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DERMO-EPIDERMAL JUNCTION IN INVASIVE SQUAMOUS CELL CARCINOMA

An electron microscopic study

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The *normal dermal membrane* is a band-like structure, which is approximately 300 Å thick and arranged almost parallel to the baseline of the epidermal cells (17). In this band a meshy substructure made up of very fine vertical and horizontal filaments has been demonstrated (10, 11).

The dermal membrane is separated from the opposed basal epidermal cells by the *subepidermal space,* which is also approximately 300 Å wide.

Across this space, bundles of very fine parallel *"anchoring filaments"* (10, 11) simulating the meshwork of the dermal membrane extend from this structure to the half-desmosomes of the basal epidermal cells.

From the dermal surface of the dermal membrane short fibrils emerge. These are the *"anchoring fibrils"* of Palade and Farquhar (16). Their endings have not been clearly visualized. It is presumed that the dermal membrane and the anchoring filaments and fibrils emerging in both directions consist of the same material.

The elastic fibrils of the corium are attached to the dermal membrane without any intermediate connection figures. These *"elastic fibril anchorings"* is the last link of the system which anchors the epidermal cells to the corium, and which seems to be of elastic nature (10, 11, 12, 13). The structures of the dermo-epidermal junction are considered to develop by unknown interaction between epidermal cells and the dermal connective tissue (12), or by simple secretion from the epithelium (8).

Cytoplasmic protrusions of human cervix carcinoma cells through the underlying junction have been observed (1, 7, 9). However, the ultrastructure of the penetration of human skin carcinoma into the dermis is insufficiently known. The present study reveals ultrastructural changes of invasion of a human squamous cell carcinoma through the dermo-epidermal junction.

Material and Methods

A squamous cell carcinoma of the vulva consisted of an atrophic scaly part and a slightly elevated papillomatous part. Numerous specimens were removed from both areas for electron microscopy. The specimens were fixed with a 4 % glutaraldehyde solution in veronal acetate buffer pH 7.4 with 4.5 % sucrose for one hour, washed in the same buffer overnight, fixed with a 1 % osmic acid solution in the buffer for 30 minutes, and washed in the buffer. The fixation and washing were carried out in a refrigerator controlled at a temperature of 4°C. The samples were dehydrated in a series of graded alcohols and emhedded in Epon 812. To select areas for ultramicrotomy, thick sections were stained with

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toluidine blue and examined under the light microscope. By an LKB ultramicrotome ultrathin sections were cut of dermal areas where oval or spindle-shaped large cells with ample cytoplasm and mitotic figures were found. The sections were stained with uranyl acetate and lead citrate. A Siemens electron microscope "Elmiskop I A" was operated at 80 kV with double condensors.

Observations

In the ultrathin sections the basal epidermal cells showed sparse bundles of short tonofilaments, ribosomes, and distinct half-desmosomes as well as short cytoplasmic protrusions into the inter-epithelial and subepidermal spaces (Fig. 1, 2, 3). In all specimens, the dermo-epidermal junction showed two pathological figures. One was characterized by an almost normal dermal membrane and a subepidermal space that appeared irregularly wide because of the presence of cytoplasmic protrusions from the basal epidermal cells. The anchoring filaments failed to show parallel arrangement, nor did they form bundles. The anchoring fibrils and the elastic fibril anchorings were like those of normal skin (Fig. 1). The other changes were an irregularly thick dermal membrane and innumerable threads extending from the dermal membrane to the basal epidermal cells and to the corium. The subepidermal space was almost filled with threads, while bundles of anchoring fibrils and elastic fibril anchorings could not be identified (Fig. 2, 3). The irregularly thick dermal membrane showed sporadic discontinuities where cytoplasmic protrusions of basal epidermal cells were penetrating. The protrusions contained no half-desmosomes and faced the corium without any specific junction structures (Fig. 3, 4).

Fragments of dermal membrane were found in the surroundings of the cancer cells in the corium and near the cytoplasmic protrusions penetrating the dermo-epidermal junction. However, the variously shaped fragments did not delimit the cancer cells from the surrounding dermis (Fig. 4, 5, 6, 7), the two structures being separated by connective tissue. Occasionally, the displaced parts of dermal membrane continued to the overlying original dermal membrane (Fig. 4, 5, 6). These dermal membrane fragments showed no anchoring filaments, and scarce elastic fibril anchorings, but numerous distinct anchoring fibrils (Fig. 6, 7, 8). Dense thread-like material was pronounced in the surrounding dermal connective tissue (Fig. 8, 9).

The cancer cells in the upper corium showed bizarre nuclei with lucent spots in the chromatin areas, and with ribosomes, mitochondria, and, in some cells, lysosome-like particles and granular endoplasmic reticulum in their cytoplasm. However, no tonofilaments and no half-desmosomes could be demonstrated. The cancer cells faced the dermal connective tissue with their cell membrane (Fig. 5, 6, 7). Occasionally, such primitive cancer cells were also found in the epidermal layer (Fig. 11).

In the deeper corium, primitive cancer cells were found single or grouped. Their mitochondria were few and their lysosome-like particles were poorly developed. Around the cancer cells, there was no dermal mebrane, only dense thread-like material (Fig. 9). Extremely long narrow cytoplasmic protrusions of the cancer cells were seen along the elastic fibrils, showing cytoplasmic ampullae (Fig. 10).

Discussion

Because the dermo-epidermal junction seems to be developed by an interaction between the basal epidermal cells and the connective tissue (12) it is logical to expect a change following malignant transformation of the epidermal cells. Carcinoma cells exhibit various stages from primitive to differentiated forms (5, 15, 19). However, no figures specific of cancer have been found in the cytoplasm of such cells (2, 4, 7). Variations in tonofilaments and desmosomes seem to indicate the grade of differentiation, the most primitive cells containing no tonofilaments, no desmosomes, no granular endoplasmic reticulum, few mitochondria, poorly developed lyso-

some-like particles, and numerous ribosomes (3, 19). In the present study of squamous cell carcinoma, the basal epidermal cells could not be characterized as differentiated cancer cells nor as precancerous cells, but irregular cytoplasmic protrusions suggested a considerable cell activity. The cancer cells located in the corium appeared primitive because of lack of tonofilaments and desmosomes.

The numerous fine threads emerging from the original dermal membranes in both directions suggest early changes of the dermo-epidermal junction occurring together with the epidermal changes. The pathologic structure and the varying thickness in the areas of invasion as well as the location of dermal membrane fragments around the cancer cells suggest that this material originated from some interaction between the primitive cancer cells and the dermal connective tissue. A displacement of the dermal membrane has previously been observed in experimental carcinogenesis of mice (18). Cytoplasmic protrusions through the dermo-epidermal junction have been observed in experimental carcinogenesis of mice (6) and hamsters (5), but have not been reported to be seen in squamous cell carcinomas in man.

One mode of invasion was characterized by signs of dedifferentiation in the cellular protrusions from the basal epithelial cells penetrating the dermo-epidermal junction, and by the existence of displaced fragments of dermal membrane. Another mode of invasion was suggested by the findings of primitive cancer cells in the middle of the epidermis. These cells may migrate into the corium through the dermo-epidermal junction.

The long and narrow cytoplasmic processes with ampullae observed in the corium indicate that the carcinoma cells extend along the elastic fibrils transporting cytoplasmic components between distant areas.

Dense thread-like material between the dermal fibers may represent destruction of dermal connective tissue (14).

SUMMARY

Invasion of primitive squamous cell carcinoma and an altered fine structure of the dermo-epidermal junction were demonstrated by the electron microscope.

One mode of invasion was characterized by penetration of cytoplasmic protrusions through the junction. Dedifferentiation was found only in these protrusions.

Another mode of invasion was suggested by the findings of primitive cancer cells in the middle epidermal layer. These cells are presumed to migrate into the corium through an altered dermo-epidermal junction. The junctional changes were an irregularly thick dermal membrane, an irregularly wide subepidermal space, and innumerable threads emerging from the dermal membrane, while the anchoring filaments and fibrils and elastic fibril anchorings were scarce and indistinct.

Fragmented dermal membrane material was located in the surroundings of the primitive cytoplasmic protrusions and of the primitive cancer cells in the corium, although not immediately bounding the cancer cells from the corium. Here and there, the fragments showed a continuity with the original dermal membrane. The displaced dermal membrane fragments showed no anchoring filaments, numerous distinct anchoring fibrils, and scarce elastic fibril anchorings. This dermal membrane material may be formed by unknown interaction between the primitive cancer cells and the corium. In the surroundings of the primitive cancer cells dense thread-like material was demonstrated in the corium.

Long narrow cytoplasmic processes of the primitive cancer cells were found along the dermal elastic fibrils.

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FIGURES

Fig. 1. Basal epidermal cells (EP) contain sparse bundles of short tonofilaments (T), and half-desmosomes with distinct lamellae (thick arrow). Short cytoplasmic elongations (P) protrude into the interepithelial and subepidermal spaces. The thickness of the dermal membrane (D) is regular and constant, while the subepidermal space (S) is irregularly wide. The anchoring filaments show an irregular unparallel arrangement (thin arrows). Anchoring fibrils (A) and elastic fibril anchorings (EA) are seen. E indicates elastic fibrils, and C collagen fibrils. × 60,000

Fig. 2. Basal epidermal cells showing sparse bundles of short tonofilaments (T) and some distinct half-desmosomes (thick arrow). The basal epidermal cells send cytoplasmic protrusions into the corium. The protrusions are bounded by an irregularly thick dermal membrane (D). The anchoring filaments, anchoring fibrils, and elastic fibril anchorings are indistinct and appear like thread-like material in continuation with the dermal membrane. S: Subepidermal space. × 60,000

Fig. 3. In the dermo-epidermal junction, the dermal membrane (D) shows an irregular thickness, and so does the subepidermal space (S). Some areas show no dermal membrane in the original place. Cytoplasmic protrusions through the dermo-epidermal junction(framed arrows). Fragments of dermal membrane are marked by FD. A half-desmosome (thick arrow) shows a definite lamella while others do not. The anchoring filaments and fibrils and the elastic fibril anchorings appear like threads. × 60,000

Fig. 4. Irregularly thick dermal membrane (D). Displaced dermal membrane (DD). Cytoplasmic protrusions reach the dermal fibers without specific junctional structures. The epidermal cells (EP) have tonofilaments (T), but, in their cytoplasmic protrusions, no distinct tonofilaments are seen, and no distinct half-desmosomes, while they are rich in ribosomes and cystic granular endoplasmic reticulum (CR). Numerous anchoring fibrils (A) and one elastic fibril anchoring (EA) are seen. FD indicates fragment of dermal membrane. × 60,000

Fig. 5. Invasion of cancer cells into the upper dermis. The invading cancer cells show mitochondria (M), lysosome-like particles (L) and granular endoplasmic reticulum (R). D indicates original dermal membrane, FD fragment of dermal membrane. DD displaced dermal membrane. High magnification of the framed areas are seen in the subsequent figures numbered respectively. × 15,500

Fig. 6. Cytoplasmic processes (P) penetrate through the dermo-epidermal junction. The profiles of the processes are primitive because of lack of tonofilaments and desmosomes. D indicates original dermal membrane, DD displaced dermal membrane. The displaced dermal membrane shows irregular thickness, distinct anchoring fibrils (A), but elastic fibril anchoring is not clear. × 60,000

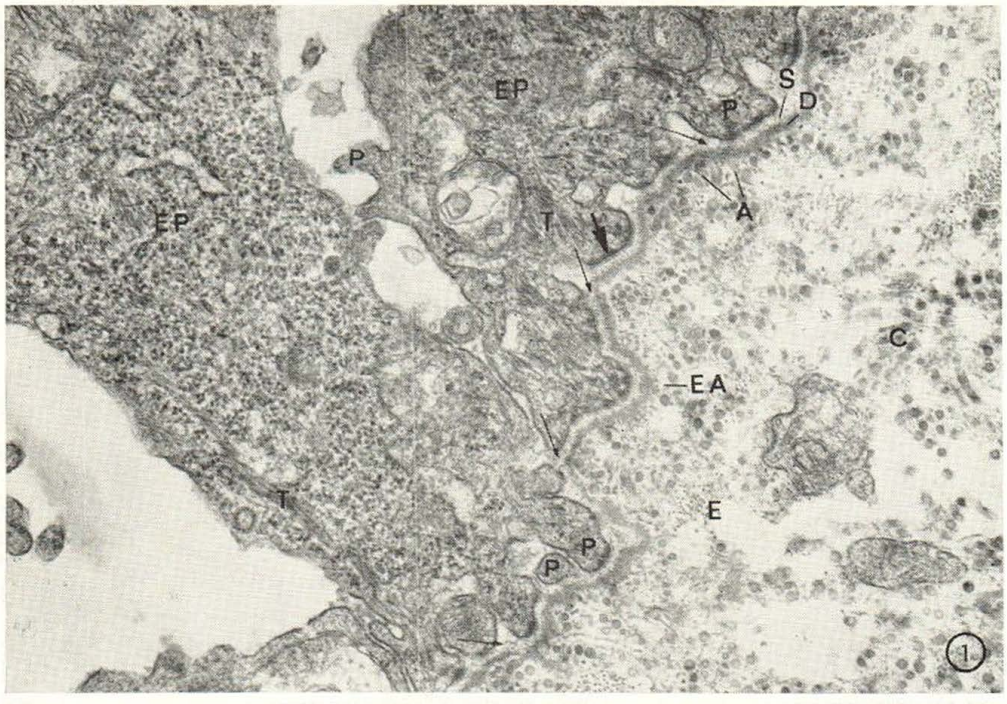
Fig. 7. Fragmented dermal membrane (FD) among primitive cancer cells (CC). The fragmented dermal membrane shows irregular thickness and numerous distinct anchoring fibrils (A). No anchoring filaments and no elastic fibril anchorings are seen. C indicates collagen fibrils. × 60,000

Fig. 8. Fragmented dermal membrane (FD) around a primitive cancer cell (CC). E indicates a bundle of elastic fibrils. In the dermal connective tissue, dense thread-like material is seen (*). × 60,000

Fig. 9. A primitive cancer cell shows mitochondria (M) and ribosomes in the cytoplasm. The cell is situated along bundles of elastic fibrils (E). Dense thread-like material (*) is seen in the dermal connective tissue. ×20,000

Fig. 10. A long narrow cytoplasmic protrusion of a primitive cancer cell is found along a bundle of elastic fibrils (E). A indicates cytoplasmic ampulla. ×30,000

Fig. 11. A primitive cancer cell in the epidermis. The profiles of the cell are similar to those of the cancer cells in the corium (Fig. 5). The cell contains a bizarre nucleus (N) with several lucent spots in the chromatin area. The cytoplasmic organelles are round mitochondria (M), ribosomes, granular endoplasmic reticulum (R), and variously shaped lysosome-like particles (L). There are no tonofilaments or desmosomes, and fine granular material coats the cell surface (arrows). ×15,000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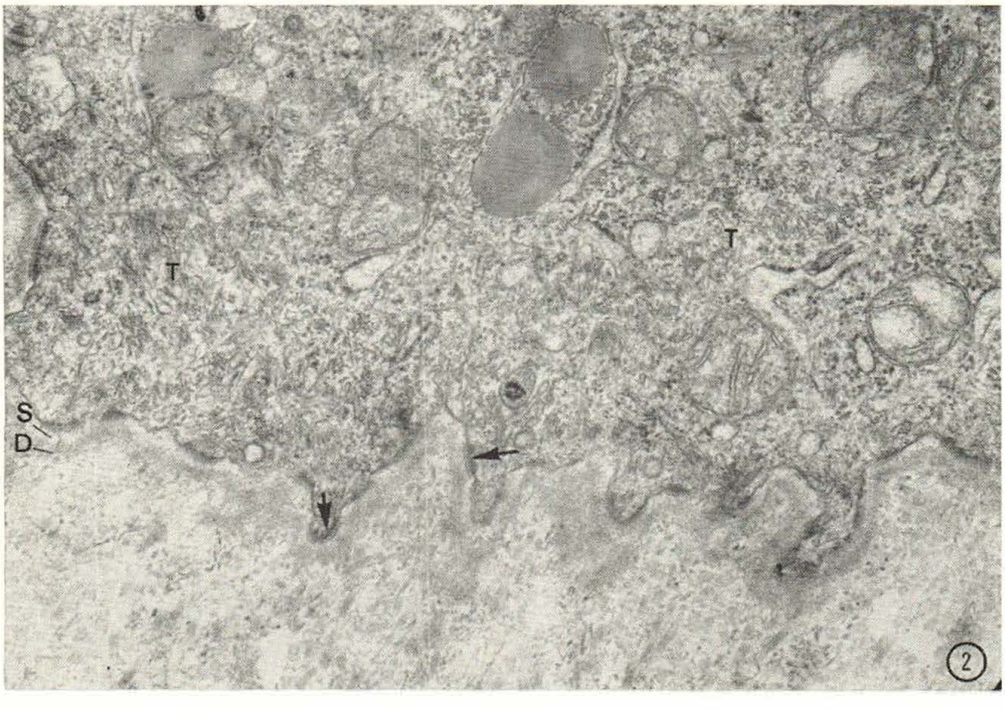
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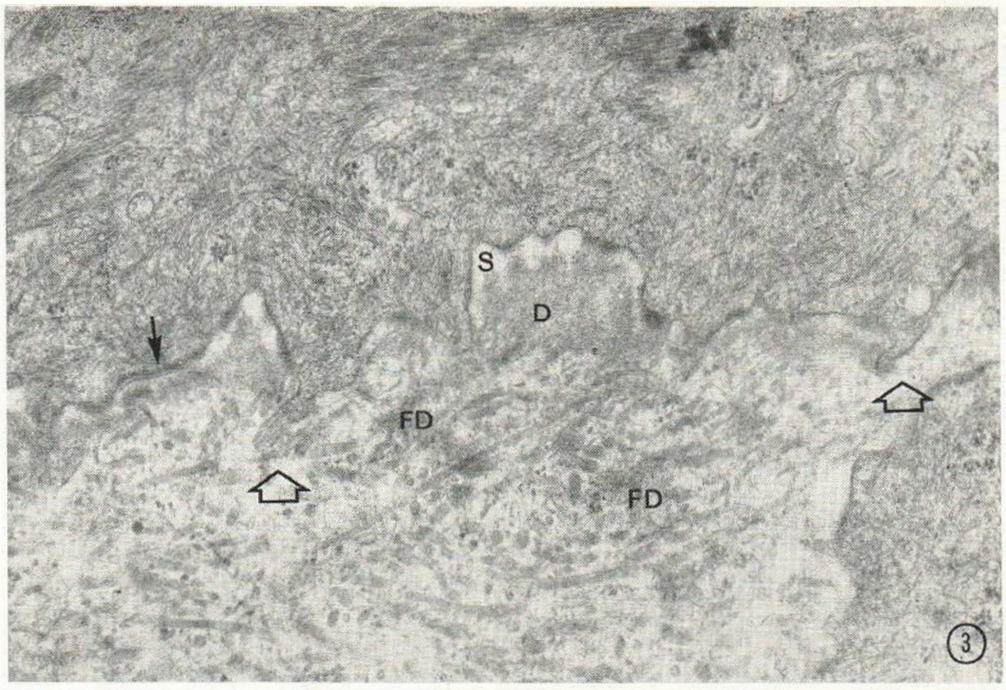
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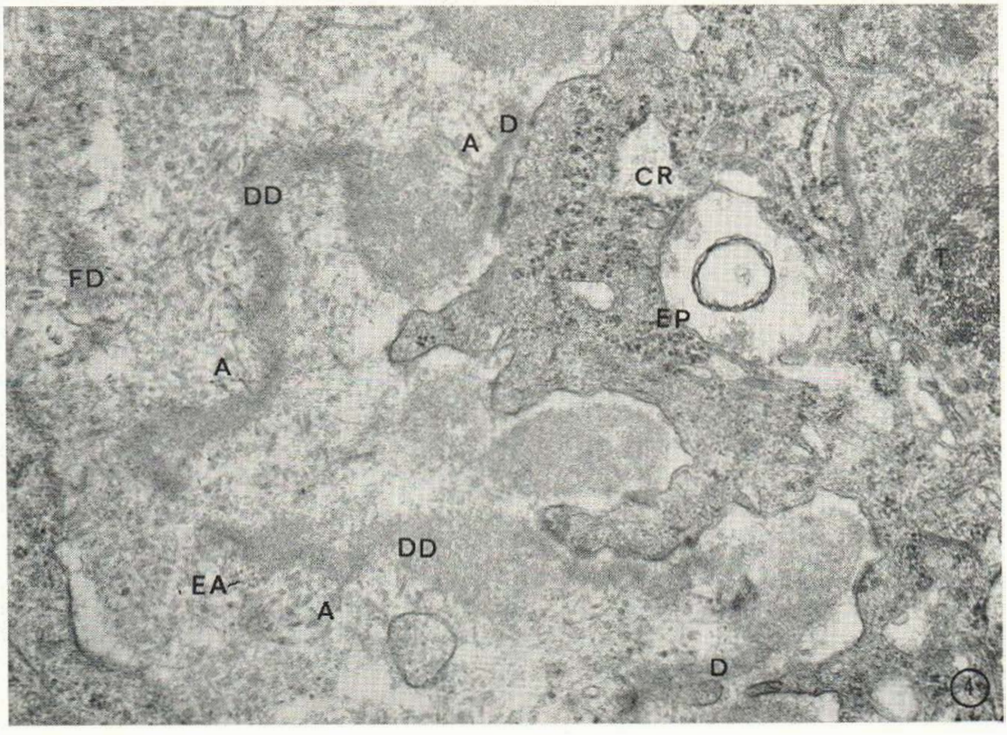
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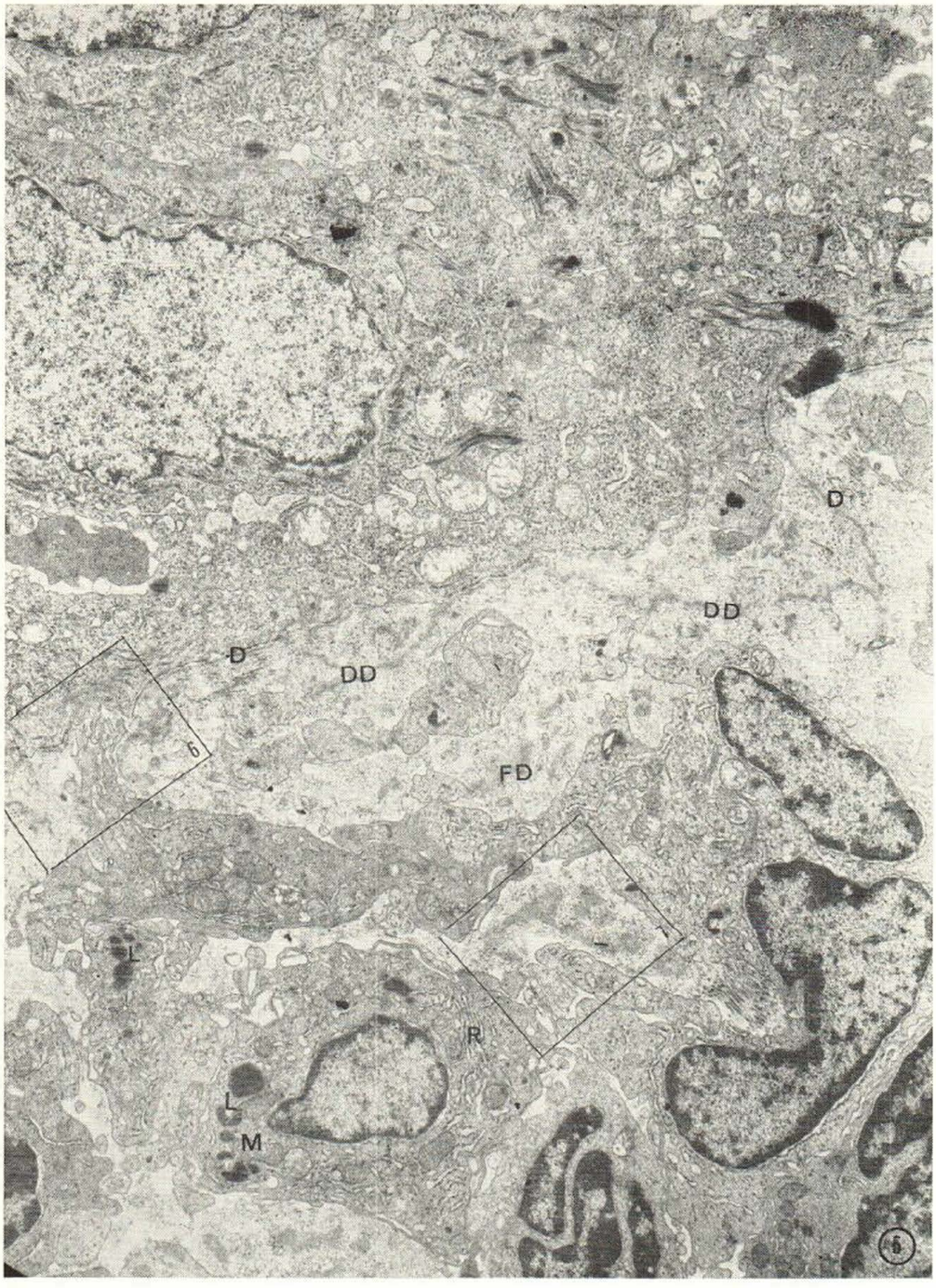
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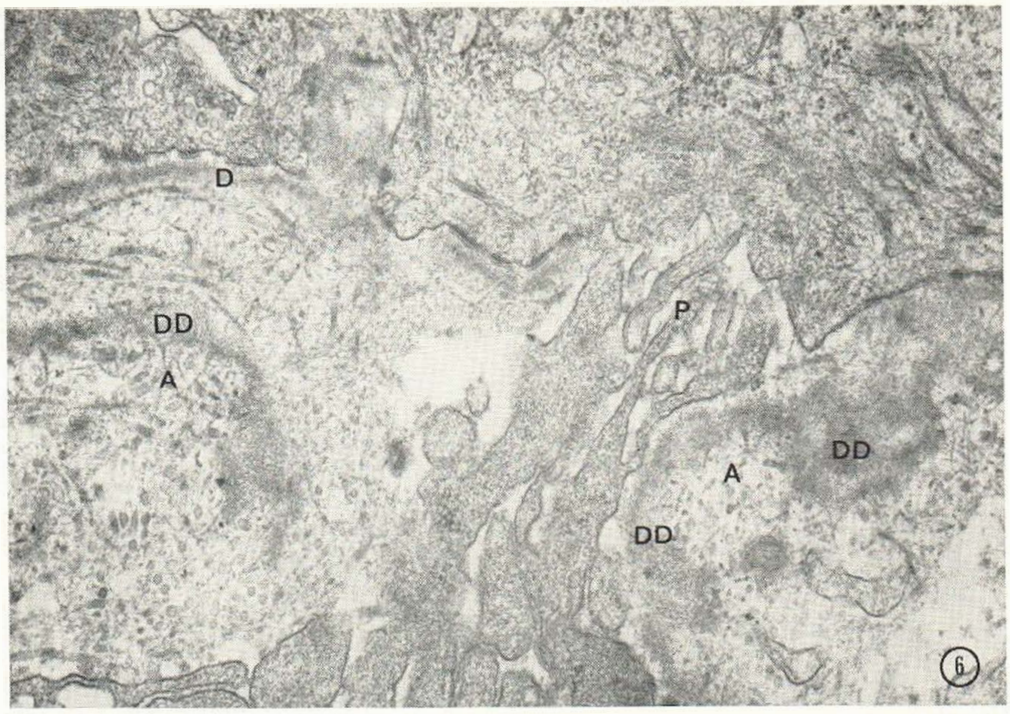
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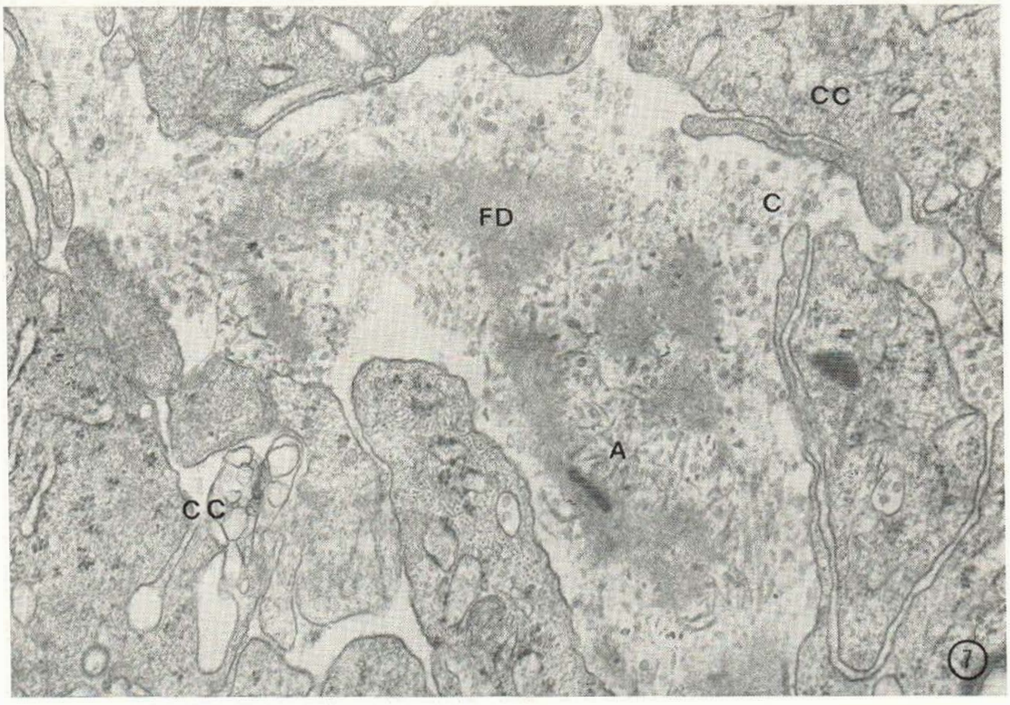
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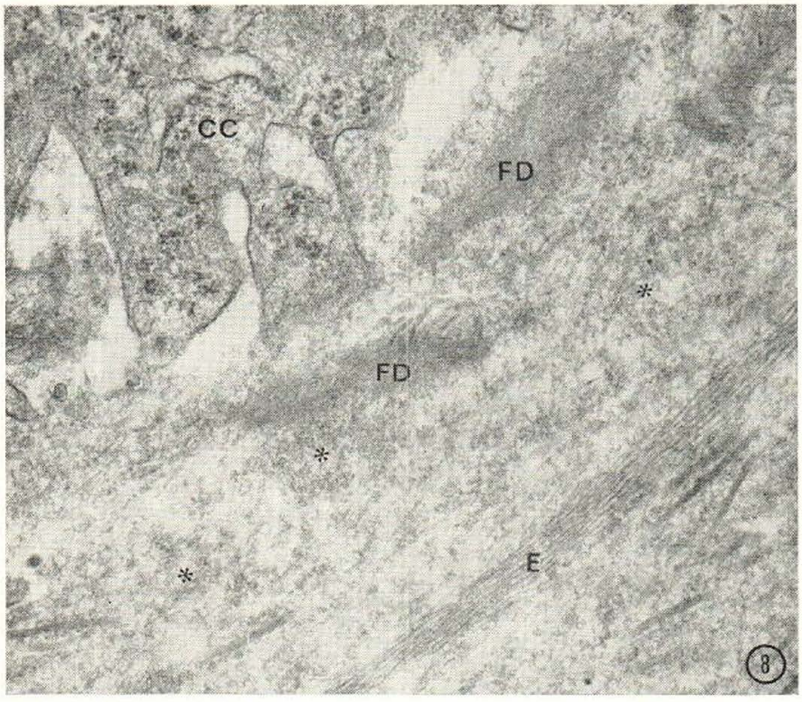
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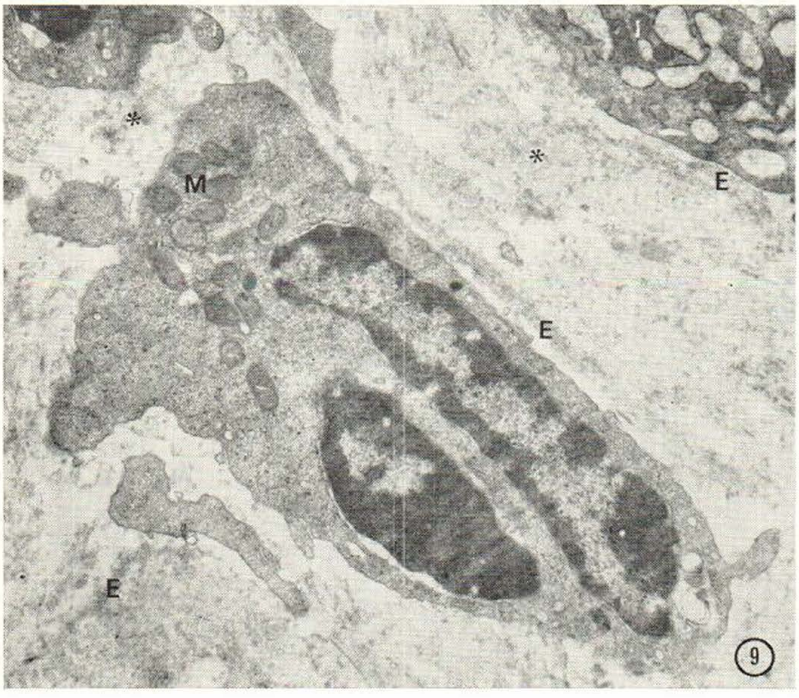
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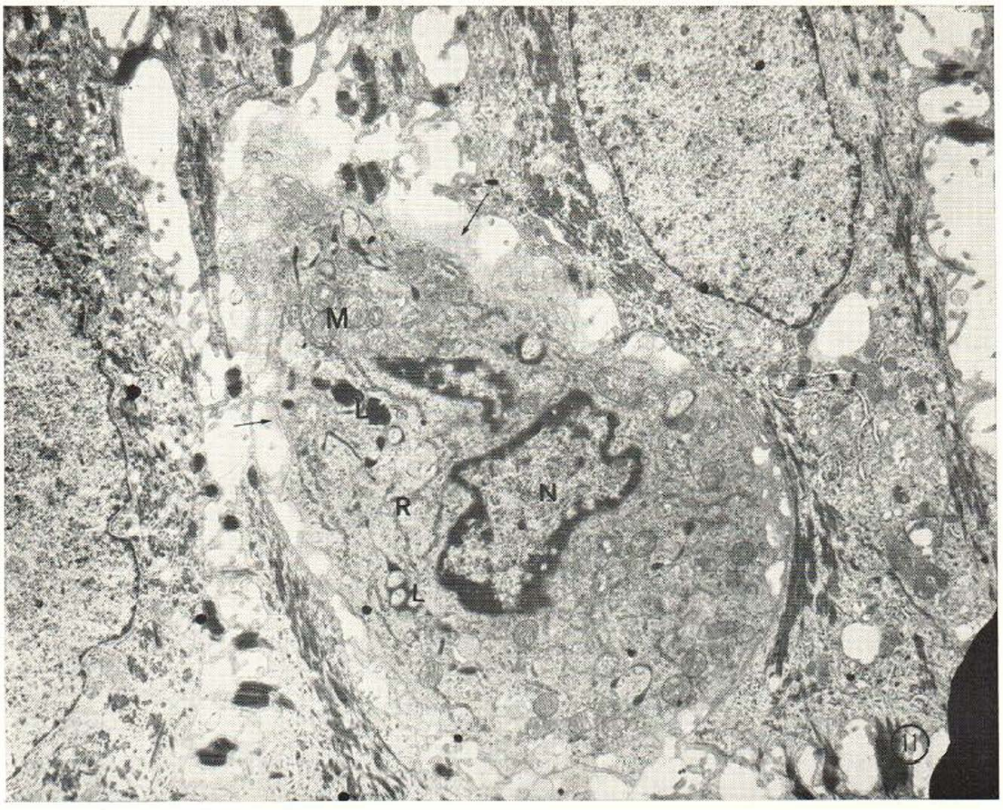
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