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# DERMO–EPIDERMAL JUNCTION IN BASAL CELL CARCINOMA

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*Abstract.* Four basal cell carcinomas of the undifferentiated type were studied by electron microscopy. The cytoplasm of the carcinoma cells varied with the maturity. Severe changes of the junctional structures were demonstrated. Constant changes were the thickening and discontinuities of the dermal membrane, and disappearance of anchoring filaments, anchoring fibrils and elastic fibril anchorings.

The dermo-epidermal junction of normal skin has several substructural characteristics (4, 5, 7), and is considered to develop by an interaction between epidermal cells and dermal tissue (6). In squamous cell carcinomas in man (2, 8) and in experimental animals (11) changes of the dermal membrane have been found to correspond with changes in the desmosome apparatus. Previously, an unbroken 400 Å wide dermal membrane and uniform figures of cancer cells have been described as characterizing basal cell carcinomas (13). Below, details of pathological junction patterns are described.

## MATERIALS AND METHODS

Four facial basal cell carcinomas in 4 patients were biopsied. The specimens were fixed in a 6.5% glutaraldehyde solution buffered with Veronal acetate pH 7.2 with 7.5% sucrose at 4°C overnight. After washing, the specimens were fixed in 1% osmic acid solution in the same buffer as used for the glutaraldehyde fixative at 4°C for 1 hour, washed again and dehydrated in graded concentrations of alcohol. A hydrophilic aliphatic polyepoxide (Durcupan) was used for embedding. For orientation, thick sections of the embedded specimens were cut and stained with a 1% aqueous toluidine blue solution. All four tumours were of the undifferentiated solid type of basal cell carcinoma. Ultrathin sections were cut of areas with different junction figures in the cancer cell nests and scattered cancer cell groups infiltrating the corium showing ortho- and metachromatic staining of the surrounding dermal areas. Ultrathin sections were stained with uranyl acetate and lead citrate and analysed by a Siemens electron microscope (Elmiskop IA) at 80 kV with double condensors.

## OBSERVATIONS

The junction structures presented various patterns, and the substructural deviation from normal was pronounced. The changes could be classified as follows.

1. *Junction structure showing a band-shaped dermal membrane and half-desmosomes.* The cytoplasm of the cancer cells contained sparse tonofilament bundles, vesicles, ribosomes, and mitochondria. The half-desmosomes were unequally broad with or without lamellar substructures (Figs. 1, 3, 4). Pocket-like spaces were seen between the convoluted carcinoma cell membranes and the dermal membrane (Fig. 4). The latter presented defects (Figs. 3, 5), localized thickenings (Figs. 2, 4) and blurred areas (Fig. 4). In the areas of deficient dermal membrane, the cancer cell membranes faced the elastic fibrils directly (Figs. 3, 5). A meshy appearance of displaced dermal membrane pieces could be seen between deep rete pegs (Fig. 3). The anchoring filaments appeared faint and blurred (Figs. 3, 4), or were entirely absent along with the change of the dermal membrane (Figs. 2, 5). The anchoring fibrils were slender and dense with faint bandings and irregularly arranged (Figs. 1, 2, 3). In most areas the elastic fibril anchorings were scarce (Fig. 4) except in the areas below the meshy dermal membrane (Fig. 3). In this layer, the elastic fibres had a granular appearance with indistinct elastic fibrils, while the collagen fibrils showed distinct bandings (Figs. 2, 4). Occasionally, masses of short straight threads were seen surrounded by collagen

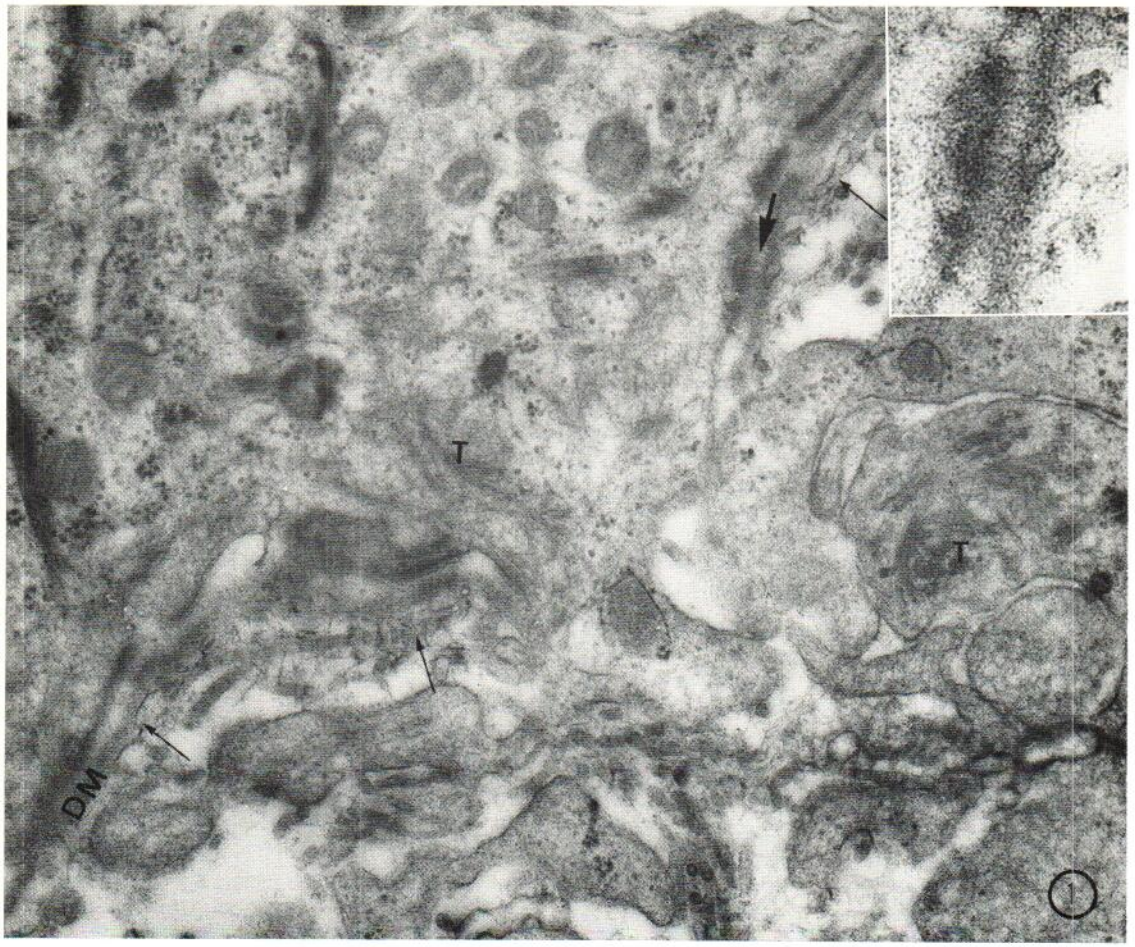


*Fig. 1.* The band-like dermal membrane (*DM*) is perforated by cytoplasmic protrusions of a carcinoma cell. The dermal membrane is connected with half-desmosomes by anchoring filaments (*thick arrow*). The same area is shown in the inset. The anchoring fibrils are slender and dense with a faint periodicity. They run in irregular directions (*thin arrows*). The carcinoma cells in the basal epidermal layer as well as under the dermal membrane contain sparse bundles of tonofilaments (*T*). × 47 000. Inset, × 94 000.

fibres and ribosome-like granules (Figs. 5, 6). In some areas, cancer cells were penetrating the regular band-shaped dermal membrane (Fig. 1). In the areas of perforation, the half-desmosomes and the anchoring filaments were distinct while the anchoring fibrils were changed as described above (Fig. 1).

2. *Junction structure presenting pronounced alterations of the dermal membrane and the half-desmosomes.* In some areas the dermal membrane was extremely thick, and no distinct half-desmosomes were demonstrated, but, here and there, the membrane of the basal epidermal cells revealed contrasty thickenings of various sizes (Fig. 7). A narrow and blurred space separated the thick homogeneous dermal membrane from the cancer cells. Such areas presented no anchoring filaments, no elastic fibril anchorings and scarce indistinct anchoring fibrils. Collagen fibrils and amorphous material as well as wide empty spaces were found under the dermal membrane. In the areas of thin or discontinued dermal membrane (Fig. 8), the carcinoma cells contained sparse and indistinct tonofilament-bundles, round mitochondria and dense masses of ribosomes (Figs. 8, 9). Half-desmosomes were rarely found, and those present mostly appeared as slight thickenings of the cell membrane (Fig. 8). The dermal membrane

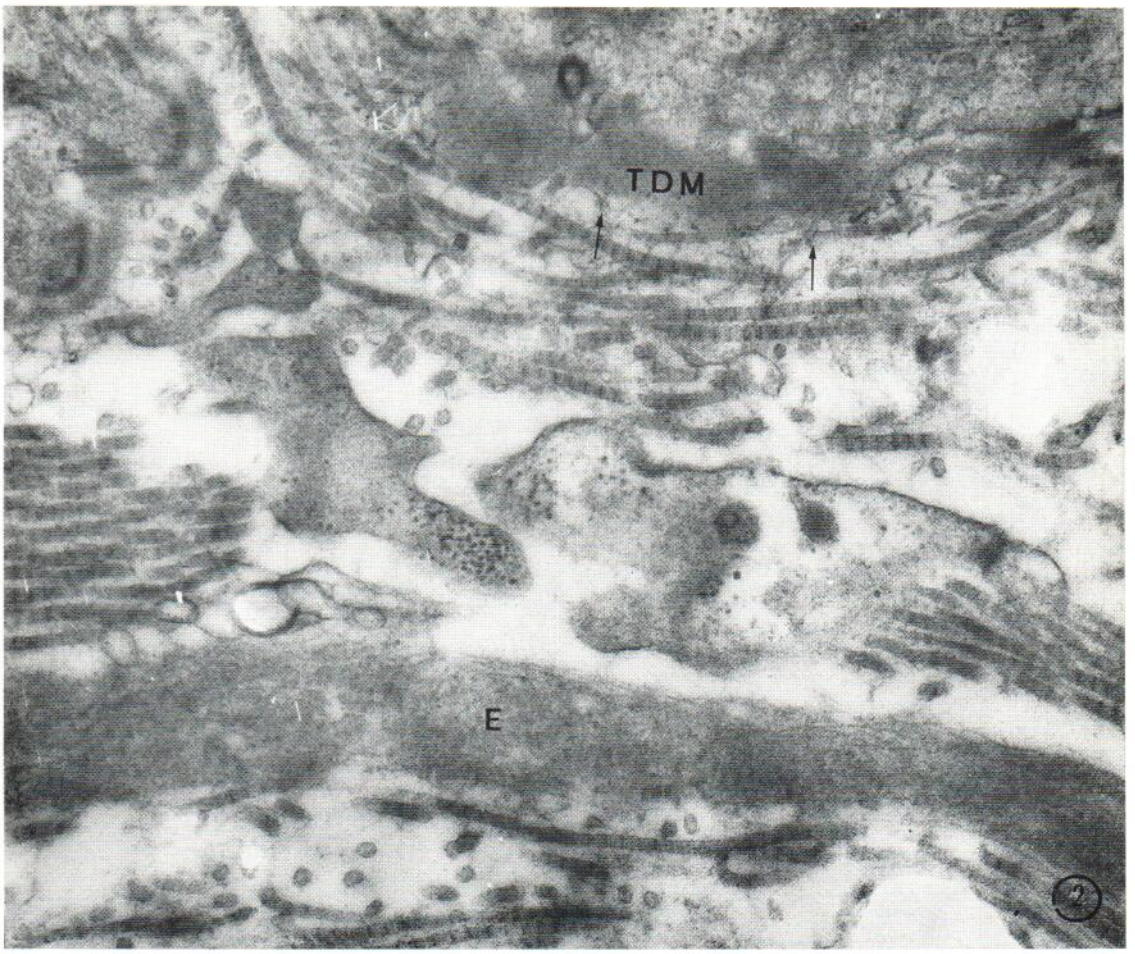


*Fig. 2.* Localized thickening of the dermal membrane (*TDM*) with anchoring fibrils (*thin arrows*). No anchoring filaments or subepidermal spaces are found in the same area. An elastic fibre in the corium has a granular appearance and indistinct elastic fibrils (*E*). × 47 000.

did not show the regular band shape as seen in normal skin, and masses of amorphous and thread-like material of irregular thickness were found on the cell membrane (Figs. 8, 9). The stroma close to the cancer cell nests contained round particles which were connected with each other by fine threads. Collagen fibres and elastic fibrils were scarce (Figs. 8, 9).

3. *Complete lack of junction structures.* In such instances, the cancer cells contained no tonofilaments; only vesicles, ribosomes, granular endoplasmic reticulum and mitochondria could be demonstrated. Finger-like cytoplasmic protrusions and homogeneous material close to the cancer cell membrane were noted (Fig. 10). Occasionally, ribosome-like granules were found in the stroma, as if they were spread out from the cancer cells (Fig. 10).

## DISCUSSION

The dermal membrane of normal skin is a regular, approximately 300 Å thick band, occasionally showing internal threads (7). The membrane is separated from the cell membrane of the basal cell layer by an almost constant subepidermal space with an approximate width of 300 Å. The half-desmosomes are connected with the dermal membrane by fine parallel threads crossing the subepidermal space (anchoring filaments, 4, 5, 7).

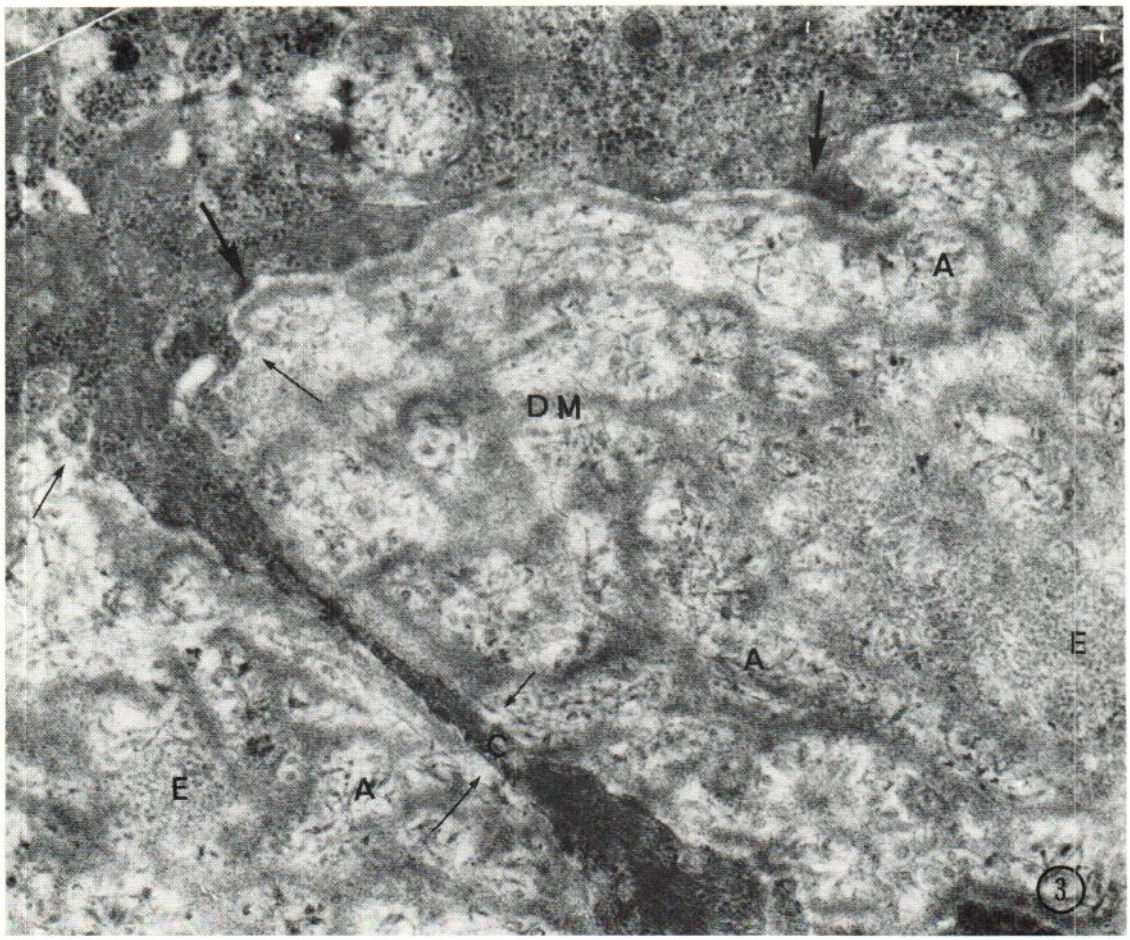


*Fig. 3.* Mesh-work of band-shaped dermal membrane (*DM*). A long cytoplasmic extension (*C*) is seen in the corium. Anchoring filaments and half-desmosomes are blurred (*thick arrows*) while anchoring fibrils (*A*) are distinct. Masses of the elastic fibrils (*E*) are found in the dermal membrane meshwork. Thin arrows indicate defects in the dermal membrane. × 47 000.

The membrane sends banded fibrils (anchoring fibrils) to the corium, and it may be in direct continuity with dermal elastic fibrils (elastic fibril anchoring, 4, 5, 7). Collagen fibrils and ground substance fill in the space under the dermal membrane (5). Usually, no perforation of the dermal membrane can be seen. In basal cell carcinoma, a variety of pathological cytoplasmic figures were observed. As mentioned in a previous paper (8), tonofilaments and masses of ribosomes and vesicles indicate maturity of the cancer cells. Distinct patterns of the dermal membrane and of the anchoring filaments were seen in areas of well-developed half-desmosomes which, in turn, seemed closely related to well-developed tonofilaments. The figures of the anchoring fibrils were pathological, even under a band-shaped dermal membrane and in the presence of distinct anchoring filaments. This fact suggests some influence from the dermal side. Scarce elastic fibrils under the dermal membrane as well as granulated elastic fibres with indistinct fibrils indicated that elastic material was partly dissolved in the stroma of the tumour. Increased masses of elastic fibrils in some areas may have existed earlier in the corium of facial skin. Previous histological studies (9, 10) have demonstrated new-formation of elastic fibres in basal cell carcinoma while in the present

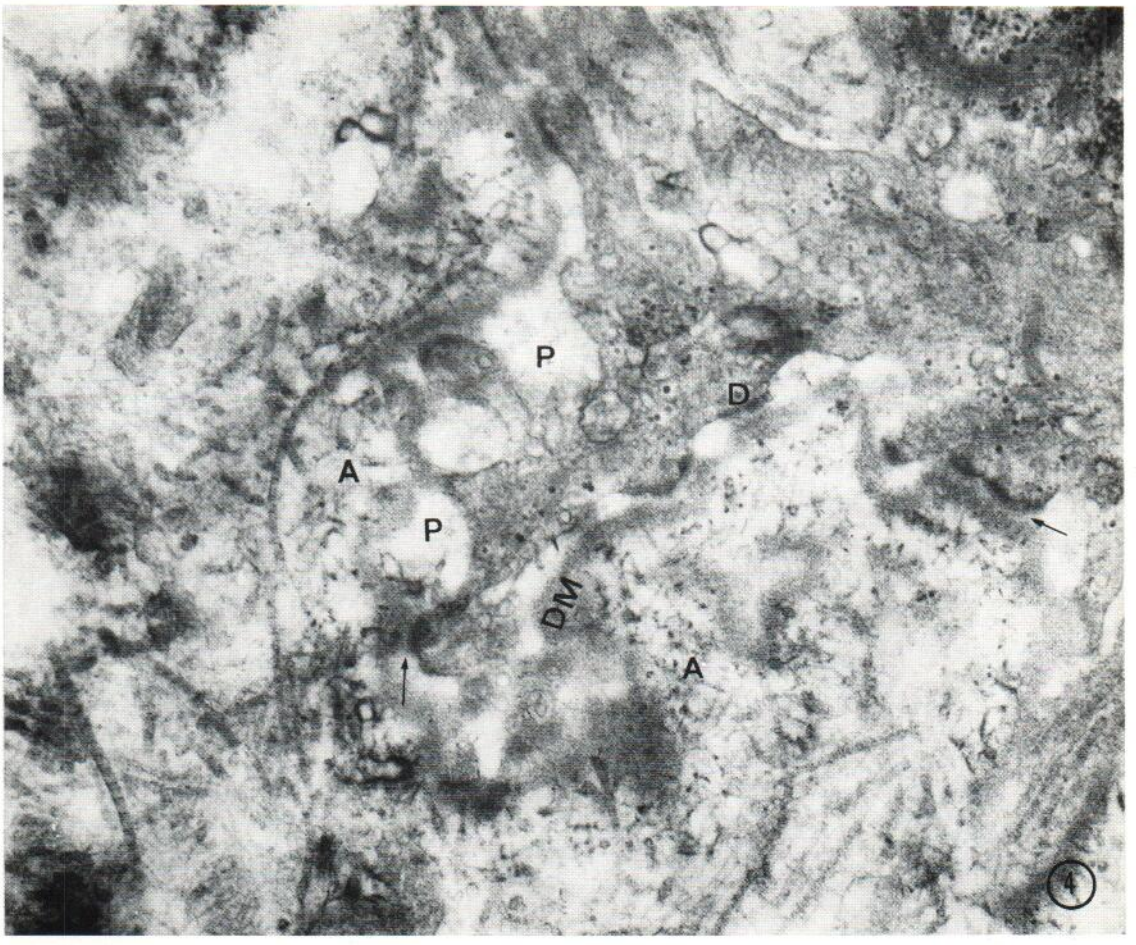


*Fig. 4.* Pocket-like subepidermal spaces (*P*). Half-desmosomes (*D*) and anchoring filaments (*thin arrows*) are blurred while anchoring fibrils are distinct (*A*). The dermal membrane (*DM*) shows irregular thickness. There are no elastic fibril anchorings. Collagen fibrils show distinct banding. × 47 000.

study no figures indicating newly formed elastic fibrils could be seen. The number of distinctly banded collagen fibers also decreased, but no figures of degradation were found. Some thread masses under the dermal membrane (Figs. 5, 6) simulate amyloid (3). The round particles with interconnecting fine threads are presumed to represent acid mucopolysaccharides (Fig. 9) mainly because of strong metachromasia with toluidine blue in the very same areas. Identical figures have been described in the aorta of chick embryos (12). No similarities between basal and squamous cell carcinomas can be demonstrated regarding stromal destruction (8). Penetration of cytoplasmic protrusions from cancer cells through the dermal membrane has previously been described in human squamous cell carcinoma of skin (8) as well as in oral mucous membrane (2) and experimental carcinomas of mice (1, 11). However, the existence of definite connections between cancer cells and dermal membrane in the immediate surroundings of the perforations militates against the probability of migration of the cancer cells. The cytoplasmic penetration observed in the present study only suggests increased cellular activity. It can be concluded now that, depending on its maturity, the basal cell carcinoma can produce pathological junctional structures. The change of the junctional structures as well as the penetration of cytoplasmic protrusions may signify early invasion of the tumour.

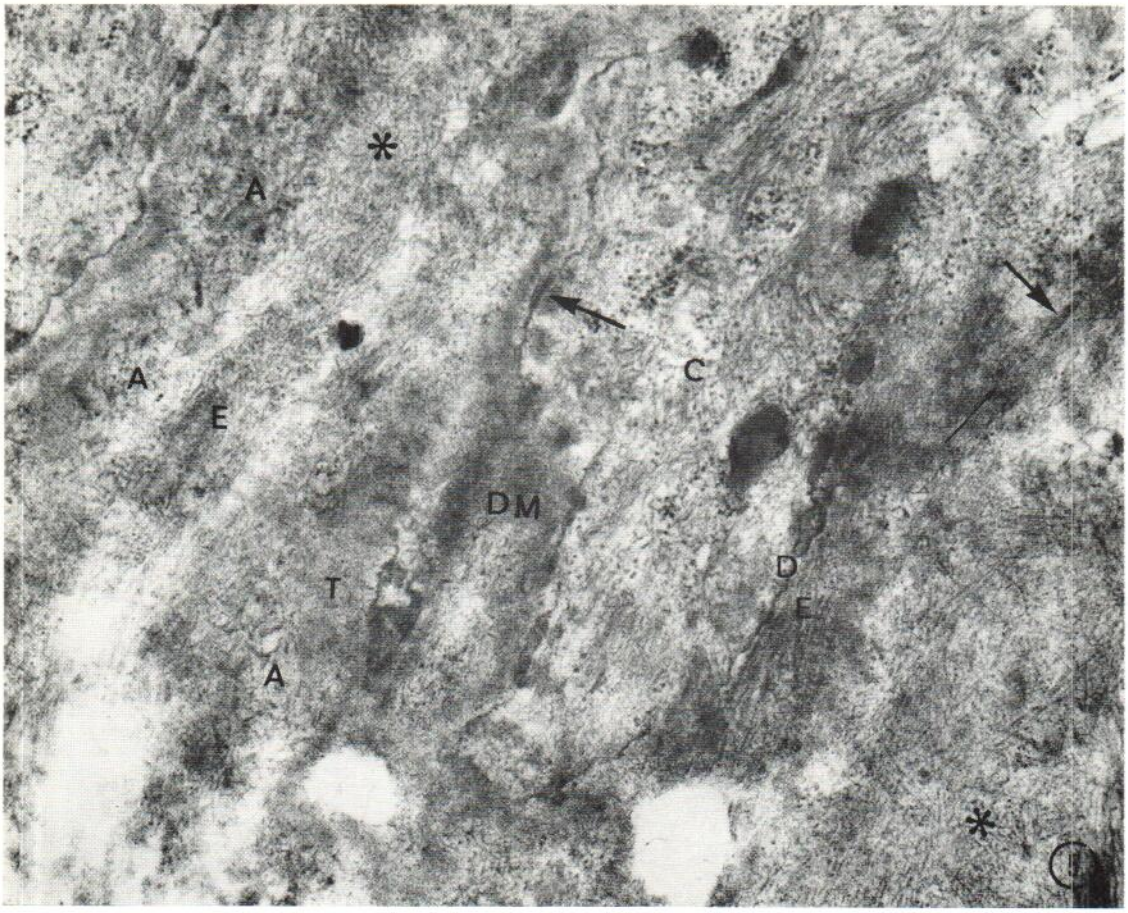


*Fig. 5.* A basal cell bordered by irregularly shaped dermal membrane (*DM*) without subepidermal space (*thin arrow*). The dermal membrane shows localized thickenings (*T*) and defects (*D*). The anchoring fibrils (*A*) accumulate here and there under the dermal membrane. Elastic fibrils (*E*) are found close to the carcinoma cell (*C*). Masses of thread-like material are prominent (*). The half-desmosomes are blurred (*thick arrow*). × 47 000.

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## REFERENCES

1. Frei, J. V.: The fine structure of the basement membrane in epidermal tumors. J Cell Biol *15:* 335, 1962.
2. Frithiof, L.: Ultrastructure of the basement membrane in normal and hyperplastic human oral epithelium compared with that in pre-invasive and invasive carcinoma. Acta Path Microbiol Scand Suppl. *200,* 1969.
3. Hashimoto, K., Gross, B. G. & Lever, W. F.: Lichen amyloidosis. Histochemical and electron microscopic study. J Invest Derm *45:* 204, 1965.
4. Kobayasi, T.: An electron microscope study on the dermo-epidermal junction. Acta Dermatovener (Stockholm) *41:* 481, 1961.
5. — Morphology of the dermo-epidermal junction. Collagen Symposium (Tokyo) *VI:* 73, 1965.
6. — Development of the fibrillar structures in human fetal skin. An electron microscope study. Acta Morph Neerl Scand *4:* 257, 1966.
7. — Electron microscopy of the elastic fibers and the dermal membrane in normal human skin. Acta Dermatovener (Stockholm) *48:* 303, 1968.
8. — Dermo-epidermal junction in invasive squamous cell carcinoma. Acta Dermatovener (Stockholm) *49:* 445, 1969.
9. Mehregan, A. H., Staricco, R. G. & Pincus, H.: Elastic fibers in basal cell epithelioma. Arch Derm (Chicago) *89:* 93, 1964.

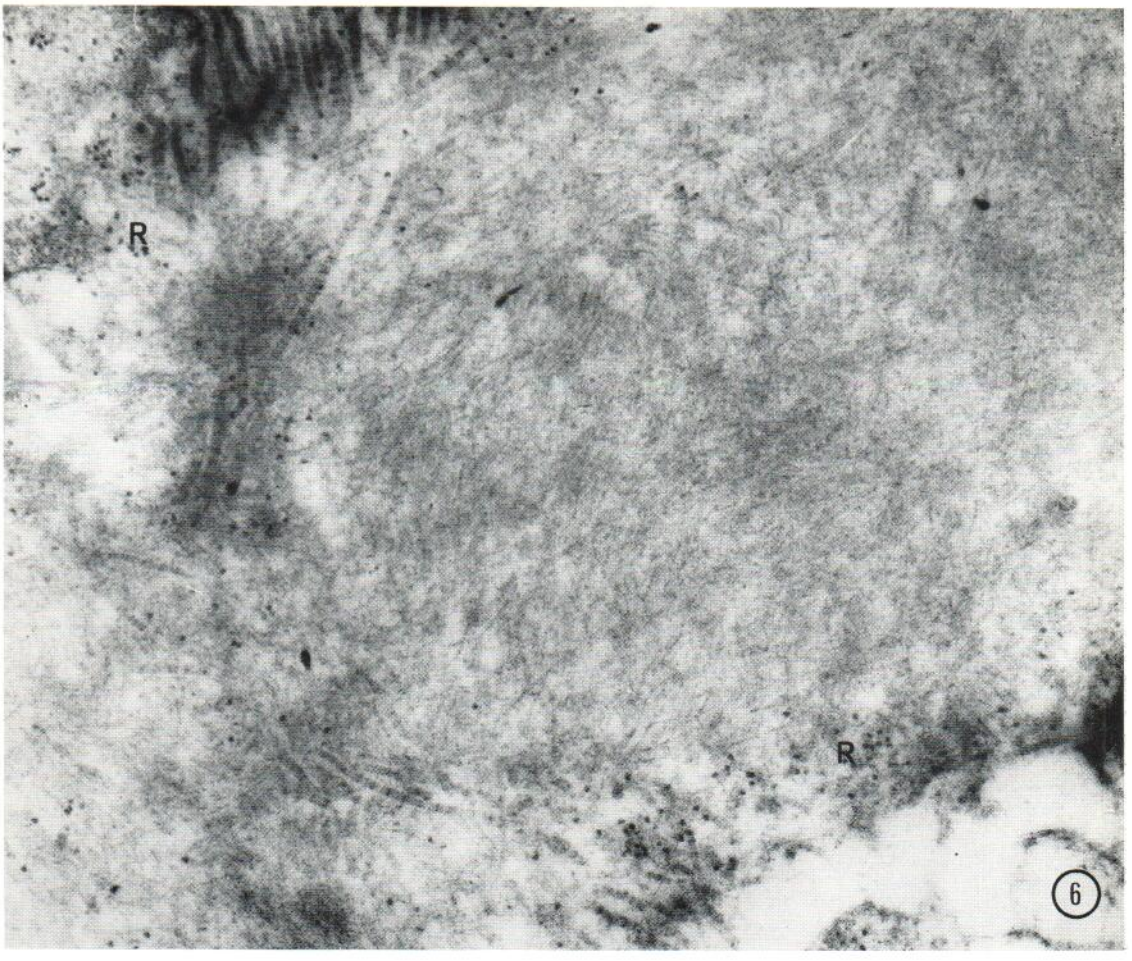


*Fig. 6.* A mass of threads is surrounded by collagen fibrils and ribosome-like granules (*R*). × 47 000.

10. Runder, E. J., Mehregan, A. H. & Pincus, H.: Elastic fibers in superficial basal cell epithelioma. Occurrence on solar protected areas. J Invest Derm *45:* 70, 1965.
11. Tarin, D.: Sequential electron microscopical study of experimental mouse skin carcinogenesis. Int J Cancer *2:* 195, 1967.
12. Takagi, K. & Kawase, O.: An electron microscopic study of the elastogenesis in embryonic chick aorta. J Electron Micro (Tokyo) *16:* 330, 1967.
13. Zelickson, A. S.: An electron microscope study of the basal cell epithelioma. J Invest Derm *39:* 183, 1962.

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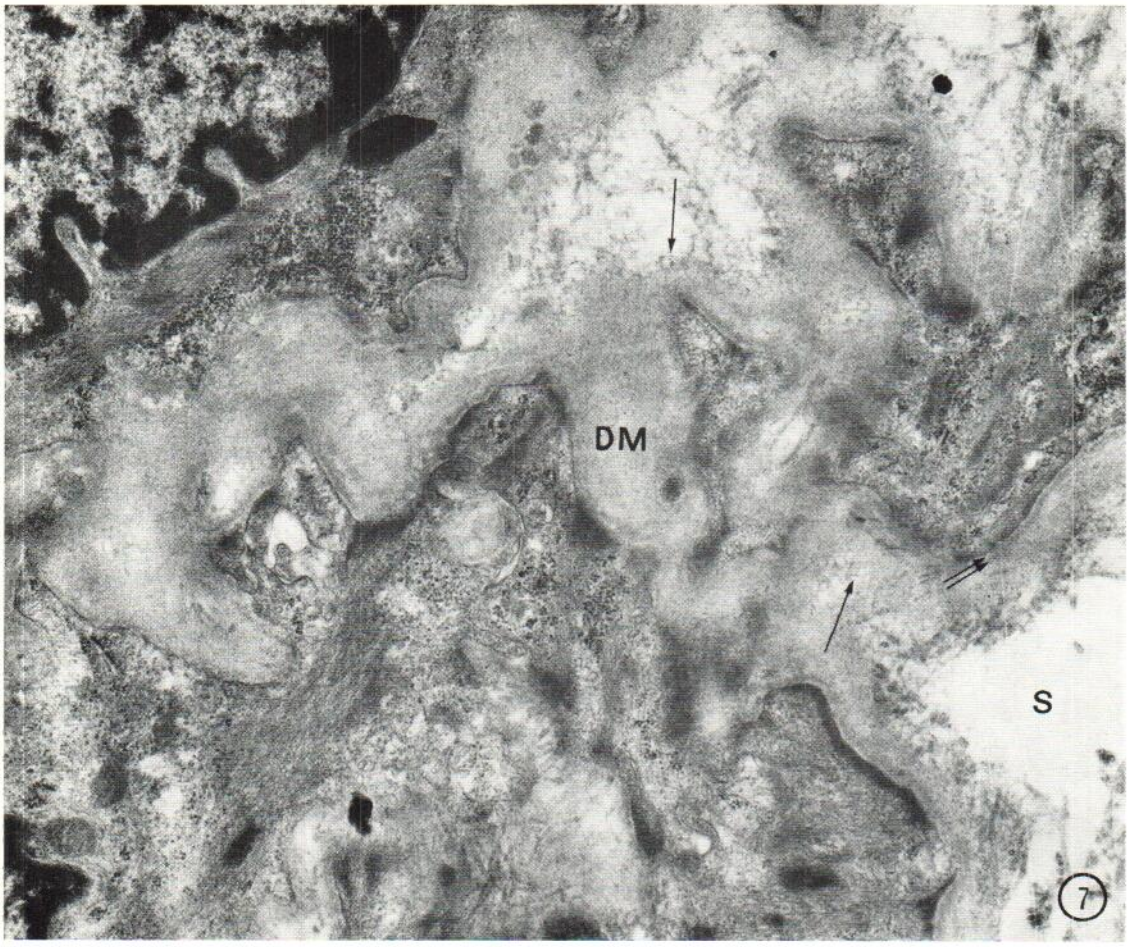


*Fig. 7.* Extremely thick dermal membrane (*DM*). Indistinct narrow bands (*two parallel arrows*) are found close to the cancer cell membrane, simulating subepidermal space and dermal membrane. The carcinoma cells contain thin tonofilaments and dense thickenings of their cell membrane, but no distinct half-desmosomes. Anchoring fibrils are scanty (*arrows*). Empty spaces (*S*) are found under the thick dermal membrane (*DM*). × 16 000.

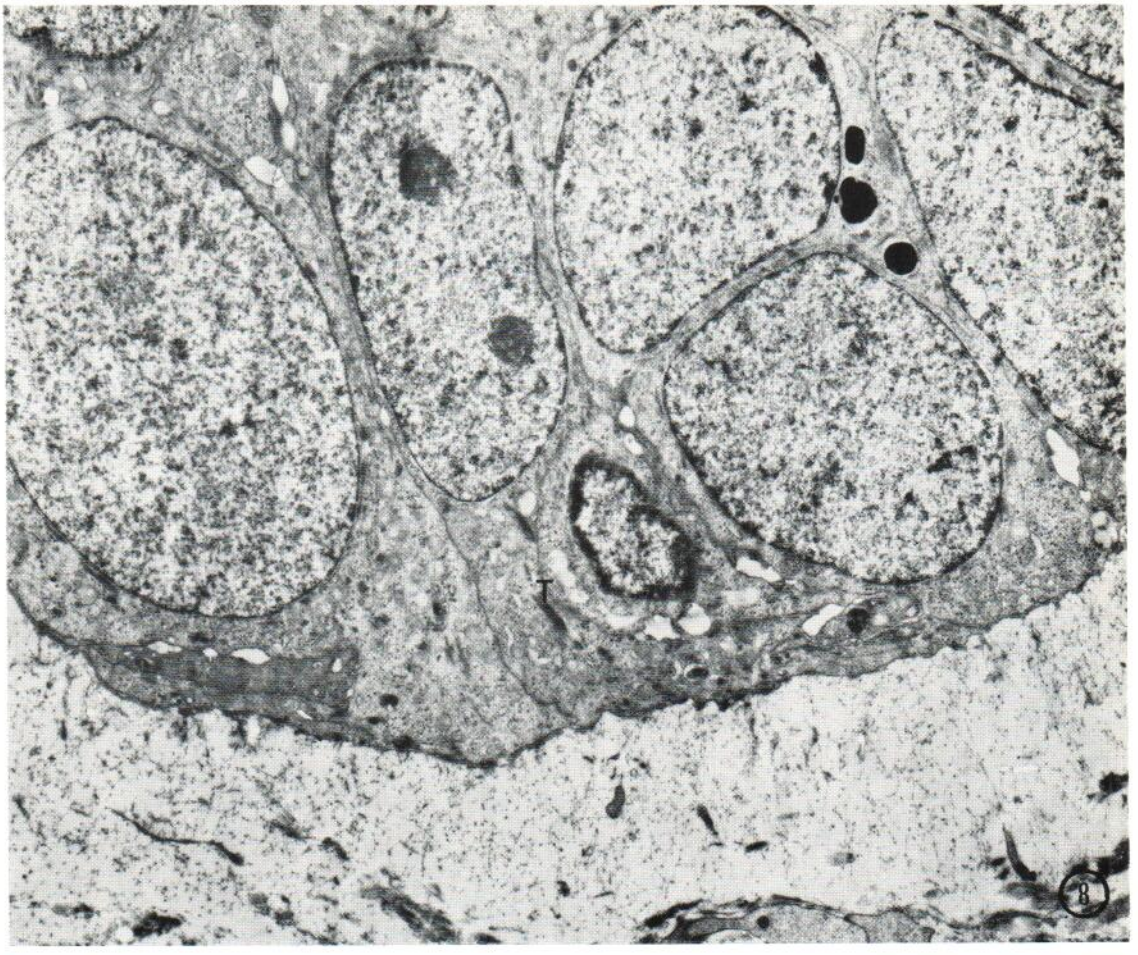


*Fig. 8.* Carcinoma cells containing a few tonofilament-bundles (*T*). The dermal membrane is partially coating the cell surfaces. Details are shown in Fig. 9. Under the dermal membrane, no distinct dermal fibrils are found. × 7 000.

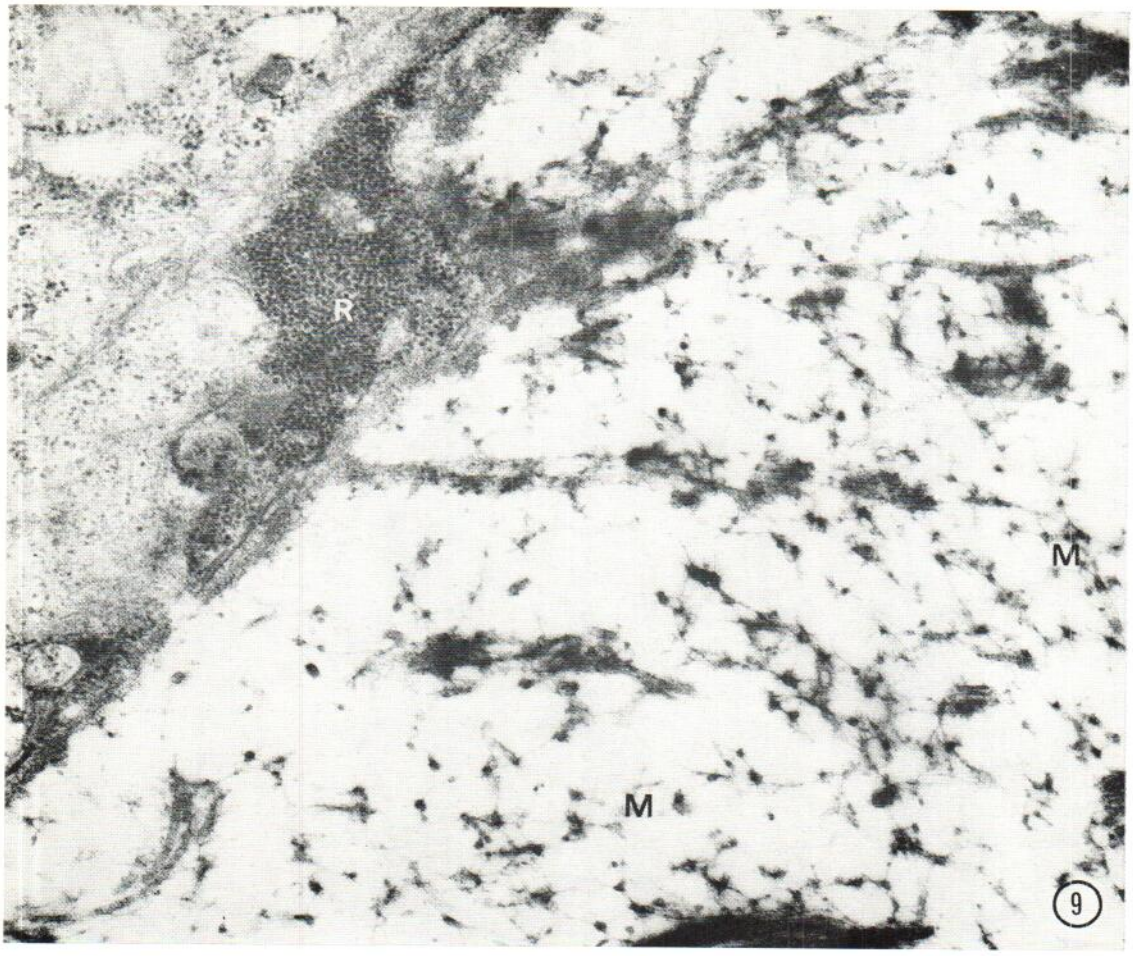


*Fig. 9.* Dermal membrane coating a cancer cell surface. No regular junction structures are seen. In the stroma, no collagen or elastic fibrils are found. Many round small particles with fine thready connections, probably mucopolysaccharides, are outstanding (*M*). Ribosomes in a carcinoma cell (*R*). × 47 000.

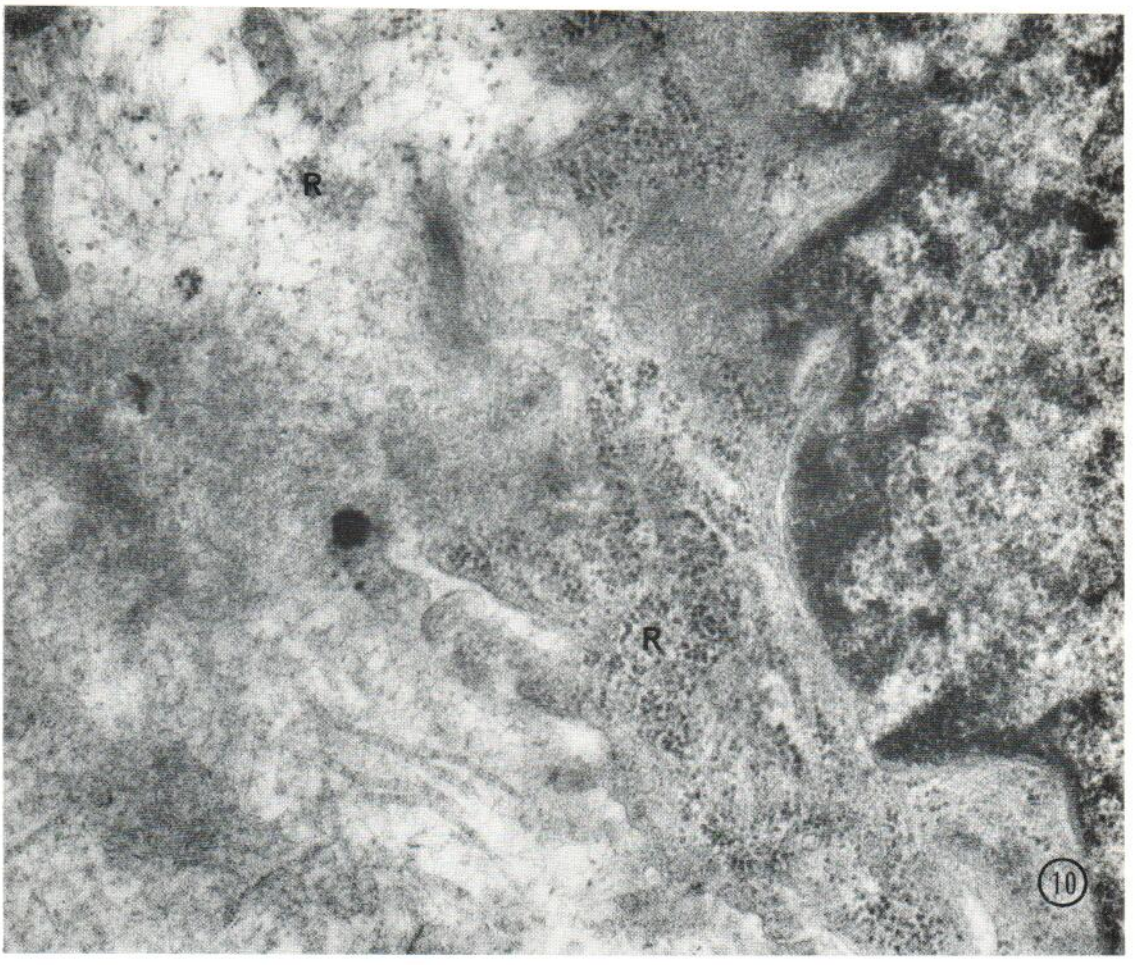


*Fig. 10.* A carcinoma cell containing masses of ribosomes (*R*) shows cytoplasmic protrusions but no junction structures. Ribosome-like granules are also found in the connective tissue (*R*). × 47 00.