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The Ultrastructure of the Integument of the American Eel, *Anguilla rostrata*

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Summary. The morphology and ultrastructure of the lateral body integument of the leptocephalus, glass eel, pigmented elver, and adult stages of the American eel, *Anguilla rostrata*, were examined with light and electron microscopy. The integument consists of an epidermis separated by a basal lamina from the underlying dermis. Three cell types are present in the epidermis in all stages. Filament-containing cells, which are the principal structural cell type, are increasingly numerous at each stage. Mucous cells, which secrete the mucous that compose the mucous surface coat, are also more numerous in each subsequent stage and are more numerous in the anterior lateral body epidermis than in the posterior lateral body epidermis of the adult. Club cells, whose function is unknown, are most numerous in the glass eel and pigmented elver. Chloride cells are common in the leptocephalus which is marine and infrequent in the glass eel. They are not present in the pigmented elver and adult which inhabit estuaries and freshwater. Lymphocytes and melanocytes are also present in some stages. The dermis comprises two layers: a layer of collagenous lamellae, the stratum compactum, and an underlying layer of loose connective tissue, the stratum spongiosum.

There is a progressive increase in epidermal thickness at each stage which is paralleled by an increase in the thickness of the stratum compactum. Rudimentary scales are present in the dermis of the adult. The increase in the number of epidermal filament-containing cells, epidermal thickness and stratum compactum thickness is correlated with an increased need for protection from abrasion and mechanical damage as the eel moves from a pelagic, oceanic habitat to a benthic, freshwater habitat. The increase in mucous cell numbers is likewise correlated with an increased need for the protective and anti-bacterial action of the mucous surface coat in the freshwater environment.

Key words: Integument — *Anguilla* — Ultrastructure.

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Introduction

The fish integument serves a variety of special functions related to its position at the interface between the organism and the environment. As the boundary layer of the fish, it is a principal barrier to the exchange of water, ions and larger molecules between the environment and the organism. The intact integument serves as a barrier to the entry of micro-organisms and resists the mechanical damage of various abrasions and shocks encountered in the environment. The integument also serves a variety of secretory and sensory functions.

Ultrastructure. Numerous ultrastructural investigations of various aspects of fish integument have been conducted. The most comprehensive ultrastructural survey of teleost epidermis was conducted by Henrikson and Matoltsy (1968 a, b, c) who contrasted the mucogenic epidermis, characteristic of teleost, with the keratinized epidermis characteristic of the predominantly terrestrial higher vertebrates. Other investigators have described the epidermis of larval and adult stages for both agnathans (Downing and Novales, 1971) and teleosts (Jimbo et. al., 1963; Jones et al., 1966; Merrilees, 1974). The whole integument has been examined in both scaleless teleosts (Fishelson, 1972, 1973) and teleosts with regions of scaleless and scaled integument (Brown and Wellings, 1970; Roberts et al., 1970; Roberts et al., 1972; Hawkes, 1974a; Harris and Hunt, 1975a). Other authors have concentrated on specific features of the integument: surface cuticle (Whitear, 1970); specializations of superficial cells (Yamada, 1968; Bereiter-Hahn, 1971); mucous cells (Kitzan and Sweeny, 1968; Pickering, 1974; Harris and Hunt, 1975b); chemosensory cells (Trujillo-Cenoz, 1961; Whitear, 1971b); free nerve endings (Whitear, 1971a); collagenous lamellae (Fujii, 1968; Nadol et al., 1969); scale formation (Brown and Wellings, 1969); and chromatophores (for review, see Fujii, 1969). In addition, ultrastructural investigations of the integument of the exclusively aquatic larval amphibians (Chapman and Dawson, 1961; Parakkal and Matoltsy, 1964; Farquhar and Palade, 1965; Kelly, 1966a, b) have demonstrated that numerous parallels can be drawn with the ultrastructure of fish integument.

Most investigations have concentrated on histological and ultrastructural aspects of integument and less attention has been devoted to the interrelationship of integumentary morphology and specific environment and behavioral characteristics of each developmental stage of the species studied. The American eel, *Anguilla rostrata*, is an especially useful fish for studies of this type. Because of its complicated life history and long distance migrations, the eel encounters a variety of marine, estuarine and freshwater environments and fills various ecological niches in each. The integument of an eel of the genus *Anguilla* has been investigated (Henrikson and Matoltsy, 1968a, b, c), but only the adult stage was examined. This study presents the histology and ultrastructure of the integument of the major life stages of the American eel and relates the observed differences to the environments encountered by and the behavioral characteristics of each stage.

Life History of Anguilla rostrata. Efforts to elucidate the life histories of American and European eels have been reviewed by Harden Jones (1968). The studies of Schmidt, beginning in the early 1900's resulted in several papers (Schmidt, 1922, 1923) in which he outlined the life histories of *Anguilla anguilla* and *Anguilla rostrata*. Other accounts of the life histories have been proposed (Tucker, 1959)

and criticisms of various aspects of Schmidt's hypothesis have been made (Vladykov, 1964; Harden Jones, 1968) but the major points of Schmidt's hypothesis have gained wide acceptance.

Schmidt proposed that American and European eels spawn in an area of the Sargasso Sea between 20° N and 34° N latitude and 50° W and 60° W longitude. *Anguilla anguilla* spawns in the northern half of this section and *A. rostrata* in the southern half. Vladykov (1964), however, believes that the distribution of *A. rostrata* larvae collected in the Atlantic is better explained by a spawning ground to the south and west of this area. Spawning occurs at depths of 200 m to 400 m throughout spring and early summer. Schmidt attributed the sorting out of the two species to their respective coasts to a differential rate of larval development. *A. rostrata* undergoes metamorphosis from the leptocephalus to the elver after one year. During this time, surface currents have carried it north and west from the spawning area to the North American coast. *A. anguilla* requires three years for larval development, during which time prevailing surface currents will have carried it to the European coast. After several years of growth in rivers and estuaries, eels presumably return to the parent spawning area, spawn and die.

Materials and Methods

Developmental Stages

The life stages of the European eel, which are similar to those of the American eel, have been described in detail. Bertin (1956), for example, notes numerous stages and substages in the course of the eel's development. For purposes of this study, a more general classification of the life history into five major stages has been adopted. These five stages are:

1. Leptocephalus: This is the initial larval form during the first oceanic phase of the eel's life. Metamorphosis to the second stage occurs at about the time the eel reaches the continental shelf off the coast it will inhabit as an adult (larva stage I; Bertin, 1956).

2. Unpigmented Elver or Glass Eel: At this stage, the eel has the elver body form (resembling a small version of the adult) but lacks pigment. It is at this stage that the eels enter estuaries and begin to ascend rivers (semi larva-stage III, IV; Bertin, 1956).

3. Pigmented Elver: During the first few months of estuarine and freshwater existence, the elvers gradually acquire the dark, greenish-brown coloration of the adult (elver-stage VI B; Bertin, 1956).

4. Yellow Eel: This stage is distinguished from the previous one by a considerable increase in size and weight. Eels spend several years in the freshwater or estuarine environment before the onset of sexual maturity.

5. Silver or Bronze Eel: When the eel becomes sexually mature, it develops a silver (European) or bronze (American) coloration, descends rivers and presumably returns to the Sargasso Sea to spawn. In the present study, the lateral body (flank) integument of the first four stages was examined.

Leptocephali of *Anguilla rostrata* were collected in the Sargasso Sea 22 mi S of St. David's Head, Bermuda at a depth of 100 m. Unpigmented elvers (glass eels) were collected from the Damariscotta estuary, Walpole, Maine. Pigmented elvers were obtained from a small tributary to the Penobscot River in the vicinity of Sandy Point, Maine. Adults were obtained from a dealer.

Technique

Histology. Whole leptocephali, glass eels, and pigmented elvers and pieces of integument from several body regions of adult eels were fixed in Bouin's fixative. After approximately 1 week in fixative, the tissue was dehydrated in an ascending ethanol series, cleared with xylene and embedded in Tissue Prep, 56.5° M.P. (Fisher). Seven micron sections of tissue were cut and stained with Ehrlich's hem-

atoxylin and 0.1% eosin. Sections of araldite and epon embedded tissue (see below) approximately 1 μ in thickness were also examined with light microscopy. This material was stained with toluidine blue (Pearse, 1960) or Mallory's Azure II-Methylene Blue (Richardson, et al., 1960).

Ultrastructure. Whole leptocephali, glass eels and pigmented elvers and large pieces of adult integument were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer. After 2 hours in the fixative, small pieces of tissue (< 1 mm^3) from the region of the lateral septum (in mid body for the first 3 stages, and anterior, mid body and posterior flank for adults) were removed and placed in the fixative for an additional hour. Following fixation the tissue was washed several times with buffer and post-fixed in 2% OsO_4 in phosphate buffer for 2 hours. After post-fixation the specimens were washed several times with buffer, dehydrated in an ascending ethanol series to propylene oxide and embedded in Araldite 506 or Epon 812. Embedded material was sectioned with a diamond knife on a Sorvall Porter-Blum MT 2-B ultramicrotome. Sections were stained with saturated aqueous uranyl acetate followed by lead citrate (Reynolds, 1963) and examined with a Philips EM 201 electron microscope.

Results

Organization of the Epidermis. Eel epidermis shows a progressive increase in thickness with age from 23 μ in the leptocephalus, 45 μ in the glass eel and 50 μ in the pigmented elver, to 260 μ in the adult (Figs. 1–6). Some regional variation in thickness is observable at each stage. A basal stratum of columnar to cuboidal cells resting on the basal lamina is present. Cells of intermediate layers do not form distinct strata and are highly irregular and variable in shape. The superficial layer of cells is squamous or cuboidal. The lateral line canal, present only in the pigmented elver and adult stages, is lined with epidermis which is continuous with surface epidermis at periodic surface openings (Fig. 4). Little intercellular space is present at any level of the epidermis of any stage. Filament-containing (FC) cells, mucous cells and club cells are the predominant cell types of the epidermis. Chloride cells, lymphocytes and melanocytes are present in certain stages. Clusters of chemosensory cells (taste buds), while numerous in the head region are infrequent on the flank in the vicinity of the lateral line. Single chemosensory cells were not observed with light or electron microscopy and if present are rare. The relative frequency of the various cell types changes with increasing age and, in some instances, from region to region of the animal. Several trends are evident. FC cells and club cells form the bulk of the epidermis in the leptocephalus (Fig. 1). The increase in epidermal thickness of the glass eel is due to an increase in the number of club cells which are the predominant cell type at this stage (Fig. 2). Club cells remain the predominant cell type in the pigmented elver (Figs. 3, 4). In the anterior flank epidermis of the adult, a considerable increase in the frequency of mucous cells and FC cells is apparent (Fig. 5), however, the adult posterior flank epidermis more closely resembles glass eel and pigmented elver epidermis (compare Figs. 2, 3, and 6). Chloride cells were observed only in the leptocephalus and glass eel stages. Lymphocytes were observed in the glass eel, pigmented elver, and adult stages. Melanocytes are present only in the pigmented elver and adult.

Filament-Containing Cells. Filament-containing (FC) cells are characterized by the presence of numerous cytoplasmic tonofilaments. The principal difference among the four stages is the relative frequency with which FC cells occur, being lowest in the leptocephalus and greater at each subsequent stage. The increase in epidermal thickness at each stage, however, is due to the increase in FC cell numbers

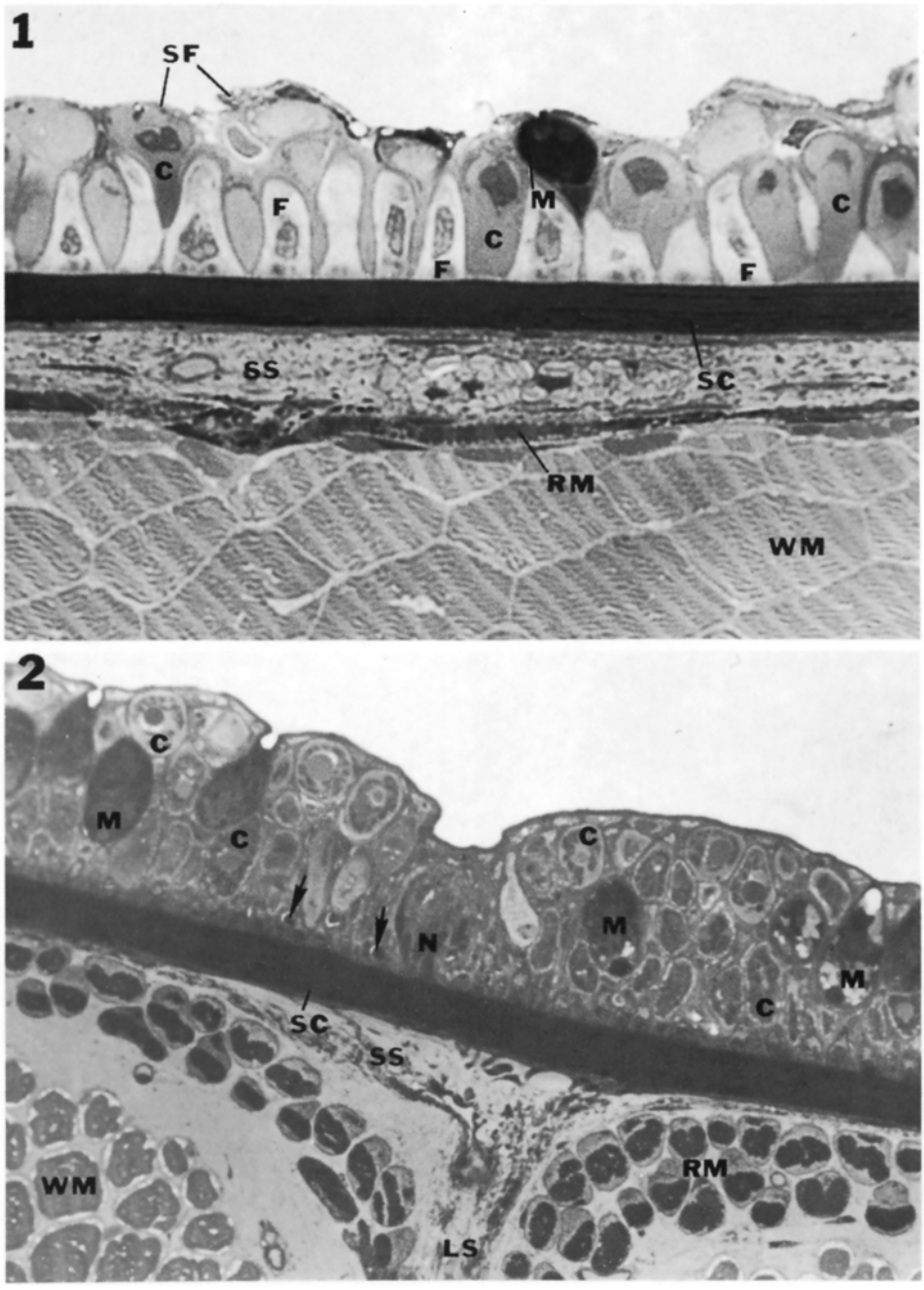


Fig. 1. Integument and subdermal muscle of the leptocephalus. Club cell (*C*), FC cell (*F*), mucous cell (*M*), red muscle fibers (*RM*), stratum compactum (*SC*), surface FC cell (*SF*), stratum spongiosum (*SS*), white muscle fibers (*WM*). ×1,000

Fig. 2. Integument and subdermal muscle of the glass eel. Club cell (*C*), lateral septum (*LS*), lymphocyte (arrow), mucous cell (*M*), neuromast (*N*), red muscle fibers (*RM*), stratum compactum (*SC*), stratum spongiosum (*SS*), white muscle fibers (*WM*). ×475

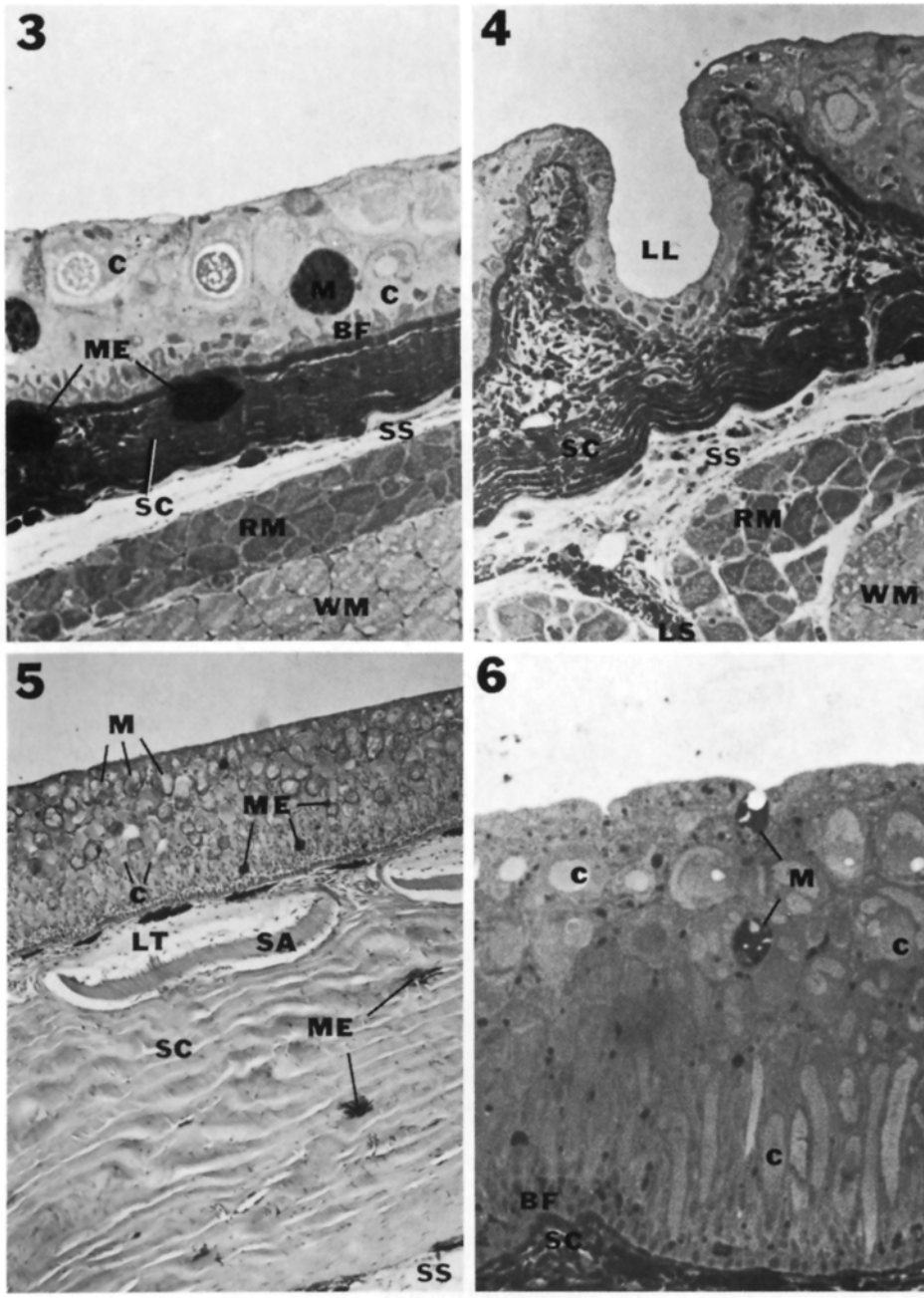


Fig. 3. Integument and subdermal muscle of the pigmented elver. Basal FC cell (*BF*), club cell (*C*), mucous cell (*M*), melanocyte (*ME*), red muscle fibers (*RM*), stratum compactum (*SC*), stratum spongiosum (*SS*), white muscle fibers (*WM*). ×425

Fig. 4. Surface opening of the lateral line canal in the pigmented elver. Epidermis lining the canal is continuous with surface epidermis at these openings. Lateral line canal (*LL*), lateral septum (*LS*), red muscle fibers (*RM*), stratum compactum (*SC*), stratum spongiosum (*SS*), white muscle fibers (*WM*). ×425

Fig. 5. Anterior flank integument of the adult. Club cell (*C*), loose connective tissue (*LT*), mucous cell (*M*), melanocyte (*ME*), scale (*SA*), stratum compactum (*SC*), stratum spongiosum (*SS*). ×90

Fig. 6. Posterior flank integument of the adult. Club cell (*C*), mucous cell (*M*), stratum compactum (*SC*). ×240

only in the adult. FC cells occur at all levels of the epidermis and are the sole constituent of the cuboidal to columnar basal cell stratum except in the leptocephalus. FC cells at intermediate levels of the epidermis are highly irregular in shape and do not form distinct strata. The surface cell stratum comprises squamous to cuboidal FC cells except where mucous cells and chloride cells reach the surface.

Basal FC cells rest on the basal lamina and numerous hemidesmosomes occur along the basal cell membrane (Figs. 8, 9). FC cells of this stratum generally resemble truncated cones, tapering at their apical ends (Figs. 7, 10). The basal FC cells contact one another laterally to varying degrees, the cell membranes forming extensive interdigitations in the zone of contact (Fig. 11, inset). In the leptocephalus, club cells and chloride cells are inserted deeply between the basal FC cells, occasionally reaching the basal lamina (Fig. 7). In the glass eel and pigmented elver, the basal FC cells contact one another over their basolateral surfaces (Fig. 10). Immature mucous cells, club cells, FC cells of intermediate levels and lymphocytes are interposed between the apical ends of the basal FC cells (Figs. 10, 18). Intercellular interdigitations and regions with numerous desmosomes are frequent along the boundary between basal FC cells and adjacent mucous cells, club cells, and FC cells of intermediate levels. Lymphocytes and basal FC cells are usually separated by intercellular space (Fig. 10). In the adult basal FC cells are contiguous over their entire lateral surface and there are no interposed cells of intermediate levels (Fig. 11).

The cytoplasm of basal FC cells shows greater affinity for electron stains than does the cytoplasm of cells of intermediate layers (Figs. 10, 11). The electron density of these regions is probably due to the presence of numerous free ribosomes in the cytoplasm (Fig. 12).

The most distinctive cytoplasmic feature of the basal FC cell is the presence of compact central skeins of parallel tonofilaments (80 Å diameter) (Fig. 12). The tonofilaments follow an irregular course from the basal lamina to the apical end of the cell (Fig. 12). In the basal region of the cell, bundles of tonofilaments from the skeins converge upon the hemidesmosomes along the basal lamina (Figs. 8, 9). The skeins are separated from the cell membrane in other regions by a zone of peripheral cytoplasm (Fig. 12). Tonofilaments are also present in the peripheral cytoplasm but are dispersed and do not follow parallel courses as in the skeins (Fig. 12). The skeins partially surround an irregularly-shaped, multilobed nucleus and a zone of perinuclear cytoplasm (Fig. 12). Most of the cytoplasmic organelles, including mitochondria and rough endoplasmic reticulum, are concentrated in the perinuclear cytoplasm. Glycogen is frequently present in all regions of the cytoplasm (Fig. 12).

FC cells of intermediate layers are highly variable in shape (Fig. 16). The configuration of the cell membrane is determined by the adjacent cell type. Borders between FC cells are irregular with extensive intercellular interdigitations. There are also regions with numerous desmosomes (Figs. 16, 17). Borders between FC cells and mucous cells are concave-convex, conforming to the oval shape of the mucous cells (Fig. 13). Occasional interdigitations (Fig. 13) and desmosomal attachments occur. Club cell – FC cell borders conform to the oval-shaped club cell (Fig. 22). Frequent FC cell cytoplasmic extensions indent the club cell membrane (Figs. 15, 22). Desmosomes are rare (Fig. 26). Club cells and mucous cells are surrounded by FC cells and do not contact one another. In regions of contact between FC cells and lymphocytes, no interdigitations or desmosomes are present (Fig. 10). Contact between FC cells and chloride cells resembles the FC cell-FC cell

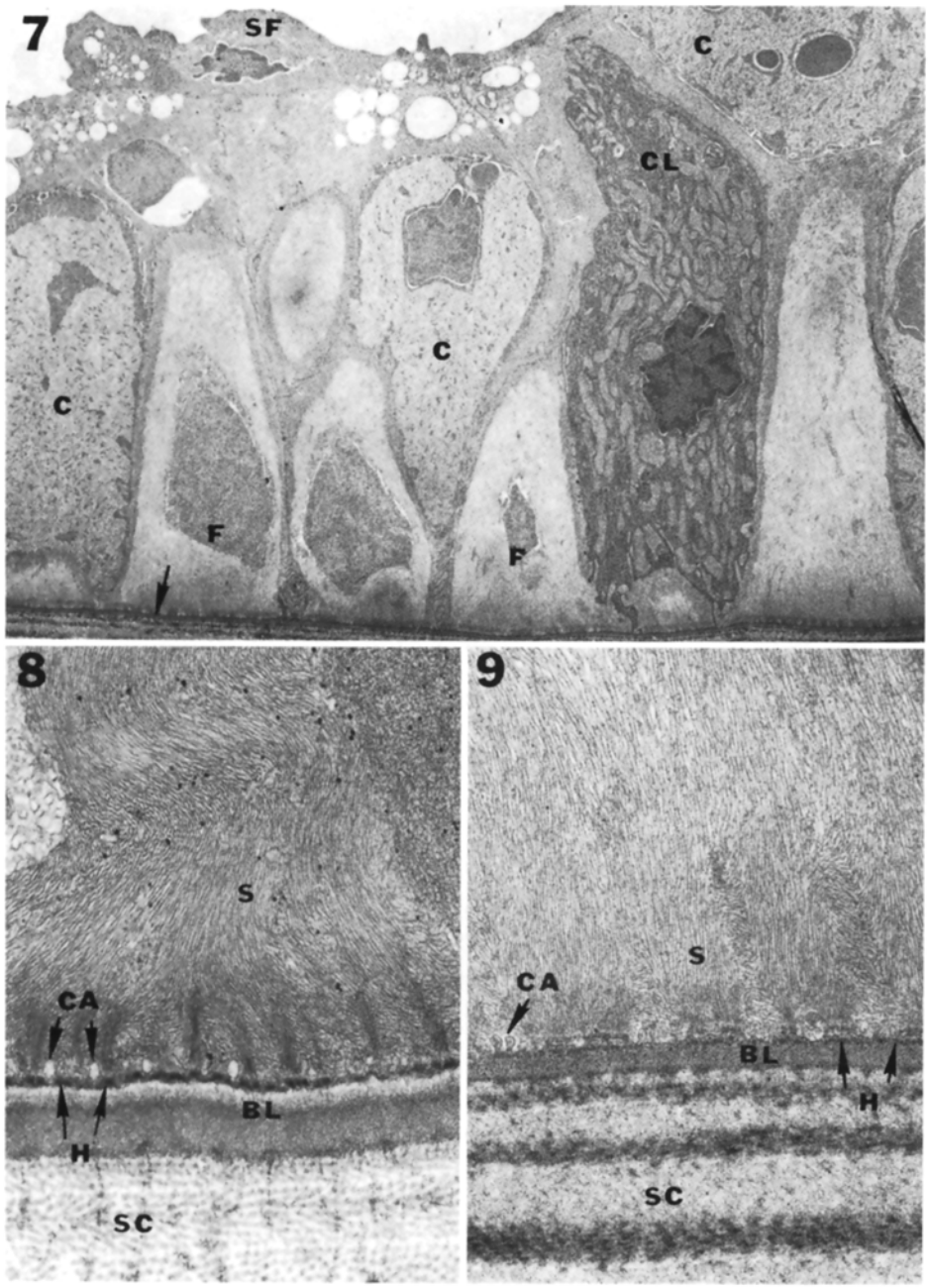


Fig. 7. Epidermis of the leptocephalus. Basal lamina (arrow), chloride cell (*CL*), club cell (*C*), FC cell (*F*), surface FC cell (*SF*). ×3,800

Fig. 8. Hemidesmosomes on the basal lamina of the glass eel. Basal lamina (*BL*), caveola (*CA*), hemidesmosome (*H*), skein (*S*), stratum compactum (*SC*). ×28,300

Fig. 9. Basal epidermal region of the leptocephalus. Basal lamina (*BL*), caveloa (*CA*), hemidesmosome (*H*), skein (*S*), stratum compactum (*SC*). ×26,500

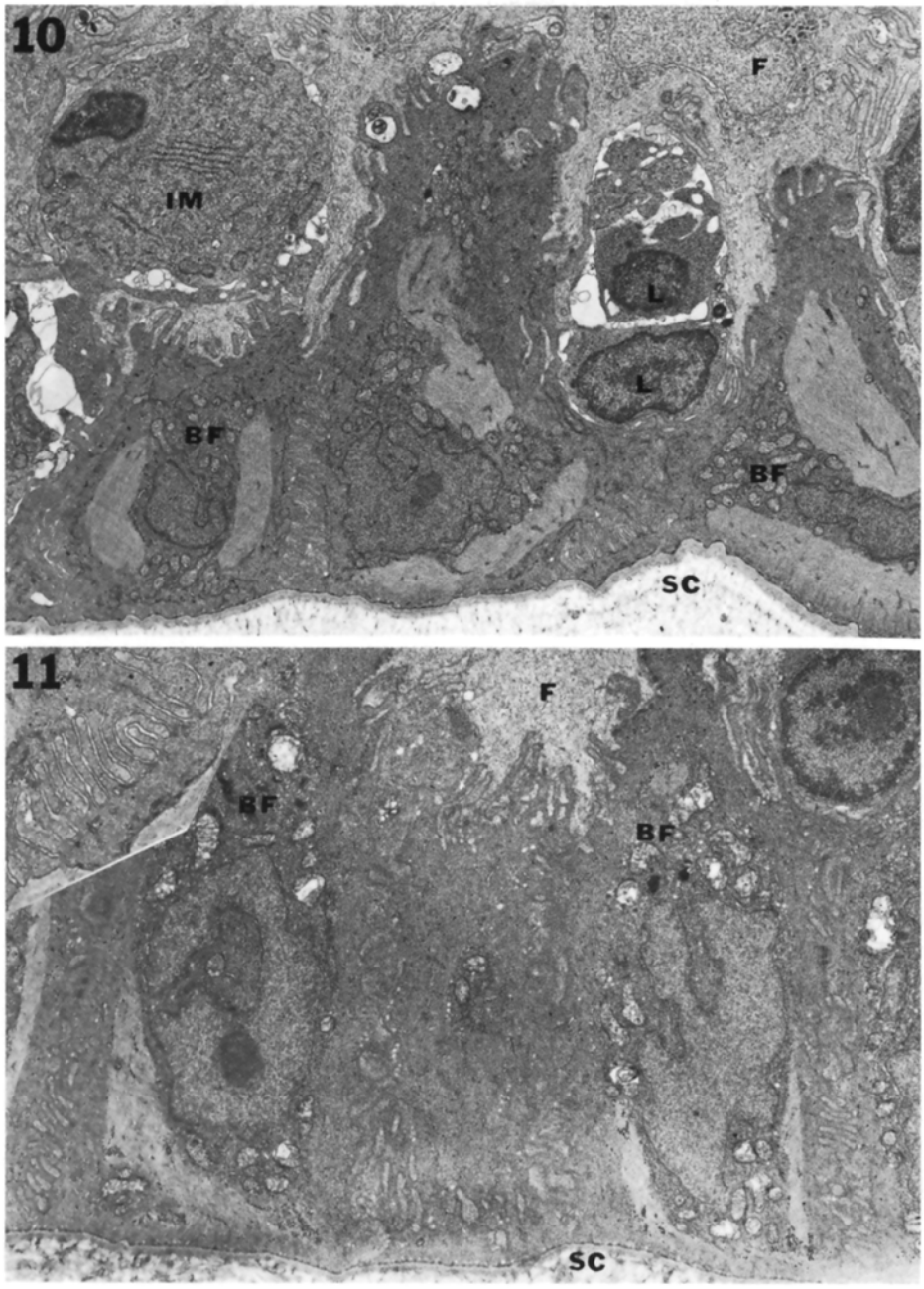


Fig. 10. Basal epidermal region of the pigmented elver. Basal FC cell (*BF*), FC cell (*F*), immature mucous cell (*IM*), lymphocyte (*L*), stratum compactum (*SC*). ×7,200

Fig. 11. Basal epidermal region of the adult eel. Basal FC cell (*BF*), FC cell (*F*), stratum compactum (*SC*). ×7,200. Inset: Region of intercellular interdigitation between basal FC cells of the pigmented elver. ×24,000

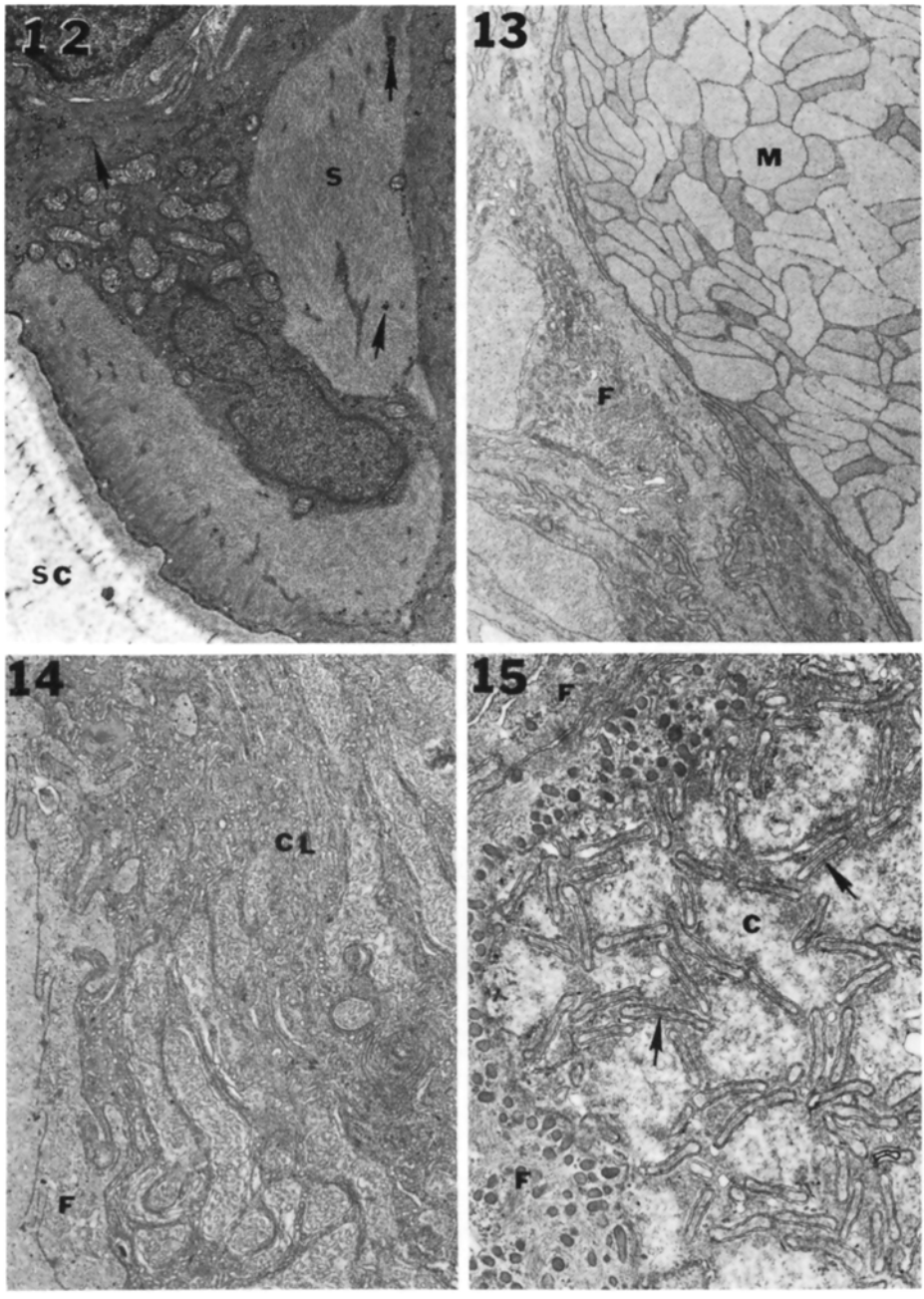


Fig. 12. Basal FC cell of the pigmented elver. Glycogen deposits (arrow), skein (*S*), stratum compactum (*SC*). ×12,700

Fig. 13. Mucous cell-FC cell boundary in the adult. FC cell (*F*), mucous cell (*M*). ×7,600

Fig. 14. Chloride cell-FC cell boundary in the leptocephalus Chloride cell (*CL*), FC cell (*F*). × 13,800

Fig. 15. Tangential section through the club cell periphery of the adult. Cytoplasmic extensions (arrows) of FC cells indenting the club cell cytoplasm are seen in transverse section. Club cell (*C*), FC cell (*F*). ×15,900

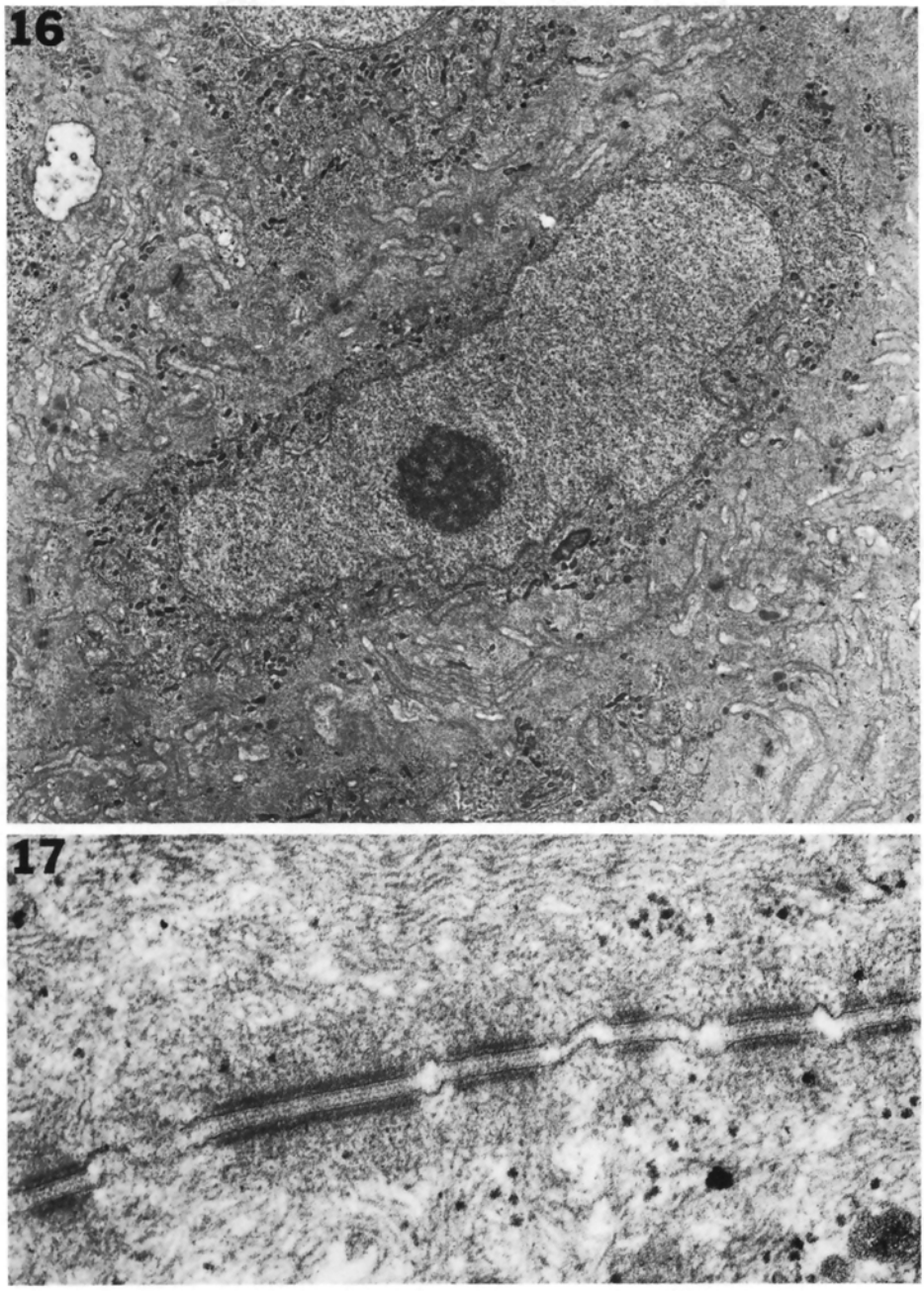


Fig. 16. FC cell of the intermediate epidermis of the adult eel. Concentric regions of peripheral and perinuclear cytoplasm surround the nucleus. The cell boundary is highly irregular with numerous intercellular interdigitations and desmosomes. ×9,600

Fig. 17. Desmosomes at the boundary between two FC cells of the intermediate epidermal layers of the adult eel. ×84,800

pattern with regions of intercellular interdigitation occurring frequently along the FC cell-chloride cell border (Fig. 14). Melanocytes have a regular border (Fig. 5) without interdigitations, cytoplasmic extensions, or desmosomes.

The peripheral cytoplasm of intermediate FC cells contains tonofilaments, numerous free ribosomes and occasional electron dense vesicles which are not apparent in the basal FC cells (Fig. 16). The tonofilaments show no particular orientation in the cytoplasm, but converge on regions of the cell membrane where desmosomes are present (Fig. 17). Organelles are concentrated in the central cytoplasm (Fig. 16). Mitochondria, rough endoplasmic reticulum, Golgi profiles, and free ribosomes are present. Frequently, concentrations of electron dense vesicles similar to those in the peripheral cytoplasm are present. The nucleus is variable in shape, conforming to the shape of the cell.

FC cells of the superficial epidermal layer of the glass eel, pigmented elver, and adult are squamous or cuboidal (Figs. 2, 3, 20 and 21). Surface FC cells of the leptocephalus are squamous (Fig. 7). Surface FC cells rarely showed evidence of sloughing except in the leptocephalus. The cell membrane configurations of the surface FC cells are identical to those described for the FC cells of intermediate layers (Figs. 20, 21). The free border of the cell is folded into microridges seen in section (Fig. 21). Microridges are less frequent or absent in the leptocephalus (Fig. 7). The surface mucous coat is largely removed by histological preparation, but remnants are present in some sections. The cytoplasmic contents of intact surface FC cells are similar to those described for FC cells of intermediate layers. Frequently a zone of rough endoplasmic reticulum surrounds the nucleus (Fig. 21). Golgi profiles are common and mitochondria infrequent. Cytoplasmic vesicles similar to those observed in the cytoplasm of intermediate FC cells are abundant in all stages but the leptocephalus.

Mucous Cells. Mucous cells in various stages of differentiation are present at all levels of the epidermis except within the basal cell layer. The most basal mucous cells, located between the apical ends of the basal stratum of FC cells in the glass eel and pigmented elver and in the overlying layers in the leptocephalus and adult, possess only a few membrane-bounded packets of mucin (Fig. 18). Rough endoplasmic reticulum and Golgi profiles are relatively undeveloped in these cells. Mucous cells of intermediate levels, in addition to the presence of considerable accumulations of mucin, have extensively developed rough endoplasmic reticulum and Golgi profiles (Fig. 19). In mucous cells at the surface accumulation of mucin packets is so great that the nucleus and cytoplasmic organelles have become restricted to a small basal area of the cell (Figs. 20, 35). This small area of cytoplasm contains extensive dilated rough endoplasmic reticulum and Golgi profiles and vesicles. The nucleus, with its prominent nucleolus, is surrounded by a zone of rough endoplasmic reticulum (Fig. 20). The mucin packets in the basal region of the cell are closely associated with Golgi profiles and vesicles (Fig. 20). Mucin packets in the apical half of the cell are clustered in a compact mass surrounded by a thin rim of cytoplasm. Membranes bounding the individual mucin packets are often incomplete and the packets appear to coalesce. Mucin is expelled from the apical end of the cell at the free surface of the epidermis (Fig. 20).

Club Cells. Club cells are large, oval cells restricted to the intermediate layers of the epidermis except in the leptocephalus where they reach the basal lamina. The

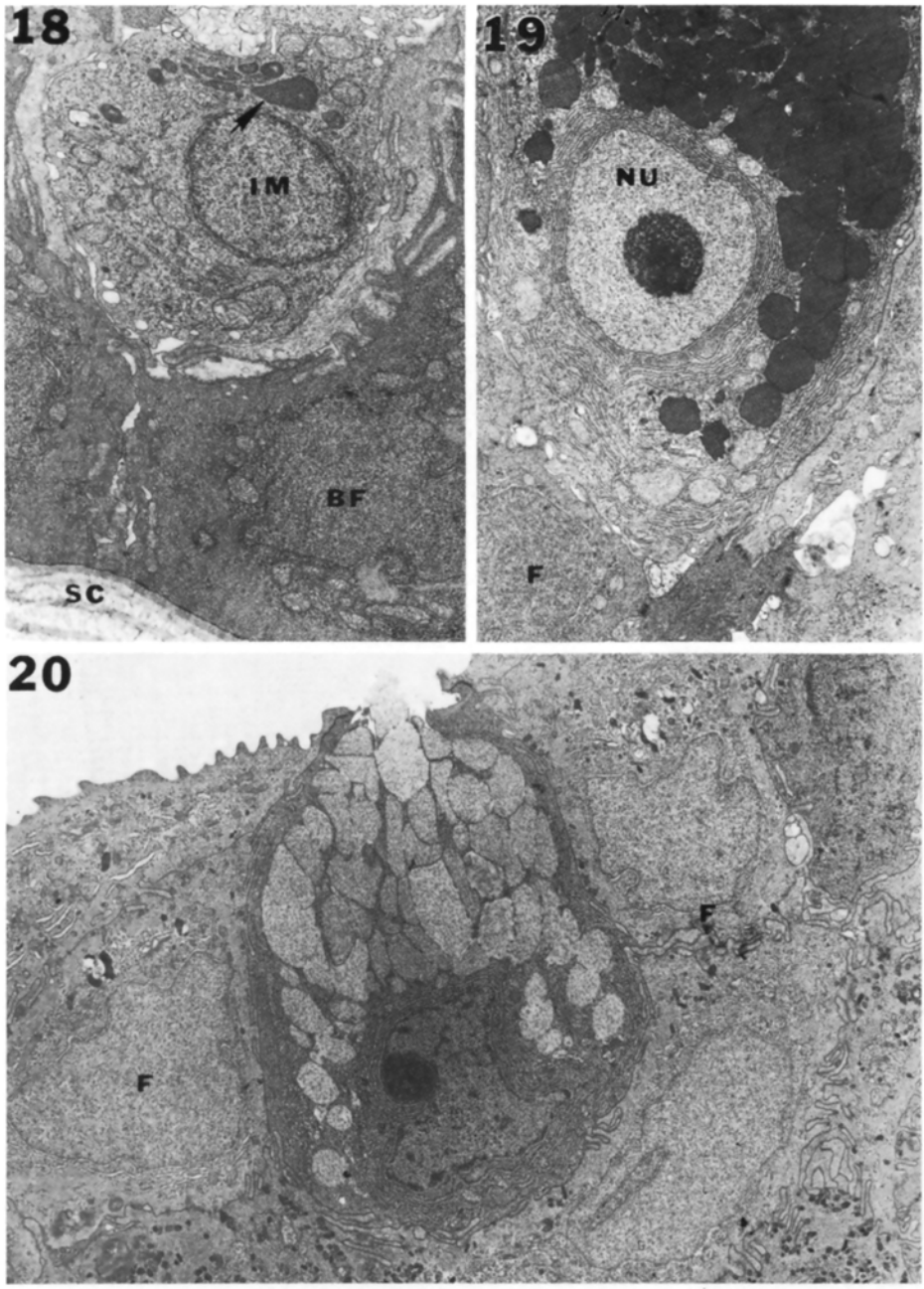


Fig. 18. Immature mucous cell of the pigmented elver. Basal FC cell (*BF*), immature mucous cell (*IM*), mucin packets (arrows), stratum compactum (*SC*). ×9,700

Fig. 19. Nucleus and surrounding cytoplasm and mucin packets of a mucous cell in the intermediate epidermis of the glass eel. A zone of rough endoplasmic reticulum surrounds the nucleus. FC cell (*F*), nucleus (*NU*). ×5,600

Fig. 20. Mucous cell of the adult at the free epidermal surface. A zone of rough endoplasmic reticulum surrounds the nucleus. Individual mucin packets associated with Golgi profiles are present in the basal region of the cell. Apical mucin packets show partial coalescence. FC cell (*F*). ×6,600

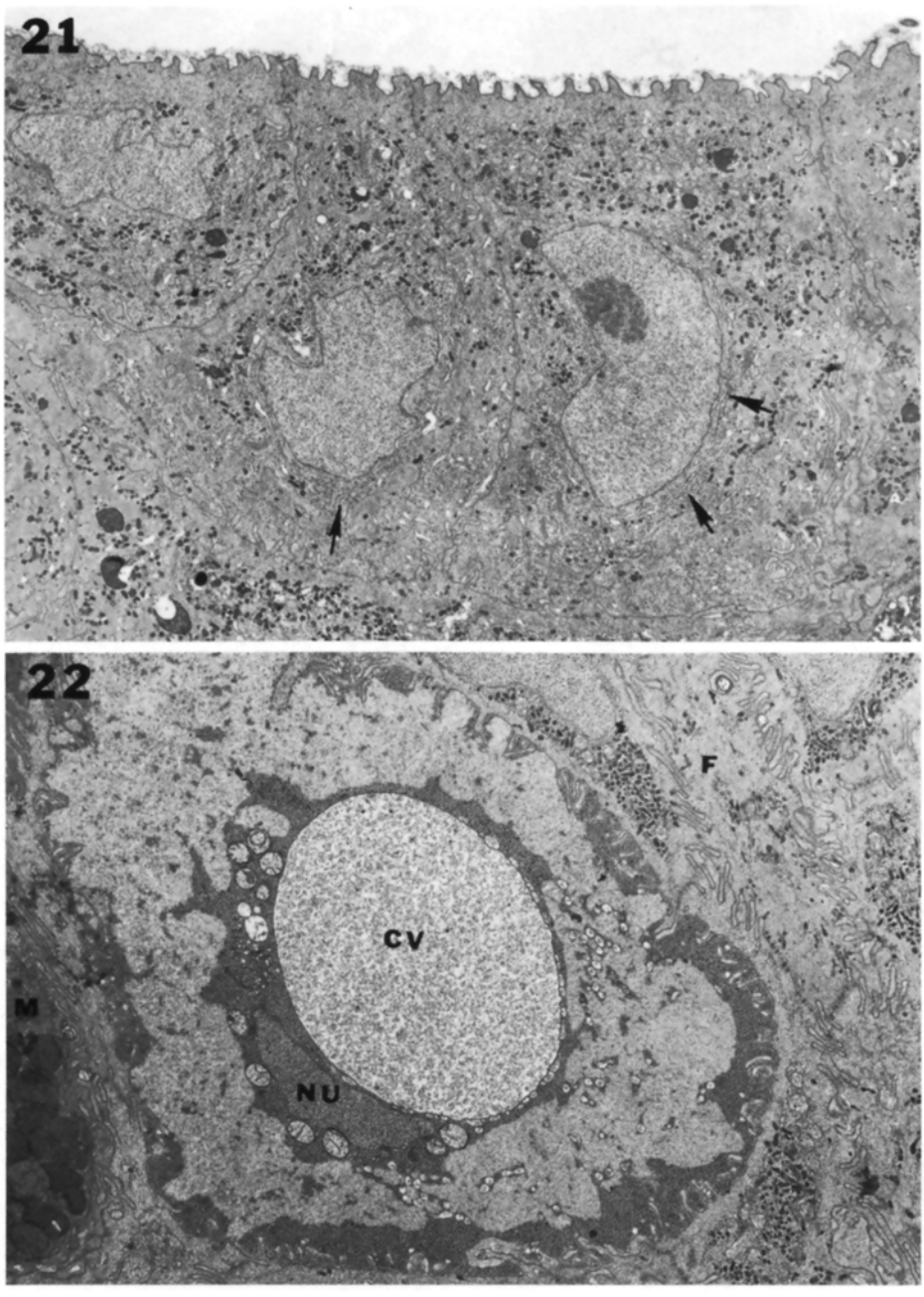


Fig. 21. Cuboidal FC cell of the adult at the epidermal surface. A zone of rough endoplasmic reticulum (arrows) surrounds the nucleus of the cell. ×6,000

Fig. 22. Club cell of the pigmented elver. Central vacuole (*CV*), FC cell (*F*), mucous cell (*M*), nucleus (*NU*). ×6,000

appearance of the club cell boundary has been described above. The appearance of the club cell as a whole is highly variable from stage to stage and within the epidermis of a single stage (Figs. 22, 26, 27, 28).

Certain features are common to all stages. The peripheral region of the cell in all stages contains electron-lucent and electron-dense cytoplasm distributed in a variety of patterns (Figs. 22, 26, 27, 28). Free ribosomes and vesicles are present throughout. The electron-lucent cytoplasm, which occupies the majority of the peripheral region, contains arrays of small circular or oval structures (Figs. 24, 25). In some regions incomplete circles or U-shaped figures are present. Short sections of tubules appear to comprise subunits with a width equal to the diameter of the circular figures (370 Å). The circular figures may be arranged in hexagonal arrays with each circle or U-shaped figure surrounded by six others.

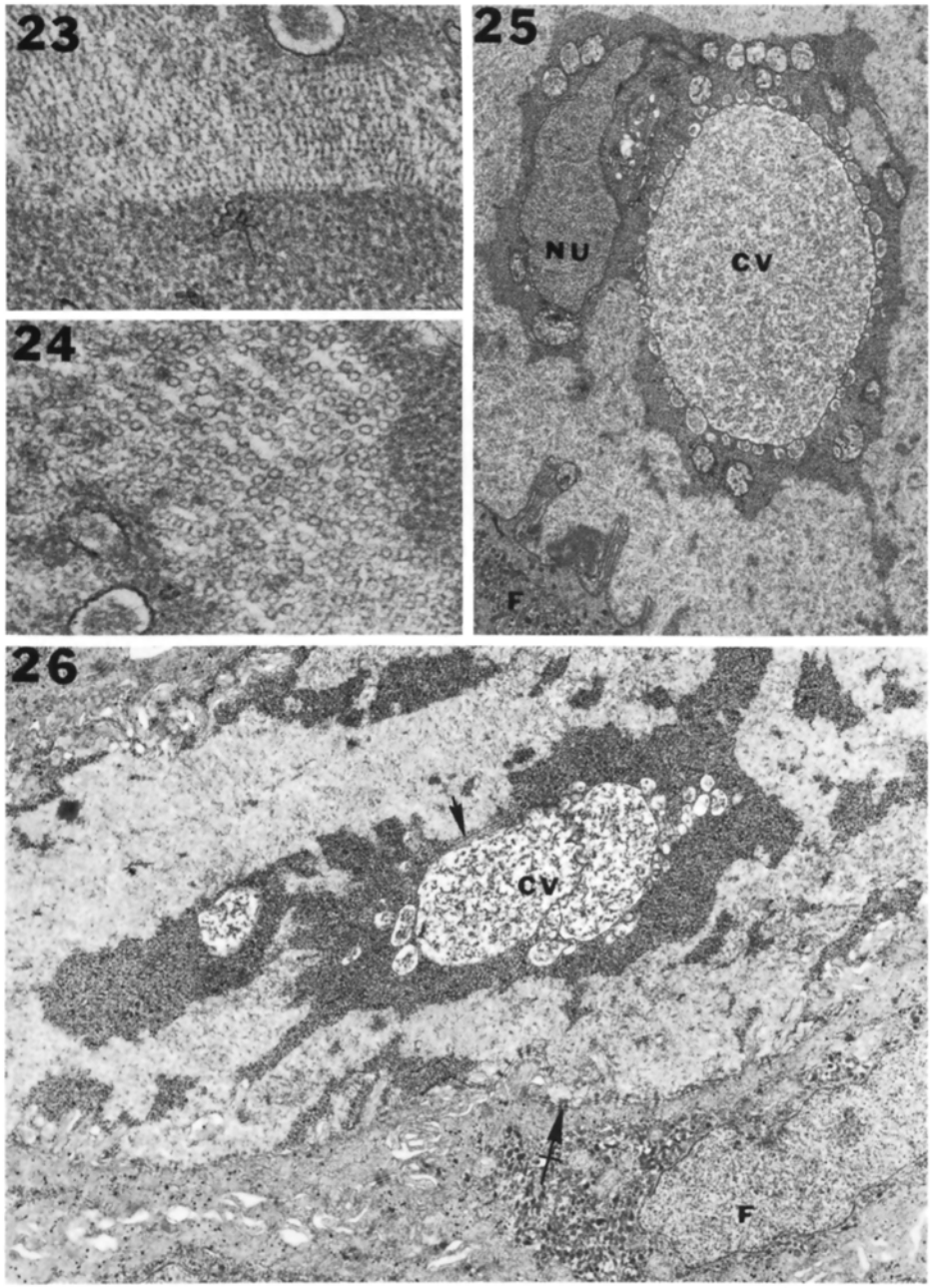
In the central region a zone of fine granular cytoplasm surrounds a large central vacuole and the nucleus (Fig. 25). A few mitochondria, Golgi profiles, and microtubules may be present (Figs. 25, 26). In the leptocephalus a single large vacuole is not usually present and several smaller vacuoles are located in this region and throughout the cytoplasm (Fig. 28). In the glass eel, pigmented elver, and adult, the central vacuole contains a flocculent, darkly staining material which is condensed in some cells (Fig. 26). A ring of small membrane-bounded vesicles surrounds the central vacuole (Fig. 25). These small vesicles are also observed in other regions of the cytoplasm (Fig. 22). The nucleus is smaller than, and displaced from the center of the cell by, the central vacuole and frequently has a concave border on the side adjacent to it (Figs. 22, 25). The nucleus of the leptocephalus club cell, however, is centrally located and larger than any of the adjacent vacuoles (Fig. 28).

Lymphocytes. Typical lymphocytes were most commonly observed in the epidermis of the glass eel and pigmented elver, less frequently observed in the epidermis of adults, and not observed in the leptocephalus. Lymphocytes are restricted to basal regions of the epidermis (Figs. 2, 3, 10). A cluster of two or three cells is located between the apical ends of the basal layer of FC cells in the glass eel and pigmented elver (Fig. 10). Lymphocytes are usually surrounded by intercellular space; junctional complexes or interdigitations are not present in areas of contact between lymphocytes and adjacent cells.

Chloride Cells and Melanocytes. Chloride cells were frequently observed in the leptocephalus and rarely in the glass eel. They were not observed in the pigmented elver or adult. The chloride cell boundary has been described above. In the leptocephalus chloride cells were observed primarily in the intermediate and surface layers, although the slender, tapering basal portion of the cell may extend to the basal lamina. In the glass eel chloride cells were observed only in the intermediate layers. The cytoplasm of the chloride cell is entirely occupied by mitochondria, a vesicular endoplasmic reticulum, and numerous small vesicles (Figs. 7, 29). A few Golgi profiles and ribosomes are present. The nucleus is centrally located, irregularly shaped and densely staining (Fig. 7).

Melanocytes are occasionally present in the epidermis of the pigmented elver and adult. Melanocytes are oval with a regular border. The cytoplasmic contents are similar to those described for the melanocytes of the dermis (Figs. 5, 39, 41).

Basal Lamina. The epidermis is separated from the collagenous lamellae of the stratum compactum by a basal lamina. The average thicknesses of the basal laminae



Figs. 23 and 24. Intermediate cytoplasmic regions of the pigmented elver club cell. The electron lucent cytoplasm contains arrays of circular figures (Fig. 24) and U-shaped figures (Fig. 23). ×48,400

Fig. 25. Pigmented elver club cell. The central region of the cell contains a large central vacuole (*CV*) surrounded by a ring of small vesicles and the nucleus (*NU*). FC cell (*F*). ×7,800

Fig. 26. Club cell of the adult eel. Central vacuole (*CV*), desmosomes (barred arrow), FC cell (*F*), microtubules (arrows). ×9,600

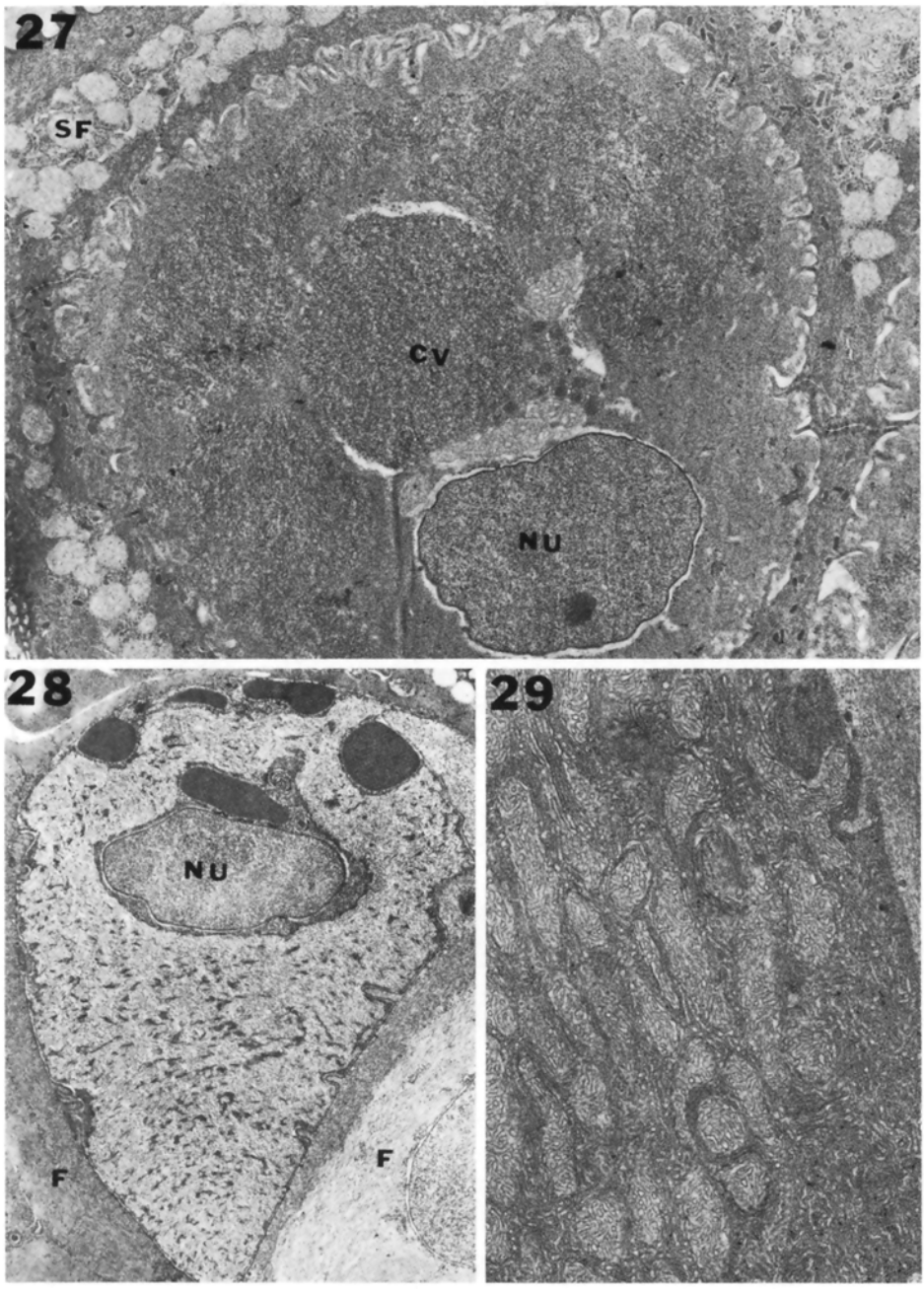


Fig. 27. Club cell of the glass eel. Central vacuole (*CV*), nucleus (*NU*), surface FC cell (*SF*). ×9,000

Fig. 28. Club cell of the leptocephalus. The large central vacuole characteristic of other stages is absent. FC cell (*F*), nucleus (*NU*). ×7,200

Fig. 29. Cytoplasm of a leptocephalus chloride cell. The cytoplasm contains numerous mitochondria and extensive vesicular endoplasmic reticulum. ×16,800

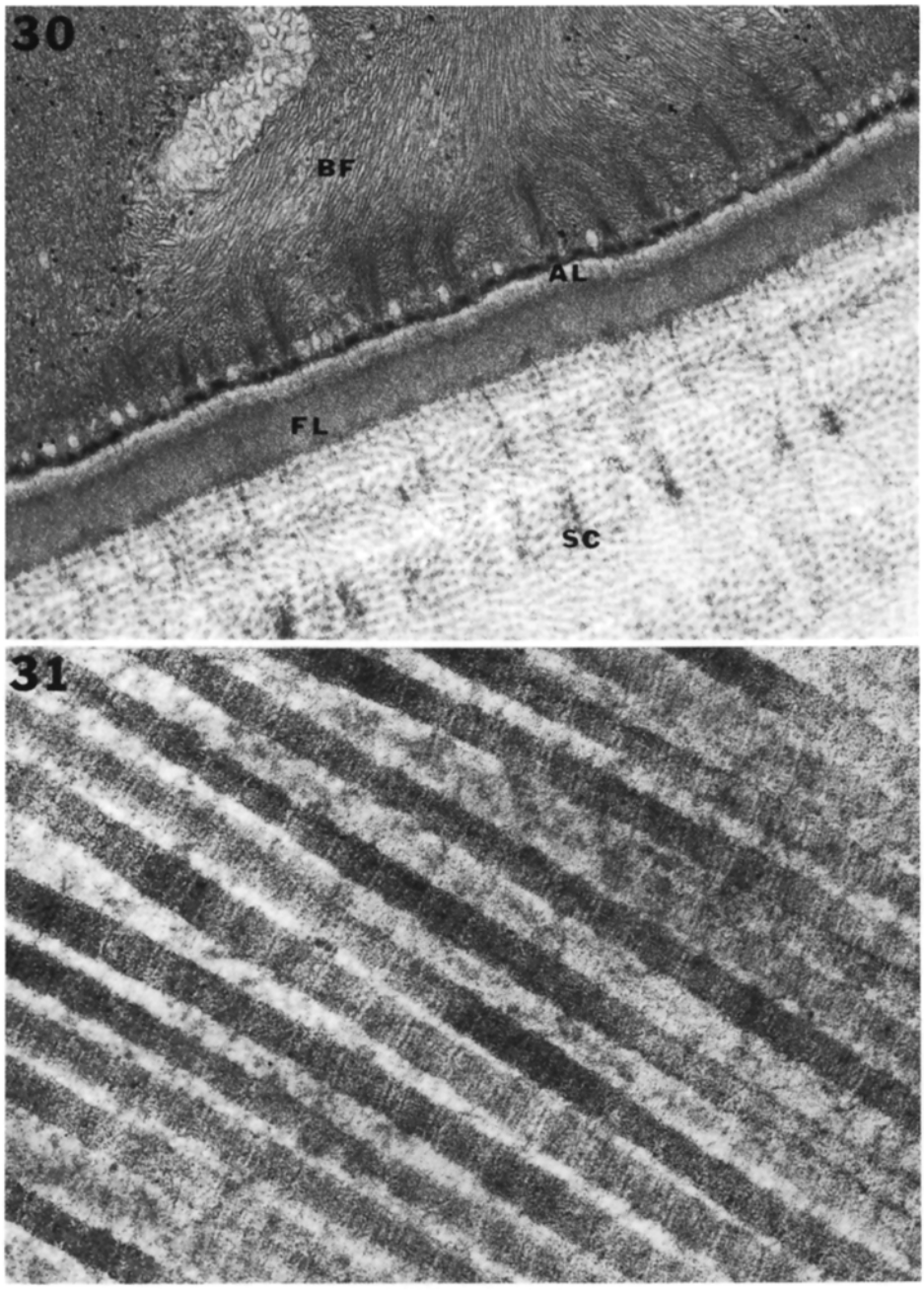


Fig. 30. Basal lamina of the glass eel. Adepidermal layer (*AL*), basal FC cell (*BF*), flocculent layer (*FL*), stratum compactum (*SC*). ×33,600

Fig. 31. Collagen fibers in the adult stratum compactum. ×154,400

of the four stages are: leptocephalus, 1500 Å; glass eel, 3500 Å; pigmented elver, 3000 Å; adult, 3200 Å. The basal lamina comprises two layers: a layer of electron-dense flocculent material and an adepidermal layer (Fig. 30). The adepidermal layer contains fibrillar elements which traverse it from the flocculent layer to the regions of hemidesmosomes on the basal epidermal cell membrane (Fig. 30). Small indentations (caveolae) of the basal epidermal cell membrane frequently occur (Figs. 8, 9, 30). The caveolae are continuous with the adepidermal layer and alternate with regions of hemidesmosomes along the basal epidermal cell membrane.

Organization of the Dermis. The dermis of the eel consists of a layer of collagenous lamellae (stratum compactum) overlying a layer of loose connective tissue (stratum spongiosum) (Figs. 1, 2, 3, 5, 32). Scales are present in the dermis of the adult (Fig. 5). The dermis is bounded by the basal lamina peripherally and the subdermal muscle centrally. In the leptocephalus and glass eel, the two strata of the dermis are separated by a layer of fibroblasts (Figs. 32, 33). The stratum compactum exhibits a trend of increasing thickness due to an increase in the number of lamellae and an increase in thickness of individual lamellae (leptocephalus, 17.2 μ; glass eel, 15.7 μ; pigmented elver, 28.7 μ; and adult, 480 μ). The stratum spongiosum is highly variable in regional thickness at each stage.

Stratum compactum. The collagenous lamellae of the stratum compactum parallel the basal lamina. Thirteen to 16 lamellae are present in the leptocephalus; 19 to 21 in the glass eel; 23 to 26 in the pigmented elver; and 28 to 33 in the adult. Lamellae closer to the basal lamina are thinner (Fig. 33). The collagen fibers within a lamella parallel one another and are oriented at an oblique angle to the longitudinal axis of the fish and to the fibers of adjacent lamellae (Fig. 34). The individual collagen fibers comprise a series of repeating subunits with a major period of 485 Å in all stages (Fig. 31). Bundles of collagen fibers, oriented normal to the lamellae and intersecting several layers, are occasionally present in the glass eel and frequently present in the pigmented elver and adult (Figs. 36, 37). They were not observed in the leptocephalus. Fibers from these transverse bundles appear to merge with fibers of the lamellae in some sections (Fig. 36). The fiber bundles also terminate on folds of the basal lamina (Fig. 36).

In the leptocephalus and glass eel a layer of fibroblasts forms the lower boundary of the stratum compactum (Figs. 32, 33). In the pigmented elver and adult this layer is not present although individual fibroblasts are frequently found at the interface of the stratum compactum and the stratum spongiosum. No fibroblasts were observed within the collagenous lamellae of the stratum compactum of the leptocephalus (Fig. 32). In the glass eel a few fibroblasts are present in the lower lamellae (Fig. 33) and in the pigmented elver and adult they are abundant at all levels (Figs. 37, 39). Fibroblasts are squamous often tapering to slender flattened cytoplasmic processes which may extend for considerable distances (Fig. 37). The fibroblast cytoplasm contains rough endoplasmic reticulum, mitochondria and Golgi profiles. Vesicles with fibrillar and granular contents are abundant (Fig. 38).

Scales, which do not overlap, occur only in the adult. Each scale is surrounded by a small pocket of loose connective tissue located between the uppermost lamella of the stratum compactum and the basal lamina (Fig. 5). The loose connective tissue surrounding a scale is not continuous with that which surrounds adjacent scales. Melanocytes are similar to those found in the stratum spongiosum (described

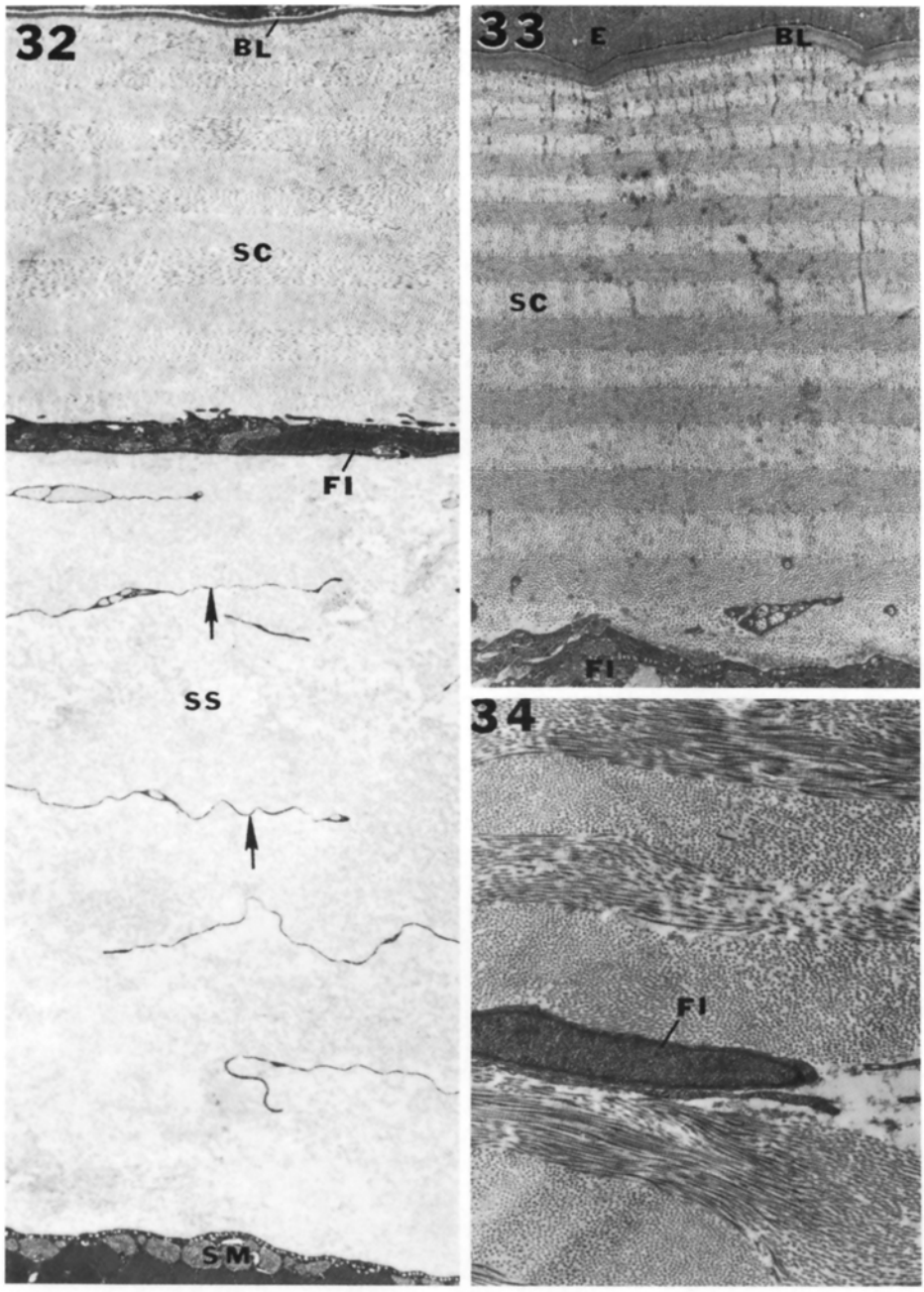


Fig. 32. Dermis of the leptocephalus. The stratum compactum and stratum spongiosum are separated by a layer of fibroblasts. Basal lamina (*BL*), fibroblasts (*FI*), flattened cytoplasmic extensions of fibroblasts (arrows), stratum compactum (*SC*), subdermal muscle (*SM*), stratum spongiosum (*SS*). ×5,500

Fig. 33. Stratum compactum of the glass eel. Basal lamina (*BL*), epidermis (*E*), fibroblasts (*FI*), stratum compactum (*SC*). ×7,200

Fig. 34. Collagenous lamellae of the stratum compactum of the adult. Fibroblast (*FI*). ×9,600

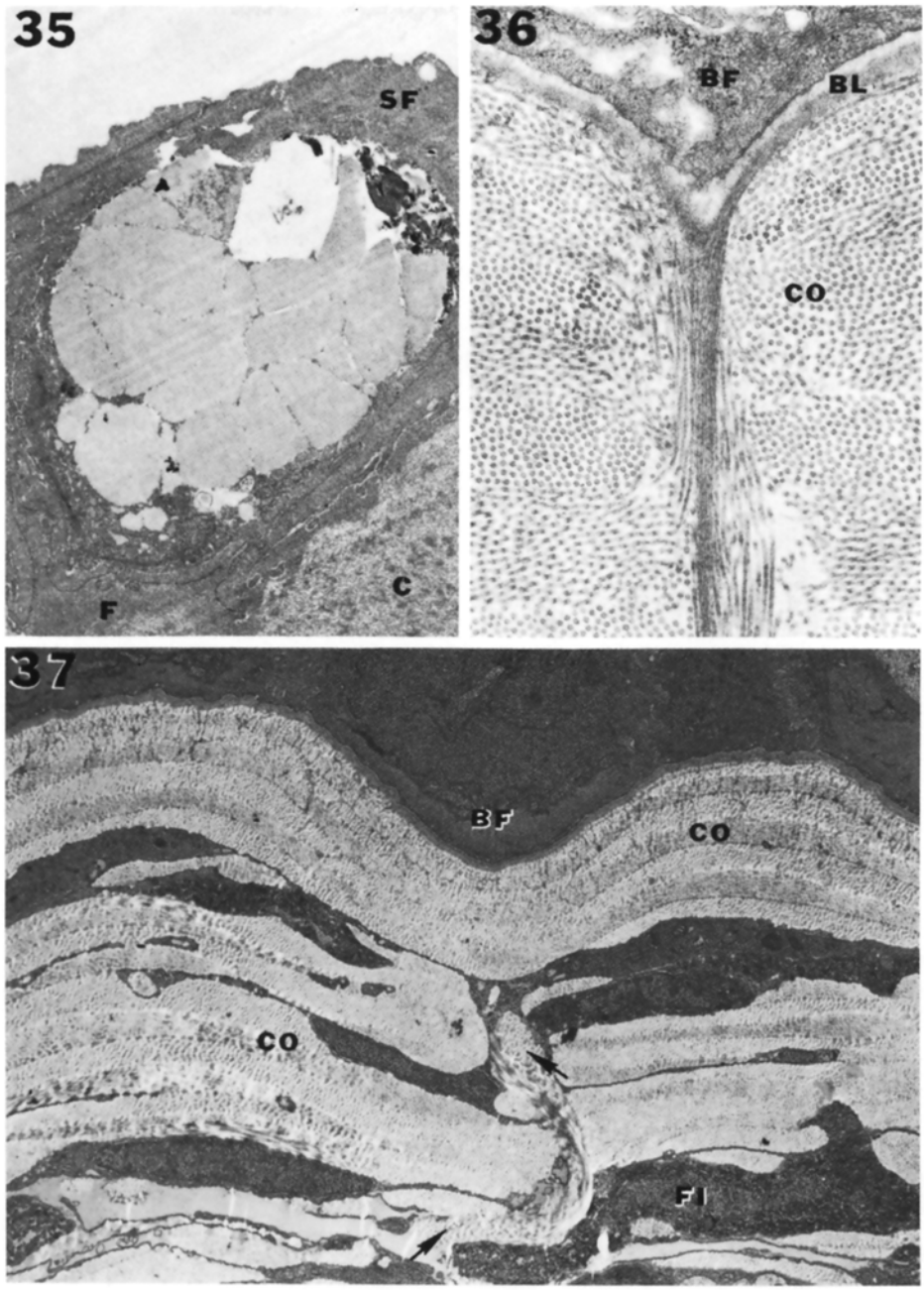


Fig. 35. Mucous cell in leptocephalus epidermis. Club cell (*C*), FC cell (*F*), surface FC cell (*SF*). ×8,100

Fig. 36. Termination of collagen fibers from a transverse bundle on a fold of the basal lamina in the pigmented elver. Basal lamina (*BL*), basal FC cell (*BF*), collagenous lamellae (*CO*). ×25,800

Fig. 37. Stratum compactum of the pigmented elver. Fibroblasts are interposed between the collagenous lamellae at all levels. Fibers of the transverse bundle turn 90° to align with fibers of collagenous lamellae in two regions (arrows). Basal FC cells (*BF*), collagenous lamellae (*CO*), fibroblast (*FI*). ×6,800

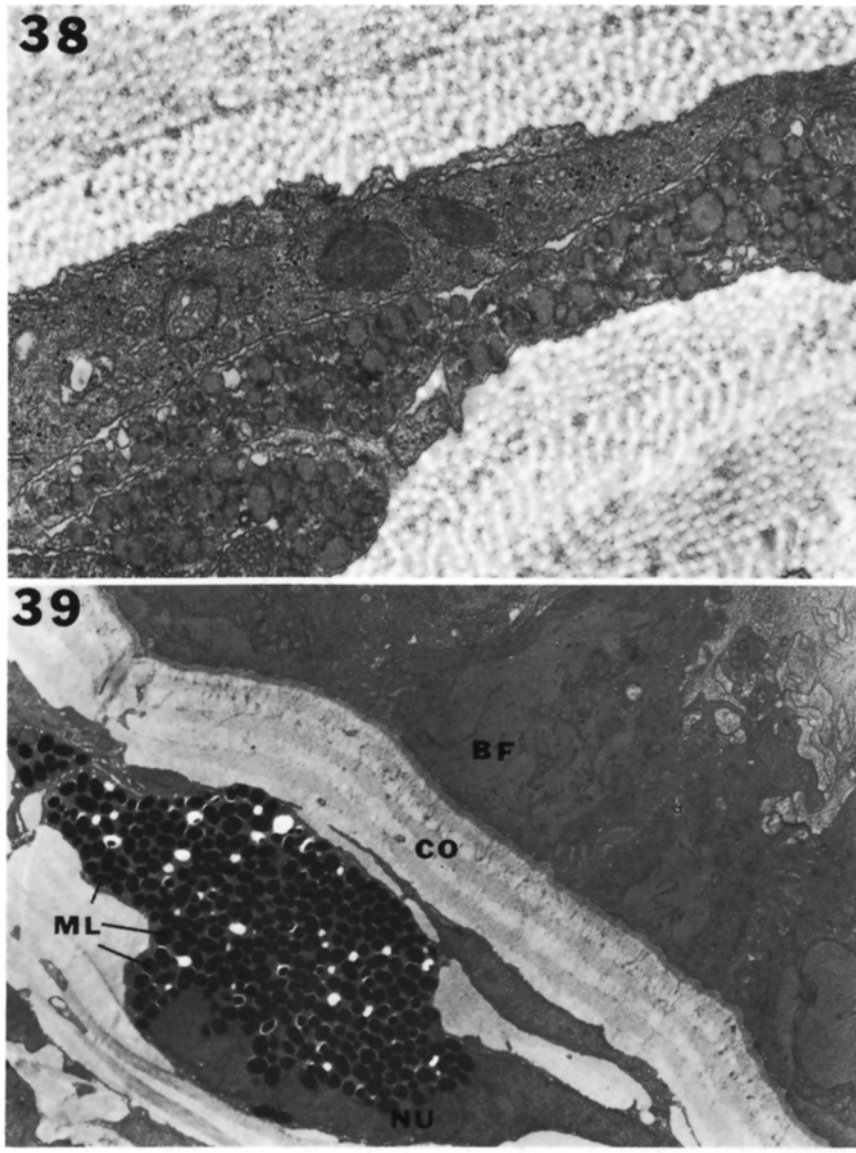


Fig. 38. Cytoplasm of fibroblasts in the pigmented elver. Large vesicles with fibrillar contents and smaller vesicles with granular contents are present. ×27,600

Fig. 39. Melanocyte of a pigmented elver. Basal FC cell (*BF*), collagenous lamellae (*CO*), melanosome (*ML*), nucleus (*NU*). ×5,600

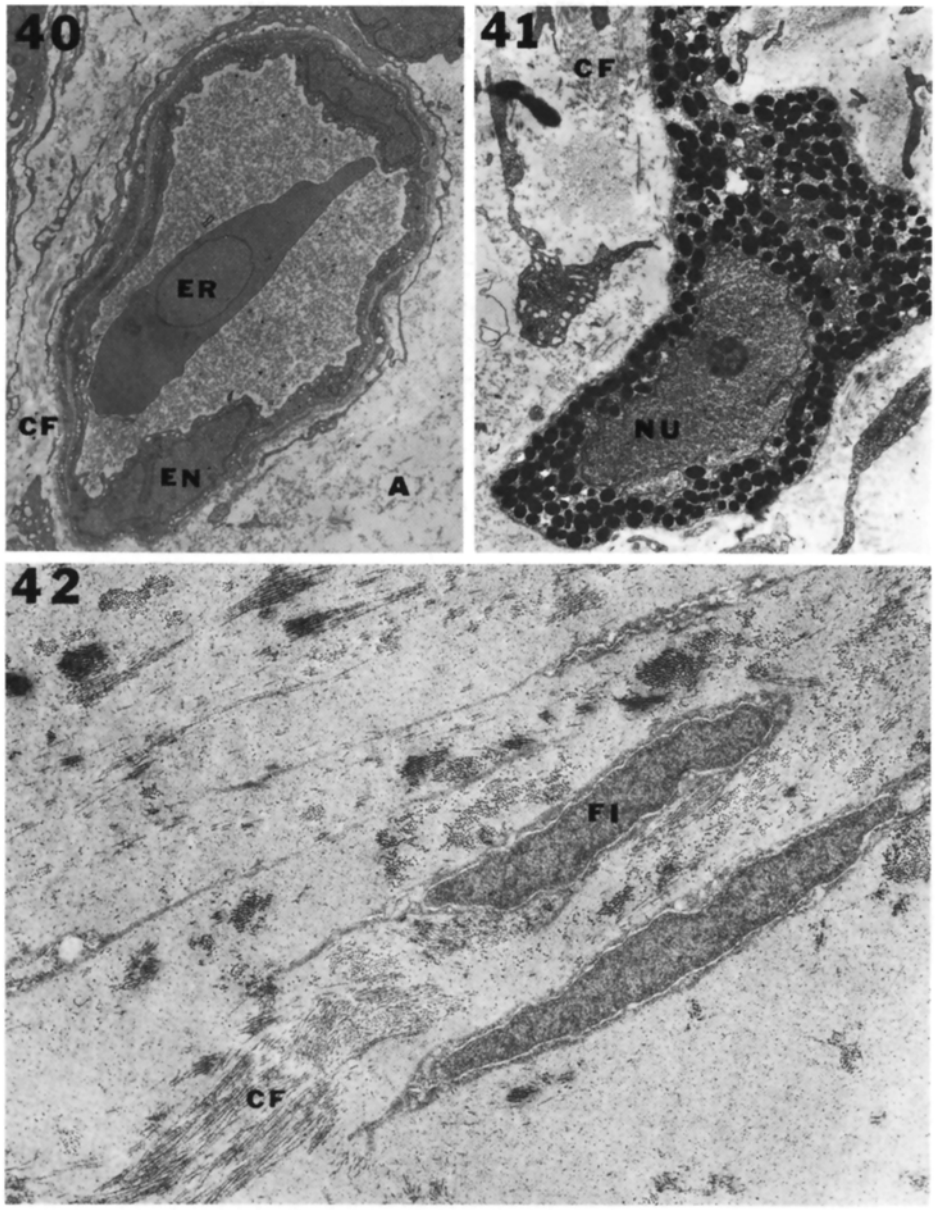


Fig. 40. Capillary in the stratum spongiosum of the pigmented elver. Amorphous matrix (*A*), collagen fibers (*CF*), endothelial cell (*EN*), erythrocyte (*ER*). ×6,600

Fig. 41. Melanocyte in the stratum spongiosum of the adult. Collagen fibers (*CF*), nucleus (*NU*). ×5,400

Fig. 42. Stratum spongiosum of the glass eel. Collagen fibers (*CF*), fibroblast (*FI*). ×9,000

below) (Fig. 39). Capillaries are present in the stratum compactum, often in association with clusters of fibroblasts.

Stratum spongiosum. The stratum spongiosum is a layer of loose connective tissue bounded by the stratum compactum and subdermal muscle (Figs. 1, 2, 3, 32). Loose connective tissue of the stratum spongiosum merges with loose connective tissue associated with the muscle and no distinct boundary is present. The stratum spongiosum contains fibroblasts, melanocytes, collagen fibers and blood vessels embedded in an amorphous matrix (Figs. 40, 41, 42). Collagen fibers may be present in bundles or as individual fibers (Fig. 42), but are not organized into lamellae and show no particular orientation within the matrix. Melanocytes are present in the pigmented elver and adult (Fig. 41). These cells are irregular in shape unlike melanocytes in the epidermis. Melanocytes are usually isolated from adjacent cells by collagen fibers and the amorphous matrix. The cytoplasm of melanocytes contains numerous electron-opaque melanosomes. A few mitochondria with sparse cristae, fibrillar elements, microtubules and Golgi profiles are present. The melanocyte nucleus is large and irregular in shape.

Discussion

Histology and Ultrastructure. The morphology and ultrastructure of the integument of the four life stages reported here conforms well to other reports of larval and adult teleost integument. In particular the adult stage is in close agreement with Henrikson and Matoltsy's (1968a, b, c) description of the same stage. Numerous parallels with the integument of larval amphibians can be drawn.

The free surfaces of the superficial FC cells of the glass eel, pigmented elver, adult and to a lesser extent the leptocephalus, are folded into microridges similar to those reported for *Lebistes reticulatus* (Bereiter-Hahn, 1971), *Oncorhyncus kisutch* (Hawkes, 1974a), *Esox americanus* (Merrilees, 1974), and in the gill epithelium of *Salmo gairdneri* (Olson and Fromm, 1973). Microvilli (which may be similar microridges in section) are present at the free edge of larval anuran epidermis (Chapman and Dawson, 1961) and larval newt epidermis (Kelly, 1966a). Microridges probably aid in retaining mucous secretions (Olson and Fromm, 1973) or may be associated with the mechanism of wound closure (Bereiter-Hahn, 1971).

FC cells in the epidermis of all stages examined showed no evidence of the progressive degeneration and keratinization which occurs in the epidermis of higher vertebrates. This is typical of teleost epidermis (Parakkal and Alexander, 1972). Henrikson (1967) reported that cells at all levels of teleost epidermis remain mitotically active as evidenced by their ability to incorporate tritiated thymidine. Surface FC cells in the eel were very rarely in the process of sloughing or degenerating.

FC cells are characterized by the presence of 80 Å diameter cytoplasmic tonofilaments. This diameter is close to values reported for other teleosts (e.g., 70 Å, Henrikson and Matoltsy, 1968a; 80 Å, Flaxmann, 1972). FC cell boundaries are highly irregular, with regions of extensive intercellular interdigitation and numerous desmosomes. These specializations for intercellular attachment plus the abundant cytoplasmic tonofilaments suggest that the FC cell is the principal structural cell type of the epidermis. In the lamprey a single cell type, the mucous cell containing

tonofilaments, subserves both the functions of structural support and mucous secretion (Downing and Novales, 1971). In the Osteichthyes the two functions are performed by distinct cell types. However, the well-developed rough endoplasmic reticulum and Golgi apparatus, as well as numerous cytoplasmic vesicles within the intermediate and surface FC cells, suggest that they retain secretory capacity in addition to their structural role. Whitear (1970) concluded that the vesicles of surface FC cells were secreted to form the surface cuticle she observed in several species. A surface cuticle similar to the one described by Whitear was not observed in any stage of the eel. Remnants of the surface mucous coat, probably disrupted by preparative procedures, were frequently observed in all stages.

The basal FC cell stratum is characterized by prominent skeins of tonofilaments. Similar skeins have not been reported for other teleosts but appear to be present in micrographs of fingerling *O. kisutch* integument (Hawkes, 1974a) and adult *E. americanus* integument (Meriles, 1974), and are present in larval anurans (Chapman and Dawson, 1961). Early investigators designated these structures "figures of Eberth" in anurans. As in eels, the figures of Eberth are associated with numerous hemidesmosomes along the basal lamina and with regions where transverse bundles of collagen fibers in the dermis terminate on the basal lamina. Figures of Eberth probably strengthen the basal FC cell stratum and with the hemidesmosomes and transverse collagen bundles, bind dermis and epidermis tightly together (Chapman and Dawson, 1961). These structural adaptations are not present in teleosts generally. Henrikson and Matoltsy (1968a) report that hemidesmosomes are generally absent from teleost epidermis other than *Anguilla*. The structural adaptations may be required by the pronounced body flexures characteristic of both anuran tadpole swimming and anguilliform motion.

Mucous cells in the eel epidermis show the usual progressive accumulation of mucin, development of Golgi profiles and rough endoplasmic reticulum, and concentration of organelles at the basal pole of the cells as the cells mature and migrate from deeper regions of the epidermis toward the epidermal surface. The rate at which mucous cells mature is apparently variable, since mucous cells at the same level in the epidermis show varying degrees of maturity. A similar observation was made for the epidermis of *Salmo salar* (Roberts et al., 1970). Coalesence of mucin packets appeared greatest in mature mucous cells near or at the epidermal surface. Kitzan and Sweeny (1968) contend that this coalescence is an artifact produced by fixation in glutaraldehyde followed by postfixation in osmium tetroxide and that fixation in osmium tetroxide alone produces packets with discrete boundaries. Although some variation in the appearance of mucous cells was apparent, this variation did not appear to correspond to Kitzan and Sweeny's (1968) identification of type A, B, and C mucous cells in *Protopterus annectens* epidermis. A second mucous cell type, identified by Merilees (1974) in *Esox americanus* epidermis, was not observed in the eel. The high frequency of mucous cells in the anterior flank skin and their low frequency in posterior flank skin of the adult are similar to the distribution of mucous cells in *Salmo trutta* and *Salvelinus alpinus* (Pickering, 1974). Pickering suggests that mucus migrates posteriorly as the fish swims and high concentrations of anterior mucous cells are required to maintain an even surface mucous coat. Rosen and Cornford (1971) have found that dilute solutions of mucus from the surface mucous coat of several teleosts reduce the friction of water flowing

in a turbulent state. They propose that turbulent flow of water along the body of the fish dissolves some of the mucous coat which dampens further turbulence by reducing the friction as water flows posteriorly along the body. A high concentration of anterior mucous cells as in the eel would be useful in producing mucus to reduce friction along the rest of the body.

The unusual cytoplasmic substructure of the *Anguilla* club cell is present in cells of all life stages although the cell varies in appearance from stage to stage and within the epidermis of a single stage. No clear pattern of variation corresponding to age, body region or level of the epidermis was apparent. Henrikson and Matoltsy (1968c) proposed that the ordered cytoplasmic substructure are helices aggregated with their longitudinal axes parallel to form filaments; transverse and oblique sections of the helices produced the arrays of circles and U-shaped figures observed in the cytoplasm. However, their suggestion that these filaments are contributed to the surface mucous coat lacks an adequate explanation for the mechanism by which the filaments are transported to the surface. Although club cells appear to have reached the epidermal surface in some material examined with the light microscope, they were never observed at the surface in material examined with the electron microscope. However, mucous cells which occur less frequently than club cells (except in the anterior flank epidermis of the adult), were commonly observed at the surface using both light and electron microscopy. There is little intercellular space in the epidermis of any stage and this does not seem a likely route for transporting the club cell fibrillar element to the surface. The appearance of club cells in other teleosts is highly variable (Pfeiffer, 1963; Henrikson and Matoltsy, 1968c). The teleost club cell is assumed to be secretory in function, (Quay, 1972), however, the club cells in none of the stages examined possessed well developed endoplasmic reticulum or Golgi apparatus. Two roles have been ascribed to the club cell in other teleost orders. It secretes and accumulates "Schreckstoff" in the Cypriniformes (Pfeiffer, 1963) and it secretes and accumulates a substance which makes the flesh unpalatable to potential predators in lampreys (Pfeiffer and Pletcher, 1964). A fright reaction induced by integumentary schreckstoff has also been reported for tadpoles of several anurans (Hrbáček, 1950), but the substance has not been identified as the product of a particular cell type. The alarm reaction does not occur in adult eels (Pfeiffer, 1963) but the possibility that schreckstoff is produced by stages other than the adult has not been examined. Either of the two suggested club cell roles might be of survival value to the eel, especially in the glass eel and pigmented elver stages when, due to their size and large numbers while ascending rivers, they are probably more vulnerable to predation than at other times. The glass eel and pigmented elver are the two stages in which the club cell occurs with greatest frequency.

Chloride cells were commonly observed in the leptocephalus and rarely in the glass eel. All the cells observed resembled "type A" chloride cells of *Anguilla japonica* gill epithelium (Shirai and Utida, 1970), but lacked apical pits and were located in the intermediate epidermis as well as at the epidermal surface.

Lymphocytes are restricted to the regions adjacent to the apical ends of basal FC cells in the glass eel, pigmented elver and adult. Lymphocytes at this same level of the epidermis have been reported in several air-breathing freshwater teleosts (Mittal and Munshi, 1971). Mittal and Munshi suggest that this results from the

presence of a lymphoid tissue with well-defined lymph spaces not merely invasion of the epidermis by free lymphocytes.

Organization of the dermis in the eel is essentially that of a scaleless fish despite the presence of rudimentary scales in the adult (compare with a dermis containing overlapping scales eg. *Lebistes reticulatus*, Henrikson and Matoltsy, 1968 a). The thickness of the stratum compactum, especially in the adult, tends to confirm the contention that there is an inverse relationship between the degree of scale development and the thickness of the stratum compactum (Mittal and Munshi, 1970). The stratum of fibroblasts separating the stratum compactum and the stratum spongiosum in the leptocephalus and glass eel is probably responsible for the initial formation of the collagenous lamellae of the stratum compactum. Subsequent addition of collagen fibers in the pigmented elver and adult is presumably made by fibroblasts interposed between the lamellae. Lamellae parallel the basal lamina and are not tilted at a 3° angle to it as in *Fundulus heteroclitus* (Nadol, et al., 1969). Transverse collagen bundles normal to the lamellae are numerous and probably serve to further strengthen the stratum compactum. Similar transverse bundles have been reported for *Monopterus albus* (Liem, 1967), which has a scaleless integument, and for larval anurans (Chapman and Dawson, 1961).

Melanocytes in the pigmented elver and adult are concentrated at the superficial and deep surfaces of the stratum compactum and are occasionally interposed between the collagenous lamellae. The melanocyte is the only pigment cell type present in the flank region. Since melanocytes are not associated with iridophores or xanthophores, the flank integument of the eel lacks the chromatophore unit described for *O. kisutch* and *Xiphophorus helleri* (Hawkes, 1974 b). Pigmentation changes in the eel are therefore morphological rather than physiological (Hadley, 1972).

Functional Morphology. One of the principal functions of the integument is protection of the organism from mechanical shocks and abrasions. Several trends are evident in integumental development of the eel which reflect the changing demands for mechanical protection placed on it. There is a progressive increase in epidermal thickness, most dramatically between the pigmented elver and adult stages. Part of this increase in thickness is due to increase in the number of FC cells, which are the principal structural cell type of the epidermis, and increase in number of mucous cells, which are responsible for the production of the surface mucous layer. There is a concomitant increase in stratum compactum thickness, again with the greatest increase occurring between the pigmented elver and adult stages. Rudimentary scales appear at the adult stage. The behavior and habitats of the eel during its first four life stages are similar to those described by Bertin (1956) for *A. anguilla*. As a leptocephalus the eel is a part of the plankton living in the upper portion of the oceanic water column. As an adult the eel lives in fresh or brackish water and is an active predator. The glass eel and pigmented elver are transitional in terms of behavior and habitat and in integumental anatomy. Increase in thickness of the epidermis and stratum compactum and development of scales are adaptations for the adult life stage where the fish's freshwater, predominantly benthic habitat and behavior require greater protection against abrasion and mechanical damage than its pelagic, oceanic habitat as a leptocephalus.

The surface mucus of the fish plays a protective role against abrasions and

infection. Van Oosten (1957) suggests that there is an inverse relationship between mucus-secreting capacity and the degree of scale development. Specific immunochemical reactions within the mucus of fish have been described (Fletcher and Grant, 1969). Stoklosowa (1966), noting a sexual dimorphism in mucous cell frequency in *Salmo trutta* epidermis, concluded that the greater mucous cell frequency in females provided protection from abrasion required for nest digging in gravel. The frequency of mucous cells and presumably mucus-secreting capacity is least in the leptocephalus and increases at each stage. As noted above, the pigmented elver and adult stages, where mucous cell frequency is greatest, have the greatest need for protection from mechanical damage.

A second important integumentary function is the use of integumentary pigments to provide protective coloration. The lack of pigmentation and the laterally compressed body form of the leptocephalus make it transparent in sea water which is an excellent camouflage for its oceanic environment. Pigmentation begins to appear during the glass eel-pigmented elver transition, and the animal acquires an increasingly dark coloration during the pigmented elver stage. The development of pigmentation coincides with the eel's transition to a freshwater, predominantly benthic habitat where dark dorsal pigmentation is a more suitable protective coloration.

A third function of the integument is its role in maintaining the electrolyte balance of the organism. The chloride cells of the gill region are the principal site of monovalent ion excretion in most teleosts (Conte, 1969) and eels of the genus *Anguilla* were among the first fish in which this was demonstrated (Keys, 1931, 1933). In the leptocephalus and perhaps to a lesser extent in the glass eel, the integument serves as a secondary excretory organ because it contains occasional chloride cells. The pigmented elver and adult lack integumental chloride cells but are probably hyper-osmotic in their environments and may not require accessory chloride cells. Actively secreting chloride cells of the gill epithelium of *A. japonica* dedifferentiate or disappear when the fish are adapted to freshwater (Shirai and Utida, 1970).

The role played by the mucous coat in helping maintain constancy of the internal chemical environment is unclear. One study showed that ions diffuse almost as rapidly through eel mucus as through water (van Oosten, 1957). However, it was noted that an intact mucous coat was apparently necessary for successful adaptation to changing salinities by the elver. In *O. kisutch* the mucous coat increases in thickness as the fish moves from salt to fresh water (van Oosten, 1957). An increase in mucous cell frequency from leptocephalus to glass eel is apparent. This increase might be associated with a possible role of the mucous coat in aiding adaptation to changing salinity.

References

Bereiter-Hahn, J.: Licht- und elektronenmikroskopische Untersuchungen zur Funktion von Tonofilamenten in den Epidermiszellen von Fischen. Cytobiologie **4,** 73–102 (1971)

Bertin, L.: Eels, a biological study. London: Cleaver-Hume 1956

Brown, G.A., Wellings, S.R.: Collagen formation and calcification in teleost scales. Z. Zellforsch. **93,** 571–582 (1969)

Brown, G.A., Wellings, S.R.: Electron microscopy of the skin of the teleost, *Hippoglossoides elassodon*. Z. Zellforsch. **103,** 149–169 (1970)

Chapman, G.B., Dawson, A.B.: Fine structure of the larval anuran epidermis with special reference to the figures of Eberth. J. biophys. biochem. Cytol. **10,** 425–435 (1961)

Conte, F.P.: Salt secretion. In: Fish physiology, Vol. 1 (Hoar, W.S. and Randall, D.J., eds.), pp. 241–292. New York: Academic Press 1969

Downing, S.W., Novales, R.R.: The fine structure of lamprey epidermis, I. Introduction and mucous cells. J. Ultrastruct. Res. **35,** 282–294 (1971 a)

Downing, S.W., Novales, R.R.: The fine structure of lamprey epidermis, II. Club cells. J. Ultrastruct. Res. **35,** 295–303 (1971 b)

Downing, S.W., Novales, R.R.: The fine structure of lamprey epidermis, III. Granular cells. J. Ultrastruct. Res. **35,** 304–313 (1971 c)

Farquhar, M.G., Palade, G.E.: Cell junctions in amphibian skin. J. Cell Biol. **26,** 263–291 (1965)

Fishelson, L.: Histology and ultrastructure of the skin of *Lepadichthys lineatus* (Gobiesocidae: Teleostei). Marine Biol. **17,** 357–364 (1972)

Fishelson, L.: Observations on skin structure and sloughing in the stone fish *Synanceja verrucosa* and related fish species as a functional adaptation to their mode of life. Z. Zellforsch. **140,** 497–508 (1973)

Flaxman, B.A.: Cell differentiation and its control in the vertebrate epidermis. Amer. Zool. **12,** 13–26 (1972)

Fletcher, P.T., Grant, J.C.: Immuno-globulins in the serum and mucus of the plaice (*Pleuronectes platessa*). Biochem. J. **115,** 65 p. (1969)

Fujii, R.: Fine structure of the collagenous lamellae underlying the epidermis of the goby, *Chasmichthys gulosus*. Annot. Zool. Jap. **41,** 95–106 (1968)

Fujii, R.: Chromatophores and pigments. In: Fish physiology, Vol. III (Hoar, W.S. and Randall, D.J., eds.), pp. 307–354. New York: Academic Press 1969

Hadley, M.E.: Functional significance of vertebrate integumental pigmentation. Amer. Zool. **12,** 63–76 (1972)

Harden Jones, F.R.: Fish migration. London: Edward Arnold Ltd. 1968

Harris, J.E., Hunt, S.: The fine structure of the epidermis of two species of salmonid fish, the Atlantic salmon (*Salmo salar* L.) and the brown trout (*Salmo trutta* L.). I. General organization and filament-containing cells. Cell Tiss. Res. **157,** 553–565 (1975 a)

Harris, J.E., Hunt, S.: The fine structure of the epidermis of two species of salmonid fish, the Atlantic salmon (*Salmo salar* L.) and the brown trout (*Salmo trutta* L.) II. Mucous cells. Cell Tiss. Res. **163,** 535–543 (1975 b)

Hawkes, J.W.: The structure of fish skin, I. General organization. Cell Tiss. Res. **149,** 147–158 (1974 a)

Hawkes, J.W.: The structure of fish skin, II. The chromatophore unit. Cell Tiss. Res. **149,** 159–172 (1974 b)

Henrikson, R.C.: Incorporation of tritiated thymidine by teleost epidermal cells. Experientia **23,** 357–358 (1967)

Henrikson, R.C., Matolsty, A.G.: The fine structure of teleost epidermis. I. Introduction and filament-containing cells. J. Ultrastruct. Res. **21,** 194–212 (1968 a)

Henrikson, R.C., Matoltsy, A.G.: The fine structure of teleost epidermis. II. Mucous cells. J. Ultrastruct. Res. **21,** 213–221 (1968 b)

Henrikson, R.C., Matoltsy, A.G.: The fine structure of teleost epidermis. III. Club cells and other cell types. J. Ultrastruct. Res. **21,** 222–232 (1968 c)

Hrbáček, J.: On the flight reaction of tadpoles of the common toad caused by chemical substances. Exper. **6,** 100–101 (1950)

Jimbo, J., Shibukawa, K., Kobayashi, K., Soda, K., Kimura, K.: Electron microscopic observation on epidermis of teleost, *Salmo irideus*. Bull. Yamaguchi med. School **10,** 49–52 (1963)

Jones, M., Holliday, F.G., Dunn, A.E.G.: The ultrastructure of the epidermis of larvae of the herring (*Clupea harengus*) in relation to rearing salinity. J. Mar. Biol. Ass. U.K. **46,** 235–239 (1966)

Kelly, D.E.: Fine structure of desmosomes, hemidesmosomes, and an adepidermal globular layer in developing newt epidermis. J. Cell Biol. **28,** 51–72 (1966 a)

Kelly, D.E.: The Leydig cell in larval amphibian epidermis fine structure and function. Anat. Rec. **154,** 685–700 (1966 b)

Keys, A.: Chloride and water secretion and absorption by the gills of the eel. Z. vergl. Physiol. **15,** 364–388 (1931)

Keys, A.: The mechanism of adaptation to varying salinity in the common eel and the general problem of osmotic regulation in fishes. Proc. roy. Soc. B **112,** 184–199 (1933)

Kitzan, M.S., Sweeny, P.R.: A light and electron microscope study of the structure of *Protopterus annectens* epidermis. I. Mucus production. Canad. J. Zool. **46,** 767–772 (1968)

Liem, K.F.: Functional morphology of the integumentary, respiratory, and digestive systems of the synbranchoid fish, *Monopterus albus*. Copeia **1967**, 375–388 (1967)

Merrilees, M.J.: Epidermal fine structure of the teleost *Esox americanus* (Esocidae, salmoniformes). J. Ultrastruct. Res. **47**, 272–283 (1974)

Mittal, A.K., Munshi, J.S.: Structure of the integument of a freshwater teleost, *Bagarius bagarius* (Ham.) (Sisoridae, Pisces). J. Morph. **130,** 3–10 (1970)

Mittal, A.K., Munshi, J.S.: A comparative study of the structure of the skin of certain air-breathing freshwater teleosts. J. Zool. (Lond.) **163,** 515–532 (1971)

Nadol, J.B., Gibbins, J.R., Porter, K.R.: A reinterpretation of the structure and development of the basement lamella: an ordered array of collagen in fish skin. Develop. Biol. **20,** 304–331 (1969)

Olson, K.R., Fromm, P.O.: A scanning electron microscopic study of secondary lamellae and chloride cells of rainbow trout (*Salmo gairdneri*). Z. Zellforsch. **143,** 439–449 (1973)

Oosten, J. van: The skin and scales. In: The physiology of fishes (ed. M.E. Brown), Vol. I., pp. 207–244. New York: Academic Press 1958

Parakkal, P.F., Alexander, N.J.: Keratinization: a survey of vertebrate epithelia. New York and London: Academic Press 1972

Pearse, A.G.E.: Histochemistry, theoretical and applied. Boston: Little, Brown and Co. 1960

Pfeiffer, W.: Alarm substances. Experientia **19**, 113–168 (1963)

Pfeiffer, W., Pletcher, F.F.: Club cells and granular cells in the skin of lamprey. J. Fish. Res. Bd. Can. **21,** 1083–1088 (1964)

Pickering, A.D.: The distribution of mucous cells in the epidermis of the brown trout *Salmo trutta* (L.) and the char *Salvelinus alpinus* (L.). J. Fish. Biol. **6,** 111–118 (1974)

Quay, W.B.: Integument and the environment: Glandular composition, function, and evolution. Amer. Zool. **12,** 95–108 (1972)

Reynolds, E.S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17,** 208–212 (1963)

Richardson, K.C., Jarett, L., Finke, E.H.: Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. **35**, 313–323 (1960)

Roberts, R.J., Shearer, W.M., Elson, K.G.R., Munro, A.L.S.: Studies on ulcerative dermal necrosis of salmonids. I. The skin of the normal salmon head. J. Fish. Biol. **2,** 223–229 (1970)

Roberts, R.J., Young, H., Milne, J.A.: Studies on the skin of plaice (*Pleuronectes platessa*, L.). I. The structure and ultrastructure of normal plaice skin. J. Fish. Biol. **4,** 87–98 (1972)

Rosen, M.W., Cornford, N.E.: Fluid friction of fish slimes. Nature (Lond.) **234,** 49–51 (1971)

Schmidt, J.: The breeding places of the eel. Phil. Trans. B. **211,** 179–208 (1922)

Schmidt, J.: Breeding places and migrations of the eel. Nature (Lond.) **111,** 51–54 (1923)

Shirai, N., Utida, S.: Development and degeneration of the chloride cell during seawater and freshwater adaptation of the Japanese eel, *Anguilla japonica*. Z. Zellforsch. **103,** 247–264 (1970)

Stoklosowa, S.: Sexual dimorphism in the skin of the seatrout *Salmo trutta*. Copeia **1966**, 613–614 (1966)

Trujillo-Cenóz, O.: Electron microscope observations on chemo- and mechanoreceptor cells of fishes. Z. Zellforsch. **54,** 654–676 (1961)

Tucker, D.W.: A new solution to the Atlantic eel problem. Nature (Lond.) **183,** 495–501 (1959)

Vladykov, V.D.: Quest for the true breeding area of the American eel (*Anguilla rostrata* LeSueur). J. Fish. Res. Bd. Can. **21,** 1523–1530 (1964)

Whitear, M.: The skin surface of bony fish. J. Zool. (Lond.) **160,** 437–454 (1970)

Whitear, M.: The free nerve endings in fish epidermis. J. Zool. (Lond.) **163,** 231–236 (1971 a)

Whitear, M.: Cell specialization and sensory function in fish epidermis. J. Zool. (Lond.) **163,** 237–264 (1971 b)

Yamada, J.: A study on the structure of surface cell layers in the epidermis of some teleosts. Annot. Zool. Jap. **41,** 1–8 (1968)

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