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ELECTRON MICROSCOPIC STUDY OF THE HUMAN ADULT ECCRINE GLAND

I. THE DUCT*

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Electron microscopic investigations of the adult human eccrine duct, both of the intraepidermal and intradermal segments, are few in number. It has been reported by Hibbs (1), Charles (2) and Zelickson (3) that the ductal lumen was lined by numerous microvilli. Hibbs (1) described a dense periluminal band of tonofilaments as being present in the luminal cells. It is still controversial whether the eccrine sweat duct remains patent in the intraepidermal segment of the duct (2) or is partially occluded (1). Hibbs (1), Charles (2) and Zelickson (3) had noted that the plasma membrane of the ductal cells showed complicated convolutions, but its significance was not certain.

In this investigation the luminal villi were observed pinching off into the lumen and often releasing their contents into the lumen. The intraepidermal segment of the duct, though often narrowed by the cytoplasmic projections, was found to be patent throughout. The convolutions of the plasma membrane were especially complex in the lateral border of the ductal cells, and were considered to be an important reserve of the membrane against stretching forces.

Zelickson (3) has previously stated that the intraepidermal portion of the eccrine sweat duct was a distinct entity composed of specialized inner cells and layers of outer cells. Keratohyaline granules were observed by Zelickson (3) and Charles (2) at different levels in the inner and outer cells of the intraepidermal duct; Zelickson (3) described them only in the inner cells near the stratum corneum and Charles (2) stated that they were present in ductal cells well below the level of the stratum granulosum.

In this study the duct was found to be composed of one layer of inner cells and two to three layers of outer cells. Keratohyaline granules were first detected in the middle section of the squamous layer in the inner cells of the duct and in the lower squamous layer in the outer cells.

The cuticle of the eccrine sweat duct observed with the light microscope has been attributed by previous authors to miscellaneous subcellular organelles localized near the luminal border, such as "granules" and villi by Zelickson (3) or as terminal webs, intracellular tonofilaments and desmosomes by Ellis and Montagna (4). In this study, however, an extraneous fuzzy coating of the luminal villi was found and interpreted as the structure which could also be responsible for the cuticle seen under light microscopy.

MATERIALS AND METHODS

Biopsy specimens were obtained from several areas of the body including the soles of the feet and the palms of the hands of adult Caucasian volunteers. The specimens were cut into 1 mm cubes and fixed for 2 hours in a 1% solution of osmium tetroxide which had been buffered to pH 7.2 with veronal acetate and then had been adjusted (5) to physiological osmolarity with 4.5% sucrose (5). The pieces of tissue thus fixed were dehydrated in increasing concentrations of ethanol and propylene oxide and embedded in Araldite. Sections were cut with an LKB Ultrotome, and stained first with 1% uranyl acetate in 50% ethanol for 1 minute and then with lead citrate of Reynolds (6) for ten minutes. This combination of staining greatly improved the contrast of the electron micrographs. Uranyl acetate in ethanol seemed to increase the staining propensity of lead citrate by facilitating its penetration into the Araldite-embedded tissue.

RESULTS

1. Intraepidermal Eccrine Sweat Duct

In the basal and squamous layers of the epidermis the eccrine duct was composed of

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several concentric layers of cells. At low magnifications the cells of the duct were readily distinguishable from the cells of the surrounding epidermis because of their semilunar configuration in cross-sections (Fig. 1). The ductal wall cells of the stratum granulosum were completely keratinized (Fig. 2), whereas the epidermal cells surrounding the ductal cells were not yet keratinized. In the upper stratum corneum, however, the ductal cells could not be recognized as such were it not for the persistent lumen (Fig. 3).

The inner lining of the duct was composed of a single layer of inner cells. They appeared semilunar in cross-section with curved long axis surrounding the lumen.

Numerous vesicles of various sizes and slightly electron dense amorphous substances filled the spaces between the microvilli (Figs. 1, 2). At low, scanning magnification as shown in Fig. 4, these vesicles and amorphous substances formed a distinct, innermost ring of the ductal wall which appeared to correspond to the cuticle under light microscopy (see below for detailed discussion).

The luminal border of each inner cell was studded with numerous cytoplasmic projections, or microvilli, which also lined the invaginations or crypts present along the luminal border in the lower epidermis (Fig. 5). Cytoplasmic elevations projected into the lumen at the junction of adjoining inner cells (Figs. 1, 2, 4).

The lateral border. In the basal layer and in the sweat duct ridge, the lateral border of each inner cell possessed an intricate series of cytoplasmic protrusions which interdigitated with those of the neighboring inner cells (Fig. 5). These interlocking cytoplasmic protrusions were connected by prominent desmosomes which, although numerous, were only present in approximately one half of the cytoplasmic protrusion. The border between the inner cells and the surrounding cells (outer cells) showed fewer cytoplasmic protrusions.

The luminal differentiations and multivesicular bodies. In appropriately sectioned specimens there were three distinct rings surrounding the nuclei (Figs. 4, 5): first, a perinuclear clear zone relatively free of tonofilaments and rich in organelles, second, peripheral to it, a narrow band of interwoven tonofilaments and third, close to the microvillous luminal border, a terminal web where cellular organelles are relatively few (Fig. 5). This band of tonofilaments became thickened along the luminal portion of the inner cell and formed a spongy meshwork of tonofilaments (Fig. 5). Both the perinuclear clear zone and spongy meshwork contained small vesicles, free ribonucleoprotein (RNP) particles and multivesicular bodies. The spongy meshwork of tonofilaments was similar to the tonofilamentous ring described by Ellis and Montagna (4) in the intraepidermal eccrine duct of *Macaca mulatta*. In agreement with Ellis and Montagna (4), the most likely function of this zone seemed to be in the formation of a tonofilamentous ring to prevent the collapse of the lumen. In this report, it will be referred to as the periluminal filamentous zone, as it has been called in the human embryo (7). The multivesicular bodies characterized the inner cells of the intraepidermal eccrine sweat ducts (Fig. 5). Multivesicular bodies were present in abundance in the intraepidermal eccrine sweat duct of human embryos (7); they appeared much more dense because of a deposition of electron dense substances (7). Small vesicles increased in number in the meshwork of the periluminal filamentous zone toward the luminal border (Fig. 5) and passed into the lumen through disruptions of the plasma membranes.

Keratinization of the Inner Cell. In most of the body areas and in the palm of the hand, keratohyaline granules were seen in the cytoplasm of the inner cells at the level of the middle section of the squamous layer of the surrounding epidermis (Fig. 4) (Table I). The inner cells accumulated more keratohyaline granules as they moved into the upper squamous layers (Fig. 5). At the level of the granular layer of the surrounding epidermis the inner cells appeared flattened, contained no nucleus but occasionally contained loosely packed keratin filaments in addition to small vesicles and fine particles (Fig. 2); keratinization seemed to have taken place since keratohyaline granules were no longer seen (Fig. 2). Along the luminal border some microvilli, though reduced in number, continued to be present (Fig. 2). In the middle section of the horny layer the inner cells began to shed into the lumen without further accretion of keratin filaments or an increase of interfilamentous cementing substances (Fig. 6). The thickness of the plasma membranes of these shedding inner

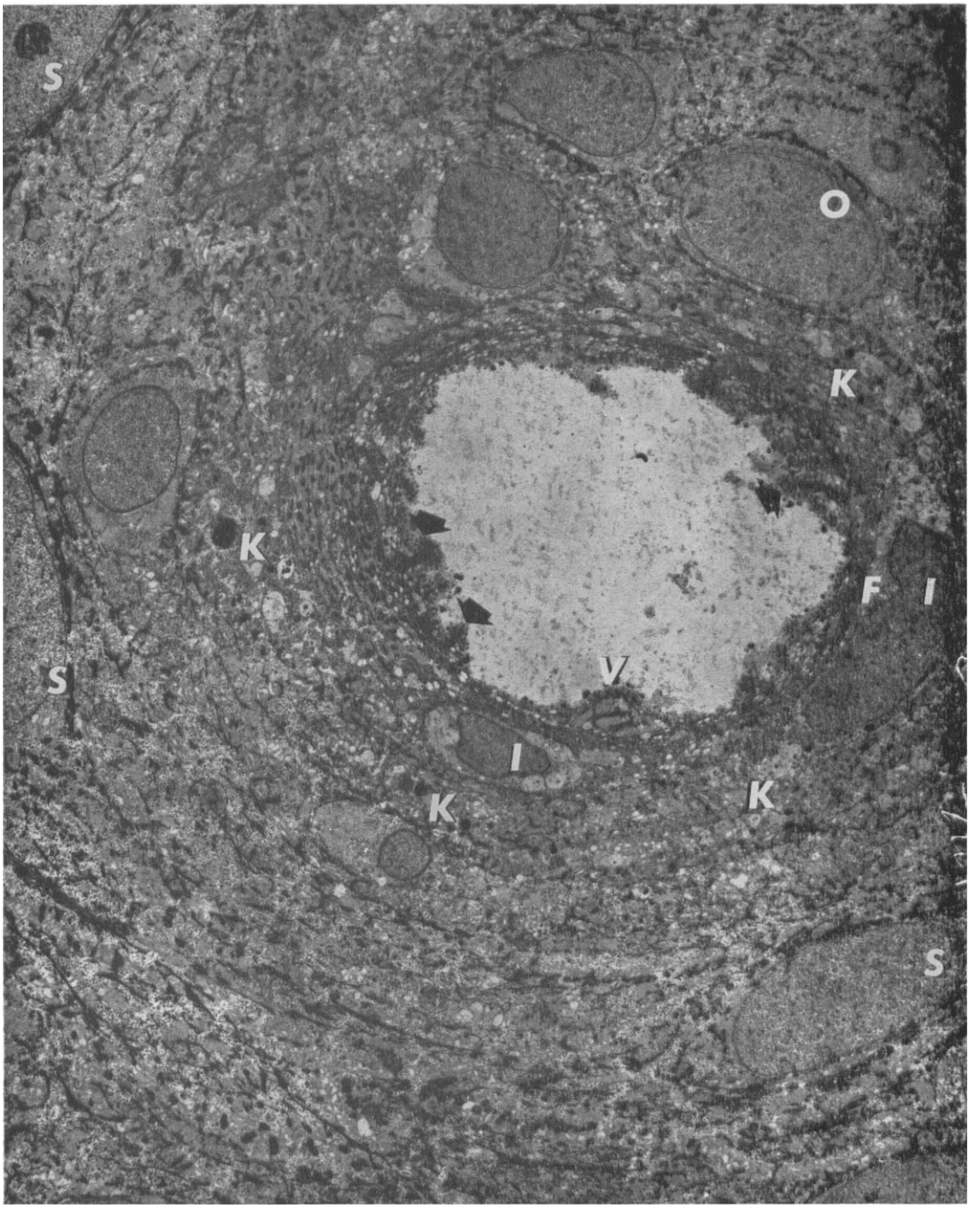

FIG. 1. *Epidermal sweat duct in the lower squamous layer.* The lumen is surrounded by inner cells (*I*) with numerous luminal microvilli (*V*), outer cells (*O*) and epidermal squamous cells (*S*). Keratohyaline granules (*K*) are already present in the outer cells, but not in the surrounding squamous cells (*S*). Arrows: elevation at the luminal junction of two adjoining inner cells. *F:* periluminal filamentous zone. From the palm. (× 3,600)

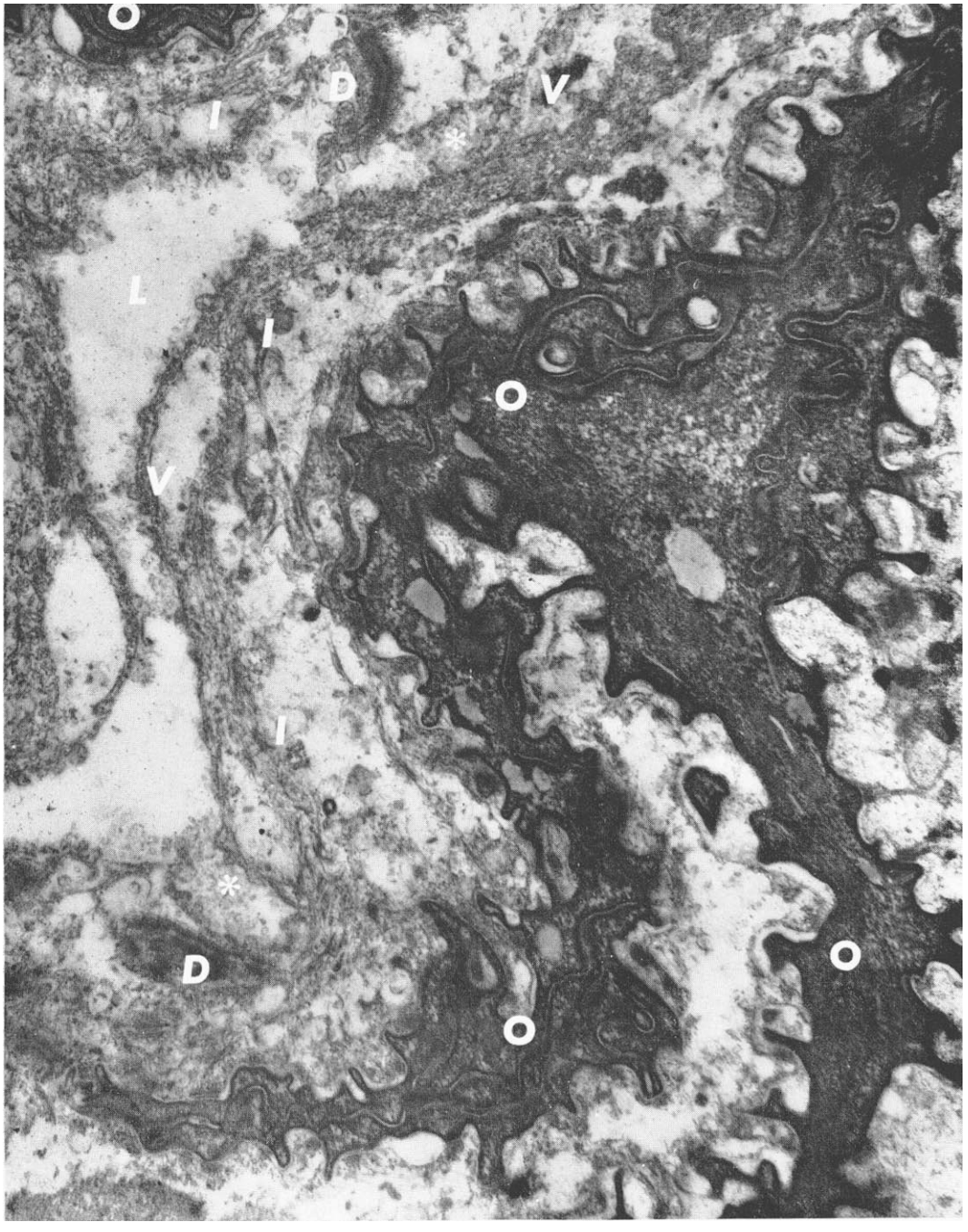

FIG. 2. *Epidermal sweat duct in the granular layer.* The inner cells (*I*) are flattened and contain loosely aggregated filaments. The surrounding outer cells (*O*) are completely keratinized. Note that the plasma membranes of the outer cells have thickened considerably while those of the inner cells have not. A junction of adjoining inner cells (upper *D*) projects into the lumen. *D:* desmosomes between adjoining inner cells. *L:* ductal lumen. *: Vesicles and slightly electron dense amorphous substances filling spaces between microvilli. *V:* microvilli. From the palm. (× 21,750)

FIG. 3. *Epidermal sweat duct in the upper horny layer.* The lumen (*L*) is surrounded by several layers of completely keratinized cells, of which the inner-most layer is shedding into the lumen (arrows). *K:* keratin filaments. From the palm. (× 4,500)

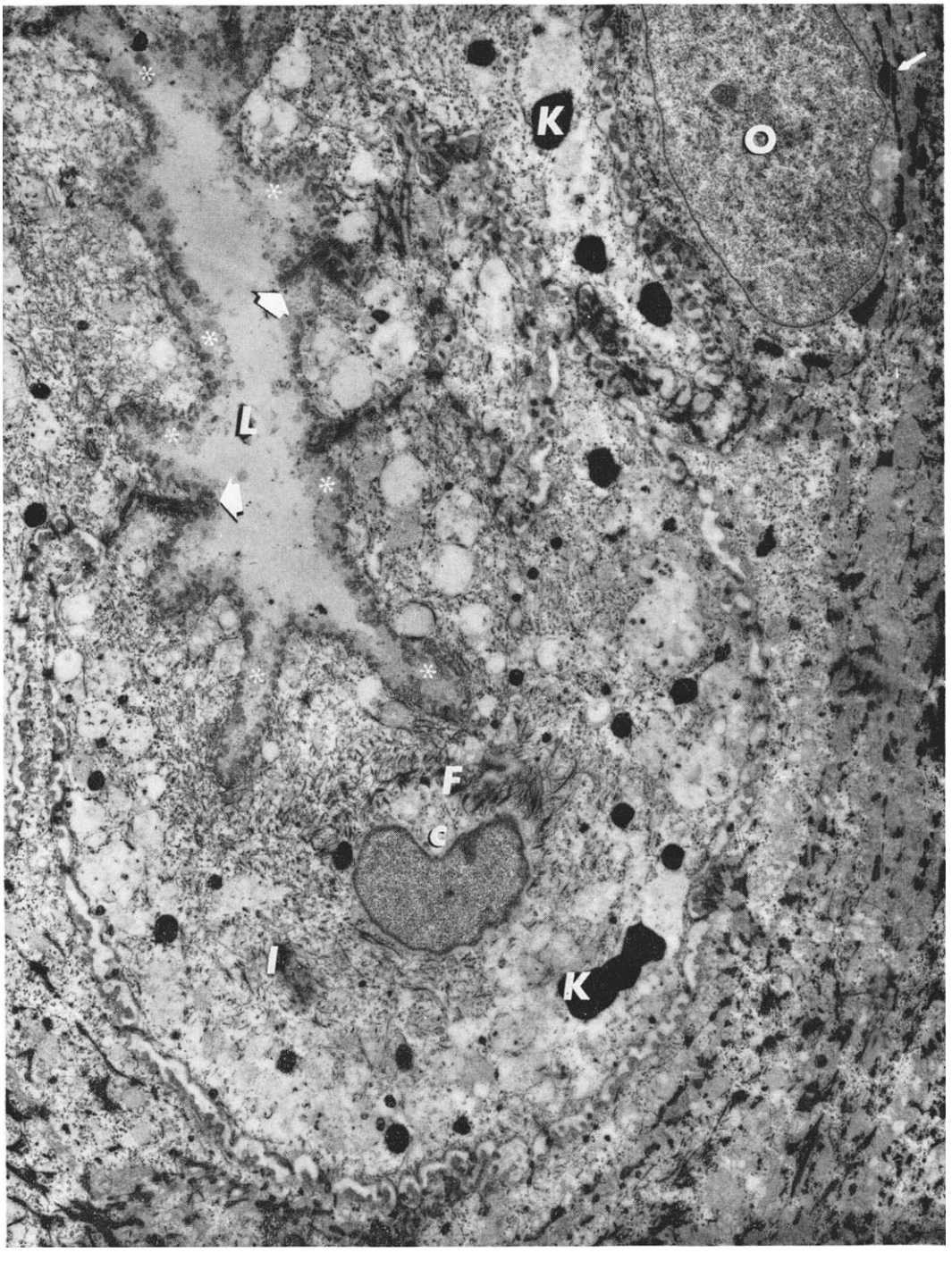

FIG. 4. *Epidermal duct in the mid-squamous layer.* A thick cuticular ring of moderate electron density continuously lines the entire luminal border *, in which microvilli are embedded. The luminal (*K*) are seen in the inner cells project into the lumen (wide arrows). Keratohyaline granules (*K*) are seen in the inner cells (*I*). A periluminal filamentous zone (*F*) surrounds a perinuclear clear zone (*c*). A narrow zone of tonofilaments surrounding a perinuclear clear zone of the outer cell (*O*) shows deposits of keratohyalin (thin arrows). *L:* lumen.

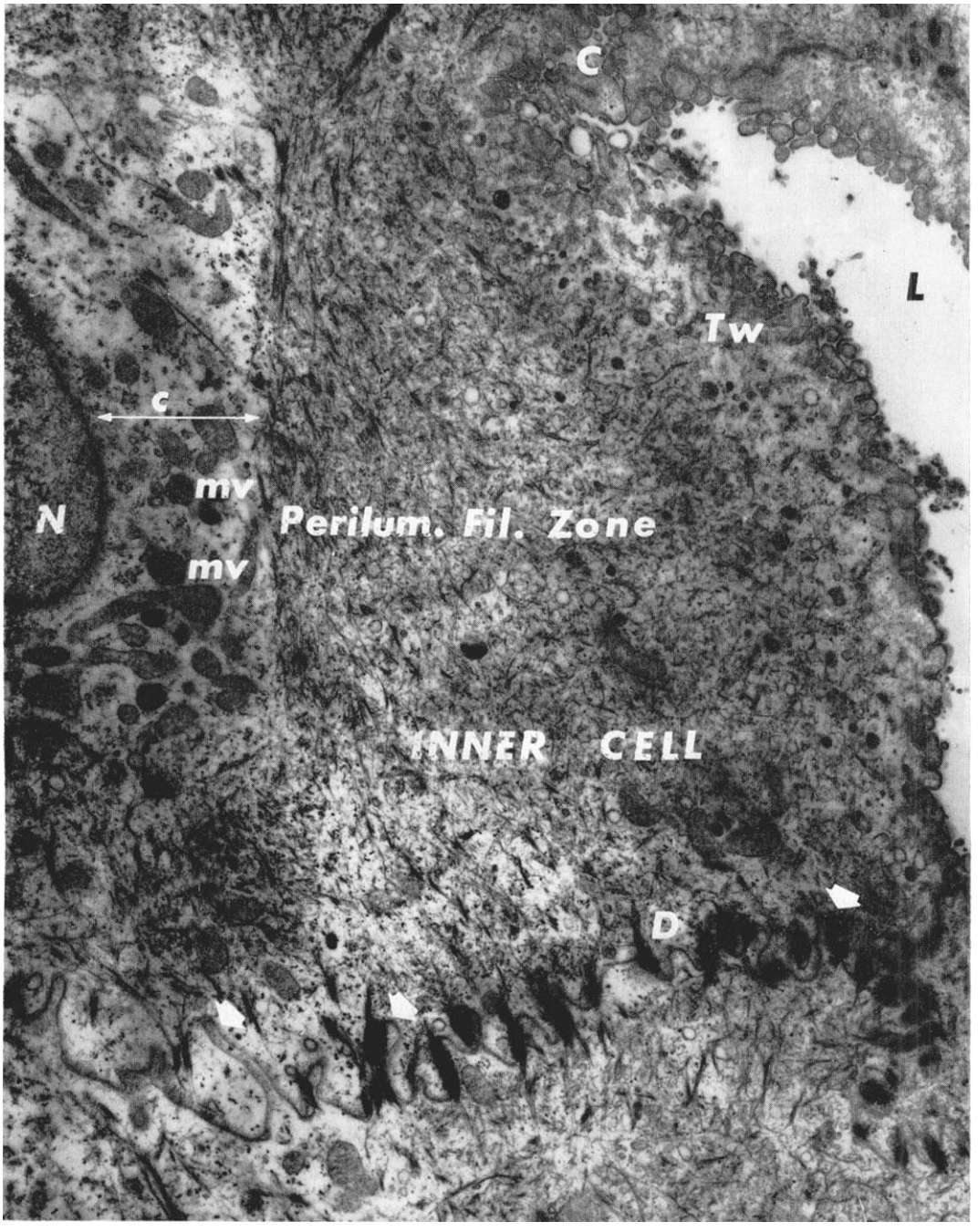

FIG. 5. *Epidermal sweat duct in the sweat duct ridge.* The ductal lumen (*L*) is surrounded by inner cells which possess a villous luminal border, cryptae (*C*), a terminal web (*Tw*), a periluminal filamentous zone, a perinuclear clear zone (*c*) and an oblong nucleus (*N*). The border between adjoining inner cells shows interdigitations (thick arrows). *D:* desmosomes. *mv:* multivesicular bodies. From the sole. (× 14,800)

TABLE I

Level of the keratinization of the intraepidermal eccrine duct

	Inner cells		Outer cells	
	Sole of the foot	Palm of the hand	Sole of the foot	Palm of the hand
Sweat Duct Ridge	−	−	−	−
Basal Layer	−	−	−	−
Lower Squamous Layer	+	−	+	+
Middle Squamous Layer	++	+	++ ∼ +++	++
Upper Squamous Layer	++	++	*k*	+++ ∼ *k*
Granular Layer	*k*	++	*k*	*k*
Lower Horny Layer	shed into the lumen	++ ∼ *k*	*k*	*k*
Middle Horny Layer		*k* ∼ begin to shed	*k* shed into the lumen	*k*
Upper Horny Layer		shed into the lumen	*k*	*k* ∼ shed into the lumen

− No keratohyaline granules; + Few keratohyaline granules; ++ Moderate amount of keratohyaline granules; +++ Abundance of keratohyaline granules; *k* Keratinization.

cells did not increase appreciably. In the upper horny layer the duct was lined by thin, completely keratinized *outer cells* which also were shed into the lumen (Fig. 3).

In the sole of the foot, keratohyaline granules were already present in the inner cells in the lower squamous layer. The process of the keratinization followed the same pattern as observed in the palm (Table I). It seemed that the inner cells of the duct in general never accumulated as many keratohyaline granules as the *outer cells* or the epidermal squamous cells; they seemed to shed prematurely into the lumen without dense condensation of the keratin filaments (Table I), (Figs. 2, 6). The ductal lumen, though frequently narrowed by the irregular outline of the luminal border and by the accumulations of vesicles, remained patent from the sweat duct ridge to the orifice on the surface of the horny layer.

The outer layer of the duct was composed of two to three layers of outer cells surrounding the single layer of inner cells. The shape of the outer cells and of their nuclei was similar to that of the inner cells. Prominent desmosomes connected the outer cells with the inner cells, with one another, and with the cells of the surrounding epidermal cells.

A perinuclear clear zone and, peripheral to it, a narrow zone of tonofilaments as described above for the inner cells, could also be found in the outer cells. Discernible in the cytoplasm of the outer cells were the same kinds of organelles as described for the inner cells except for the absence of the multivesicular bodies and the presence of an abundant amount of round granules of the dimension of 1500–5000 Å in the upper squamous layer of the surrounding epidermis. These granules often contained cristae-like inner structure (Fig. 7) and were delimited by double membranes. They were thus identical with those granules described by Odland (9), Selby (10) and recently by Matoltsy and Parakkal (11). Matoltsy and Parakkal (11) called them "membrane-coating granules" since they believed that they eventually fused with the plasma membranes of the host cells, empty their contents into the intercellular spaces and coat the plasma membranes from outside. Small, round keratohyaline granules were seen in the lower squamous layer in the specimens from most of the body areas and from the palm of the hand (Fig. 1); at the level of the middle (Fig. 4) and upper squamous layers (Fig. 7) they became larger, had multifaceted borders, and appeared to be typical keratohyaline granules. At the level of the stratum granulosum, the outer cells of the eccrine duct were already completely keratinized with an increased electron density and thickening of their plasma membranes (Fig. 2) (Table I). In the sole of the foot keratohyaline granules appeared in the outer cells in the lower squamous layer and the outer cells were already

FIG. 6. *Epidermal duct in the middle section of the horny layer.* The inner cells (*I*) which are shedding into the lumen can readily be distinguished from the surrounding outer cells (*O*) because they appear lighter and amorphous due to their incomplete keratinization. (× 4,470)

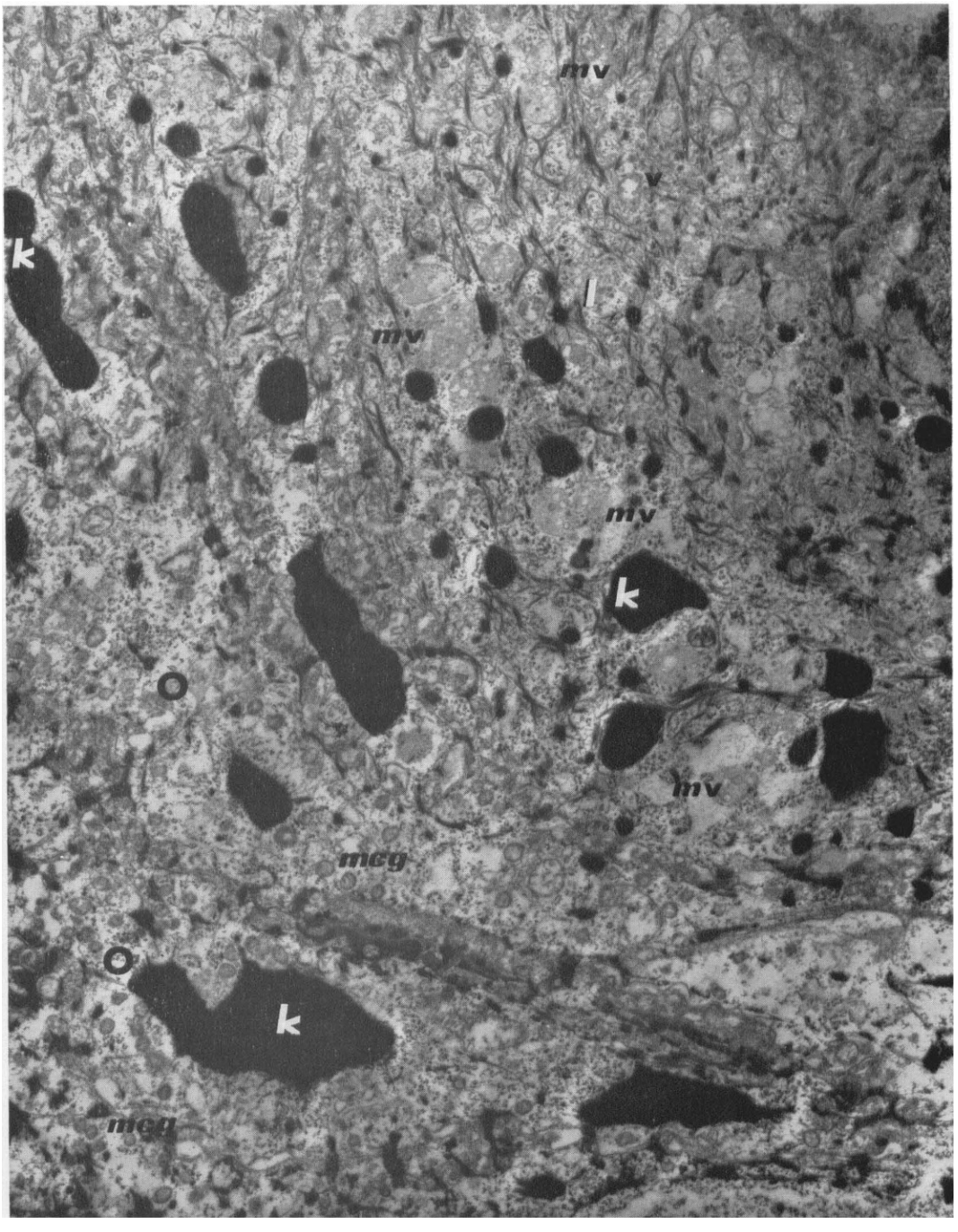

FIG. 7. *Epidermal duct in the upper squamous layer.* A part of the lumen with numerous microvilli is cut at the right upper corner. Enmeshed in a spongy meshwork of the periluminal filamentous zone of the inner cells (*I*) are small vesicles (*v*) and multivesicular bodies (*mv*). Outer cells (*O*) contain numerous membrane-coating granules (*mcg*); *k:* keratohyaline granules. From the arm. (× 10,440)

completely keratinized at the level of the upper squamous layer (Table I). The outer cells in the sweat duct ridge, especially in the sole of the foot, usually contained a moderate to abundant amount of glycogen as previously reported (8).

2. Intradermal Eccrine Sweat Duct

In the straight as well as in the coiled portions of the intradermal sweat duct, the ultrastructural features were essentially the same as described above for the intraepidermal sweat duct seen in the basal layer and in the sweat duct ridge of the epidermis. Thus, extremely complicated invaginations of the lateral borders of the inner cells were also present (Fig. 8). The outer cell layer consisted of only one layer of cells, which was continuous with the basal cell layer of the epidermis and rested upon a basement membrane.

The microvilli lining the luminal border of the inner cells of the intradermal sweat duct were larger, especially flatter and wider, than those of the intraepidermal segment (Fig. 8). The "fuzzy" external coating of the microvilli was thicker and more electron dense than in the epidermis, and often revealed a filamentous inner structure (Fig. 9). In some areas, microvilli were narrowed at their bases or lay free in the lumen (Fig. 9) indicating that a process of pinching-off of villi was taking place. In many areas fragments of cytoplasm and plasma membrane were seen in the lumen, particularly near the luminal border (Fig. 8). As in the intraepidermal eccrine duct, small vesicles, the extraneous fuzzy coating, pinched-off microvilli and their debris made up a distinct, moderately electron dense innermost layer of the ductal wall.

While in the intraepidermal portion of the eccrine duct adjoining inner cells showed cytoplasmic elevations at the luminal border, no such feature was seen in the dermis. On the contrary, in the intradermal sweat duct shallow fissures were present at the luminal junction of adjoining inner cells (Figs. 8, 9).

Except for the differentiation along the luminal border of the inner cells there was no significant morphologic difference between the inner cells and the outer or basal cells. This is not surprising in view of our observation (12) that the inner cells of the intradermal eccrine duct of the human embryo arise from the basal cell layer.

DISCUSSION

In general, the ultrastructure of the various types of cells of the *adult* eccrine sweat duct is identical to each corresponding type of cell of the embryonic eccrine sweat duct except that multivesicular bodies are more numerous and much denser in the inner cells of the intraepidermal embryonic duct (7).

Although absorption is the main function of the human eccrine duct (13) a certain amount of secretion by means of the excretion of vesicles and pinching-off of villi seems to exist.

The eccrine sweat duct lumen, when examined under the light microscope, is lined by an eosinophilic cuticle which is PAS reactive (diastase-resistant). When the electron microscope is used to examine the lumen at low magnification, an amorphous band of moderate electron density is seen lining the luminal border. Upon examination at high magnification, however, this amorphous material is resolved into a so-called fuzzy extraneous coat with a filamentous structure, small vesicles and debris of the plasma membranes of the luminal microvilli. It is our impression that the cuticle of the eccrine sweat duct seen with the light microscope corresponds to these components just mentioned above. A similar histologic and ultrastructural correlation is seen in the villi of the intestinal mucosa (14). However, if one considers the birefringent border of the eccrine sweat duct as a part of the cuticle as Schmidt (15) and Ellis and Montagna (4) do, the periluminal filamentous zone, as well as the luminal microvilli must also correspond to the cuticle as seen with the light microscope.

The presence of shallow fissures at the luminal junction of adjacent inner cells in the intradermal eccrine duct has not been previously described. It is possible that because of this special device the luminal border, rigid because of its thick periluminal filamentous zone, can bend at regular intervals and thus maintain patency of the lumen when pressure is applied perpendicular to the epidermis, as it occurs to the sole of the foot when standing. In addition, the marked folding of the interdigitated plasma membranes of ductal cells plus the absence of desmosomes in approxi-

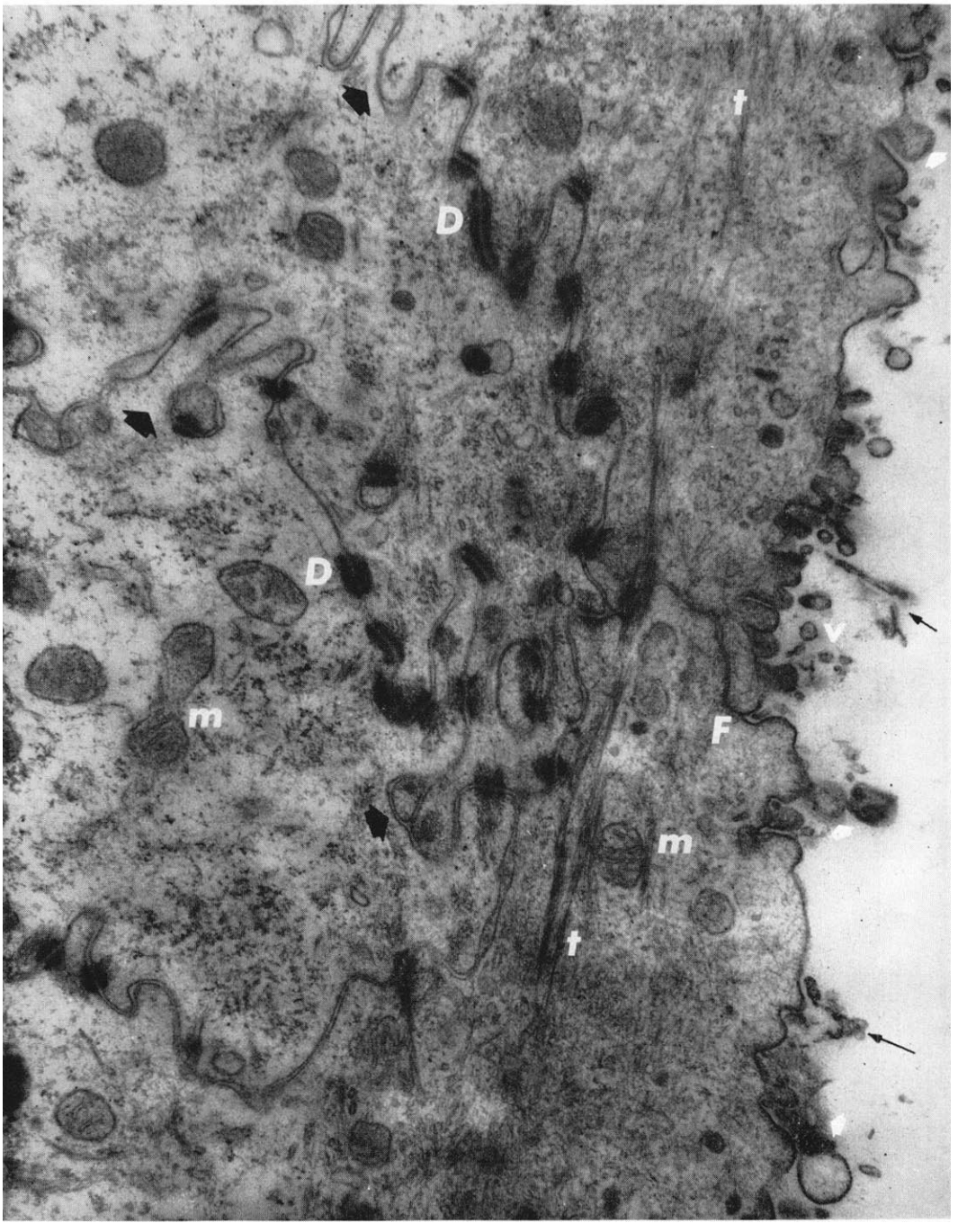

FIG. 8. *Intradermal sweat duct.* Numerous, well-developed microvilli line the lumen. Pinched-off microvilli, a thick extraneous coating (thick white arrows), small vesicles (*v*) and debris of the plasma membranes of the microvilli (thin arrows) form a cuticular border of the lumen. A shallow fissure (*F*) is present at the luminal junction of adjoining inner cells. Note an extremely convoluted lateral border of inner cells (thick black arrows). *D:* desmosome. *m:* mitochondria. *t:* tonofilaments of a periluminal filamentous zone. From the sole. (× 30,240)

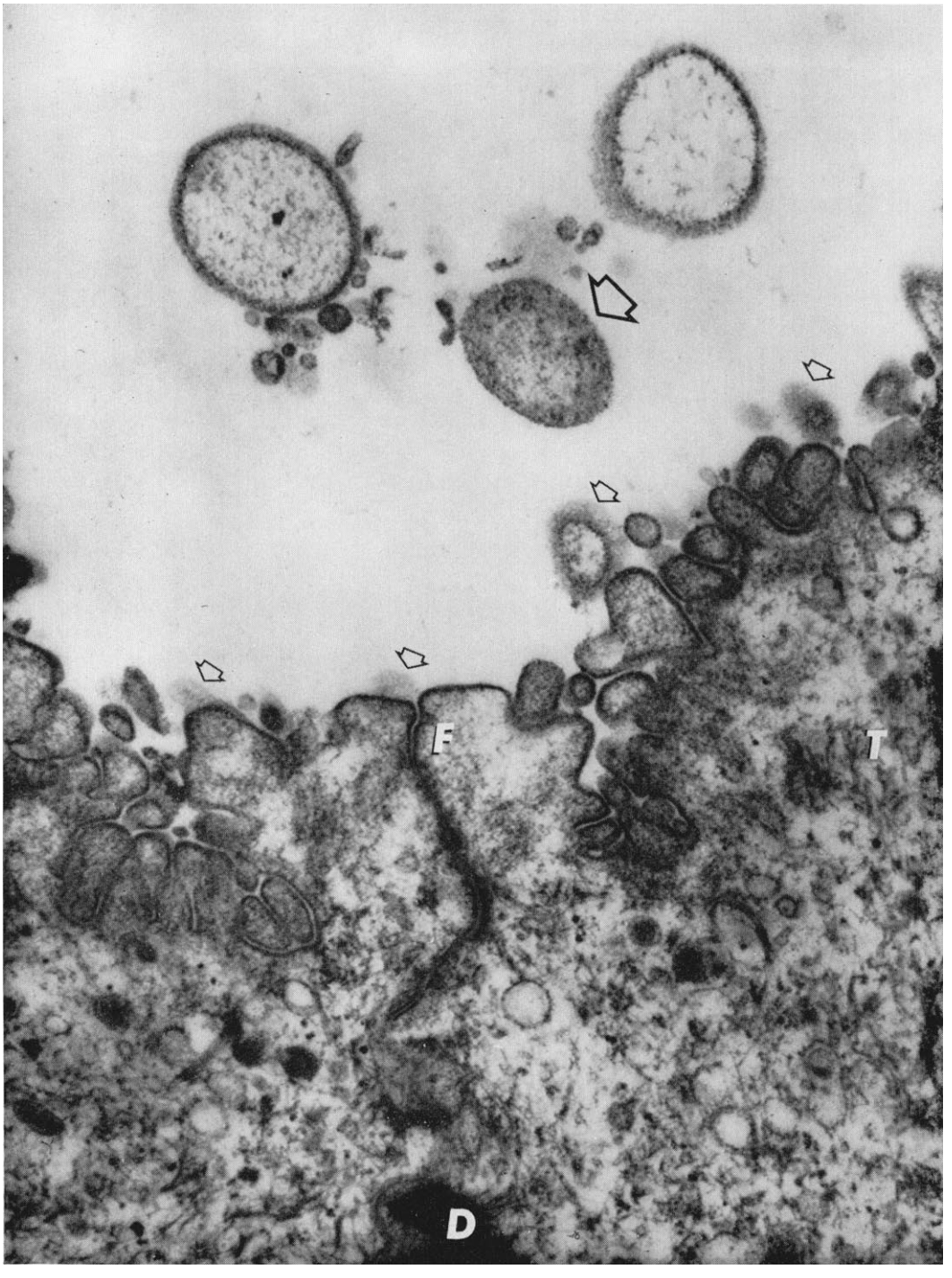

FIG. 9. *Extraneous fuzzy coat* of the dermal eccrine sweat duct. There are three pinched off villi in the lumen. One of which (large arrow) was sectioned through the extraneous fuzzy coat and reveals that it has a filamentous structure. The other two were sectioned through the body of the villi and reveal a filamentous coating at the periphery. The filamentous fuzzy coat is also observed on the villi lining the luminal border (small arrows). *D:* desmosome. *F:* a shallow fissure at the luminal junction of the adjoining inner cells. *T:* tonofilaments composing a spongy meshwork of the periluminal filamentous zone. From the palm. (× 45,820)

mately half of these folds could serve as a reserve if the skin is stretched (as in the sheering force of walking).

In the intraepidermal segment of the eccrine sweat duct the fissures at the luminal border are lacking. This may be due to the fact that the intraepidermal lumen is surrounded by strong ductal wall cells which undergo keratinization much earlier than the rest of the epidermis, thus providing further support for the duct, and sits above a shock absorbent cushion of dermal connective tissue. Near the orifice in the granular layer the outer cells are completely keratinized and further strengthen the ductal wall. Thus, the lumen would not require addiitonal measures to maintain its patency.

The fact that the membrane-coating granules of Matoltsy and Parakkal are present in the outer cells and absent in the inner cells is compatible with the finding that the outer cells are completely keratinizing cells with thickened plasma membranes while the inner cells are not.

SUMMARY

Electron microscopic study of the adult eccrine sweat duct revealed within the epidermis one layer of inner cells and two to three layers of outer cells. The intradermal portion was composed of one layer of inner cells and one layer of outer cells. In the stratum corneum all layers of the intraepidermal duct had become keratinized and could not be distinguished from the surrounding keratinized epidermal cells. The inner cells of both the intraepidermal and the dermal portion of the eccrine duct possessed a perinuclear clear zone surrounded by a band of tonofilaments. The luminal portion of this band formed the periluminal filamentous zone. The function of this zone and shallow fissures present at the luminal junction of the inner cells of the intradermal duct, appeared to be the maintenance of luminal patency. Microvilli present along the luminal border possessed an extraneous fuzzy coating. The periluminal cytoplasm and the microvilli contained small vesicles similar to those found in the lumen. There was evidence that these vesicles passed from the inner cells into the lumen through disruptions of the plasma membrane and also as a result of pinching-off of microvilli.

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