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CELLULAR CHANGES IN THE PSORIATIC EPIDERMIS

VIII. *Observations on the Submicroscopic Cytoplasmic Differentiation of Epidermal Cells of Primary Tissue Cultures from Psoriatic Lesions treated in vivo with Ammoniated Mercury*

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Abstract. Electron microscopic observations on epidermal cells of primary tissue cultures from psoriatic lesions treated *in vivo* with ammonium mercuric chloride are reported. After 7 days of treatment with an ointment containing 5% ammoniated mercury, skin biopsies were taken and epidermal tissue cultures established and maintained for 5 weeks. Electron microscopic analysis of this material reveals high cytoplasmic content of tonofilamentous material randomly dispersed in loose bundle-like formations with a structural appearance similar to that of normal epidermal cells cultured identically. In comparison with cultured psoriatic cells from non-treated lesions, the content of tonofilamentous material of the cultured pretreated cells is indubitably increased and a higher state of tonofilamentous structural differentiation is observed. The desmosomes also appear comparatively well differentiated. The significance of the observed altered cellular differentiation is discussed.

Electron microscopic analysis of psoriatic and normal epidermis shows distinct differences in the differentiation of the epidermal cells. In comparison with normal epidermal cells, the psoriatic cells of stratum basale, spinosum and granulosum are characterized by a pauperism of tonofilaments, by few and poorly developed desmosomes and an abundance of cell organelles (1, 4, 13). In primary tissue cultures and subcultures from normal and psoriatic epidermis these differences are maintained to a considerable extent. In both primary cultures and subcultures the normal epidermal cells exhibit a striking profusion of tonofilaments and tonofilament bundles. Psoriatic epidermal cells show an increase in tonofilamentous material when cultured for 9 weeks. The tonofilaments in the cultured cells are often seen forming curls (8).

Electron microscopic investigation of the localization of mercury in the psoriatic and normal epidermis after treatment in vivo with an ointment containing ammonium mercuric chloride has indicated a considerable selectivity in the affinity of mercuric compound to certain chemical constituents of the epidermal cells, i.e. the ribosomes, the chromatin material and certain intranuclear particles, reflected by a marked increase in electron density of these structures after this treatment. A specific combination of mercury with certain of these cellular components might indicate a blocking of the functional organization of the ribosomes. Action on intranuclear structures, indicated by the enhancement in contrast, may either inhibitively influence the nucleolar role in RNA-synthesis causing a suppression of ribosomes and protein synthesis and thus impairing the cellular division rate, or amend an assumable imperfection of messenger RNA synthesis (2, 9). A comparison of normal and psoriatic epidermal cells treated in the same way revealed no differences regarding the submicroscopical localization of mercury (2). In the normal cells, however, no intranucleolar particles could be identified but intranuclear particles of a size and contrast behaviour similar to ribosomes were found in the karyoplasm. The role of these particles is unknown, but it is conceivable that they precede and herald the formation of ribosomes.

The influence of mercury on the cellular differentiation of psoriatic cells was studied using electron microscopic analysis of double-stained

specimens from lesions treated for 7 and 15 days with an ointment containing 5% ammoniated mercury (3). After treatment in vivo for 7 days, amorphous masses resembling tonofilamentous material in contrast behaviour were observed with juxtaposed ribosomes. The ribosomes were numerous and seen in polysomal configurations, often forming circles and rosettes or in a staggered arrangement indicating a helical structure. The material treated for 15 days with ammoniated mercury showed obviously well differentiated desmosomes and was strikingly rich in randomly spread tonofilament-bundle structures often forming verticilated aggregates.

The present study was undertaken to see whether epidermal cells from psoriatic lesions treated in vivo with the ammoniated mercury ointment can be grown in primary tissue cultures and whether any ultrastructural changes of cellular differentiation induced by this treatment are traceable.

MATERIAL AND METHODS

The material consisted of skin biopsies from the forearms of 5 adult male patients, aged 28–49 years, with clinically manifested and histologically verified psoriasis. No patient had received any treatment within the previous 5 months. The biopsies were taken from lesions 1–3 weeks of age, after 7 days of treatment with 5% ammoniated mercury ointment.

The specimens for culture were obtained by punch biopsy technique without anesthesia. They were separated from the dermis and cut in small blocks in isotonic sodium chloride solution and transferred to Leighton tubes. The cultures were performed on insetsplates and in a medium consisting of 70% medium 199, 20% homologous serum and 10% chick embryo extract as earlier described (8). After 5 weeks, aggregates of the outgrowths were removed by dissection for electron microscopic analysis. Fixation was carried out in 6.5% glutaraldehyde buffered with a phosphate solution of pH 7 for 6 hours at 4°C (12). Postfixation was performed in 2% osmium tetroxide buffered with the phosphate solution for 2 hours at 18°C, and the specimens were rinsed in phosphate buffer solution. After dehydration in increasing concentrations of acetone the specimens were imbedded in Vestopal W. Ultrathin sections were cut with the LKB-Ultratome I and III, and after double-staining with uranyl acetate and lead citrate the sections were examined and photographed in a Siemens Elmiskop I and Hitachi HS-7S.

RESULTS

The primary cultures of psoriatic epidermis from lesions treated in vivo with the ammoniated mercury ointment showed continuous growth for 5 weeks and behaved in a similar way as described earlier (8). Control cultures of untreated normal and psoriatic epidermis were also established. The submicroscopic structure of an epidermal cell from the outgrowth areas of a culture of untreated normal skin, as appears after glutaraldehyde–osmium tetroxide fixation, is depicted in Fig. 1. The cytoplasm is loaded with tonofilaments sectioned at different angles, and randomly oriented. The arrangement into distinct bundles forming a network with relatively large cytoplasmic interspaces, as seen in uncultured normal cells, is lacking and only indicated loose aggregations can be traced. The micrograph in Fig. 2 shows the cytoplasmic organization of cells from an untreated psoriatic lesion, cultured for 5 weeks. The structural features of uncultured psoriatic epidermal cells characterized by a paucity of tonofilaments, poorly developed desmosomes and large intercellular spaces are largely retained. Examples of the submicroscopic differentiation of the cytoplasm of psoriatic epidermal cells of cultures established from lesions pretreated with ammonium mercuric chloride, are presented in Figs. 3–8. There is a striking difference between the submicroscopic morphology of cultured pretreated cells and that of untreated cells. An alteration of the differentiation is mostly indicated by a transformation of the tonofilament synthesis.

In the cytoplasm of the psoriatic cell depicted in Fig. 3 tonofilamentous material is randomly dispersed in loose bundle-like formations. The discontinuous arrangement of the bundle-like structures assumes the structural appearance observed in normal epidermal cells cultured identically. The individual tonofilaments are only indicatively distinguished. The cytoplasmic content of bundle-like tonofilament material is indubitadly increased in comparison with cultured psoriatic cells from non-treated lesions (cf. Fig. 2). A higher state of tonofilamentous structural differentiation is repeatedly perceived as shown in Figs. 4–6.

A high magnification of the structural tonofilamentous organization in the cytoplasm of a cultured psoriatic cell is shown in Fig. 4. Individual tonofilaments are unequivocally traced and in many places a double contoured substructure suggested, conjecturing a tube-like structure of this material. The tonofilaments are grouped into loose bundles. The tonofilament bundles form

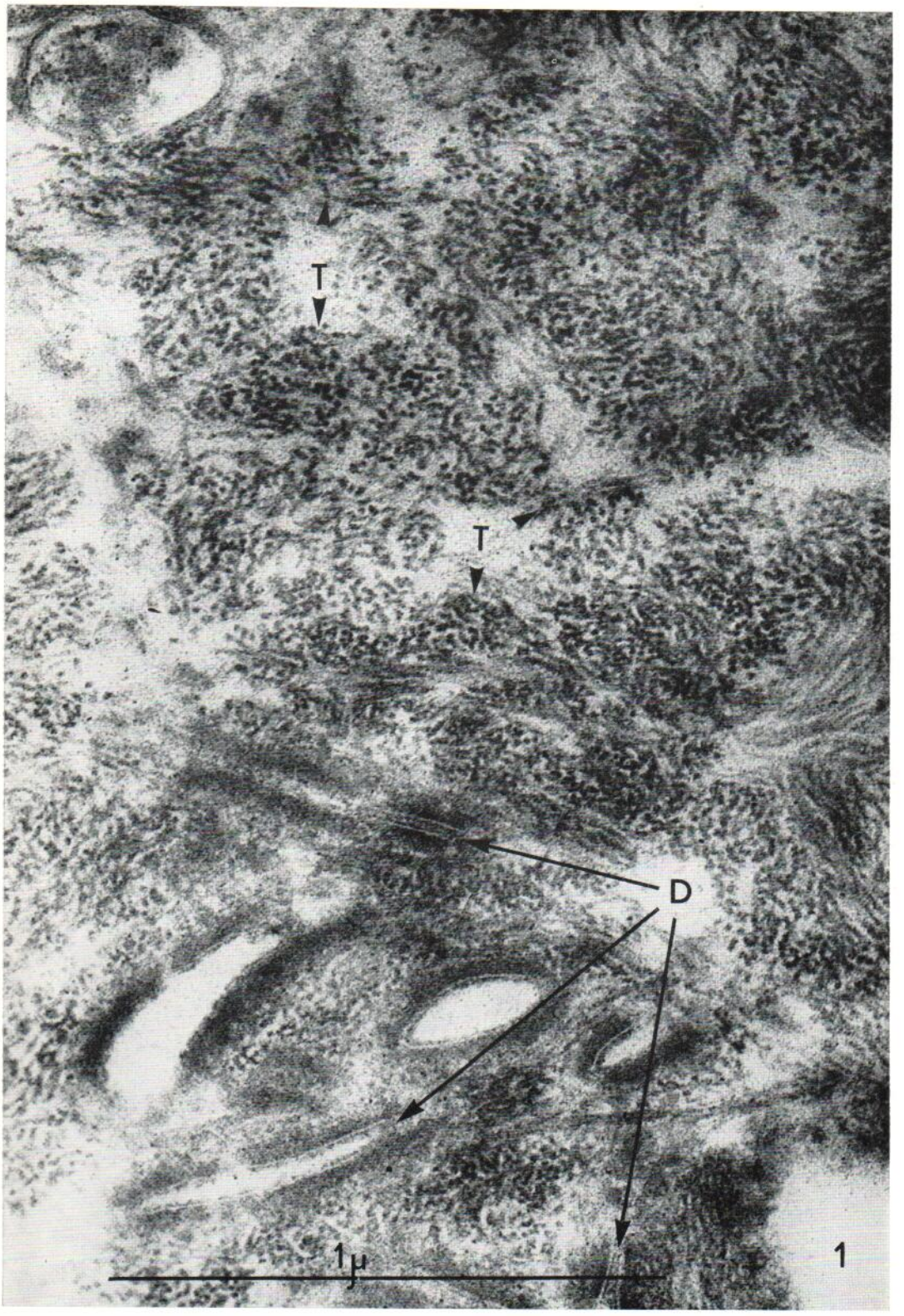

Fig. 1. Parts of cytoplasm of cultured normal epidermal cells depicting desmosomes and large numbers of tonofilaments. × 89 000.

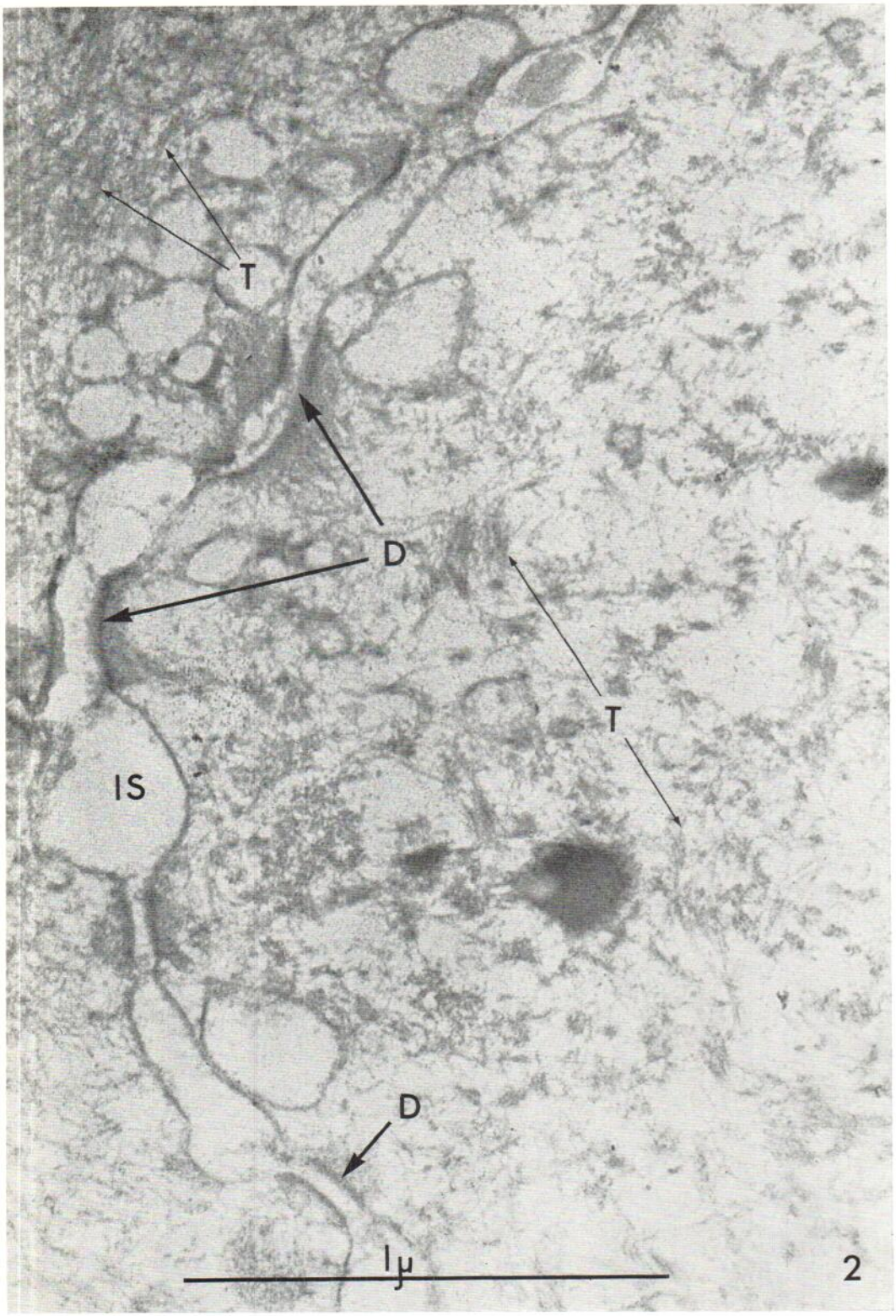

Fig. 2. Cytoplasmic structure of cultured untreated psoriatic cells revealing the paucity of tonofilaments and the poorly developed desmosomes. × 76 000.

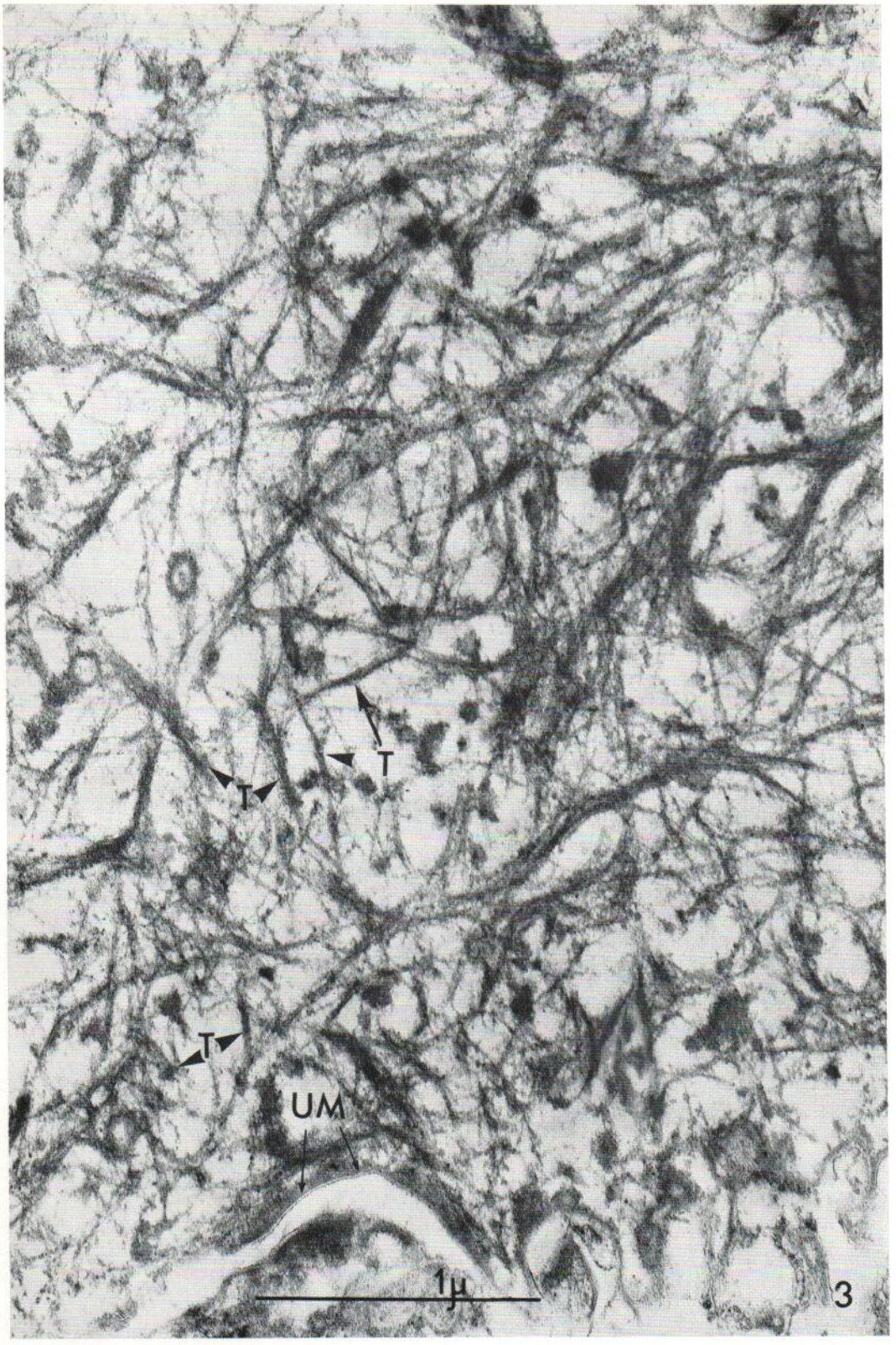

Fig. 3. Electron micrograph of cytoplasm of a pretreated psoriatic cell culture for 5 weeks, demonstrating the structural appearance of the tonofilaments, slightly network-like. × 45 000.

Fig. 4. Details of tonofilaments of a cultured pretreated psoriatic cell. × 101 000.

Fig. 5. Part of cytoplasm of a cultured pretreated psoriatic cell showing the higher state of tonofilament differentiation. × 79 000.

Fig. 6. Tonofilaments of the same material as in Fig. 5.
× 128 000.

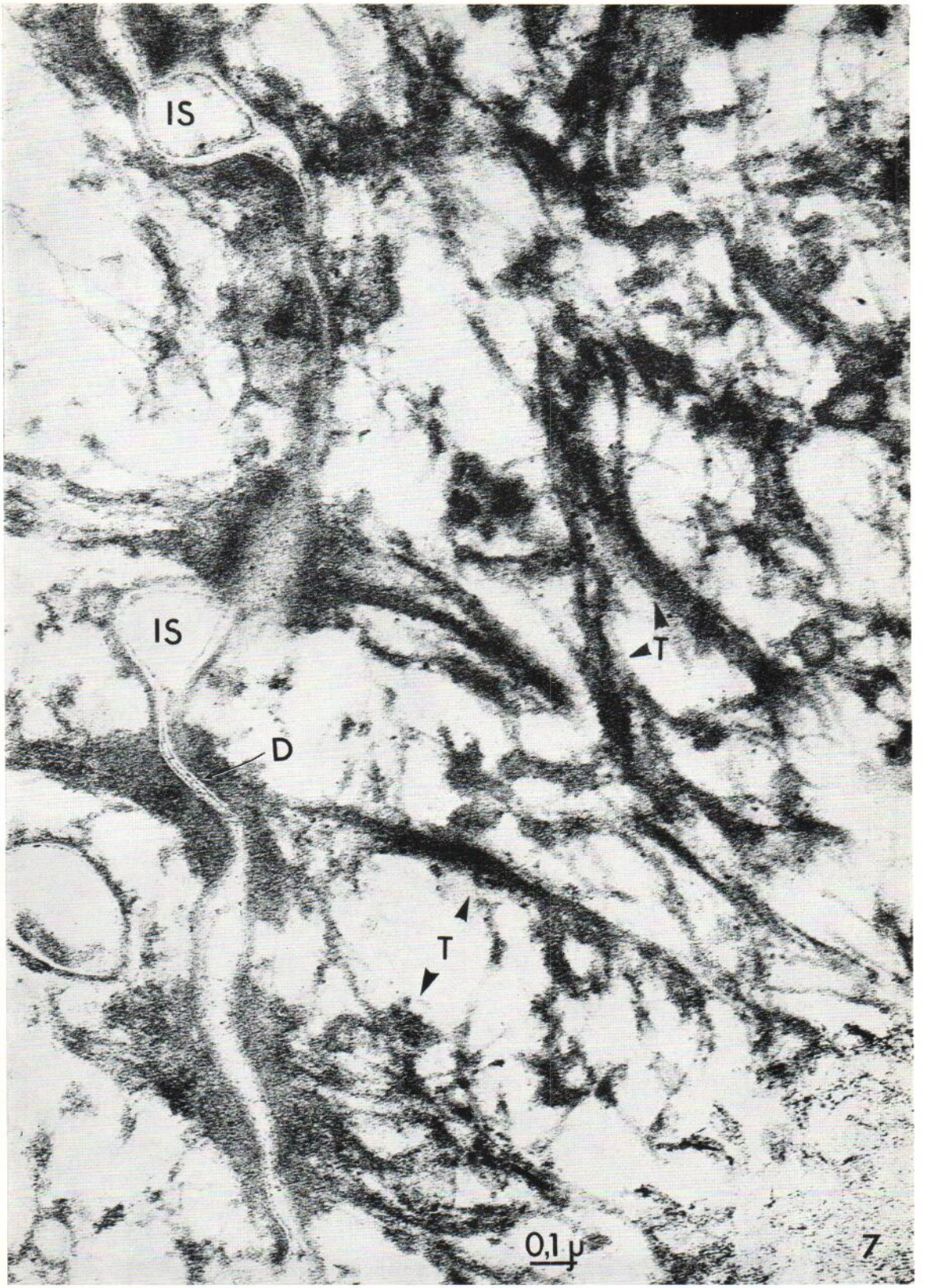

Fig. 7. Portion of cytoplasm and cell borders of cultured epidermal cells from pretreated lesions showing comparatively well differentiated desmosomes and moderately enlarged intercellular spaces. × 78 000.

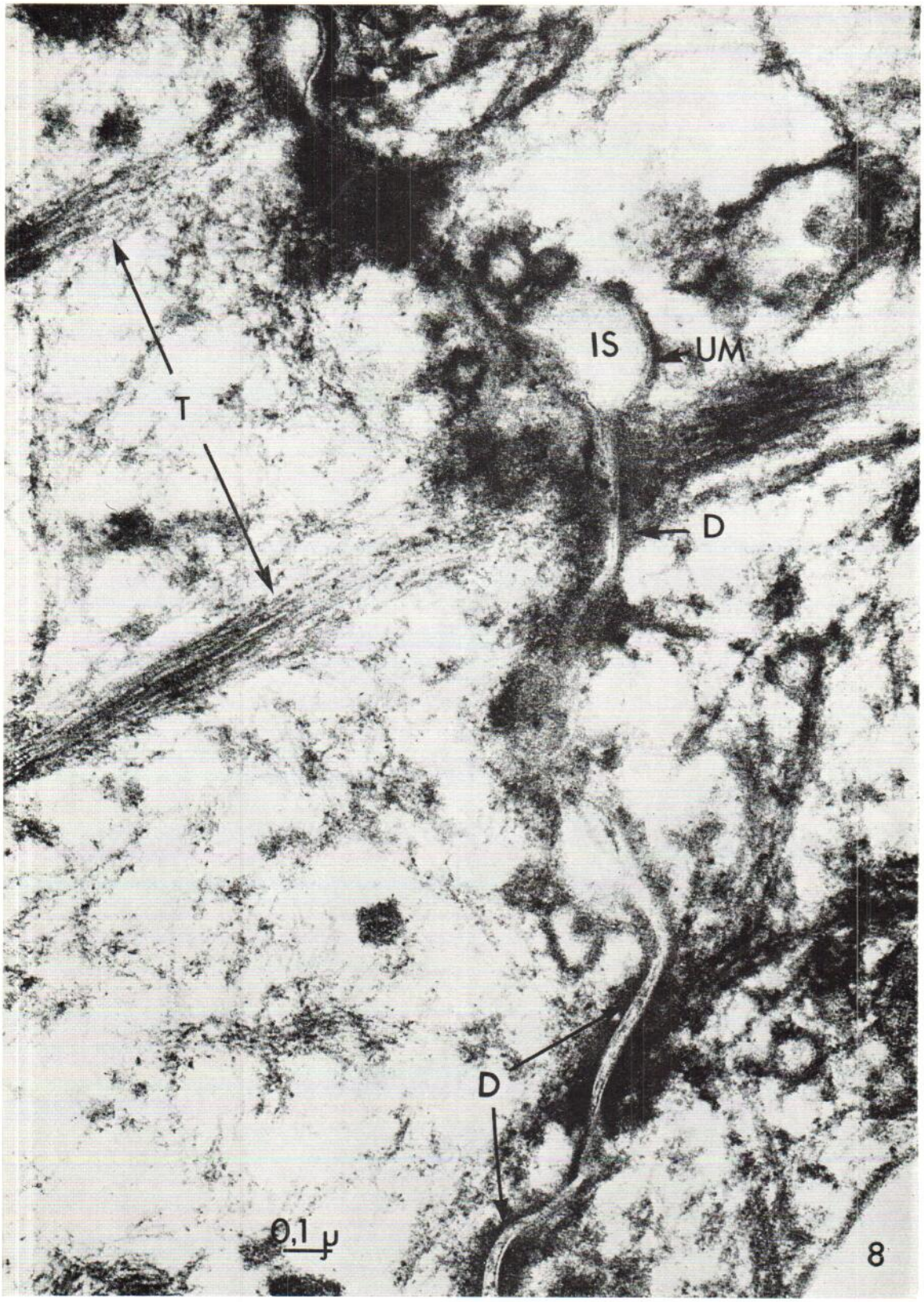

Fig. 8. Part of cell borders of the same material, showing fairly well differentiated desmosomes, unit membrane, and clearly distinguishable individual tonofilaments in the associated tonofilament bundles. × 82 000.

an indicative network. No tendency to verticilation is detectable. The higher magnifications of the cytoplasmic structural organization in Figs. 5–6 reveal further salient details.

The tonofilament material is aggregated into loose bundles with a number of anastomoses. The indicated double-contoured substructure of the tonofilaments is ubiquitous. In Figs. 7–8 the submicroscopic structure of the desmosomes of a psoriatic cell from an outgrowth area is depicted. The desmosomes appear comparatively well differentiated. The tonofilament bundles associated to the desmosomes are cut longitudinally as well as cross-sectioned. Moderately enlarged intercellular spaces are found, containing haphazardly distributed electron-dense material. The tonofilament material is found mostly in bundles associated with the desmosomes but also dispersed in the cytoplasm without any recognizable single tonofilaments. No clearly defined ribosomes could be identified unequivocally in the present material, since it is difficult to distinguish them from oblique cross-sectioned tonofilaments.

DISCUSSION

In the normal epidermal cells the tonofilaments form a dense network preferentially along an axis perpendicular to the skin surface (5). The tonofilaments in the cells of the basal layer show less tendency to aggregate in bundles, and, simultaneous with the progressive epidermal cellular differentiation and perceptible as part of this process, the formation of bundles and the orientation of the tonofilament bundles to the skin surface is accentuated (7, 11). The paucity of tonofilaments and tonofilament bundles of the uncultured psoriatic cells is earlier demonstrated (1, 4, 13). The tonofilament content of the psoriatic epidermal cells increases significantly in higher layers although never reaching the insuperable values of normal skin. Psoriatic cells in primary cultures 5 and 9 weeks old are characterized by numerous dispersed tonofilaments, suggesting excessive synthesis of tonofilaments during the cultivation period. When cultured for 9 weeks the psoriatic cells frequently show a grouping of the tonofilaments into loosely aggregated bundles.

The multitudinous tonofilamentous material of cultured psoriatic cells from lesions treated *in vivo* with ammonium mercuric chloride, perceivably factitiously increased, indubitably surpasses that of cultured epidermal cells from non-treated lesions as well as that of non-cultured psoriatic cells whether non-treated or treated *in vivo*. The organization of the tonofilament material of the pretreated psoriatic cells indicates a more differentiated state, occurring as loose bundle-like formations with the structural appearance of cultured normal epidermal cells. As considered in earlier reports (2, 3, 9), the effect of mercury compound on psoriatic epidermal cells treated in vivo conjectures an influence on the cellular protein synthesis by interference of either ribosomal functional properties or synthesis of ribosomes or messenger RNA. This amendatory effect on the cellular function is maintained when the pretreated psoriatic cells are transferred to in vitro cultures and the development of the tonofilamentous material seems to be better differentiated in comparison with cultured epidermal cells from untreated psoriatic lesions. A surmised effect of the ammonium mercuric chloride is an action on the part of the genome which regulates the tonofilamentous synthesis, producing a succession of synthesis, where earlier-formed substances may trigger the formation of their successors (6, 10). Although no definite evidence permitting any omnifarious functional significance to be ascribed to the tonofilaments in the keratinization process, an amended synthesis of tonofilaments indicates change appurtenant to normal differentiation. The appropriative development of desmosomes in the outgrowth areas is maintained and relatively numerous desmosomes are observed. Their morphological appearance shows no differences in comparison with uncultured cells in cultures from normal epidermis and psoriatic cells from untreated lesions.

The desmosomes as they appear after the culture procedure, and the fixation method used, do not permit any substructural analysis. The psoriatic cells of the present cultures have not yet shown any distinguishable signs of cornification and keratin formation but the amended functional organization of the tonofilamentous system is indicative of a presumable ability to perform the first steps in the normal cornification process. It is conceivable that the continuous studies of long-term cultures and subcultures will provide considerable information on the factitiously amended differentiation and supposed appurtenant amendment of keratinization.

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Abbreviations used: D, Desmosome; *IS,* intercellular space; *T,* tonofilament bundle; *TF,* tonofilament; *UM,* unit membrane.

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