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ULTRASTRUCTURAL CHANGES OF ZINC DEFICIENT RAT EPIDERMIS: AN ELECTRON MICROSCOPIC STUDY

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Abstract. Zinc deficiency was produced in rats by feeding them a diet containing 0.5 mg zinc per kg. After 2 weeks the zinc-deficient rats showed markedly stunted growth as compared with control rats receiving an identical diet supplemented with 50 mg zinc per kg. After about 3 weeks all rats in the zinc-deficient group developed a greasy, coarse and scaly coat with dispersed, denuded, eczematous patches. After 32 days the animals were sacrificed and skin specimens were obtained from the abdominal area. Electron microscopy showed two different patterns of epidermal changes: (A) edematous cell bodies and cell organelles, degenerate nuclei, while desmosomes were well preserved; (B) parakeratotic and dyskeratotic cells with sparse keratohyalin granules, abnormal keratinosomes, poorly developed desmosomes, and disintegrated mitochondria in the upper part of the epidermis. In the lower part, keratinocytes contained numerous ribosomes and thick chromatin aggregates in the nuclei. Dendritic cells were disintegrated in pattern A, while they appeared normal in pattern B. In both patterns mitochondria showed vacuoles and a lack of DNA granules. A transient form of type A and B changes was also observed.

Key words: Acantholysis; Cell organelles; Dyskeratosis; Epidermis; Parakeratosis; Zinc deficiency

Follis et al. (2) have performed extensive light microscopic studies on zinc deficiency dermatitis in rats. The changes included edema of the prickle cells, and, occasionally, acantholytic and necrotic cells. The outer epidermal layer appeared parakeratotic, especially over areas of irregularly widened prickle cell layer. Similar changes have been observed in zinc deficiency in patients suffering from acrodermatitis enteropathica (4) and in patients receiving total parenteral nutrition devoid of zinc (1). The present investigation was undertaken to study ultrastructural changes in the epidermis of zinc-deprived rats.

MATERIAL AND METHODS

Experimental animals

Twenty-four male albino rats having an initial weight of about 75 g were used. The rats were divided into three groups of 8 individuals each. One group received a zinc-deficient rat test diet *ad libitum* (Table I). Two groups received an identical diet supplemented with 50 mg zinc per kg. One of the control groups received the diet *ad libitum* while the other received a restricted amount as

Table I. *Composition of zinc-deficient rat test diet (0.5 mg zinc/kg)*

In the control diet zinc carbonate ($ZnCO_3$) is added at a level of 89.2857 g/kg (adds 50 mg zinc/kg) and the dextrose is reduced to 636.4090943 g/kg of diet

Egg white solids, spray dried		200.0
Dextrose, hydrate, technical		636.49838
Corn oil		100.0
Non-nutritive fibre (cellulose)		30.0
Sodium chloride	NaCl	5.55198
Potassium phosphate	K_2HPO_4	10.68784
Calcium phosphate	$CaHPO_4$	2.48948
Magnesium sulphate	$MgSO_4$	1.6513
Calcium carbonate	$CaCO_3$	9.94405
Ferric citrate	(16.7% Fe)	0.91154
Potassium iodide	KI	0.02652
Manganese sulphate	$MnSO_4 \cdot H_2O$	0.00879
Cupric sulphate	$CuSO_4 \cdot 5H_2O$	0.00994
Cobalt chloride	$CoCl_2 \cdot 6H_2O$	0.00185
Biotin		0.004
Vitamin B_{12} (0.1% trituration in mannitol)		0.02
Calcium pantothenate		0.016
Choline chloride		1.5
Folic acid		0.0005
Niacin		0.025
Pyridoxine HCl		0.004
Riboflavin		0.006
Thiamin HCl		0.01
Dry vitamin A palmitate (500.000 U/g)		0.02
Dry vitamin D_2 (500.000 U/g)		0.0025
Dry vitamin E acetate (500 U/g)		0.22
Menadione		0.00033
Chlortetracycline HCl		0.39

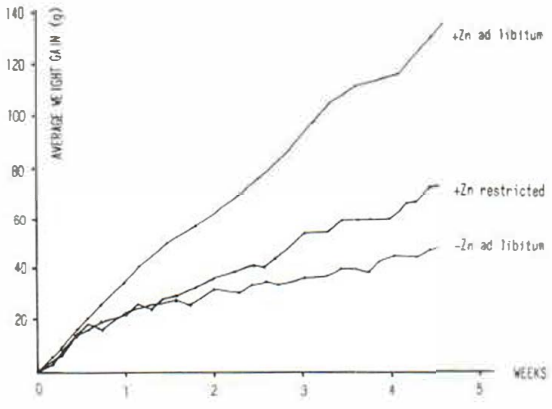

Fig. 1. Average weight gain of zinc-deprived and zinc-supplemented groups of rats ($n=8$).

ingested by the anorectic zinc-deficient rats (pair-fed control group). The animals were housed in cages made of plastic and stainless steel. Zinc-free deionized water was offered freely.

Skin specimens

After 32 days 2 rats from each group were selected for the present investigation, while the remaining animals were used for other purposes. The animals were carefully shaved on the abdominal area with an electric razor. Then under aether narcosis the rats were sacrificed by decapitation. The abdominal skin was excised and a piece of about one cm^2 was cut into minor fragments and immediately fixed in 6% glutaraldehyde in 0.2 mol cacodylate buffer, pH 7.2, with 7.5% sucrose. The specimens were dehydrated in a series of ethanol and embedded in Epon 812. Ultrathin sections were cut with a Reichert ultramicrotome OM 2 and OM 4 and stained with uranyl acetate and lead citrate. A JEOL electron microscope 1-0 CX was operated for the observations.

RESULTS

Clinical observations

The effect of zinc deficiency on the growth rate appears from Fig. 1. After about 3 weeks the zinc-deprived rats, apart from showing stunted growth, developed a scaly coarse coat which became thinned and unkempt (Fig. 2). Well-defined eczematous lesions were seen in major skin folds and annular or circular lesions appeared in the dorsal and abdominal areas. The skin and pelt of the zinc-supplemented control groups appeared normal.

Ultrastructural findings

Electron microscopy of zinc-deficient rat epidermis revealed two different pathological patterns,

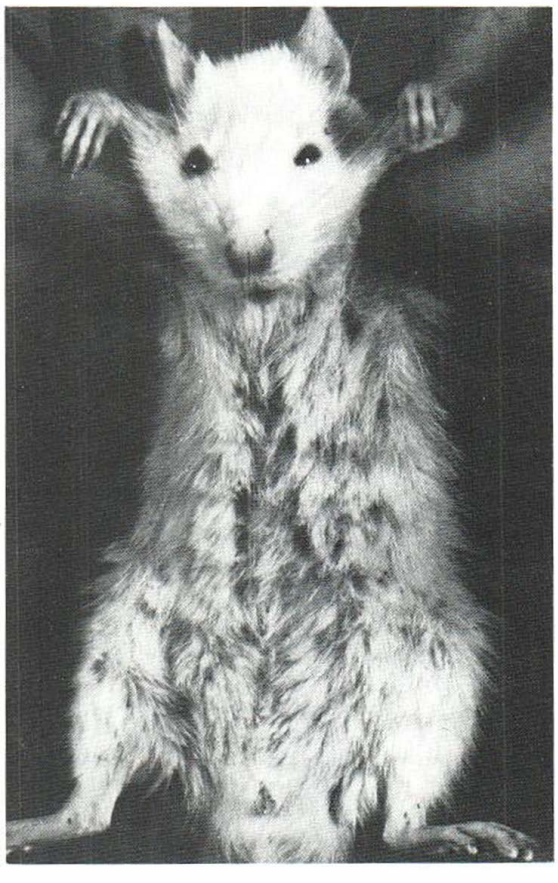

Fig. 2. Nine-week-old albino male rat after 30 days' deprivation of zinc.

whereas the control rats showed essentially normal features.

Pattern A. In the whole of the epidermis, keratinocytes were edematous, especially in the periphery of their cytoplasm where tortuous cytoplasmic protrusions could be seen (Fig. 3). Mitochondria were cystic, lacking cristae and DNA granules. The granular endoplasmic reticulum showed dilated cisternae with aggregates of ribosomes, and the Golgi apparatus was small. Multivesicular bodies and small primary lysosomes were seen (Fig. 4). The nuclei contained coarse chromatin granules without peripheral condensates. The intercellular spaces were intact, with a normal fine structure. Keratinosomes were slightly reduced in size, and keratohyalin granules were few. Some horny plates contained remnants of ribosomes in the keratin masses. Dendritic cells were strongly

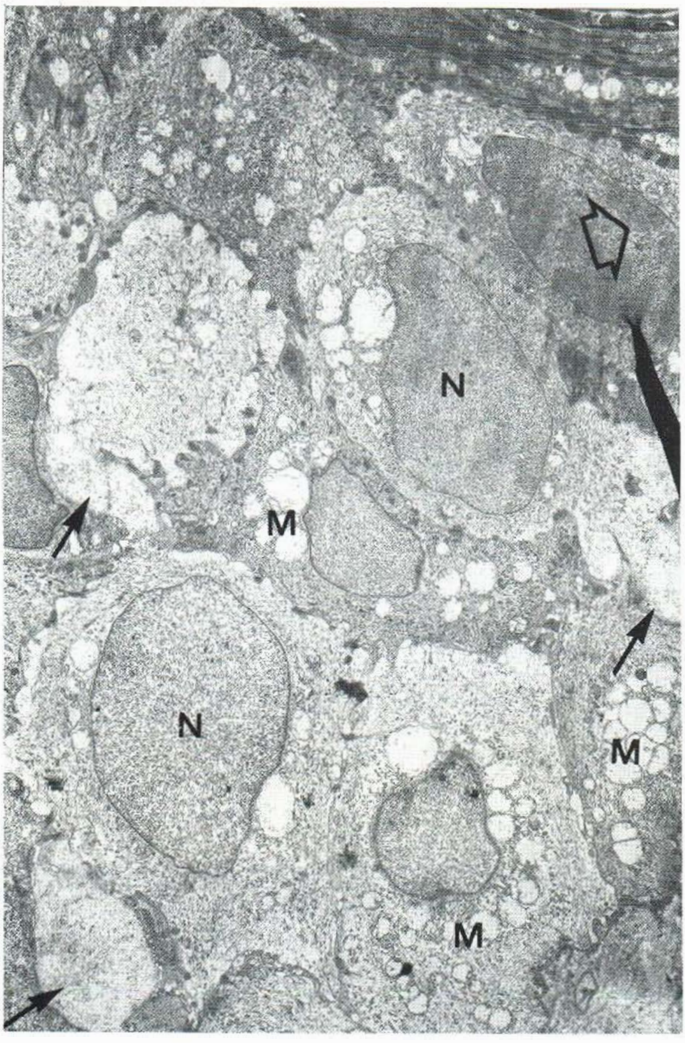

Fig. 3. Pattern A. Edematous keratinocytes show tortuous cytoplasmic protrusions (massive arrows), vacuolated mitochondria (M) and degenerate nuclei (N). Intercellular space is not dilated. The area indicated by a framed arrow is shown in Fig. 4. ×7740.

edematous, containing debris of cell organelles. The cell membrane was intact.

Pattern B. Keratinocytes in the basal cell and spinous cell layers contained numerous ribosomes and mitochondria (Fig. 5). The mitochondria were gathered and confluent in the perinuclear area of the upper spinous cell layer and contained no DNA granules (Fig. 6). The tonofilament bundles were sparse in the lower part of epidermis, but in the upper part thick bundles were present at the periphery of the cells. The granular endoplasmic reticulum and the Golgi apparatus was dilated. Nuclei from different layers of epidermis had strongly condensed chromatin masses and enlarged nucleoli (Fig. 5). Desmosomes of basal cells and of lower spinous cells were poorly developed. These cells showed finger-like cytoplasmic protrusions in the widened intercellular space. In contrast, the space in the upper part of epidermis was narrow and desmosomes were normal. Keratohyalin granules were not seen. In some cells fat droplets could be seen (Fig. 6). Keratinosomes varied in number and size. Horny plates contained remnants of cell organelles in varying amounts and had a thick cell membrane. In some areas of the upper spinous cell layer degenerate keratinocytes were observed. These cells appeared dyskeratotic, with sparse desmosomes. Filaments, vacuoles and dense bodies were located in the central part of the cytoplasm and tonofilaments at the periphery (Fig. 7). Den-

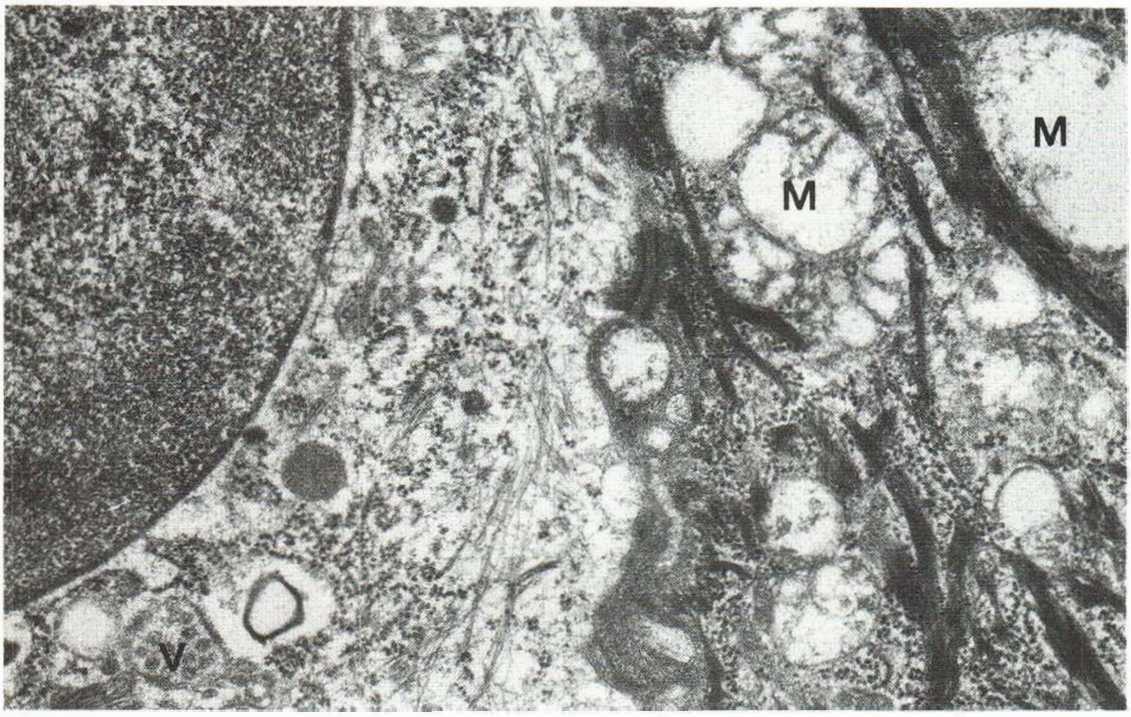

Fig. 4. Pattern A. Vacuolated mitochondria (M) and multivesicular body (V). No peripheral condensates of chromatin are seen in a degenerate nucleus. ×30 000.

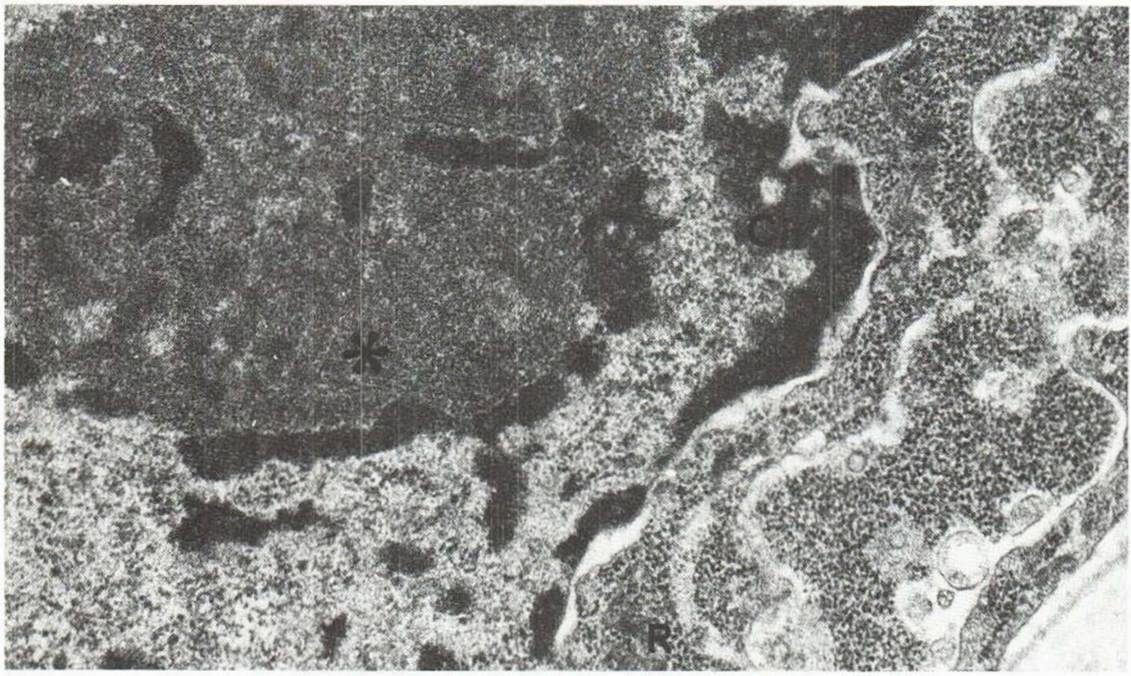

Fig. 5. Pattern B. Keratinocyte in basal cell layer. The cytoplasm is filled with ribosomes (R). Nucleus contains distinct chromatin condensates at the periphery (Ch) and a large nucleolus (asterisks). Intercellular space is dilated and contains ribosome-like granules. ×30 000.

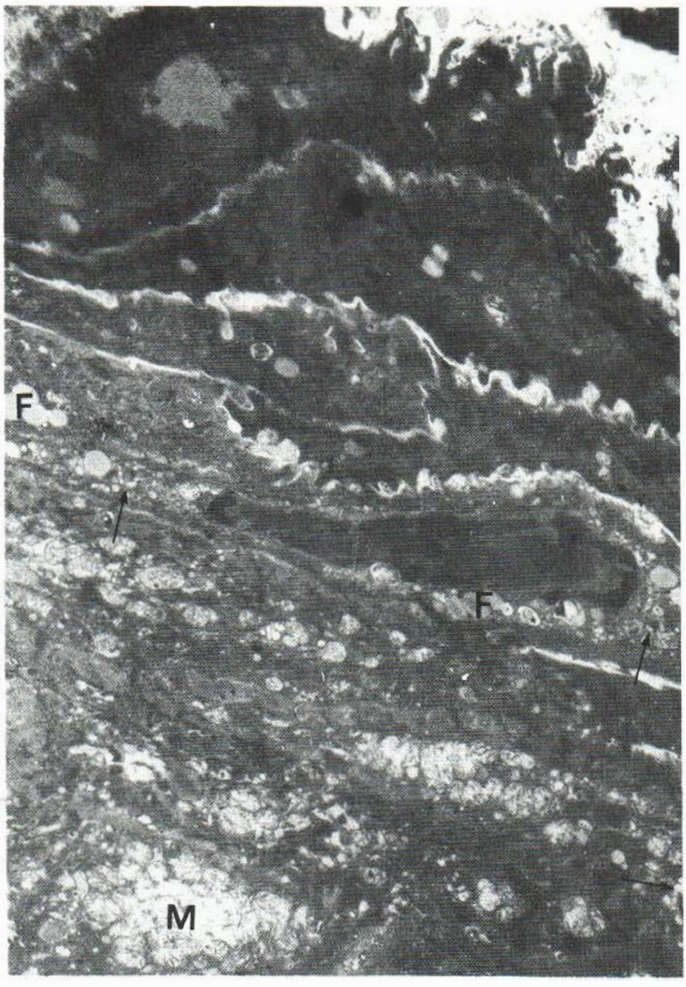

Fig. 6. Pattern B. Parakeratotic horny layer and upper spinous cell layer. Remnants of nuclei and cell organelles are seen in the horny plates. Keratinocytes in the upper spinous cell layer show numerous vacuolated and confluent mitochondria (M), dilated cisternae of endoplasmic reticulum and nuclear envelopes (arrows) and fat droplets (F). ×7740.

dritic cells appeared normal, some of them containing Langerhans cell granules.

Intermediate pattern. An intermediate form of the two patterns described was observed, displaying a gradual transition from type A to type B changes. Cellular edema of the lower spinous cell layers was present to a mild degree and the basal cells resembled those of pattern B. The horny layer was orthokeratotic, except in a few areas with parakeratotic horny plates. Dyskeratotic cells were not present and dendritic cells were normal.

DISCUSSION

The present study shows that zinc deficiency causes marked ultrastructural changes of the epidermis. One main type of abnormality included swollen cell bodies and cell organelles and degenerate nuclei, while the desmosomes remained intact. The other type of change was mainly characterized by para- and dyskeratosis. It is conceivable that the two types of abnormality represent various stages of injury, pattern A representing early and pattern B late changes induced by zinc deficiency.

It is noteworthy that in both patterns described, the vacuolated mitochondria contained no visible DNA granules such as are seen in similar conditions, e.g. seborrheic dermatitis (3). It is conceivable that the lack of DNA granules reflects a depressive effect of zinc deficiency on the DNA synthesis of the epidermal cells (5). Multivesicular bodies in keratinocytes and acantholytic cells may be observed in other pathological conditions. The present study was performed after about one

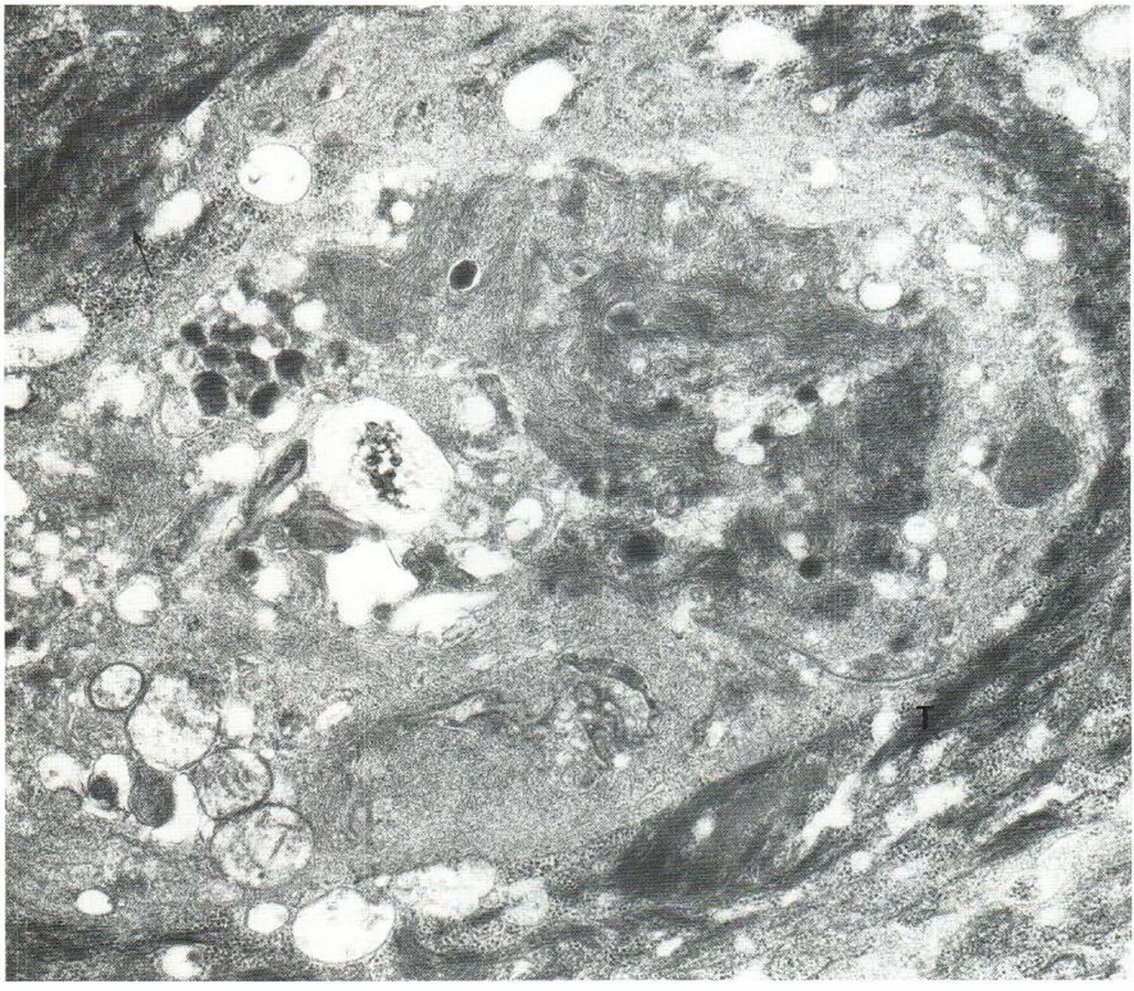

Fig. 7. Pattern B. A degenerate keratinocyte, like an acantholytic cell, shows a few desmosomes (arrow). Tonofilaments (T) encircle degenerate cell organelles but do not reach the desmosomes. ×24000.

month's zinc deprivation. It will be interesting to further define the epidermal changes occurring during earlier and later stages of zinc deficiency.

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