FINE STRUCTURE OF AN UNUSUAL INTRACELLULAR SUPPORTING NETWORK IN THE LEYDIG CELLS OF *AMBLYSTOMA* EPIDERMIS

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The early literature of light microscopy was rich in excellent descriptions of the large and beautiful cells of amphibians. The mucous cells of the epidermis of larval salamanders, rarely mentioned in the cytological literature today, received considerable attention in this earlier era. Described by Leydig (1) in 1857, these large, round cells are so common over the body of the larva that in some regions they form a continuous middle layer between the basal cells and the outer cellular layer of the epidermis. They are characterized by the presence of large cytoplasmic granules that give the staining reactions of mucus (see reviews by Heidenhain (2) and Dawson (3)). The ground cytoplasm around the granules appears pale and devoid of visible structure when viewed with the light microscope, but a denser area can be seen adjacent to the nucleus (Fig. 1). Langerhans (4) described a supporting network in the peripheral cytoplasm of Leydig's "Schleimzellen" and early investigators (2) speculated that the shape of this large, rather empty cell was maintained by the rigid, basket-like, peripheral net of Langerhans (Fig. 1). The present study confirms Langerhans' observations of a structure which in its dimensions is at the limit of resolution of the light microscope, and adds several observations of interest to students of the fine structure of cytoplasmic filaments and desmosomes.

MATERIALS AND METHODS

Limbs and tails of larval *Amblystoma punctatum* and *A. opacum* were fixed in buffered osmium tetroxide, dehydrated, and sectioned in methacrylate as described in a previous report (5). The larvae were 18 to 40 mm in length at the time of fixation. Unstained sections and sections stained with lead hydroxide (6) were examined in an RCA EMU-3E electron microscope. Thicker plastic sections of the same material were stained with the periodic acid–Schiff technique and studied with the light microscope. Whole mounts of formalin-fixed skin stained with Sudan black B were also studied with the light microscope.

OBSERVATIONS

Fig. 2 is a low magnification electron micrograph showing portions of three Leydig cells in the epidermis of the limb. The outer layer of the epidermis is a single stratum of cells which have at their free surface a "cuticular margin" (3) composed of microvilli and regularly arranged mucous granules (*C*, Fig. 2). The Leydig cells are situated between these surface epidermal cells and a single row of basal epidermal cells (*Ba*, Fig. 2) that rest directly on a thick basement membrane (*BM*, Fig. 2). The fine structure of the basement membrane and many other features of *Amblystoma* epidermis were described by Porter (7) and Weiss and Ferris (8), and the reader is referred to this earlier work for further details (see also 5).

The two Leydig cells (*Le*, *Le'*) in the center of Fig. 2 illustrate well the structural variations seen in this cell type. These large cells (25 μ in diameter) are in close contact with each other and with adjacent Leydig cells in certain areas (*B*, Fig. 2). The lobed nucleus of cell *Le'* is similar to the nuclei of ordinary epidermal cells, whereas the round nucleus of cell *Le* is smaller and denser. The cytoplasmic granules (approximately 2 μ in diameter) are less dense and more uniform in size in cell *Le* than in *Le'*. These granules are the most prominent feature of the Leydig cell cytoplasm, but their function is obscure. They give a positive reaction with the periodic acid–Schiff technique for mucopolysaccharides. In the cytoplasm, they are enclosed in membrane-bounded vesicles (*g*, Fig. 4). Smaller vesicles are associated with the membranes bounding the granules and with the cell membrane. The cell membrane (*CM*, Figs. 3 and 4) is often broken in the course of fixation and dehydration.

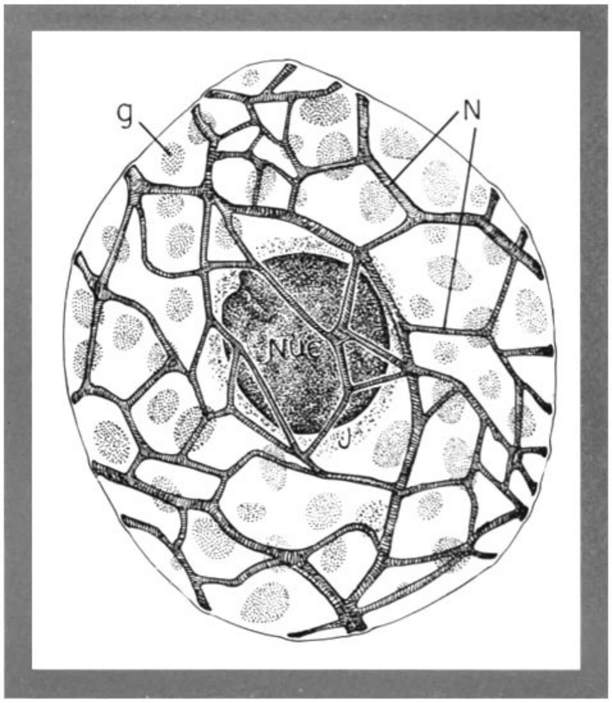


FIGURE 1

Semischematic drawing of a Leydig cell showing Langerhans' net as it would appear on one-half of the cell in a whole mount of *Amblystoma* skin fixed in formalin and studied with the light microscope.

Abbreviations Used to Label Figures

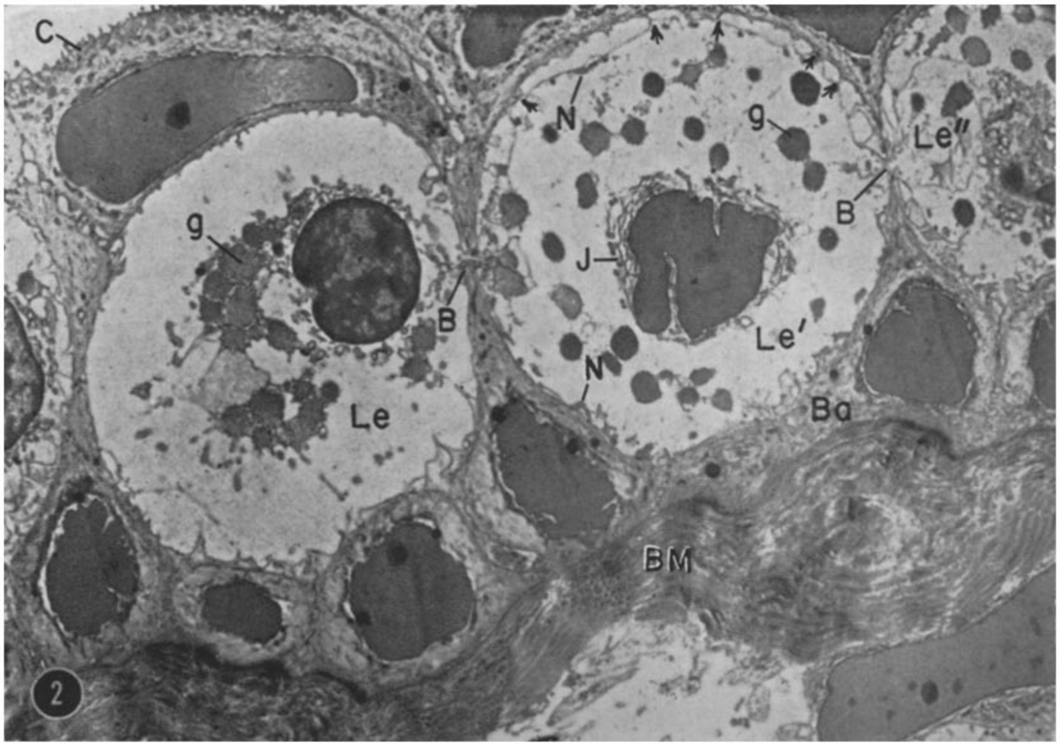
B, Region of close contact between Leydig cells
Ba, Basal epithelial cell
BM, Basement membrane
C, Cuticular margin
CM, Cell membrane
D, Desmosome
D′, Desmosome between Leydig cell and ordinary epidermal cell
Ep, Epidermal cell
ES, Extracellular space
f, Filaments of Langerhans' net
g, Mucous granule
J, Juxtanuclear cytoplasm
Le, Leydig cell
M, Osmiophilic material
N, Langerhans' net
Nuc, Nucleus
P, Particles in cytoplasm of Leydig cell
Tf, Tonofilaments

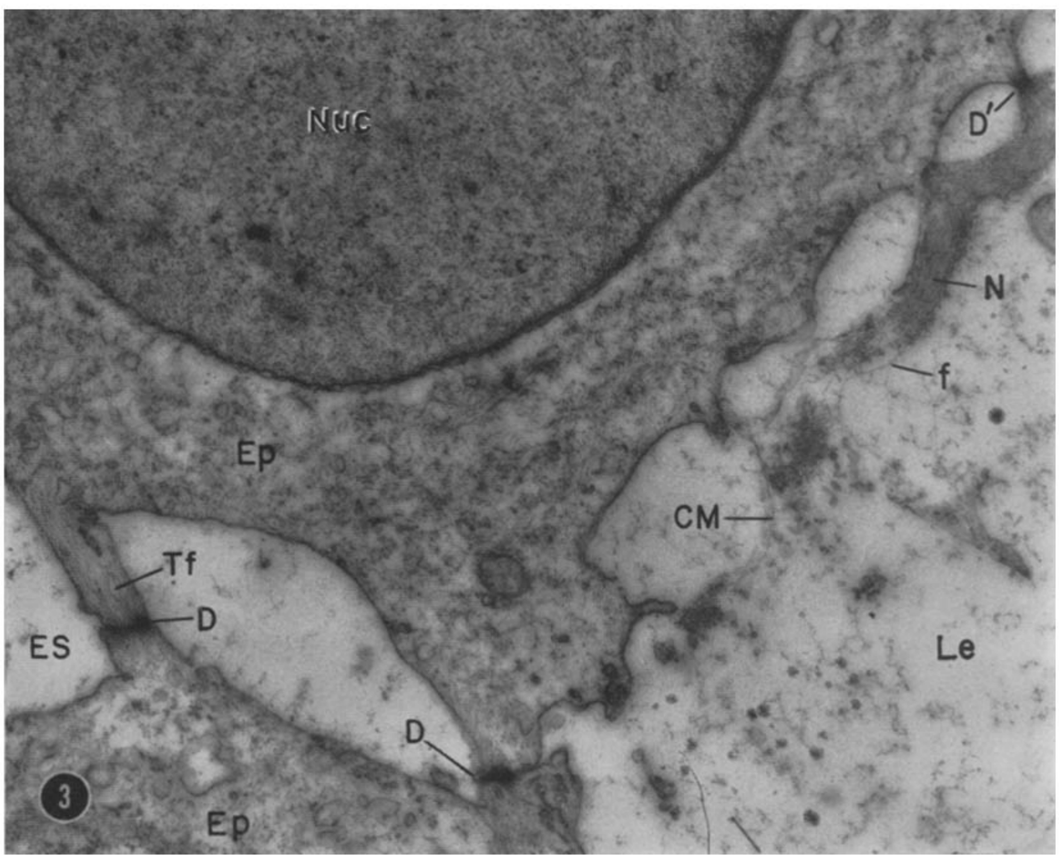
FIGURE 2

Low magnification electron micrograph of an unstained thin section showing parts of several Leydig cells (*Le*, *Le′*, *Le″*) in the epidermis of an *Amblystoma punctatum* larva. Sections of narrow bands of Langerhans' net (*N*) may be seen in the peripheral cytoplasm of cell *Le′*. × 2000.

FIGURE 3

Higher magnification electron micrograph of a stained section showing a portion of the peripheral cytoplasm of a Leydig cell (*Le*). Langerhans' net (*N*) is composed of fine filaments (*f*). A desmosome between Leydig cell and epidermal cell is shown at *D′* and two desmosomes between epidermal cells at *D*. × 16,000.


C
N
g
Le″
B
g
J
B
Le′
N
Ba
Le
BM
2


Nuc
D′
N
f
Ep
CM
Tf
D
ES
Le
D
3
Ep

The major part of the cytoplasm of the Leydig cell is remarkable for its very low electron density (Figs. 3 and 4). In addition to the granules and membrances described above and a few mitochondria, it contains minute particles (*p*, Fig. 4) which are similar to those contained in the extracellular space (*ES*, Fig. 3 and 4). Around the nucleus, there is a narrow rim of cytoplasm (*J*, Fig. 2) containing endoplasmic reticulum and associated ribosomes, mitochondria, and clusters of lamellae possibly representing the Golgi apparatus. The cytoplasm of the ordinary epidermal cell, (*Ep*, Figs. 3 and 4), in contrast to that of the Leydig cell, is rich in tonofilaments, organelles, and granules of various sizes.

The network described by Langerhans in the peripheral cytoplasm of the Leydig cell can be visualized in whole mounts of *Amblystoma* epidermis as a web of branching fibers encompassing the entire cell (Fig. 1). In the electron microscope, each fiber (*N*, Fig. 2) is resolved as a band (approximately 0.5 μ in diameter) composed of numerous, closely aggregated filaments arranged more or less parallel to one another (*f*, Figs. 4 and 6). The individual filaments (*f*, Figs. 3 and 7) are approximately 50 A in diameter and are similar in size to the tonofilaments of the epidermal cell (*Tf*, Fig. 5), but they often appear denser in sections stained with lead hydroxide, especially in areas where they are closely aggregated. In unstained sections of material fixed in osmium tetroxide, the bands of filaments comprising Langerhans' net are no denser than the tonofilaments of epidermal cells. In preparations for light microscopy, these bands show no affinity for lipid (Sudan black B) or carbohydrate (periodic acid–Schiff) stains.

In low magnification electron micrographs, the Leydig cell appears to be attached to adjacent epidermal cells at numerous points (arrows, Fig. 2). At higher magnification, each of these sites of apparent attachment can be recognized as a desmosome (*D'*, Figs. 3 and 4) similar to those found (*D*, Fig. 3) between contiguous epidermal cells (7). At each desmosome, the extracellular space between Leydig cell and adjacent epidermal cell is minimal (approximately 200 A, *ES*, Fig. 6) and filaments of the dense meshwork of Langerhans' net insert into a layer of osmiophilic material (*M*, Fig. 6) associated with the plasma membrane of the Leydig cell (*CM*, Fig. 6). The tonofilaments of the adjacent epidermal cell (*Tf*, Fig. 6) insert into an osmiophilic material under the cell membrane of the epidermal cell, as in other desmosomes (Fig. 5).

DISCUSSION

The Langerhans net in the peripheral cytoplasm of the Leydig cell has a fine structure consistent with the conclusion of earlier cytologists that it is a supporting framework for a cell type whose ground cytoplasm is no denser in its appearance than the contents of the extracellular space.

FIGURE 4

Electron micrograph of a stained section showing a mucous granule (*g*) in the cytoplasm of a Leydig cell (*Le*). The filaments of Langerhans' net (*f*) are related to a desmosome at *D'*. × 18,000.

FIGURE 5

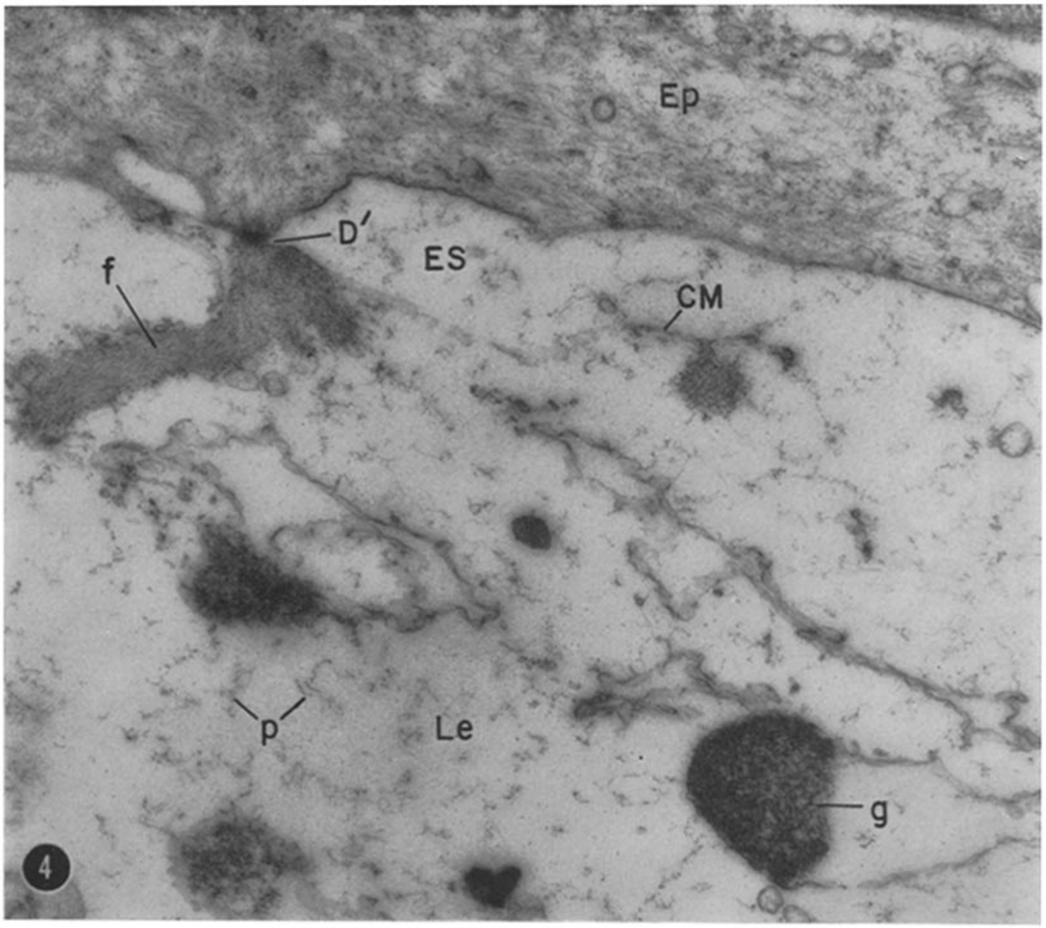
Higher magnification electron micrograph of a desmosome between two epidermal cells. Section stained with lead hydroxide. × 30,000.

FIGURE 6

Similar electron micrograph of a stained section showing a desmosome between Leydig cell and epidermal cell. Filaments of Langerhans' net appear at *f*. × 30,000.

FIGURE 7

Electron micrograph of a stained section showing the filaments (*f*) composing a band of Langerhans' net. The cell membrane appears at *CM*. × 30,000.


Ep
D
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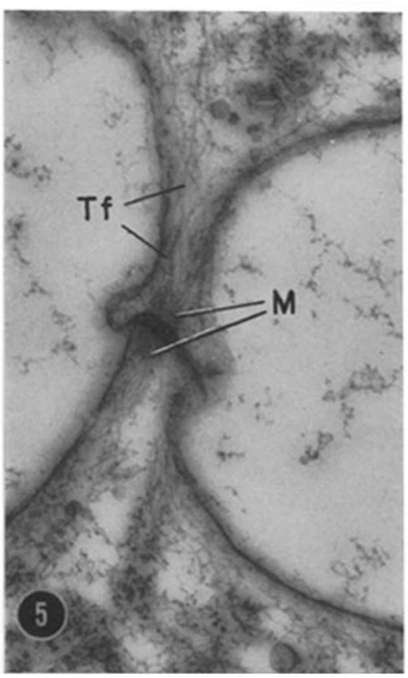
Tf
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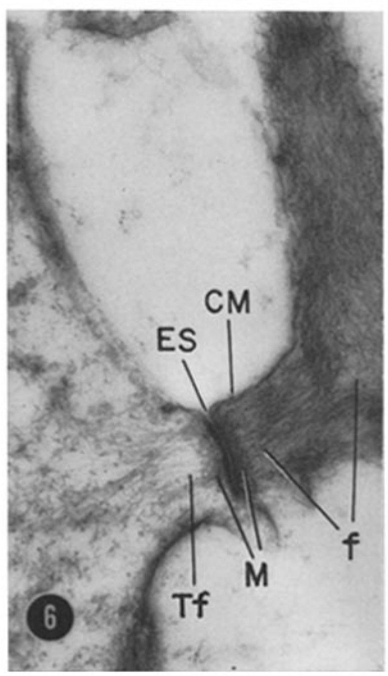
CM
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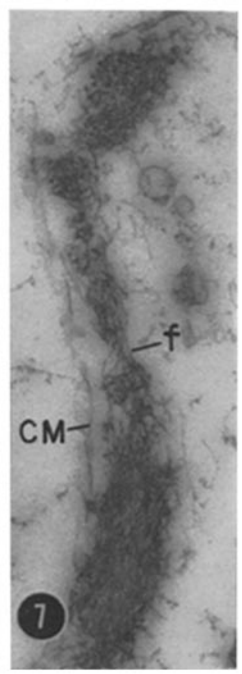
f
CM
7

Presumably of epidermal origin, this specialized cell retains filaments resembling the tonofilaments of ordinary epidermal cells. These filaments are not dispersed throughout the cytoplasm, but instead are condensed into bands at the periphery of the cell. The bands connect to form a basket-like network which appears well adapted to its presumed role of giving the Leydig cell considerable stability of cell shape. The significance of the Leydig cell's clear, filament-free ground cytoplasm and the function of its interesting mucous granules remain obscure.

The present description of the fine filaments composing Langerhans' net adds to the existing literature an unusual example of specialization of intracytoplasmic fibrous proteins in epithelial cells. Fine filaments of similar dimensions (50 A in diameter) have been described in the cytoplasm of many types of epithelial cells and larger intracytoplasmic fibers showing a periodicity comprise the rootlets of many cilia (see 9). Similar fine filaments occur in the cytoplasm of neuroepithelial cells (10). In certain columnar epithelia, cytoplasmic filments may be aggregated in the apical cytoplasm to form a dense terminal web (11, 12). The external limiting membrane of the sea gull retina is composed of similar filaments aggregated within the cytoplasm of Müller's cells (13). A supporting role is generally ascribed to these filamentous components of epithelial cells and such filaments are often associated with desmosomes and terminal bars. Cytoplasmic filaments also occur in certain connective tissue cells (14) and in muscle cells. In cardiac muscle, myofilaments insert into desmosomes and desmosome-like specializations, the intercalated discs. Using the light microscope, Leblond *et al.* (15) demonstrated that myofibrils do exhibit a staining reaction similar to the terminal web of epithelial cells. They suggested the general term, "cell web," for these fibrils and others that seem to play a role in shaping the cell.

Porter (7) and Fawcett (9) speculated that the fine 50 A filaments of epidermal cytoplasm visualized with the electron microscope may be keratin or a precursor fibrous protein. This speculation is not unreasonable in the light of histochemical (16) and x-ray diffraction (17) studies of epidermal proteins. Indeed, Astbury (18) has suggested that native fibrous proteins fall into only two general classes, the collagen class and the keratin-myosin class (k.m.e.f.). Future cytochemical studies may clarify the important similarities and differences between the fibrous proteins comprising the intracytoplasmic class of filaments visible with the electron microscope. It is tempting at the present time to speculate that the 50 A filaments composing Langerhans' net and found dispersed throughout the cytoplasm of most epithelial cells are related to keratin in composition and are supportive in function.

SUMMARY

The supporting network of fibers in the peripheral cytoplasm of the Leydig cells of *Amblystoma* epidermis is composed of fine filaments resembling tonofilaments in size and in their relation to desmosomes. It is speculated that the filaments composing the intracytoplasmic fibers of Langerhans' net are special aggregations of the same fibrous protein that comprises the filamentous component found dispersed throughout the cytoplasm of other epithelial cell types, and that this protein may be a form of keratin.

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