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Changes in the ventral dermis and development of iridophores in the anadromous sea lamprey, *Petromyzon marinus*, during metamorphosis: an ultrastructural study

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Summary. The ultrastructural changes that take place in the ventral dermis along with the development of iridophores were examined in the anadromous sea lamprey, *Petromyzon marinus*, during metamorphosis. There is a disruption of all components of the ventral dermis and a reformation that results in a sturcture very similar to that prior to metamorphosis. Although not a dermal component, a layer of iridophores develops directly beneath the dermis during late metamorphosis. The dermal endothelium is lost by mid metamorphosis (stage 4) and the highly organized collagenous lamellae making up the bulk of the dermis become disrupted by the migration of fibroblasts into the region. Many of these fibroblasts are involved in the degradation of the lamellae. By stage 5 of metamorphosis some fibroblasts become highly active collagen synthesizing cuboidal shaped cells that align to form a layer above the reformed dermal endothelium. New lamellae are formed by these cuboidal cells which then divide and migrate into the lamellae where they assume the characteristic attenuated appearance of fibroblasts in the adult dermal lamellae region. Iridophores first appear during stage 5 directly beneath the dermal endothelium. Reflecting platelets develop from double membraned vesicles associated with the Golgi apparatus. By late metamorphosis, stacks of trapezoidal shaped platelets fill the cytoplasm of the iridophores. The significance of the changes in the dermis during metamorphosis are discussed. This work is part of a continuing series of studies on the connective tissues in the anadromous sea lamprey.

Key words: Ventral dermis, Iridophores, Lamprey, Metamorphosis

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Introduction

Histological studies of the skin of the lamprey to date have concentrated on the organization and cytology of the epidermis, specifically that of the dorsal region (for reviews, see Lethbridge and Potter, 1981; Whitear, 1986). Variations observed in the morphology of lamprey skin during metamorphosis have been based primarily on light microscopic studies of the dorsal area (Johnels, 1950; Lethbridge and Potter, 1980). Ultrastructural studies have concentrated on the epidermis (Downing and Novales, 1971a,b,c; Lane and Whitear, 1980).

One of the obvious external changes that occurs during metamorphic development of the lamprey is a silvering of the ventral and lateral skin as a result of the deposition of guanine (Youson and Potter, 1979; Lethbridge and Potter, 1980; Potter et al., 1978). The silver and iridescent coloration in other vertebrates has been attributed to the presence of iridophores, a type of dermal chromatophore whose pigmentary organelles consist of stacks of reflecting platelets containing one or more of the purines: guanine, adenine, hypoxanthine and uric acid (Bagnara, 1966; Taylor, 1969; Harris and Hunt, 1973). The silvering of lamprey skin is only one of presumably many developmental changes that are occurring in the dermis of the ventrolateral skin during metamorphosis. However, no ultrastructural studies have been made of the development of the ventral dermis, including the formation of iridophores within this region, during metamorphosis of the lamprey.

The dorsal dermis in larval and adult lampreys is a dense collagenous connective tissue. Johnels (1950) notes that during metamorphosis there is considerable change in the organization of dermal connective tissue in the head of the lamprey. It is noteworthy to mention here that the ventral silvering in the skin of the sea lamprey, *Petromyzon marinus*, during metamorphosis appears first in the anterior head

region and progresses posteriorly (personal observation).

Recently, a number of ultrastructural studies have been made of connective tissues in larval, metamorphic and adult sea lampreys, particularly those tissues in the head region (Wright and Youson, 1982, 1983; Wright et al., 1983; Armstrong et al., 1987; Wright et al., 1988). These studies have revealed some unusual characteristics that may be unique to lamprey connective tissues.

Lethbridge and Potter (1981) note that some progress has been made towards an understanding of the role the skin may have in the lampreys adaptibility to the different environments it experiences during its life cycle. This progress, however, has been hindered by the lack of ultrastructural studies on the components of the skin other than the epidermis.

The present ultrastructural study of the ventral dermis during metamorphosis provides new information on the cellular and extracellular components of this connective tissue component of the skin. This work represents part of a continuing series of studies on the characterization of connective tissues in one of the most primitive extant vertebrates, the anadromous sea lamprey, *P. marinus*.

Materials and methods

Larval and young parasitic adult (juvenile) anadromous sea lamprey, *Petromyzon marinus*, were collected during the period of mid May to early June of 1989 and 1990 from tributaries of the Saint John and St. Croix Rivers, New Brunswick and Lake Washademoak and Grand Lake, New Brunswick, respectively. Larval animals were maintained in plastic 76L or 122L containers holding approximately 5 cm of mud and 20 cm of water from the lampreys' natural environment. Juveniles were housed in 400L, rectangular, gel-coated, fiber-glass tanks containing aerated, continous flow ground water. Rainbow trout, *Oncorhynchus mykiss* and/or brook trout, *Salvelinus fontinalis*, were provided as the food source for the juveniles. All animals were maintained at 11° C under controlled lighting conditions; 12 hours of darkness alternating with 12 hours of fluorescent lighting.

Larval animals with a condition factor of 1.5 or greater were considered as candidates to undergo metamorphosis (Potter et al. 1978). They were separated and regularly checked for external signs of metamorphosis from early July through November. Resulting metamorphic animals (stages 1 - 7) were identified by external features according to Youson and Potter (1979).

A total of 4 larvae (2.1 - 2.7 g in weight; 110 - 121 mm in length), 6 juveniles (1.5 - 2.0 g in weight; 120 - 125 mm in length) and 21 metamorphic animals representing all stages 1 - 7 (2 - 6 g in weight; 112 - 155 mm in lenght) were used.

All animals were anaesthetized in a 0.05% solution of tricaine methanesulfonate before removal of tissues for electron microscopy. Ventral skin from the region between the mouth and first gill slit was excised from all animals. In many cases these tissue pieces were identified as being anterior, mid or posterior.

Tissues were fixed for 2 hours at 4° C with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.35) and post-fixed at room temperature in buffered 2% osmium tetroxide. Tissues were dehydrated through a series of ethanols and embedded in Spurr's resin via propylene oxide. Thick (0.5 µm) sagittal and transverse sections were cut and stained with 1% toluidine blue in a 1% sodium borate aqueous solution. Ultrathin sections were mounted on uncoated or formvar coated copper grids. Sections were 1) stained with a saturated solution of uranyl acetate in 50% ethanol and Sato's lead stain (pH 13), 2) stained with lead stain or uranyl acetate only or 3) left unstained.

Ventral skin from two parasitic adults was used to isolate reflecting platelets (crystals) for electron microscopic examination. Pieces of skin, approximately 10 mm x 5 mm, were lightly macerated in distilled water in depression slides. After 3 hours, 5 µl of this water was placed on formvar coater copper grids and allowed to air dry. Sections and extract preparations were examined and photographed using either a Hitachi H-600 or H-7000 electron microscope.

Results

Larval and metamorphic stages 1 - 2

The ventral dermis of the larval lamprey consists of three major components: 1) basal lamina, 2) collagenous lamellae also referred to as stratum compactum and 3) dermal endothelium (Fig. 1). The basal lamina forms the outer boundary of the dermis. In the larval and first two metamorphic stages is typically consists of: the lamina lucida, an electron lucent area adjacent to the basal membrane of the epidermis; the lamina densa, a continuous horizontal sheet of electron dense material; and a pars fibroreticularis of vertically arranged microfibrils (10 to 12 nm in diameter) connecting the basal lamina to the underlying connective tissue (Fig. 2). In the larvae, the lamina lucida is somewhat obscured by the presence of many small membrane bound structures (35 - 54 nm in diameter). Vertical cords of filamentous material extend from the lamina densa through the lamina lucida towards the basal cell membrane. In stage 2 the small membrane bound structures within the lamina lucida are no longer discernable. Cytoplasmic extensions of the basal epidermal cells occasionally pass through the lamina lucida to fuse with the lamina densa (Fig. 3).

The collagenous lamellae form the principle component of the dermis of the larval and early metamorphic stages (Fig. 1). These layers of densely packed collagen fibrils are arranged more or less


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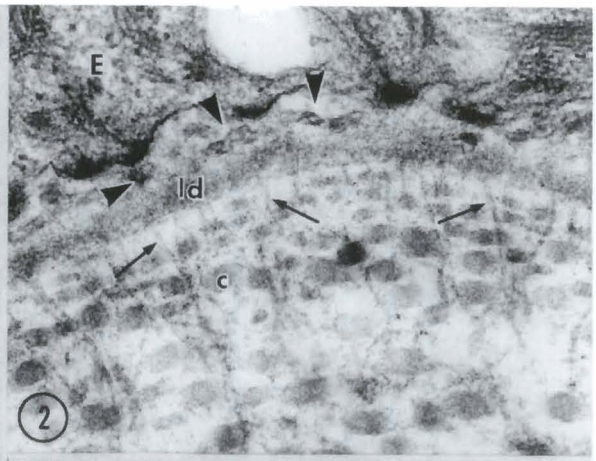
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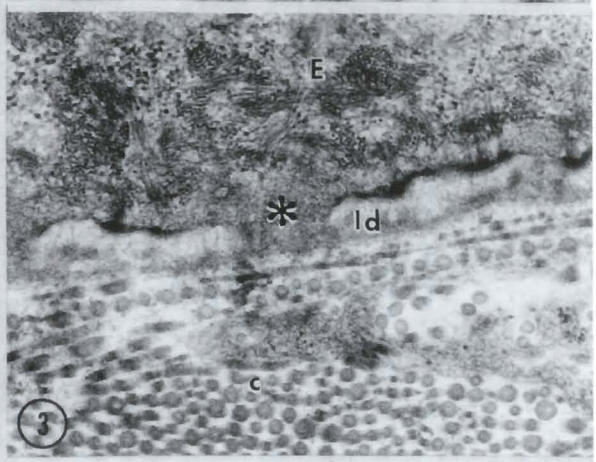
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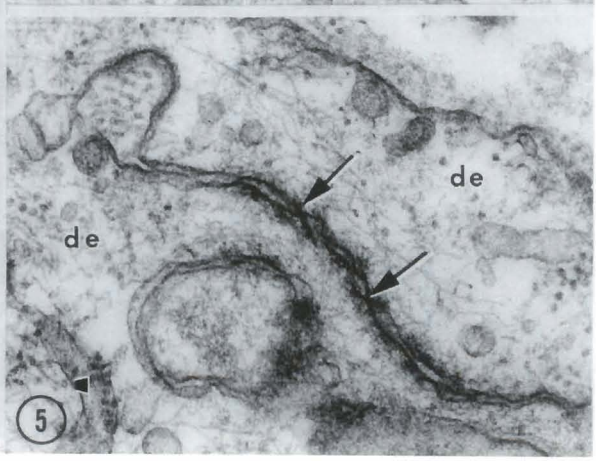
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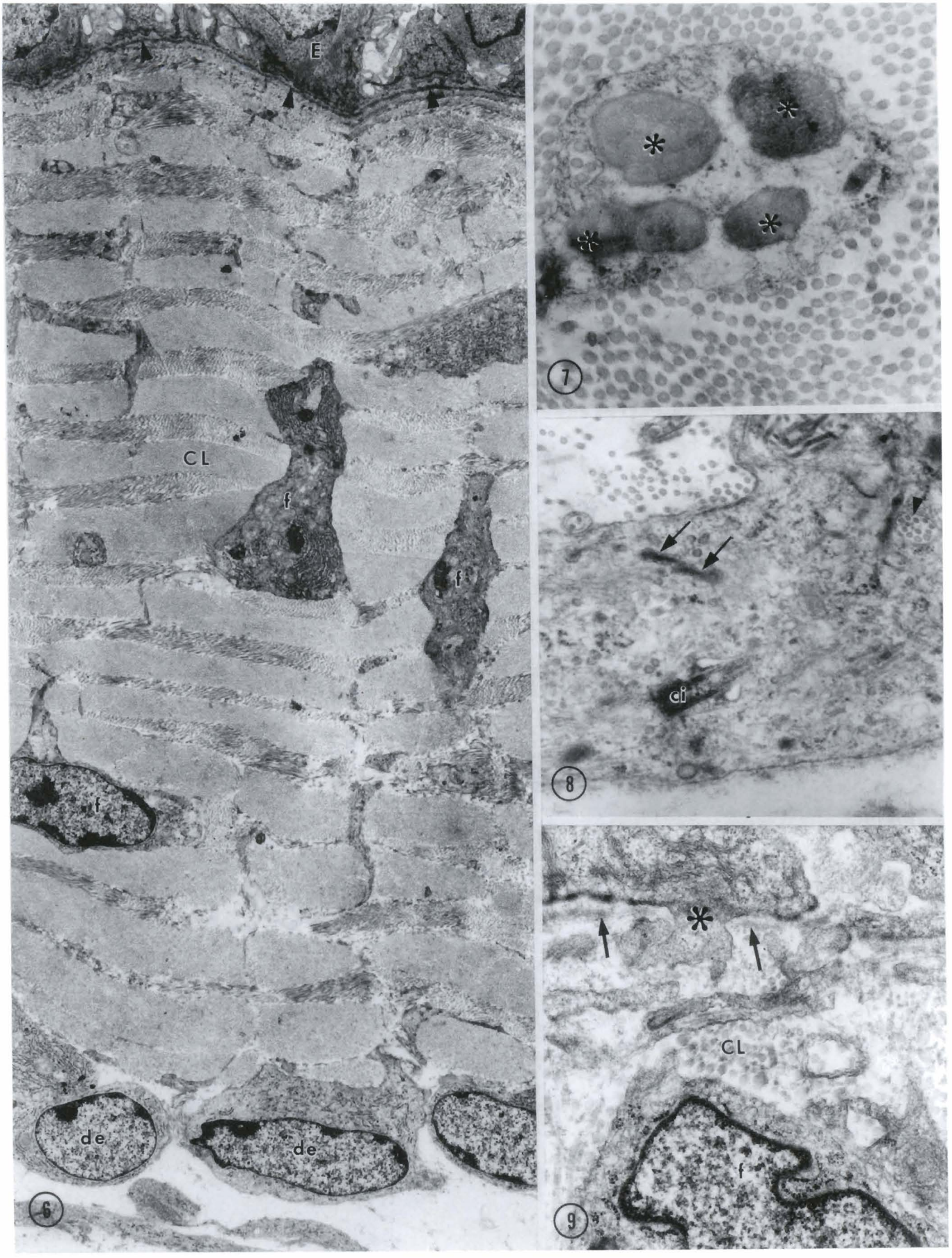
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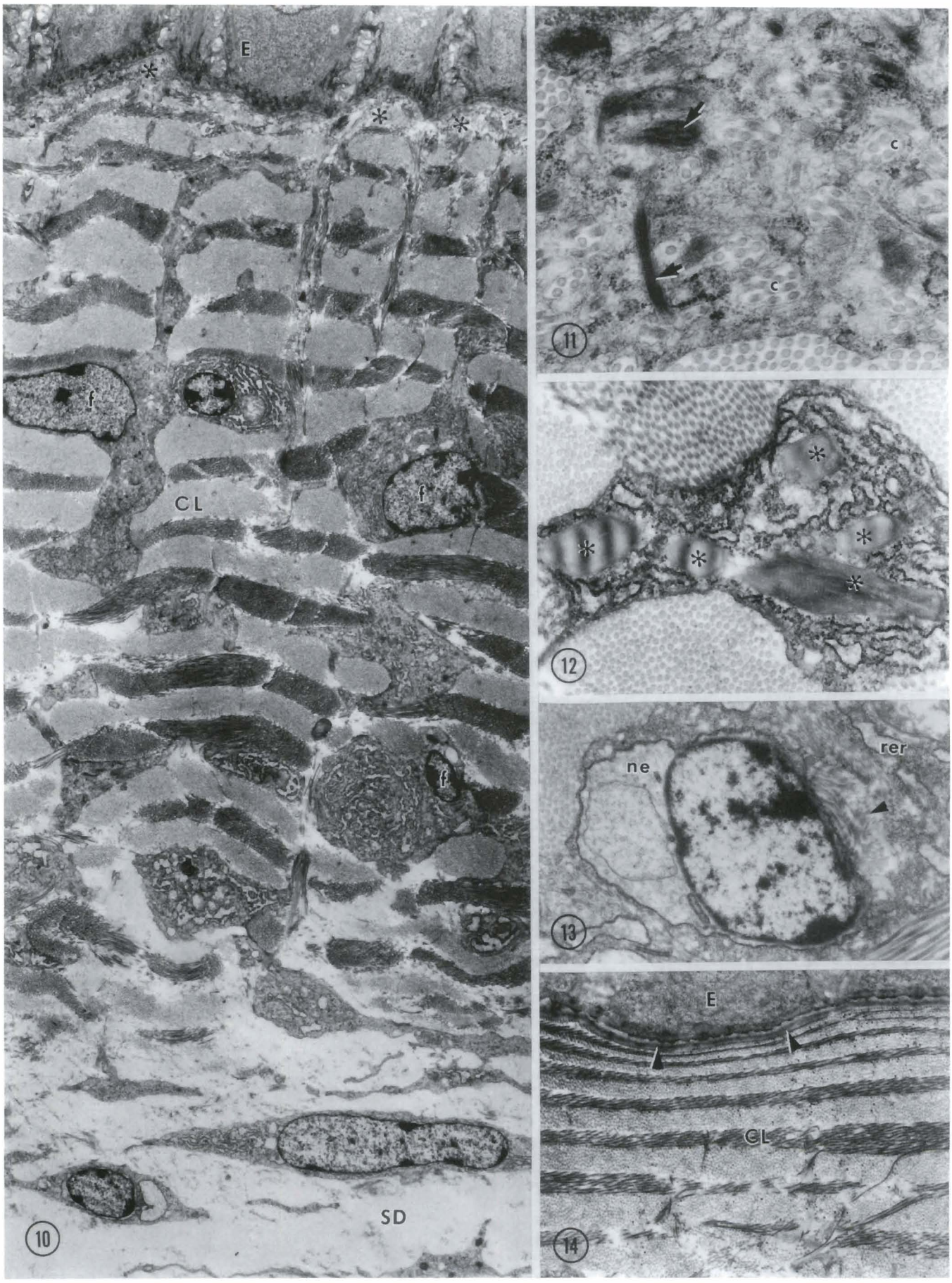
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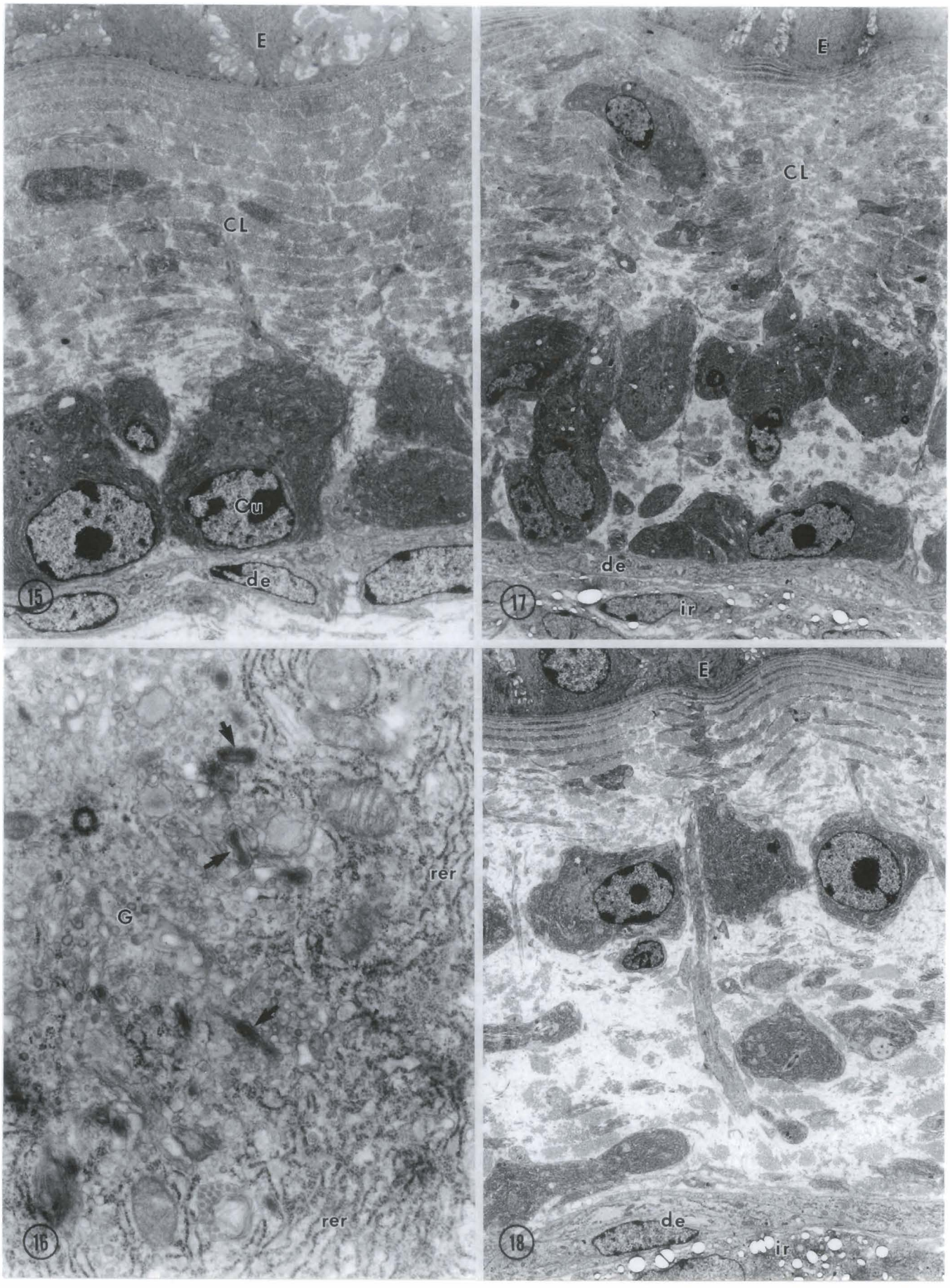
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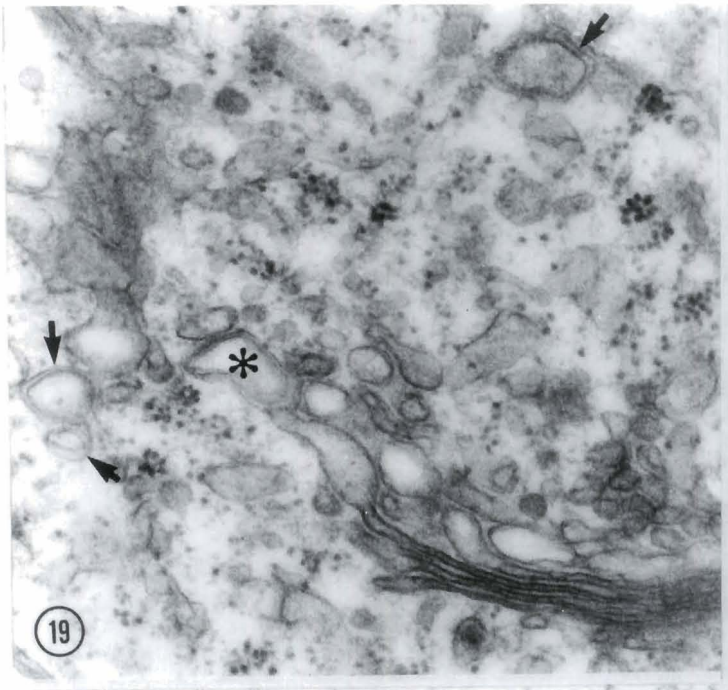

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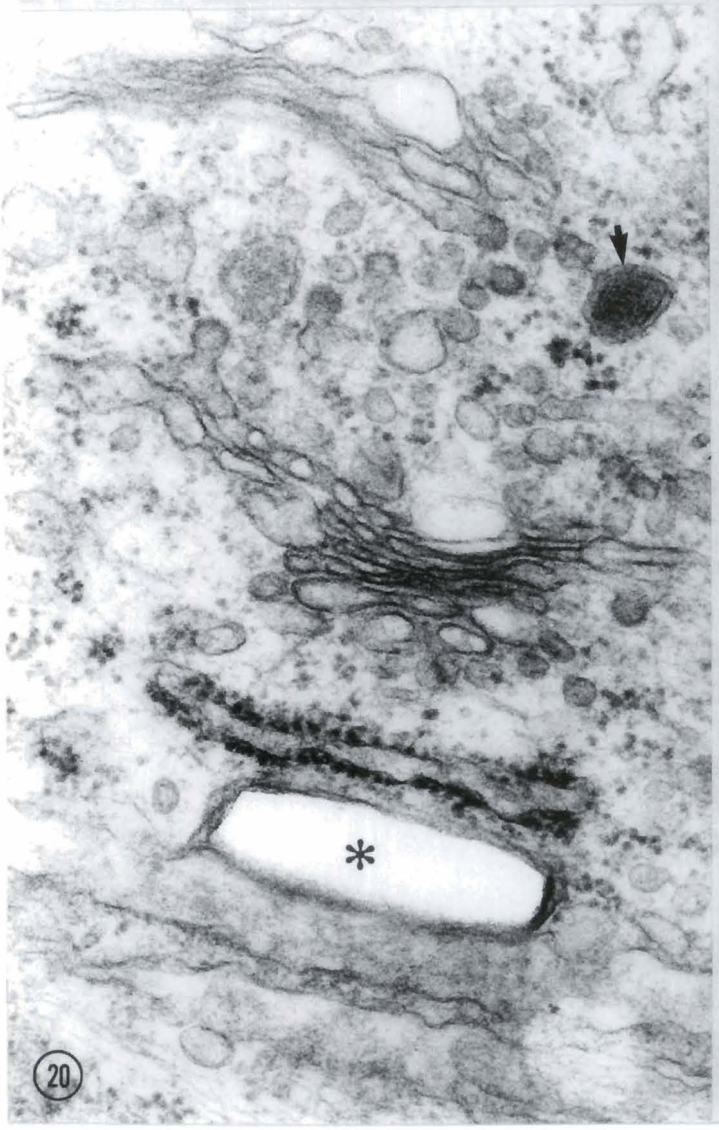

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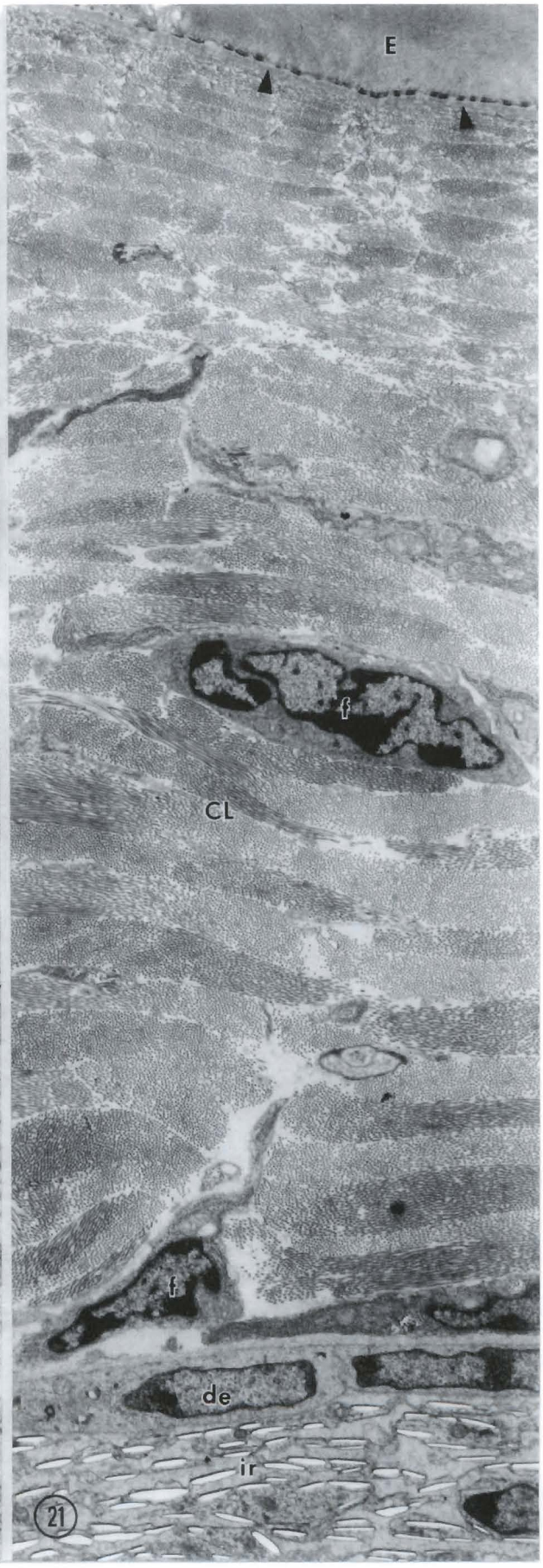

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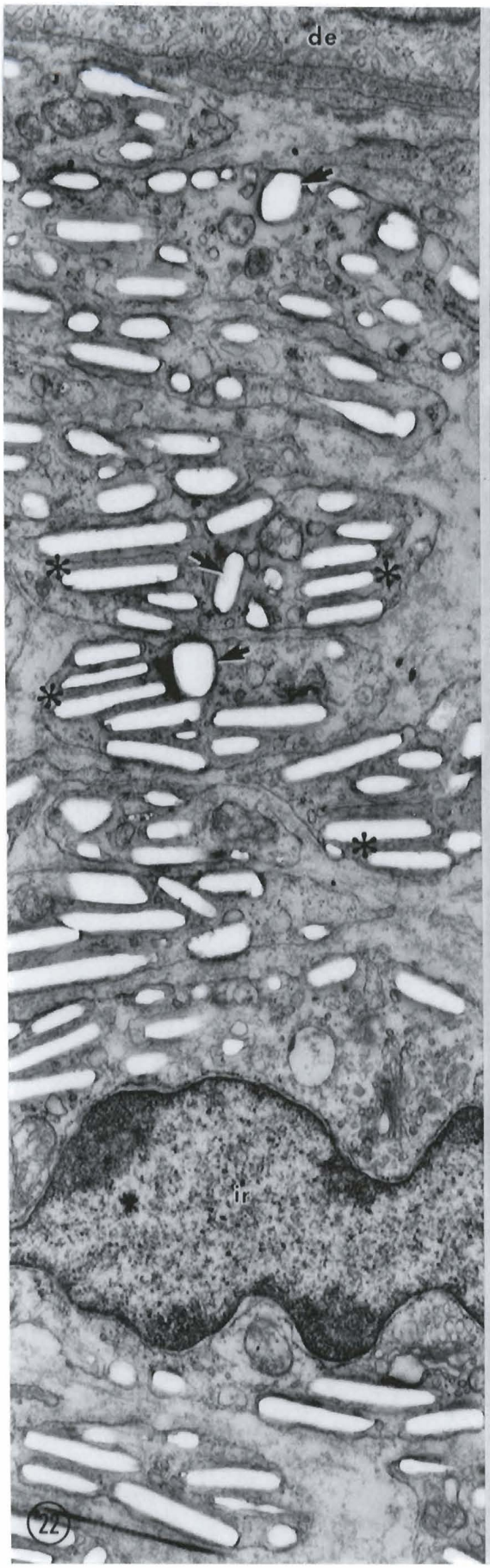
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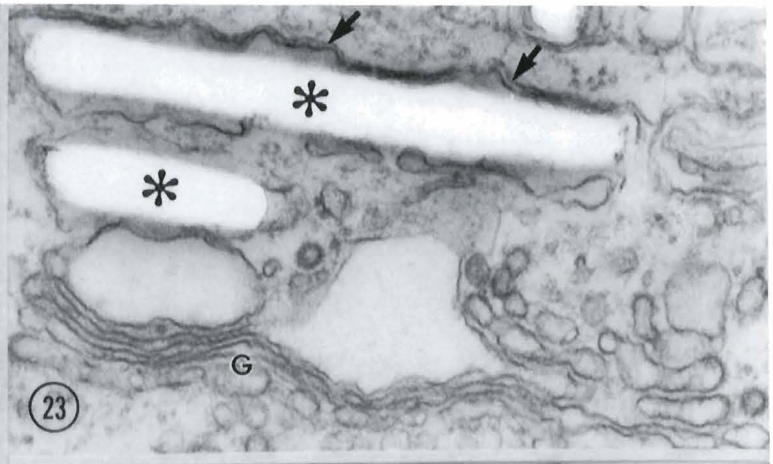
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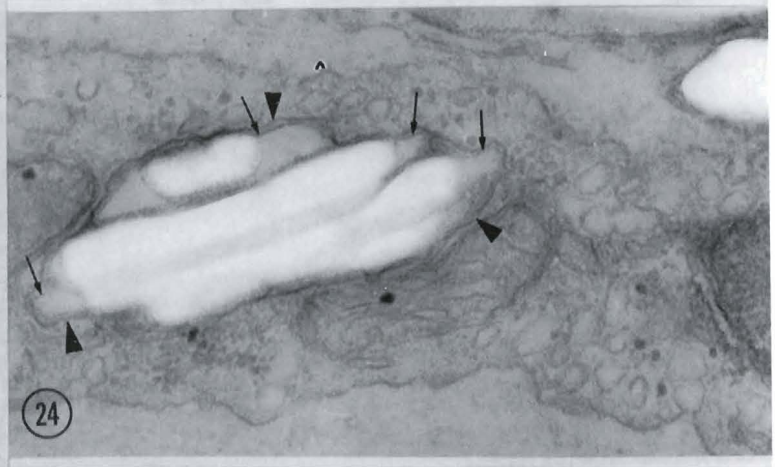
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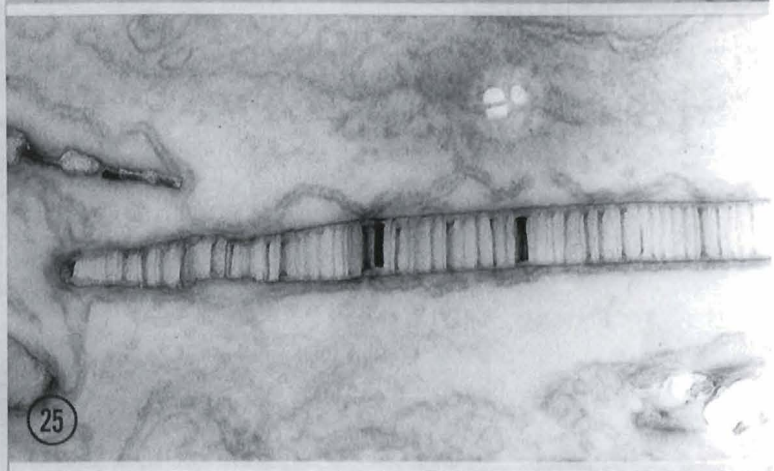
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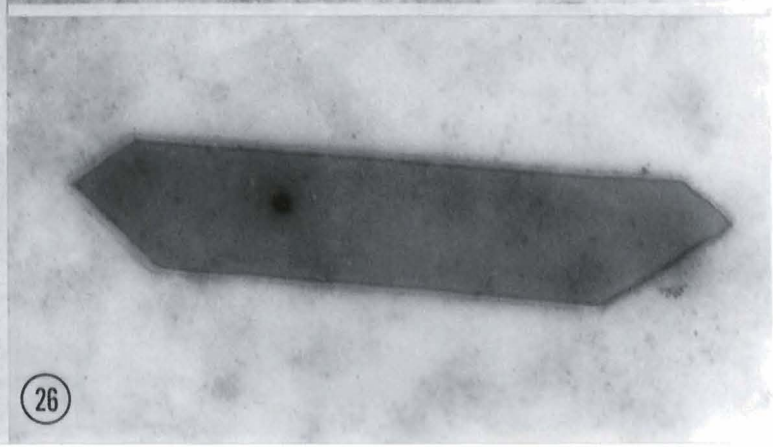
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Fig. 1. Ventral dermis of the larval lamprey showing the basal lamina (arrowheads), plywood pattern of the collagenous lamellae region (CL), and dermal endothelium (de). Some bundles of microfibrils and collagen are oriented perpendicular to the lamellae (arrows). Epidermis, E; fibroblasts, f. × 6,300

Fig. 2. Basal lamina of larval dermis consists of a lamina lucida containing many small membrane bound structures (arrowheads), lamina densa (ld), and microfibrils of the pars fibroreticularis (arrows). Epidermis, E; collagen fibrils of the collagenous lamellae region, c. × 53,000

Fig. 3. Basal lamina region of the ventral dermis of a stage two animal showing a portion of the basal epidermis (*) extending through the lamina lucida onto the lamina densa (ld). Note the lack of membrane structures in the lamina lucida. Epidermis, E; collagen fibrils, c. × 36,000

Fig. 4. Dermal endothelial cell of a larval animal showing the numerous caveolae intracellulares (arrows). Filamentous material sometimes clumped in large patches (*) is associated with both surfaces of the cell (arrowheads). × 53,000

Fig. 5. Gap junctions (arrows) between dermal endothelial cells (de) from a larval lamina. × 53,000

Fig. 6. Fibroblasts (f) and dermal endothelial cells (de) of the ventral dermis during stage 3 of metamorphosis show an increase in cell volume and change in cell shape as a result of increased cellular activity. Epidermis, E; collagenous lamellae, CL; basal lamina (arrowheads). × 5,400

Fig. 7. Portion of a fibroblast within the collagenous lamellae region of a stage 3 animal has large vacuoles containing coalescing collagen fibrils (asterisk). × 37,000

Fig. 8. Golgi region of a dermal endothelial cell from a stage 3 animal contains dumbbell shaped secretory granules (arrows) and collagen filled vacuoles (arrowhead) characteristic of a collagen synthesizing cell. Cilium, ci. × 25,000

Fig. 9. The lamina densa (arrows) of the ventral dermis of a stage 4 animal is fragmented allowing a portion of an epidermal cell (*) to extend into the collagenous lamellae region (CL). Fibroblast, f. × 24,000

Fig. 10. The collagenous lamellae (CL) of the ventral dermis of a stage 4 animal are disrupted by an increase in cells within the region. No dermal endothelium separates dermal and subdermal (SD) areas. Note the loose arrangement of collagen just beneath the basal lamina (*). Epidermis, E; fibroblast, f. × 3,500

Fig. 11. Fibroblast within the collagenous lamellae region of a stage 4 animal demonstrating many vacuoles containing groups of collagen fibrils (c) some of which are in a state of degradation (arrows). × 29,000

Fig. 12. Fibroblast within the collagenous lamellae region of a stage 4 animal which contains profiles of fused collagen fibrils in more extreme states of degradation (*). × 17,000

Fig. 13. Fibroblast within the collagenous lamellae region of a stage 4 animal showing signs of cell death with condensed cytoplasm and highly dilated rough endoplasmic reticulum (rer) and nuclear envelope (ne). Also note the vacuoles containing partially degraded collagen (arrowhead). × 13,000

Fig. 14. Stage 5 animal showing newly formed lamellae (CL) beneath the basal lamina (arrowheads). Epidermis, E. × 8,700

Fig. 15. A layer of large, synthetically active cuboidal shaped cells (Cu) is found beneath the newly formed collagenous lamellae (CL) and above the reformed dermal endothelium (de) during stage 5 of metamorphosis. Epidermis, E. × 32,000

Fig. 16. Portion of a cuboidal cell with many cisternae of rough endoplasmic reticulum (rer) and a large Golgi region (G) showing many saccules and associated secretory granules (arrows) characteristic of a collagen synthesizing cell. × 23,000

Fig. 17. A second, less polarized cuboidal cell layer forms during stage 5. Epidermis, E; collagneous lamellae, CL; dermal endothelium, de; iridophores, ir. × 2,600

Fig. 18. By late stage 5 the cuboidal cells migrate out into the collagenous lamellae region. Developing iridophores (ir) are present directly below the dermal endothelium (de). Epidermis, E. × 2,900

Fig. 19. A dilated portion of a Golgi saccule (*) and a few associated vacuoles (arrows) appear as double membrane structures within the Golgi region of a developing iridophore in a stage 5 animal. × 52,000

Fig. 20. Developing iridophore in a late stage 5 animal showing a peripherally located mature reflecting platelet (actually a space where a platelet was) bounded by a double membrane (*) and a Golgi associated developing platelet × 74,000

Fig. 21. The ventral dermis of a stage 7 animal consists of a basal lamina (arrowheads), collagenous lamellae (CL), dermal endothelium (de) and a subdermal layer of iridophores (ir). Epidermis, E; fibroblasts, f. × 6,300

Fig. 22. Subdermal iridophore layer from a stage 7 animal showing the arrangements of reflecting platelets which fill the cytoplasm. Note that many small stacks of platelets (*) sit at slight angles to each other and the skin surface, only a few individual platelets are perpendicular to the skin surface (arrow). Dermal endothelium, de; nucleus of iridophore (ir). × 18,000

Fig. 23. Higher magnification of developing reflecting platelets from the Golgi body (G) within an iridophore from a parasitic adult lamprey. Lead staining has solubilized the components of the associated reflecting platelets leaving trapezoidal spaces (*) surrounded by double membranes (arrows). × 63,000

Fig. 24. Four reflecting platelets grouped within a common outer membrane (arrowhead) and each surrounded by individual inner membranes (arrows). Lead stain only. × 50,000

Fig. 25. Unstained section of an iridophore from a parasitic adult lamprey showing the striated substructure of a fractured platelet. × 59,000

Fig. 26. Unstained, platelet extracted from the ventral skin of a parasitic adult lamprey. × 48,000

orthoganally creating a plywood pattern. Bundles of collagen fibrils and microfibrils are frequently observed extending perpendicular through these lamellae. Randomly dispersed between the lamellae is a small population of fibroblasts and occasional nerves. The fibroblasts (referred to as fibrocytes by Whitear et al., 1980) are commonly seen as attenuated, synthetically inactive cells similar in appearance to those previously described in connective tissue of the ventral head region of larval lamprey (Wright and Youson, 1982). Many surface vesicles, some forming long tubules are found associated with all parts of the cell membrane. Beneath the collagenous lamellae lies a layer of modified fibroblasts forming the dermal endothelium which delineates the inner boundary of the dermis (Whitear et al., 1980; Lethbridge and Potter, 1981). This layer is often interrupted by vertically arranged collagen

fibrils and microfibrils as well as nerves which penetrate through intercellular spaces. Numerous caveolae intracellulares are a characteristic feature of these thin, enlongated cells (Fig. 4). Hemidesmosome-like structures are present on both the dermal and subcutaneous cell surfaces. Endothelial cells are attached to each other by desmosomes. Gap junctions (with intercellular spaces of 3.5 - 4.0 nm) were also observed between adjacent cells (Fig. 5). Crystalline-like inclusions identical to those found in fibroblasts amongst the collagenous lamellae occur in many dermal endothelial cells. Discontinuous patches of filamentous material sometimes associated with microfibrils are seen along both boundaries of this cell layer.

Stage 3

There are subtle differences in the appearance of the collagenous lamellae region and dermal endothelium during stage three from that seen in the larval and stage 1 and 2 metamorphic animals (Fig. 6). There is an increase in cell volume and change in cell shape of some fibroblasts; they become thicker and have more rough endoplasmic reticulum (RER) and vacuoles containing cross sections of collagen fibrils in various states of coalition (Fig. 7). Dermal endothelial cells are more rounded demonstrating extensive RER and prominent Golgi apparatus with associated dumbbell-shaped secretory vesicles and vacuoles containing collagen fibrils (Figs. 6, 8). Junctions between the cells are lost and no hemidesmosome-like structures are present.

Stages 4 - 5

The mid-metamorphic stages are characterized by a massive reorganization of the ventral dermis in the head region. Restructuring of the dermal layers commences in the anterior ventral region and is seen to progress posteriorly.

During stage four the basal lamina shows much irregularity. The lamina densa is fragmented and cytoplasmic extensions of the basal epidermal cells pass through the resultant gaps (Fig. 9). The tightly packed linear arrangement of collagen fibrils forming the lamellae region in earlier stages is disrupted during stage four by a pronounced increase in the number of cells in the region (Fig. 10). The majority of cells are fibroblasts but other cell types with morphological characteristics typical of neutrophils and lymphocytes are also present. Fibroblasts demonstrate an extensive increase in cell volume. The cytoplasm of many fibroblasts is filled with vacuoles containing groups of collagen fibrils that are often bent or wavy with faint, indistinct banding (Fig. 11). Groups of fibrils in profile exhibit partial fusion with the sharpness of the banding varying along the length. Extreme forms of this appear in which complete fusion results in broad bands of alternating dense and lucent regions (Fig. 12). Residual bodies containing electron dense inclusions are a common component of these fibroblasts. In other fibroblasts the cytoplasm is condensed and both the cisternae of RER and the nuclear envelope are highly dilated (Fig. 13). Synthetically active cells similar in appearance to the endothelial cells of stage three are present within the modified lamellae region. Collagen fibrils within the lamellae region particularly those directly beneath the basal lamina are now seen to form a loose random arrangement. No dermal endothelial cells or endothelial layer is recognisable during stage four. The base of the dermis and subcutaneous layer are in continuum (Fig. 10).

By stage 5 the basal lamina is once again a well defined unit with a continious lamina densa and overall appearance like that of the larval animal. Beneath the basal lamina is a narrow region of newly synthesized collagenous lamellae with little and in some regions no evidence of fibroblasts (Fig. 14). Concomitant with and situated directly beneath the reestablished lamellae is a continuous, single cell layer of large, cuboidal shaped cells that juxtaposes the dermal surface of the reformed endothelium (Fig. 15). The cuboidal cells are polarized with spherical nuclei containing a prominent nucleolus positioned at the base of the cell. The apical cytoplasm is filled with an extensive RER and large Golgi apparatus with associated dense dumbbell-shaped secretory vesicles typical of collagen synthesizing cells (Fig. 16). Although the cuboidal cells are closely apposed to each other no junctions were observed between cells. The cuboidal cells divide forming a second layer of cells similar in appearance to the first but lacking an obvious polarity (Fig. 17). Neutrophils and lymphocytes are present amongst the cell layers. By late stage 5, the majority of cuboidal cells are dispersed within the reforming collagenous lamellae region, only a few cuboidal cells remain juxtaposed to the dermal endothelium (Fig. 18).

A layer of developing chromatophores, 1 - 2 cells thick first appears during stage 5 directly below the reformed dermal endothelium (Figs. 17, 18). The principle cell type of this stratum is the iridophore. These spindle-shaped cells have moderate amounts of RER and a well developed Golgi apparatus. The cisternae of the Golgi exhibit extensive dilations and blebbings. The most conspicuous feature of these developing iridophores is the presence of Golgi associated, double membrane vesicles and larger double membraned vacuoles having varying internal densities (Figs. 19, 20). The configuration of these structures ranges from round to square to rectangular. Fusion of these structures was often observed. During the initial developmental stages of the iridophores only a few apparently mature pigmentary organelles (platelets) were observed per cell and these tended to be located along the cell periphery (Fig. 20). Infrequent melanocytes occur within the iridophore layer and more rarely, melanin within an iridophore.

Stage 6-Parasitic adult

During the late metamorphic stages all the dermal layers are reestablished and superficially resemble that of the larvae. The most obvious difference being the addition of the newly formed iridophore layer directly beneath the endothelium (Fig. 21). The basal lamina of the new adult is similar to that of the larval stage although the lamina densa is twice as thick as that in the larval animal. The collagenous lamellae are analogous in form but thicker than those of the larvae. In stages 6 and 7 the fibroblasts between lamellae demonstrate moderate amounts of RER and small Golgi regions. Fibroblasts in the young adults are relatively inactive, attenuated cells with little RER and a small Golgi apparatus. No cuboidal cell layer is present above the dermal endothelium. Cells present in this area are indistinguishable from the fibroblasts found elsewhere in the lamellae region. The dermal endothelium of late metamorphic and parasitic adult animals is similar to that of the larval animal. However, the dermal surface is now covered with a distinct layer of microfibrils which separates the endothelial cells from the collagenous lamellae region, and the subcutaneous surface is covered with a thin, continuous, dense, flocculent, lamina densa-like layer.

The layer of iridophores is quite extensive by stage seven being 4 to 7 cells thick (Fig. 22). The predominant cell organelle, the reflecting platelet, fills the cytoplasm to the extent that few other organelles except the nucleus, mitochondria and occasional Golgi area are apparent. The arrangement of platelets in the iridophore layer has a very regular appearance. Individual platelets and stacks of 2 - 5 parallel reflecting platelets are often arranged at slight angles to each other and to the surface of the skin. A few individual platelets, but no stacks, lay perpendicular to the skin surface.

The mature pigmentary organelle is a double membrane structure within which lies a single trapezoidal or rectangular platelet that is usually represented by a space when lead stain is employed (Fig. 23). More infrequently, a single membrane encompasses a group of 2 - 4 reflecting platelets each of which are enveloped in their own membrane (Fig. 24). Sections left unstained or stained only with uranyl acetate demonstrate fragments or entire reflecting platelets in their membranes. The substructure of these platelets is defined by striations of varying densities which run perpendicular to the long axis (Fig. 25). Unstained platelets, extracted in entirety from parasitic adult animals, occur as trapezoidal, rhombic, rectangular and six-sized polygonal shapes (Fig. 26). The dimensions range from 0.16 to 1.1 μm in width and 1.1 to 5.7 μm in length. Thickness is estimated to range from 20 to 50 nm.

Discussion

The metamorphic changes in the lamprey dermis are unusual and possibly unique in that they do not result in the formation of a dermis different from that of the larval animal. There is a disruption of the dermis and reformation resulting in a structure exactly the same as that prior to metamorphosis. The only major change associated with the dermis as a result of metamorphosis is the formtion of a subdermal layer of iridophores.

In amphibians, metamorphic changes in the dermis of the body results in the formation of a dermis different in structure than that prior to metamorphosis (Fox, 1985). The dermis of larval amphibians consists of a basal lamina, collagenous lamellae region and a layer of dermal mesenchymal cells (fibroblasts) (Chapman and Dawson, 1961; Kinoshita et al., 1991). During metamorphosis, the collagenous lamellae region becomes disorganized as a result of migration of dermal fibroblasts into the region and phagocytosis of collagen; subsequently a new stratum spongiosum develops beneath the basal lamina and collagenous lamellae reform from newly synthesized collagen and fibroblasts (Fox, 1985). The new froglet has a dermis different in structure to that of the larvae. The formation of the new stratum spongiosum may be necessary for the adaptation of the adult to the new habitat it enters at the end of metamorphosis (ie. to accomodate the glands associated with the dermis which help keep the skin of the adult moist and allow for gas exchange when the animal is out of the water). Although the sea lamprey enters a new environment (salt water) at the end of metamorphosis there may be no need for a change in structure of the dermis for the animal to adapt to this new habitat since the skin plays no role in osmoregulation (Lethbridge and Potter, 1981). The formation of iridophores within the subdermis however in an important adaptive feature which provides camouflage ventrally to the free swimming adult.

The general organization of the dermal basal lamina of the lamprey is similar to that of mammals (Leblond and Inoue, 1989). The small structures observed in the lamina lucida of larval, late metamorphic and adult stage lamprey appear to be membrane bound electron lucent vesicles unlike the dense granules and lamellated bodies found in the adepidermal space (lamina lucida) of the skin of teleosts and larval amphibians (Brown and Wellings, 1970; Watanabe and Tachibana, 1973; Nako, 1974). The granules in teleosts are thought to be related to the deposition of the lamina densa. The function of the vesicles is unknown. It is interesting to note however that these vesicles disappear early in metamorphosis just prior to the disruption of the lamina densa. No vesicles, granules or lamellated bodies are associated with the lamina lucida of mammalian skin (Inoue and Leblond, 1988).

The basal lamina associated with epithelium of transforming tissues during lamprey metamorphosis typically demonstrate extensive thickening, folding and pleating (Wright and Youson, 1980; Sidon and

Youson, 1983) which may be related to loss of epithelial cells. This process would not be expected in the epidermis since hyperplasia rather than cell loss normally occurs (Lethbridge and Potter, 1981). Rather than folding, the dermal basal lamina fragments during metamorphosis, allowing the protrusion of portions of the epidermal cells into the collagenous lamellae region. Youson and Ogilvie (1990) observed fragmentation along with folding and pleating of the basal lamina of the degenerating gallbladder which allowed contact between epithelial cells and subepithelial cells. They suggest that such contact may be important for inducing changes in the epithelium. Although epidermal cells protrude into the collagenous lamellae region no contact with dermal cells was observed but the epidermal cells were in intimate contact with the extracellular matrix constituents. Such interaction may be necessary for the changes in the epidermis during metamorphosis.

A dermal endothelium consisting of modified fibroblasts joined together by desmosomes has been described in larval and adult lampreys and a wide variety of teleosts (Whitear et al., 1980; Whitear, 1986). Previous studies of lamprey dermal endothelial cells have demonstrated only desmosomes between cells (Whitear et al., 1980; Whitear, 1986). The present study shows the presence of typical gap junctions in addition to the more frequently observed desmosomes in larval, early (stage 1 and 2) and late (stage 6 and 7) metamorphic and parasitic adult animals. The presence of gap junctions would allow for direct communication and possibly coordination (in the form of ionic, metabolic or electrical coupling) between endothelial cells. The endothelium provides a physical barrier, partially separating the dense collagenous dermis from the more loosely arranged connective tissue of the subdermal region, perforated by nerves, microfibrils and collagenous fibrils. The function of this layer, however, is unknown (Whitear et al., 1980). During stage 3 many of the normally attenuated dermal endothelial cells become more rounded in shape and demonstrate increased synthetic activity indicated by the abundance of RER, and well developed Golgi regions with many dumbbell shaped Golgi associated bodies typical of collagen synthesizing cells. A dermal endothelium is no longer present by mid metamorphosis (stage 4). Endothelial cells can no longer be distinguished from other cells which populate the disrupted collagenous lamellae region.

The most obvious changes in the dermis during metamorphosis take place in the collagenous lamellae region. A light microscopic study of the dermal connective tissue in the head of *Petromyzon* by Johnels (1950) was the first and only work done to date on metamorphic changes of the dermis. The limiting resolution of the light microscope and the lack of animals representative of all metamorphic stages allowed only an overall impression of the changes. Nevertheless Johnels (1950) describes «a loosening of the dermal tissue» accompanied by an increase in nuclei within the collagenous lamellae region, followed by a loss of dermal tissue and reformation. The present study confirms, clarifies and adds substantially more detailed information to these earlier observations.

The disruption of the collagenous lamellae takes place during mid metamorphosis (stage 4) and is the result of an increase in cells within the regions by cell division and migration from the subdermal region which at this point in metamorphosis is in continuum with the dermis due to the loss of the dermal endothelium. The loss of organization of the collagen component may be attributed to an overall increase in phagocytosis and digestion of collagen by the many fibroblasts within the region since these cells contain collagen in various states of degradation. Fibroblasts are known to be involved in the degradation of collagen (Melcher and Chan, 1981; Kinoshita et al., 1991). Other phagocytic cells such as neutrophils are present in the region and may contribute to this process. Along with collagen degradation many fibroblasts appeared to be undergoing apoptosis, a form of cell death (Wyllie, 1981) characterized by the condensed nature of the cytoplasm and highly dilated nuclear envelope and RER. Still some fibroblasts are actively involved in collagen synthesis rather than degradation evident by the abundance of RER and characteristic Golgi associated dumbbell-shaped secretory granules.

By stage five a thin, new collagenous lamellae layer is established directly beneath the basal lamina. It is interesting that collagen lamellae were initially lost closest to the basal lamina and it is in this region that new lamellae are first formed. The lamellae appear to be formed from newly synthesized collagen produced by large cuboidal-shaped cells that initially form a distinct single cell layer above the reformed dermal endothelium. Johnels (1950) alluded to these large cells as an epithelial-like layer due, no doubt, to their size and alignment within the dermal tissue. The origin of these cells is unknown but it is possible that they may originate from the subpopulation of fibroblasts synthesizing collagen. Unlike the other fibroblasts which are either degrading collagen and/or dying these fibroblasts may align themselves beneath the basal lamina, enlarge and become highly specialized in collagen synthesis. The differentiation of the cuboidal cells may be dependent on the presence of an intact dermal endothelium since cuboidal cells are always seen in association with the dermal endothelium. Alternately, the formation of the endothelium may be dependent on the presence of the cuboidal cells. The appearance and arrangement of the cuboidal cells in the reformation of the collagenous lamellae is unusual. The authors could find no reference to anything similar in other developing or reforming dermal tissues. These cuboidal cells are reminiscent of osteoblasts (Wright and Leblond, 1981) with their polarized cytoplasm and cytoplasmic organelles characteristic of collagen synthesis, depositing fresh

layers of collagen at their apical surface. During stage 5 these cells divide to form a two cell layer, the cells then migrate away from the dermal endothelium into the new lamellae region transforming (dedifferentiating) into the typical attenuated fibroblasts found between collagenous lamellae in late metamorphic (stages 6 and 7) and adult animals.

Iridophores are reflecting chromatophores which contain guanine and other purines as their pigmentary material. It is known that dermal chromatophores of amphibians and reptiles are of neural crest origin and arise from a common stem cell (Bagnara, 1966; Alexander, 1970). This pleuripotent cell is thought to differentiate into a specific chromatophore type by producing the necessary characteristic pigmentary organelles. With respect to iridophores, the pigmentary organelles are the reflecting platelets. The origin of reflecting platelets within iridophores may be species dependent. Early studies of chondrichthyes (Arnott et al., 1970) and amphibians (Taylor, 1971) suggested that reflecting platelets originated from the Golgi apparatus. Later evidence led Bagnara et al. (1979) to propose that, in amphibians, the reflecting platelets are of endoplasmic reticulum origin. More recently, Gundersen and Rivera (1982) suggest that both the endoplasmic reticulum and Golgi apparatus contribute to platelet formation in iridophores of the guppy *(Poecilia reticulata)* .

In most lower vertebrates, purines are believed to be synthesized *de novo* in the cytoplasm (Martin et al., 1991). Reflecting platelets of iridophores contain one or more pruines (Harris and Hunt, 1973). In lampreys these platelets appear to develop from double membrane saccules and vesicles associated with the Golgi apparatus. The double membrane structures may arise from the enclosure of purine rich areas of cytoplasm by portions of Golgi saccules and vesicles. Further fusion of these structures would eventually result in increasing concentrations of purine and the development of a crystal surrounded by a double membrane, i.e. a reflecting platelet. Platelets isolated from the lamprey are very similar in size and shape to those extracted from the skin of Atlantic salmon *(Salmo salar)* (Harris and Hunt, 1973). Their regular appearance suggests that they are crystalline.

Like the iridopohores of many amphibians and fish (Taylor and Bagnara, 1972; Harris and Hunt, 1982), those of the lamprey have their reflecting platelets grouped in stacks. The orientation of these stacks at various angles to one another within the iridophores allows for the reflection of light and provides the silvery appearance to the ventral dermis. This metallic sheen of the ventral skin of late metamorphic and parasitic adult animals would mirror the bottom substrate and thus provides camouflage for the animal.

The dense regular arrangement of the larval dermis may be too resistant to allow for changes which take place in the head of the lamprey during metamorphosis (Johnels, 1950). The disruption and degradation of dermal tissue during metamorphosis may be necessary to allow for the considerable changes such as the development of cartilage, muscle and glands which takes place in the connective tissues of the head of the lamprey at this time (Johnels, 1948; Hardisty, 1979, 1981; Armstrong et al., 1988). Yet the original dermal organization must be necessary for proper resilience of the skin of the adult and thus has to be reformed by the end of metamorphosis.

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