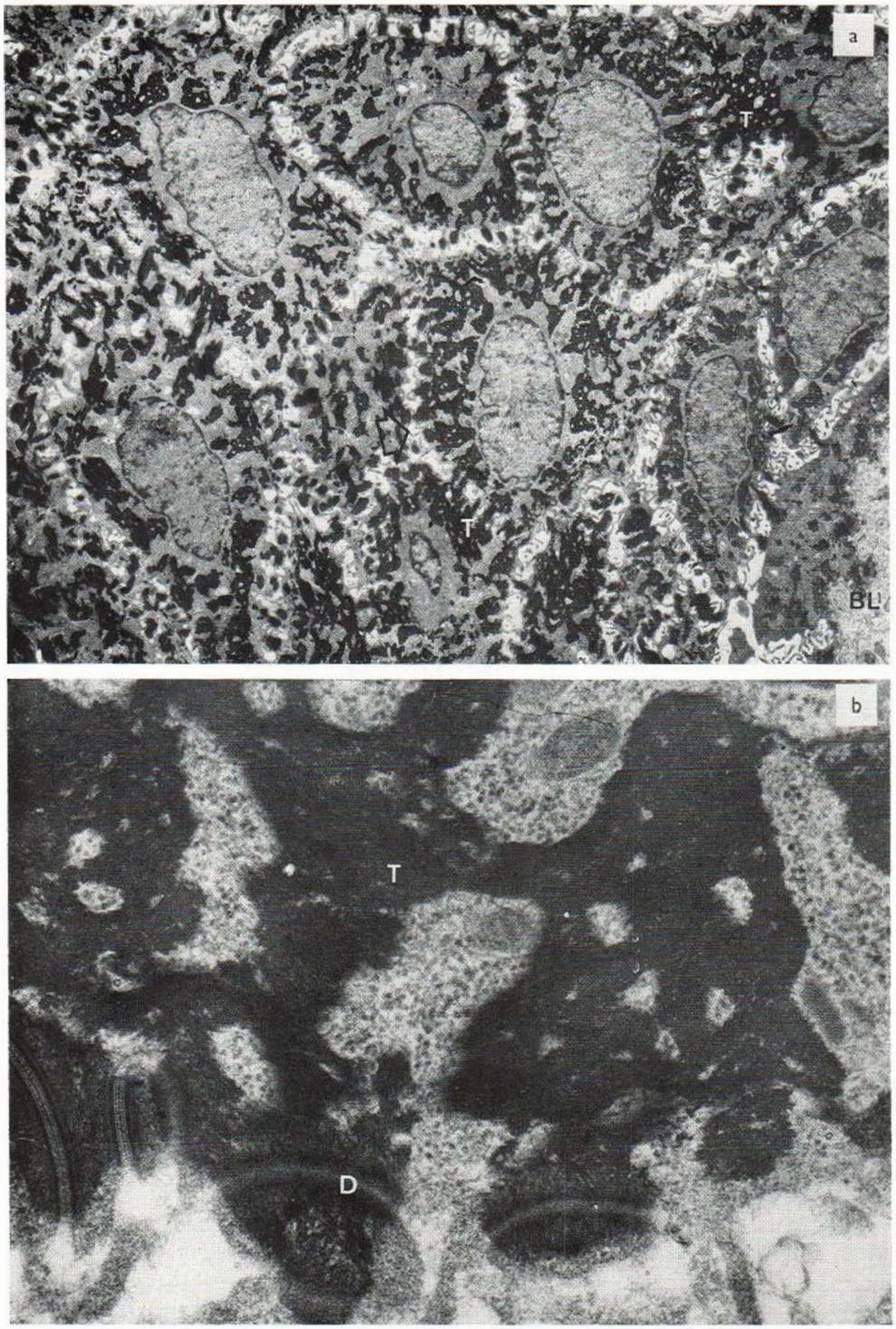

Fig. 2 (*a*) Dense lumpy masses of tonofilaments (*T*) are seen in the bottom of a pit, leaving a free perinuclear space. Basal lamina (*BL*) (× 4 000). The arrow-pointed area is presented in Fig. 2*b*. (*b*) Lumpy bundles of the tonofilaments (*T*) and desmosomes (*D*) (× 40 000).

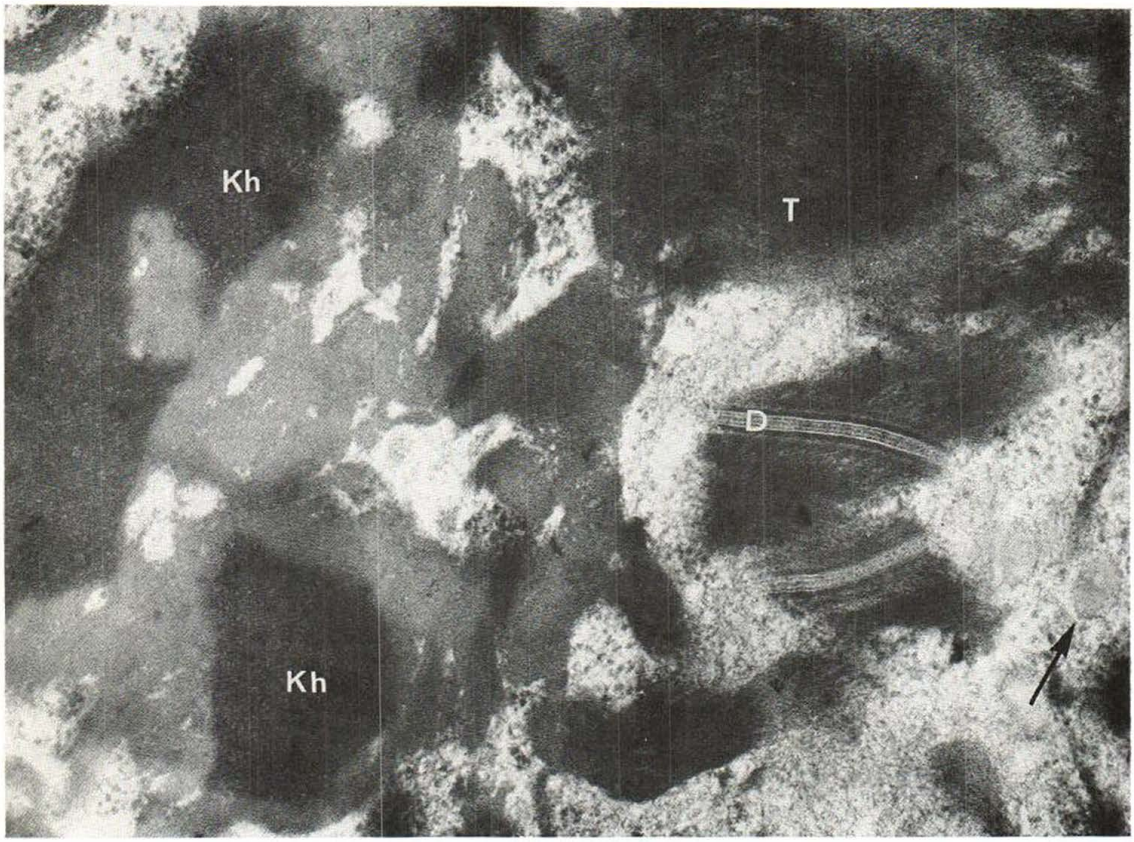

Fig. 3. The granular cell layer in the bottom of a pit. Dense irregular masses of keratohyalin (*Kh*) are seen inside the bundles of tonofilaments (*T*). *D* is a normally laminated desmosome, and a keratinosome is indicated by an arrow. Keratohyalin granules appear with numerous white, fine speckles (× 46 000).

the basal lamina by widened, subepidermal spaces (Figs. 1, 2 *a*).

The basal lamina was regular, band-shaped (Fig. 1) and the upper part of dermis appeared normal (Fig. 1).

Scanning electron microscopy revealed a slightly irregular bottom of the pits (Fig. 5) consisting of cornified epidermal cells. The walls of the pits were almost circular, slightly inclining, and shaped as a somewhat irregular cone. Around the pits a circular mound-like formation was slightly elevated above the surrounding surface. The surface of the mound and the walls of the pit were scaling with the scales directed towards the centre of the pit.

Under higher magnification (Fig. 6), the exfoliating scales gave the wall an irregular, tattered appearance. Some layers were protruding into the pits overhanging the layers beneath. The pit bottoms were elevated over an annular furrow in the periphery (Fig. 6). There was no obvious columnar arrangement in the stacking of cells. The individual cells (Fig. 7) of the wall were flat, polygonal, with abundant well-developed villi. Most of the cell surface was free and only a small part seemed attached to neighbouring cells.

DISCUSSION

Lumpy masses of tonofilaments have been described in Darier's dyskeratosis (12), Hailey-Hailey's benign familial pemphigus (19) and congenital ichthyosiform erythroderma (9). In Darier's dyskeratosis and Hailey-Hailey's pemphigus, the tonofilaments aggregate around nuclei, forming "corps rond". However, in the pits, the lumpy masses were compact and located in the

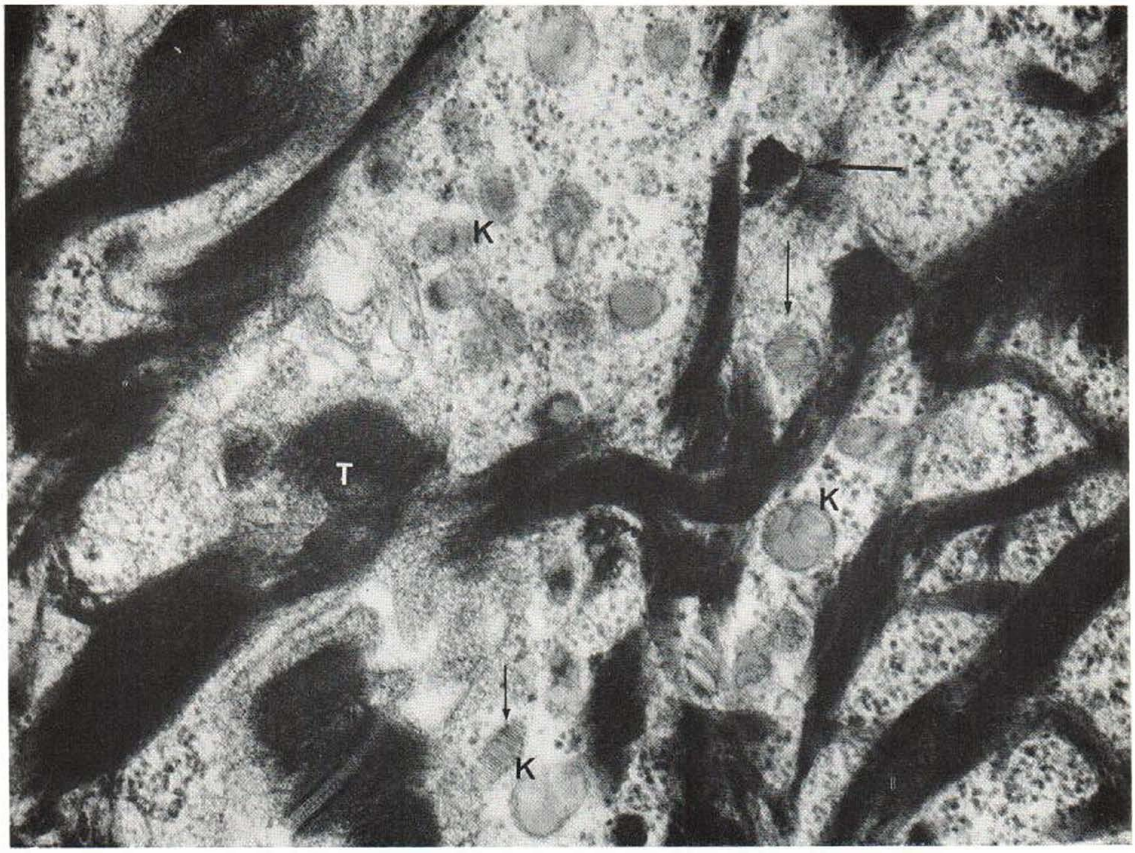

Fig. 4. The upper prickle cell layer of the pit bottom. Masses of tonofilaments (*T*) are seen between numerous keratinosomes (*K*). Some keratinosomes show distinct lamellar patterns (thin arrows), while others contain a homogeneous substance. Keratohyalin granule (thick arrow) (× 46 000).

peripheral zone of the cytoplasm. In congenital ichthyosiform erythroderma, compact lumpy masses of tonofilaments have been observed but they were seen close to the nucleus. Large keratohyalin granules with white speckles, as seen in the pits, were not seen in the other dyskeratotic diseases (9, 12, 19).

Keratinosomes are considered to be epidermal lysosomes (20, 21) and they may take part in the keratinization process (13, 15, 19). In some dyskeratotic diseases the number of keratinosomes is increased (9, 19), but large pathological keratinosomes containing homogeneous masses as observed in our patients have not been reported in other diseases. The observed changes in tonofilaments, keratohyalin granules and keratinosomes indicate a disturbed keratinization process in the pits.

Wide intercellular spaces and stretched tonofilament–desmosome complexes seen in the pit bottoms as well as finger-like cytoplasmic protrusions and pocket-like subepidermal spaces are also observed in diseases associated with intercellular oedema (2, 10) and acantholysis (12, 19).

Degenerative changes of the cytoplasm may explain the presence of the cells containing dark cytoplasm, present mainly in the bottom of the pit.

No evidence of carcinoma (11, 22) was found in the present study.

Scanning electron microscopy revealed a defect in the horny layer. Irregularly stacked horny cells and thin villous protrusions, present in the walls of the pits, have been observed in normal skin of palms and soles (14). The reduced thickness of the epidermis overlying the scaling bottom of the pits may be due to the observed abnormal keratinization.

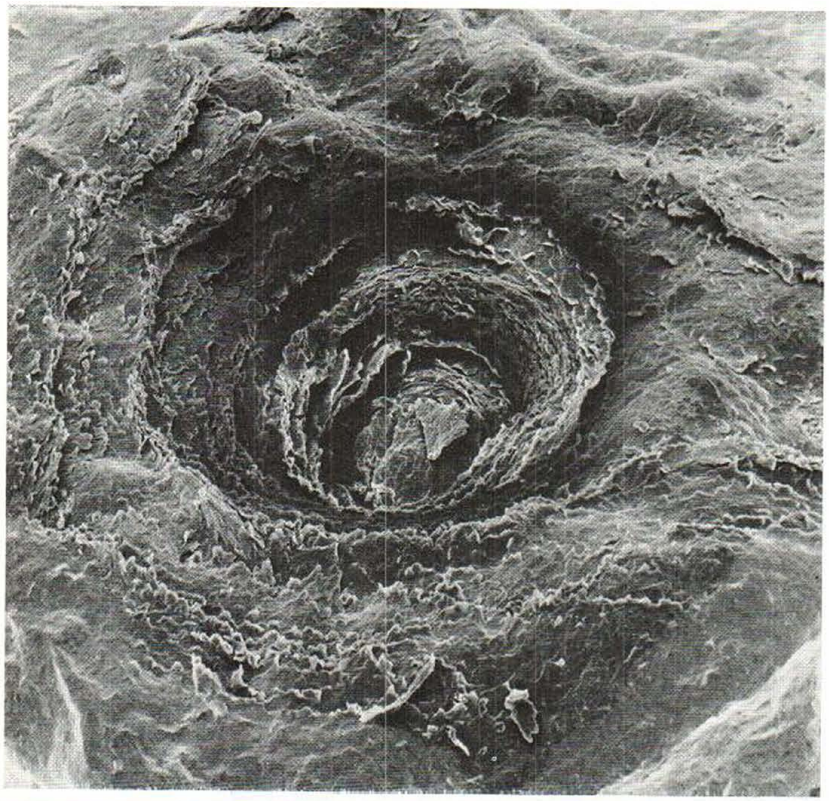

Fig. 5. Scanning electron micrograph. A view into a pit. A mound-like formation is seen around the pit (× 112).

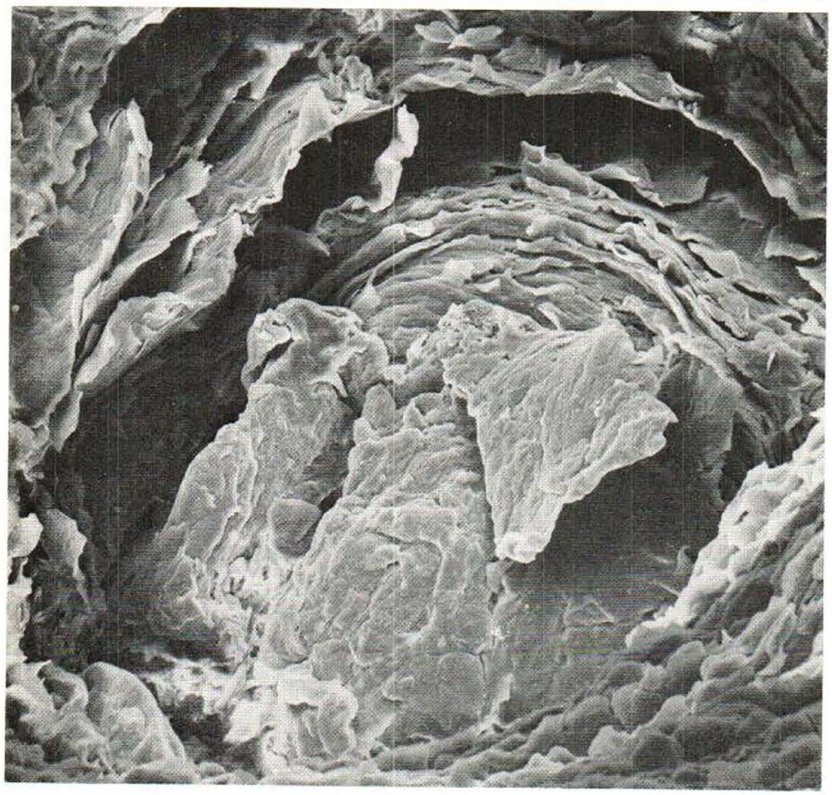

Fig. 6. Scanning electron micrograph. The bottom of the pit shows an area in the centre elevated over an annular furrow in the periphery (× 450).

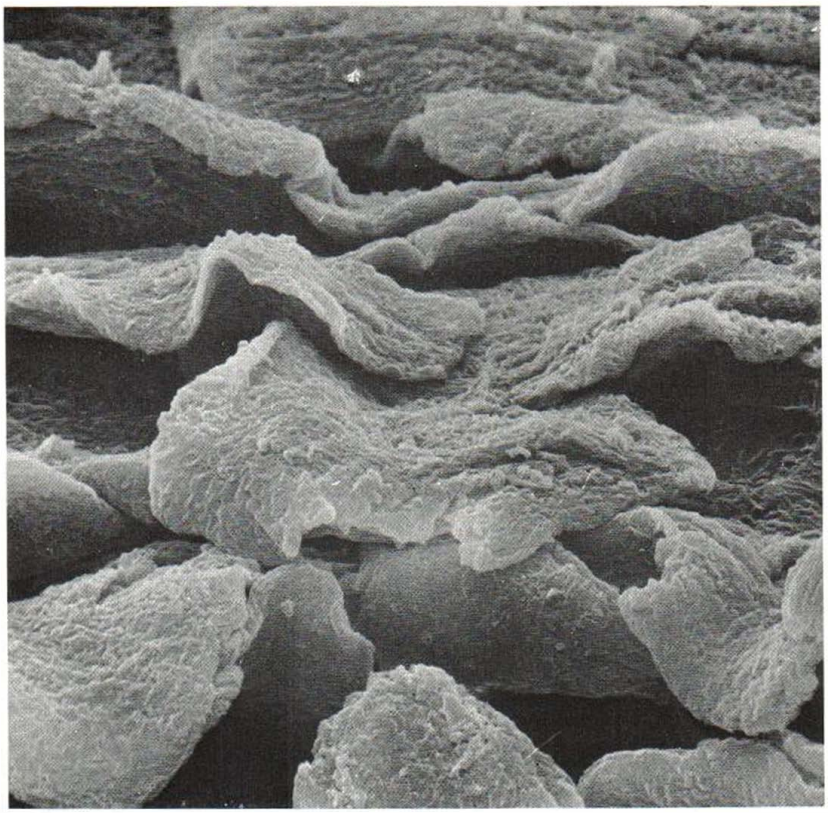

Fig. 7. Scanning electron micrograph. The individual flat, polygonal cells are stacked with the free edges of the cells protruding into the lumen of the pit (× 4 500).

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