ELECTRON MICROSCOPY OF THE SKIN OF THE FLATHEAD SOLE, *HIPPOGLOSSOIDES ELASSODON*

by

George A. Brown, B.S.

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INTRODUCTION

STATEMENT OF THE PROBLEM

The skin of fishes is like that of other vertebrates in that it is composed of two layers: an outer cellular component, the epidermis, and an inner fibrous component, the dermis, or corium (138). This external covering protects the organism from the environment and preserves the milieu interieur. A great deal of information has been obtained about the structure and function of the skin of mammals, birds, and amphibia (49, 50, 68, 69, 79, 121, 143, 159, 206). However, relatively little is known about the skin of fishes. The present thesis attempts to fill some of the gaps in our knowledge by presenting a descriptive light and electron microscopic study of the skin of the marine teleost fish, *Hippoglossoides elassodon*. The skin of this species is of added interest because it commonly gives rise to epidermal papillomas which have been studied in some detail (21, 33, 213-216).

REVIEW OF THE LITERATURE

Fish Skin General.

The skin of various teleosts (bony fishes) was studied extensively during the late nineteenth and early twentieth centuries (46, 70, 101, 160, 161, 193, 194). An extensive bibliography relating to this early work on skin and many other aspects of ichthyology has been compiled by Dean (37-39) and Wood (223). These early works contained many photomicrographs and drawings depicting the relationship of epidermis to dermis and the organization of the scales within the dermis. Recent reviews

on fish skin and scales give organized presentations of the earlier work, but have added relatively little new information (3, 15, 92, 109, 138, 174).

Fish Epidermis.

a) Squamous cells:

The fish epidermis is composed primarily of two components: the more numerous squamous cells, and the mucous cells. The entire thickness of fish epidermis consists of living cells, and is therefore analogous to the oral epithelium of man (226)* and unlike the epidermis of land vertebrates where the outer layers become cornified (138, 174). Romer (174) believes that the superficial cell layers in fish contain a small amount of keratin, while Van Oosten (130) states that keratin is absent in these cells. Burgess (23) was perhaps the first to prove the absence of keratin in the teleost epidermis by demonstrating a negative tetrazolium reaction for the sulfhydryl groups of keratin. Electron microscopic studies also demonstrate the lack of any keratin-like material (33, 83, 119, 215).

It is felt by most authors that the stratified epithelium of all vertebrates is produced by cell divisions within the basal cell layer (3, 92, 138, 174). However, autoradiographic studies on goldfish (*Carassius auratus*) show that tritiated thymidine is incorporated into epithelial cells at all levels in the epidermis (82). Other studies indicate that in the epidermis of 11-day chick embryos incorporation

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*Recent ultrastructural studies have demonstrated the presence of keratohyaline-like granules within the oral epithelial cells of mammals. However, this surface is not keratinized (116).

also occurs at all levels, while in 14-day embryos only the basal cells incorporate the thymidine (219).

The fine structure of *Salmo* epidermis was the subject of a brief report in 1963 (100), and the fine structure of teleost oral epithelium has been studied (1). However, the first detailed studies on teleost epidermis were made by Wellings, Chuinard, and Cooper (215), Chuinard (33), and later McArn (119) in connection with epidermal papillomas occurring on various species of flatfish collected in the San Juan Archipelligo in Washington State. A similar ultrastructural study on various species of fresh-water teleosts was carried out by Henrikson and Matoltsy (83).

For descriptive purposes teleost epidermis has been divided into three layers: basal, intermediate, and superficial (215).

The basal cell layer comprises a single row of cells adjacent to the basement membrane. The cytoplasm of these cells contains two morphologically distinct zones: perinuclear and peripheral. The perinuclear zone possesses a small Golgi complex, most of the mitochondria and agranular endoplasmic reticulum, and a few free ribosomes. The peripheral zone contains a few cytoplasmic filaments. The filaments are a more prominent feature in the superficial epidermal layers. Free ribosomes are present in large numbers in the peripheral cytoplasmic zone of the basal cells in those marine species studied so far. In the fresh-water species (goldfish, *Carassius auratus*; guppy, *Lebistes reticulatus*; eel, *Anguilla sp.*; catfish, *Corydoras aeneus*) studied by Henrikson and Matoltsy (83), cytoplasmic filaments are the predominant feature of the peripheral cytoplasmic zone at all epidermal levels.

Microvillous interdigitations of the cell membranes of adjacent basal cells are prominent.

The intermediate cell layer varies in thickness from species to species and at different locations on the same fish. The cells of this layer are morphological transitions between the basal and superficial layers.

The superficial cells have broad-based, short microvilli along their free, surface border, but are otherwise very similar to the cells of other layers. The perinuclear cytoplasmic zone is reduced and contains most of the mitochondria, the Golgi complex, the agranular endoplasmic reticulum, and free ribosomes. The peripheral zone is composed almost entirely of cytoplasmic filaments which have a diameter of about 80 Å. The degree of membrane interdigitation between superficial cells is decreased.

Desmosome-like structures (48) are a prominent feature of the cell membrane of the marine species reported by Wellings, Chuinard, and Cooper (215). The cytoplasmic filaments appear to be associated with these structures, and both the number of desmosomes and the number of cytoplasmic filaments increases toward the surface. Both, however, are present at all levels. Henrikson and Matoltsy (83) report some densities along the plasma membranes which are suggestive of desmosomes, but these are not a prominent feature in the fresh-water species they studied. Terminal bars (zonula occludens) (49) are observed at the outer aspect of the lateral membranes of adjacent superficial cells.

b) Mucous cells:

The epidermis of fish was shown to contain unicellular glandular mucous cells by Klaatch in 1890 (92) and Reid in 1894 (161).

These cells have since been studied extensively at the level of the light microscope (3, 70, 101, 138, 193). The smallest mucous cells are found in the basal region of the epidermis. The cells increase markedly in size until they reach the surface where they appear to burst open and discharge their contents (193).

Mucus production by goblet cells of the mammalian gastrointestinal tract has been studied extensively (60, 90, 98, 133, 134). The predominant opinion is that the carbohydrate component of mucus is first produced by the Golgi complex and is formed into mucous droplets. Freeman proposes than additional protein component is produced in the rough endoplasmic reticulum and that this protein is then transported to the Golgi complex where it becomes a component of the mucous droplets (60). This is in agreement with studies on protein-producing cells such as mammary alveolar and exocrine pancreatic cells in which protein synthesis apparently occurs in the granular endoplasmic reticulum, followed by formation of the product into droplets in the Golgi apparatus (28, 40, 142, 217).

The ultrastructure study of fish mucous cells by Wellings *et al.* (215) demonstrates that the smallest mucous droplets are observed in the region of the Golgi complex and that abundant, well developed granular endoplasmic reticulum is also present within the cells. Similar ultrastructural findings are also characteristic of the mucous cells in the skin of the eel, *Anguilla anguilla* (84).

c) Other cell types within the epidermis:

Pigment cells or chromatophores occur in both the epidermis and dermis of fishes and amphibians (3). The epidermal chromatophores vary

in shape from round and globular to stellate, with long thread-like processes. In fishes the majority of pigment cells are observed primarily in the dermis, and will be discussed in detail later (page13). Electron microscopic studies comparing epidermal and dermal chromatophores of fish appear to be lacking.

"Wandering cells", usually interpreted as lymphocytes, are found in the epidermis of many teleost species (3). These cells are usually located in the intercellular spaces of the deeper layers of the epidermis.

A "mitochondria-rich" cell has been described in toad bladder epithelium (32, 145) and in the epidermis of the leopard frog, *Rana pipiens* (49). Similar cells have not been described in fish. The function and origin of the "mitochondria-rich" cell is unclear at present.

Nerves and presumed sensory cells are also described within the epidermis of various phyla (79, 86, 127, 128, 203, 221, 222).

Other specialized cells occur in localized regions of the epidermis in certain species of teleost fishes. Because they are not generally observed, these cells will not be covered in detail, but are mentioned below for completeness.

"Pearl organs" are localized, hypertrophic, stratified squamous areas that appear keratinized on their outer surfaces. "Pearl organs" occur in the males of some teleost species and are thought to be under hormonal control (15, 162, 199).

"Chloride cells" are specialized epidermal cells found predominantly in the gill epithelium. They are thought to function in osmoregulation (24, 84, 106, 147, 192, 205).

"Cupula" are gelatinous, transparent structures that jut out from the epidermis along the lateral line in elasmobranchs, and many teleost species. The "cupula" may be formed from specialized mucous cells within the epidermis (26, 198).

Dermal-Epidermal Junction - Basement Membrane.

The basement membrane (as defined by Farquhar and Palade [49]) is a thin, finely fibrillar, continuous sheet of proteinaceous material located just beneath the base of the basal epidermal cells and superficial to the dermal collagen fibers. The cellular origin of this extracellular membrane has long been a question.

Perhaps the best evidence at present suggests that all or at least part of the membrane is secreted by the epidermal cells (81). By injecting tritiated proline into salamanders while they were regenerating limbs, Hay (81) showed that the basal epithelial cells are labelled strongly at first and later the label is more concentrated in the basement membrane. Hay also feels that the fibroblasts, which lined up along the underside of the membrane and also contained some label, might have some role in formation of the basement membrane.

Fawcett states that immunological investigations demonstrate the presence of an antigen common to most basement membranes and separate from connective tissue components. He also feels that the bulk of evidence suggests that the basement membrane is of epithelial origin (51).

Dermis.

a) General:

The dermis is composed of two layers: an outer, loose region

(stratum spongiosum) and a deeper, more dense region (stratum compactum) (92, 138).

The loose connective tissue region begins just below the basement membrane and is the thicker of the two layers. This zone contains the scales, collagen, fibroblasts, chromatophores, and some blood vessels.

Just beneath this scale-containing layer the collagen becomes more densely packed. In some species a thin subcutaneous layer, containing fat cells, is located between the dense collagenous layer and the still deeper layer of skeletal muscle.

b) Collagen and fibroblasts:

Collagen is a large molecular protein complex, abundant throughout the animal kingdom from sponges to mammals (75). This protein is synthesized primarily by fibroblasts and is a major component in skin, tendon, cartilage, and bone (40). Collagen is probably the most plentiful protein in the animal kingdom, comprising 25 to 35 percent of mammalian protein and even larger percentages in other phyla (77).

Collagen has been studied extensively by electron microscopy, x-ray diffraction, chemical analysis, and other techniques (30, 52, 57, 61, 67-69, 75-78, 97, 148-151, 153, 154, 158, 159, 173, 191, 224). The wide angle x-ray diffraction pattern, which is a reflection of both the intra- and inter-molecular arrangement of the collagen protein, is much less variable than the histologic or electron microscopic appearance of the fibers, and is therefore the best criterion for identification of the protein (63).

By 1872 it was known that collagen fibers will dissolve reversibly in acid, reconstituting when the solution is neutralized (76). In 1942 Schmitt *et al.* demonstrated that both natural collagen and this

reconstituted form had a 700 Å periodicity. It was therefore logical to assume that the length of the molecular building blocks was 700 Å (76).

Continuing their work, Schmitt, Highberger, and Gross (76) found that by adding a large, negatively charged molecule (like glycoprotein) to a dilute acetic acid solution of purified collagen, fibers with 2800 Å spacing were seen. This type fiber was termed "FLS" to indicate that it was fibrous and had long spacing. By adding ATP to an acid solution of collagen another form of collagen was produced. This was termed "SLS" (segmental long spacing) because it did not form long fibers typical of collagen, but instead formed segments of fiber 2800 Å long. From this information it was proposed that the structural unit for collagen was about 2800 Å long and under 50 Å in diameter. This unit was named "tropocollagen" (Greek, meaning turning into collagen) (76). Studies of the physical properties of collagen solutions, and electron microscopy of individual fibers, are in agreement with a molecular size of 2800 Å by 14 Å.

Based on this information, postulation as to the structure of the three basic forms of collagen were made. In the native collagen molecule, tropocollagen molecules are lined up in the same direction and overlap by about one-fourth their length, establishing the observed periodicity of about 700 Å. In the FLS form the molecules are parallel, but not facing the same direction, and not overlapped; therefore, fibers form with 2800 Å periodicity. In the SLS form the molecules are again parallel, but are all facing the same direction. Because the "heads" of one group of molecules do not attach to the "tails" of another, fibers are not formed (76).

Work on the ultrastructure of bone suggests that spaces of "hole zones" may exist between the tail of one molecule and the head of the next within the native collagen fiber (62-64). Glimcher and Krane show that the hydroxyapatite crystals, which form the calcified matrix for bone, may be primarily located in these "hole zones" (64).

The organization of the amino acids within the tropocollagen molecule was studied originally by Ramachandran in 1956 (171) and later by Rich and Crick (170, 171). Rich and Crick now feel that tropocollagen is formed by three protein chains in a helical arrangement--therefore a triple helix (171).

Studies of amino acid composition have shown that all collagens studied to date contain 30 ± 5 percent glycine and up to 25 percent proline plus hydroxyproline (76). Studies on various tissues from teleost fishes agree with this ratio (149-151).

The fact that collagen fibers are extracellular and can be precipitated from a cell-free system (76, 77) does not solve the problems of how tropocollagen is produced, how it is released from the cell, how it forms collagen fibers *in vivo*, or how the fibers are oriented within the tissue.

It is proposed that the tropocollagen molecule is produced by the rough endoplasmic reticulum and transported via vesicular elements of granular endoplasmic reticulum to the cell surface. The membranes of the vesicles are believed to fuse with the cell membrane and discharge the tropocollagen into the extracellular space (52, 67, 165, 176). Molecular aggregation then occurs at the cell surface by accretion of monomeric units onto the fibers already present (56, 153, 154, 224).

The orientation of collagen fibers within the tissue is discussed in some detail by Fitton-Jackson (56) and shows the confusion existing in this area. Porter and Pappas (153) suggested that the direction adopted by the fibers is normal to an axis of polarity passing through the centrosome and the nucleus. Gross (76) feels that collagen molecules may be secreted in a discontinuous or "pulsed" manner and that this might help to explain the organization of the fibers within the tissue.

c) Scales:

Scales are the bone-like supportive structure of fish skin. There are six kinds of fish scales (92):

(1) The placoid scales found in elasmobranch fibers are analogous to teeth in having both enamel and dentin-like components (described by Hertwig in 1874) (92).

(2) The cosmoid scale occurs only in extinct crossopterygian and extinct dipnoian fishes. It consists of three layers: an outer layer of dentin, an intermediate layer of vascular bone, and an inner layer of lamellar bone (based on Goodrich, 1870) (92).

(3) The palaeoniscoid scale is found in *Polypterus* and extinct palaeoniscoid fishes. It also consists of three layers: an outer layer of *ganoin* (a hard, shiny substance secreted by the dermis), an intermediate reduced cosmine layer, and an inner layer of lamellar bone (based on Goodrich, 1870) (92).

(4) The true ganoid scale of gar pikes and sturgeons is identical to the palaeoniscoid scale except that the middle cosmine layer is absent (based on Goodrich, 1870) (92).

(5) The cycloid scales occur in Holostei, living Dipnoi and many Teleostei (bony fishes). They are thin and flexible and consist of two layers: an inner layer of fibrous connective tissue and an outer hard bony layer (based on Hase, 1907) (92).

(6) The ctenoid scale occurs on about half of the bony fishes (Teleostei). It is identical to the cycloid scale except that the free, caudal part is covered with small teeth (hence the name ctenoid) (based on Hase, 1907) (92).

The teleost scale is a flexible, translucent, and usually overlapping or imbricating structure that is felt to be composed of an inner fibrillar layer and an outer calcified layer, and to be entirely derived from the mesoderm (3, 92, 138, 155, 174). However, a few authors believe that part of the scale may be of ectodermal origin (124).

According to current theories, scale-forming cells, called osteoblasts (129, 130, 138) or scleroblasts (132, 138), are derived from mesodermal cells along the lateral line. The mesodermal cells move out along the collagen bundles (which are arranged at 45° to the lateral line), forming clusters at major points of intersection of the collagen bundles. Here they give rise to the scales (56). Older theories suggest that the scales are organized in relation to the underlying myotomes, which are also arranged at 45° to the lateral line (128, 129, 204). Kassner (104) states that the scleroblasts migrate out from the lateral line along the course of the nerve fibers in this region.

Scale growth continues throughout life by addition of new material at the edges, causing rings analogous to the annual rings formed as trees grow (130, 132, 138). The distance between the rings appears to

be directly related to the nutritional status of the fish so that the growth rate of the entire fish is proportional to the growth of the scales and the width of the rings (17, 71, 187).

There is variation in the composition of teleost scales between species. Scales contain mineral matter varying from 16 to 59 percent of their dry weight (184). The remaining 41 to 84 percent consists mainly of two types of protein: collagen and an insoluble albuminoid called ichthylepidin (72). These two proteins are present in approximately equal proportions (184). Seshaiya *et al.* (184) studied the amino acid composition of the ichthylepidin of the scales in six species of teleosts, and compared his values with those of three other species studied by other investigators. It was observed that there was relatively little difference between the species studied, and that glycine (25%), proline (13%), and hydroxyproline (9.7%) predominated, as they do in collagen.

Studies on the deposition of collagen and minerals (calcium) in extracellular locations by *fibroblasts* (20, 30, 31, 52, 55, 57, 61, 67-69, 73, 74, 76, 78, 97, 151, 153, 154, 158, 159, 167-169, 175, 176, 191), *chondroblasts* (66, 108, 165, 167, 168), *osteoblasts* (6, 27, 45, 54, 63, 69, 108, 122, 125, 168), *odontoblasts* and *ameloblasts* (4, 7, 16, 59, 62, 65, 107, 123, 124, 126, 135, 139, 157, 163, 164, 181, 197, 209, 225), and cells producing the exoskeleton of crustaceans (200, 201) are described in some detail in various species. There are as yet no such ultrastructural studies of teleost scales.

d) Pigment cells - chromatophores:

The various colors seen in fish and animals in general are produced by a special group of pigment-containing cells called chromato-

phores. These cells are derived from the neural crest tissues of the embryo and migrate to the superficial region of the dermis, just below the epidermis (174).

Because chromatophores vary with respect to color, chemical composition, physical properties, and ultrastructural morphology, and because they have been studied by numerous investigators, the terminology is complicated and perhaps confusing. An attempt to clarify this nomenclature is presented in Table 1. Chromatophores can be divided into three main types: 1) melanin-containing cells called melanocytes or melanophores; 2) lipid-containing cells called lipophores; and 3) cells containing crystalline iridescent material and therefore called iridophores (also called guanidophores) (8, 58).

1) Melanocytes and melanophores--

According to terminology proposed in 1965 for melanin-containing cells (58), the term melanocyte is used for any cell which produces melanin. Melanophore is used to describe the melanocytes of poikilothermic vertebrates which differ from mammalian melanocytes in that their melanin granules are able to aggregate and disperse so as to cause changes in coloration.

Melanin is a protein complex synthesized from the amino acid tyrosine, and is located within cytoplasmic granules. The granules are easily visible with the light microscope, but until the advent of the electron microscope, little was known about their internal morphology. It is now known that the melanin granule is a specialized organelle which appears to originate from the granular endoplasmic reticulum and the Golgi complex as a vesicle with a unit membrane (58, 113, 183, 218).

TABLE 1

DEFINITION OF TERMS USED IN DESCRIBING PIGMENT CELLS

Melanin Granule*:	Melanin-containing particles seen with light microscopy.
Melanosome*:	Melanin-containing organelle seen with electron microscope. Melanization is complete so organelle lacks tyrosinase activity.
Premelanosome*:	Melanin-containing organelle during all stages of maturation -- active tyrosinase system.
Melanocyte*:	Cell that synthesizes melanosomes.
Melanophore*:	Cell in which aggregation and dispersion of melanosomes occurs.
Melanoblast*:	Precursor cell of melanocyte or melanophore.
Lipophore:	Lipid-containing pigment cell. (Includes xanthophores, erythrophores, and pterinophores.)
Xanthophore#:	Yellow colored pigment cell.
Erythrophore#:	Red colored pigment cell.
Pterinophore#:	Pigment cell containing pterinosomes#. (Either yellow or red.)
Iridophore#:	(guanidophore) Pigment cell containing crystalline, iridescent material.
Mixed types#:	
Xantho-erythrophore#:	Found in swordtail, *Xiphophorus helleri* (a teleost fish).
Xantho-leucophore#:	Amphibian cell containing both yellow lipid and crystalline pigments.
Melanolipophore†:	A goldfish xanthophore that develops melanosome-like granules under ACTH stimulation.

* Fitzpatrick, Miyamoto, and Ishikawa (58).
# Bagnara (8).
† Loud and Mishima (113).

Melanin is deposited on "membranes" or "coiled filaments" or "rods" within this vesicle. The granule eventually becomes so dense that the internal morphology is obliterated (44, 143). It is proposed that the term melanosome be applied to this terminal product of melanin synthesis and that the term pre-melanosome be used for all preceding stages. The term melanin granule is then reserved for any melanin-containing granule seen with the light microscope (58) (see Table 1).

In mammalian skin, melanin pigment is transferred from melanocytes into the epidermal cells within the malpighian layer (58). This transfer does not appear to occur in the skin of poikilothermic vertebrates in which melanophores are found both in the dermis and epidermis (3).

2) Lipophores--

Lipophores or lipid-containing pigment cells can be grouped according to the color of their pigment granules. Lipophores appearing yellow are termed xanthophores and red lipophores are termed erythrophores (8). While most yellow and red pigments are carotenoids, in recent years high concentrations of pteridines (close relatives of purines and flavins; containing both pyrmidine and associated pyrazine rings) (8) have been observed within some yellow and red chromatophores (8). Matsumato (118) was the first to describe the morphology of a new granule found in lipophores of the swordtail, *Xiphophorus helleri*. Because these granules contain pteridine he called them pterinosomes. He showed that the pterinosome is composed of ten or more concentric membranous spheres or spheroids. Other pterinosome-like granules have been demonstrated in several amphibian species (8) and in the teleost, *Platichthys stellatus* (119).

Lipophores containing carotinoid material are shown to possess large membrane-bound inclusions filled with amorphous material and also smaller vesicles with more electron-dense membranes, in the goldfish, *Carassius auratus* (113). These cells are similar to other lipid-containing, non-pigmented cells described by Fawcett (51). Fawcett (51) states that lipid-containing cells may vary in their ultrastructural appearance because of the difficulty in preserving lipids during fixation and dehydration. Unsaturated fatty acid droplets should be osmophilic, but this varies with the composition of the lipid. Droplets should also retain their spherical form, but they are often distorted and irregular after fixation and dehydration.

3) Iridophore (guanidophore)--

Iridophores are refractile, iridescent pigment cells which contain crystals of purines arranged in a highly ordered fashion (8). With reflected light the crystals become brilliantly scintillating, and appear white to the unaided eye. The cells are readily demonstrated using polarized light (186). Early studies showed that the crystals were composed of guanine, and therefore the cells were termed guanidophores. However, recent studies in both fishes and amphibians have shown that these cells may contain three different purines: guanine, hypoxanthine, and adenine. Therefore, Bagnara (8) feels that the term iridophore is more descriptive and appropriate for this group of cells.

Ultrastructural studies of iridophores in the skin of *Hyla* (185) show that the crystals are absent from typically fixed and sectioned material, and that only needle- or plate-like empty spaces or clefts remain. However, electron-dense material is observed within the

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membrane-bound clefts in thicker sections. It is suggested, therefore, that the purine material is either physically pushed out, or leached out, during thin sectioning.

4) Mixed chromatophores--

Chromatophores containing more than one type of pigment granule have been described (see Table 1). The origin of naturally occurring mixed chromatophores, such as the xantho-erythrophores of amphibians, is unknown. However, Loud and Mishima (113) were able to incubate lipophores on xanthic goldfish scales in a solution of ACTH, and demonstrate the subsequent formation of melanin-like granules within these cells. The identification of melanin granules was based on ultrastructural morphology, and on ammoniated silver nitrate stains for melanin and pre-melanin.

5) Physiology of color change--

The intracellular mechanisms and physiologic control of color change in the skin of cold-blooded vertebrates have been studied by many authors (15, 18, 47, 53, 87, 91, 115, 137, 140, 152, 195, 196, 208).

Early studies dealt with the motion of melanin granules within the cell during color change. At first it was felt that pseudopodia or cell processes of the melanophores were pulled in when the skin became pale (91). Later work showed these processes were relatively fixed in position, but the granules moved reversibly between the center and the periphery of the cells during color changes (115). Shanes and Nigrelli (186) studied the granules in chromatophores that polarize light (iridophores) and describe motion similar to that observed in melanophores.

Falk and Rhodin (47) propose that a basket-like meshwork of fibrils, 80 Å diameter, are located in the periphery of the cell. Contraction and relaxation of this meshwork is responsible for color change. Bikle, Tilney, and Porter (18) have demonstrated microtubules, having a diameter of 225 Å, within melanophores of *Fundulus*. These microtubules appear to run parallel to the direction of melanosome movement, suggesting they are somehow related to this movement. At present more work is needed to solve this problem of granular migration within the cell.

Others have studied the control of color change by nervous and hormonal influences (15, 53, 87, 140, 208). These studies have demonstrated great species variation in control mechanisms.

In many studies flatfishes have been adapted to various backgrounds. Sumner (196) shows good responses to changes in black, white, brown, and grey-colored background, but no response to changes in reds or yellows. Flatfish adapted to checkerboard backgrounds with variable size of squares are able to nearly approximate the background (15, 87). However, blind fish are not able to adapt, indicating that visual stimuli are involved (137, 140).

6) Chromatophore units--

Bagnara, Taylor, and Hadley (9) have shown that rapid color changes in amphibia are due to movement of granules within a complex of three different pigment cells that are closely associated. This cellular complex consists of a superficial xanthophores, an intermediate iridophore, and a deep melanophore. These cells form a morphologically and functional unit. These workers show the melanosomes concentrated below the iridocyte in frogs adapted to a light background. With

adaption to dark backgrounds the melanosomes become concentrated in cellular processes located more superficially between the xanthophores and the underlying iridophore. It is apparent that the gross appearance of color in animals is dependent upon both the type and the organization of cells within the skin.

e) Blood vessels and blood cells:

Blood vessels in fish skin predominate in the spongy layer or stratum spongiosum just deep to the epithelium. In the eel, *Anguilla anguilla*, the vessels are more numerous superficial to the scales, whereas in the blenny (*Zoarces viviparus*) they are located in the region between the overlapping scales (96).

Ultrastructure studies of the cytoplasm of endothelial cells of blood vessels reveal numerous vesicles (19, 98, 144, 167, 220). Jennings *et al.* (98) have shown in the rat that colloidal particles of thorium dioxide, and saccharated iron oxide, are transported across the endothelial cells within the vesicles, and are then released into the perivascular spaces. Rhodin (167) also observed vesicles opening to the inner and outer surfaces of the endothelial cells. This is consistent with classic theories on pinocytosis (111), and with the apparent mechanisms of uptake of yolk protein by the oocyte of the mosquito *Aedes aegypti* (178).

Also of interest is the observation that desmosome-like structures similar to those seen between epidermal cells are also noted between adjacent endothelial cells (98, 144, 167).

Studies of the peripheral blood of the goldfish reveal blood cells somewhat similar to those found in mammals (210). The red cells possess

pyknotic nuclei, but are otherwise similar. Lymphocytes are the most numerous leukocyte. Neutrophils, eosinophils, basophils, and platelets are also present. Ultrastructurally these fish cells are similar to their mammalian counterpart. (Compare Weinreb's study [210] on fish blood with Bernard and Leplus' study [14] of normal and pathologic blood cells in the human.)

Larval Skin.

Ultrastructural studies of developing teleost embryos (*Fundulus heteroclitus*) (110, 202) have shown that during cleavage the cells contain only a few poorly developed organelles and are relatively unspecialized. During the blastula stages the endoplasmic reticulum, Golgi complex, primary nucleolus, and polyribosomes first appear. During the gastrula stages the endoplasmic reticulum develops further and the nucleolus adds a granular component. Desmosomes between adjacent epidermal cells first appear in the mid-gastrula stage. Ectodermal cells in amphibian embryos (*Triturus pyrrhogaster*) have also been studied in some detail (103, 117). Matsumoto (117) believes that a surface coat may be important for morphogenetic movements in these embryos. Cloney (36) has demonstrated 50 to 70 Å diameter cytoplasmic filaments in two tunicates, *Amaroucium constellatum* and *Distaplia occidentalis*. He feels these may be responsible for contraction of epidermal cells during tail resorption.

Relatively little information was found concerning the structure of larval (post-hatching) skin in any phylum. An early study of epidermal cells in larval amphibia (*Amblyostoma opacum* and *Rana pipiens*) was done by Weiss and Ferris (211).

Priegel (153) studied early scale formation in the walleye (*Stizostedion vitreum vitreum* [Mitcheil]). He found that scales first appeared between the 24 and 29 mm stages and that by 45 mm, scale formation was essentially complete.

MATERIALS AND METHODS

Flathead sole were collected by means of an otter trawl in East Sound of Orcas Island, as previously described (214). Fish were aged by counting the number of translucent rings on the auditory ossicle.

LARVAL STUDIES

Ripe male and female flathead sole were artificially spawned (by applying gentle pressure to the abdomen) into one-quart jars partially filled with filtered sea water at 9° C. The water was changed after the first half hour and daily thereafter. Eggs which were not transparent, or which did not float, were removed and discarded with each water change.

Hatching occurred in approximately seven to nine days. Attempts to feed brine shrimp nauplii and sediments of centrifuged sea water were unsuccessful. The yolk sacs disappeared on about the seventh day post-hatching, and the larvae died.

It was possible to study and photograph the larvae in the living state by placing them in a drop of sea water on a large glass slide. A coverslip with a small amount of wax at each corner was placed over the larva in such a way that movement was partially, but not completely restricted.

Larvae were also fixed for light and electron microscopic studies (see Materials and Methods, page 24).

SCALES AND SCALED SKIN

The scales and scale-bearing skin from both the pigmented (eyed) and non-pigmented (blind) sides of 1- and 2-year-old fish were studied.

The scale arrangement was best observed with a dissecting microscope.

Individual scales were obtained by scraping the skin in a caudal to cranial direction with a sharp instrument. The scales were then mounted in glycerine and examined under the microscope. Strips of skin measuring approximately 5 x 30 mm were also peeled from formalin-fixed fish and mounted in glycerine.

Individual scales and sections of scale-bearing skin were decalcified in Bouin's fluid and embedded in paraffin for histology (see below).

Sections of scale-bearing skin measuring 1 x 1 mm or less were fixed in 1.33% osmium tetroxide, buffered with s-collidine, for electron microscopy (114) (see below). Scales were thin-sectioned by means of a diamond knife.

LIGHT MICROSCOPIC STUDIES

Tissues for light microscopy were fixed in Bouin's fluid or 10% formalin and embedded in paraffin. Sections were cut at a thickness of 5 μ on a model "820" rotary microtome (A. O. Spencer), and stained by one of the following methods: hematoxylin and eosin, Masson's trichrome, periodic acid Schiff (PAS), Gridley's reticulum, and orcein stains (5). A few formalin-fixed specimens were sectioned on a cryostat and stained for neutral fat with sudan IV (112).

ELECTRON MICROSCOPIC STUDIES

Tissues for electron microscopy were fixed in 1.33% OsO_4, buffered with s-collidine (12) or in Lentz and Trinkaus' fixative (110). The

blocks, measuring about 1 mm, were embedded in Swiss araldite (114) and polymerized at 60° C in an oven for 3 to 8 days. Thick (1 μ) and thin (50-100 mμ) sections were cut on an LKB ultrotome (LKB Instruments, Inc., Washington, D. C.), equipped with a glass or diamond knife. The thin sections were mounted on 100- to 150-mesh copper grids coated with parlodion (Mallinckrodt Chemical Works, New York, New York) and carbon. Thick (1 μ) sections were stained with Mallory's azur II and methylene blue (172), mounted in Histoclad (Clay-Adams, Inc., New York, New York) and viewed with a light microscope. The thin sections were triple stained, first with lead citrate (166) for 5 min, then with saturated uranyl acetate for 1 min, and then once again with lead citrate for 8 min. Sections were examined with an RCA EMU-3G electron microscope operated at 50 KV, or a Phillips EM 200 electron microscope operated at 60 KV.

RESULTS

Hippoglossoides elassodon, the flathead sole, is shown in Figures 1 and 2. This fish is distinguished by 1) uniform grey to olive brown color (sometimes blotched with dusky brown) on the eyed side, dusky blotches on the dorsal and anal fins, and white on the blind side; 2) dextral symmetry (eyes on the right hand side); 3) small scales with ctenoid (tooth edged) scales on the eyed side and cycloid (smooth edged) scales on the blind side, except on the caudal peduncle, and in a band on each side of the lateral line. The flathead sole reaches a length of about 18 inches (46 cm) and can be found from the State of Washington to the Bering Sea (35).

LARVAL SKIN

Artificially spawned and fertilized eggs of the flathead sole, kept at a water temperature of 9° C, developed to a two-cell stage in 5 hr, 4-cell stage in 6 1/2 hr, and early blastopore stage in 48 hr. Kupffer's vesicle appeared in about 84 hr, and hatching occurred in 160 to 216 hr. The newly hatched larva (Figs. 3, 5, 6, and 7) has a large yolk sac, prominent eye, two otic capsules containing 3 ossicles each, heart, primitive gastrointestinal tract, and brain. Numerous pigment cells or micromelanophores are visible in their subepidermal location along the body and head and are less numerous on the fins (Figs. 5, 6, and 7). These micromelanophores have stellate cytoplasmic processes and measure approximately 0.07 mm in diameter, as do the micromelanophores found in adult skin (Fig. 58). The larvae were essentially transparent except for the pigment cells.

By 7 days post-hatching the yolk sac had nearly disappeared and the larvae had grown slightly. The most obvious change, except for the absence of a yolk sac, was the increase in pigmentation, especially within the eyes (Fig. 2) and in localized spots along the body (not shown). The above findings indicate that development is apparently normal under the laboratory conditions of the experiment*.

Several stages of the developing egg, newly hatched larva, and seven day post-hatching larva were fixed for electron microscopy. All larval sections were taken in the tail region, caudal to the anal opening (see light micrograph, Fig. 8).

a) Squamous Cells.

The epithelium of the newly hatched larva measures from 5 to 8 μ in thickness and usually consists of two cell layers (Figs. 9-12).

The epidermal cells are broad and flat with marked interdigitation of the membranes between adjacent cells and narrow intercellular spaces. Terminal bars (zonula occludens) (43) are present between adjacent epithelial cells at their free (external) surfaces. The intercellular space of the developing epidermis appears open toward the subepithelial space (Fig. 10), as it does in the adult fish (Figs. 32 and 23).

Small desmosome-like structures are seen along the cell margins of adjacent cells (Figs. 10, 11, and 13). The desmosome-like structures of the larva are like those of the adult (Fig. 28) in the following ways:

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*Larvae and eggs collected with a 1 meter plankton net were identical to those raised in the laboratory, again indicating that the development under laboratory conditions was normal.

the opposing cell membranes are parallel along the length of the desmosome; filamentous material is present in the adjacent cytoplasm and does not appear to cross the intercellular space; and an electron-dense line (intermediate line) is observed in the intercellular space. However, larval and adult desmosomes differ in the following respects: the larval desmosomes are shorter on cross-section (smaller in diameter), less numerous, and the intercellular space along the course of the larval desmosome appears proportionately wider than in the adult (compare Fig. 13 and Fig. 28, both at the same magnification). However, measurement of this space reveals that it is about 250 Å in width, and therefore approximately the same as adult epidermis in this regard.

The external surfaces of the epithelial cells are essentially flat with only occasional microvilli. There is no evidence of a mucous coat or of fine filamentous material along the surface (compare with adult, Figs. 31 and 32).

The most prominent features of these cells are the large amount of granular endoplasmic reticulum and ribosomes within the cytoplasm and the absence of zonation (perinuclear and peripheral) which is typical of adult epidermal cells (Figs. 10 and 11). The amount of endoplasmic reticulum is greater in the deeper cells adjacent to the basement membrane. The ribosomes appear to be in greatest concentration around the endoplasmic reticulum, but are numerous throughout the cytoplasm except in the region just below the surface. This surface region contains numerous cytoplasmic filaments which appear to be identical to those described in various species of adult fishes (33, 83, 119, 215). These filaments are also found within the deeper cell layers; this finding is

at variance with the observations of McArn in larval starry flounders (119) (Figs. 11-13). The cytoplasmic filaments measure approximately 80 Å in diameter and appear to be associated with the desmosomes. Mitochondria are also present in the cytoplasm (Figs. 9, 10, and 13). A few smooth-walled vesicles are observed. In Figure 3 one of these vesicles appears to be opening into the intercellular space. No Golgi complexes were noted in the sections studied.

The nuclei of the larval epidermal cells are broad and flat, as are the cells containing them (Fig. 11) and have a rather smooth surface limited by a nuclear membrane. The material within the nucleus is relatively pale with only a small amount of more electron dense material around the periphery. No nucleoli were seen in the sections studied.

During mitosis (Fig. 12) the nuclear membrane is incomplete and chromosomes appear as electron dense aggregates. The nucleoplasm also contains numerous small granules of high electron density, interpreted as ribosomes. Microtubules, measuring approximately 250 Å in diameter and up to 250 mμ in length, are numerous. The microtubules probably represent spindle fibers connecting the chromosomes with the centrioles or extending between the two centrioles (40, 51).

b) Mucous Cells.

The larval epidermis, like the adult, contains mucous cells (Fig. 8) which are less numerous than the squamous cells. Morphologically, the larval mucous cells are identical to those of the adult (compare Figs. 9 and 12 with Figs. 34-36). Immature or developing mucous cells contain abundant granular endoplasmic reticulum, well developed Golgi

complexes, and membrane-bound vesicles containing fine, filamentous material, interpreted as mucus. The cells also contain typical nuclei and numerous mitochondria.

c) Dermal-epidermal Junction.

Immediately deep to the epidermis is a space 200 to 300 Å wide, which is limited by a continuous line of finely fibrillar appearing material believed to represent a primitive basement membrane (Figs. 12 and 13) (compare with adult, Fig. 23). Within the space are a few granules, 150 Å in diameter (Fig. 13). These granules are smaller and less electron dense than similar granules in adult skin (Fig. 23). The identity of these granules and the larger ones of older skin is unknown. The basement membrane measures approximately 250 Å in thickness and is thinner and less electron dense than its adult counterpart.

d) Dermis.

The large space deep to the basement membrane contains loose fibrous material believed to represent collagen. The fibers measure up to 150 Å in diameter and are most numerous in the region adjacent to the basement membrane. In some areas these fibers appear to have aggregated, forming structures with approximately 600 Å periodicity, which is consistent with the periodicity of collagen (Figs. 10 and 13).

Mesenchymal cells probably representing fibroblasts are located deep to the space containing the collagen-like material (Figs. 14 and 15). These cells have a large nucleus, mitochondria, numerous ribosomes, and a large amount of granular endoplasmic reticulum.

The chromatophores are located in the space between the early collagen and the deeper mesenchymal tissue (Fig. 14). The micromelanophores have many large homogeneous-appearing, electron-dense granules identical to those of the adult (Figs. 60-62). Some of the smaller granules have a fine lamellar appearance similar to the granules seen in a cell interpreted as an immature melanophore in adult skin (Fig. 63) and also similar to an immature, membrane-bound melanosome in the retinal pigment epithelium of a human fetus demonstrated by Fawcett (51). The micromelanophores also contain numerous mitochondria, small Golgi complexes, agranular endoplasmic reticulum, vesicles, and ribosomes. Microtubule-like structures are also seen (Fig. 14).

Light microscopic examination of newly hatched larvae (Figs. 5-7) has not demonstrated macromelanophores or iridocytes. However, on sectioning (Fig. 9), one finds a peculiar cell which is composed primarily of small, membrane-bound empty vesicles. A similar cell is also seen in adult skin (Fig. 60) and is interpreted as a possible lipophore.

A layer of muscle cells is seen deep to the cells interpreted as dermal fibroblasts (Figs. 9 and 15). These myoblasts have central nuclei, numerous large mitochondria, small granules (interpreted as ribosomes or possibly glycogen), and myofibrils. The myofibrils are located at the periphery of the cells and are composed of numerous myofilaments arranged in parallel rows. The filaments are approximately 100 Å in diameter (compare with muscle in adult, Fig. 66).

Blood vessels are not seen in the larval skin.

ADULT SKIN

a) Light Microscopy.

The morphology of the skin of adult flathead sole corresponds to previous descriptions (83, 138). The skin is composed of three distinct layers (Fig. 16): 1) an epidermis consisting of stratified squamous epithelium and mucous cells; 2) an outer portion of the dermis (stratum spongiosum) composed of loosely arranged collagen fibers, scales, and most of the pigment cells; and 3) a portion of dermis deep to the scales where the collagen is more dense or compact (stratum compactum). The web between the fin rays (Figs. 17 and 18) does not contain scales. Here the dermis consists primarily of dense collagen, pigment cells, and small blood vessels. The epidermis consists of 5 to 9 layers of ovoid, polygonal, and squamoid cells with numerous intercellular bridges. The number of layers appears to depend upon location on the fish and the species (83). Mucous cells in various stages of maturation are interposed between these squamous cells. A few pigment-containing melanophores are also seen within the epidermis (Fig. 16). In this species the epidermal cells do not extend down between the scales. This compares with scales of the lung fish, *Lepidosiren* (3), but differs from the yellow perch, *Perca flavescens* (138) and the guppy, *Lebistes reticulatus* (83), where the epidermis extends down between the scales.

b) Epidermis - Electron Microscopy.

1) Squamous cells:

As described previously, the epidermis of this species (33, 215) and other teleost species (83-85, 119) is composed of mainly two

cell types: squamous cells and mucous cells. Unlike the epidermis of higher vertebrates, there is no evidence of keratinization, cornification, or keratohyaline granule formation in the epidermis. However, for descriptive purposes the epidermis can be divided morphologically into three squamous cell layers: basal, intermediate, and superficial. The basal cell layer consists of a single row of ovoid cells adjacent to the basement membrane. The superficial cell layer is the single layer of cells at the surface of the epidermis. All cells between these two layers are termed intermediate cells (Fig. 19).

The squamous cells in all layers are typified by the presence of numerous intercellular bridges or desmosomes and by the presence of fine (80 Å diameter) cytoplasmic filaments. The cytoplasm of cells in all layers appears to be separated into two morphologically distinct zones: perinuclear zone and peripheral zone. The perinuclear zone contains most of the cytoplasmic organelles, including a Golgi complex, mitochondria, agranular endoplasmic reticulum, and some 100 to 150 Å particles interpreted as free ribosomes (Figs. 20, 26, 27, 30, and 31). The peripheral zone is composed of cytoplasmic filaments and most of the ribosomes with only a few mitochondria and agranular vesicles.

The differences noted between the basal cell layer and the more superficial layer are as follows: the cell shape changes from ovoid to polygonal, and finally to flattened or squamoid at the surface (Fig. 19). The amount of interdigitation between adjacent cells decreases, and the number of desmosomes increases (Fig. 19). The nuclei move from their central location in the basal and intermediate layers to a more basal region within the superficial cells. The number of cytoplasmic

filaments increases and tends to predominate in the region superficial to the nucleus (compare Figs. 20, 26, 27, and 30).

The nuclei in all epidermal layers are generally oval with a slightly irregular outline and a finely granular content. Nucleoli are composed of electron-dense granular material (Figs. 21 and 27). Structures interpreted as nuclear pores are seen along the nuclear membrane (Fig. 21) (compare with Merriam's work on the nuclear envelope [120]).

The perinuclear cytoplasm is similar at all levels in the epidermis. There are prominent Golgi complexes (Fig. 31), mitochondria, smooth endoplasmic reticulum, and agranular vesicles (Fig. 30). The cytoplasmic filaments, which predominate in the peripheral zone of the cytoplasm, are much more numerous in the superficial cells. In the superficial cell layer the filaments are concentrated in a zone just below the epidermal surface (Fig. 30), and appear to be the only cytoplasmic component within the short, broad-based microvilli which are found along the epidermal surface (Fig. 32).

There is a suggestion that these cytoplasmic filaments enter the "cytoplasmic plaque" (49) or "attachment plaque" (105) region of the desmosome, making a 180° loop and returning into the deeper cytoplasm of the same cell. This observation is similar to that of Kelly's (105) in his work with newt (*Taricha torosa*) epidermal desmosomes. No cytoplasmic filaments are seen crossing between the cells; however, there is a suggestion that they extend between desmosomes within the same cell (Fig. 33). Similar observations were made by Singer and Salpeters (188) and Chapman and Dawson (29) with regard to cytoplasmic filaments in larval amphibians. Desmosomes appear identical to those described in

frog epidermis (49) and newt epidermis (105) and they appear to have an intermediate line of electron-dense material (Fig. 33). Although the cytoplasmic filaments are found within basal cells (cells just superficial to the basement membrane) the cytoplasmic membrane adjacent to the basement membrane lacks hemidesmosome-like structures (Figs. 20, 22-25). The adult epidermal cells are characterized by a minimal amount of granular endoplasmic reticulum and well developed agranular endoplasmic reticulum (Figs. 20, 26, 27, and 30).

2) Epidermal surface and intercellular space:

The cell membranes of all the cells consist of a thin continuous sheet with a total thickness of approximately 80 Å. On cross-section this sheet appears as a typical three-layered "unit membrane" with electron-dense outer and inner layers and a relatively electron-lucent layer between. This is best seen in the terminal bar (*zonulae occludens*, Farquhar and Palade [49]) of the superficial cells (Fig. 32) where the cell membranes along the surface meet and fuse, suggesting that the terminal bar forms a continuous band around the superficial cells. In this region the outer leaflets are fused and form a faint electron-dense line between the two inner leaflets.

The surface of the superficial cells has short, broadly based microvilli (approximately 50 μ tall and 30 μ wide at the base [Fig. 30]). At high magnification, fine hairs 30 to 50 Å in diameter (Fig. 32), are seen adjacent to or protruding from the cell surface. These are similar to the so-called "pile or nap" demonstrated on the surface of toad bladder epithelium, *Bufo marinus* (32). The presence of microvilli and these fine hairs is the usual pattern seen with the

collidine-buffered osmium fixation used on these tissues. However, in one specimen the surface layer is covered by a dense fibrillar coating (Fig. 31) which is very similar to the mucous coating described by Farquhar and Palade (49) on the outer surface of the epidermis of the leopard frog, *Rana pipiens*.

The interdigitation between adjacent cells is most marked in the basal cell layer (Fig. 19). Large intercellular spaces containing numerous finger-like cytoplasmic processes from adjoining cells are seen. Terminal bars are only seen at the outer edge of adjacent cells in the superficial layer.

Agranular vesicles are observed adjacent to the cytoplasmic membranes. In some instances the vesicles appear to be opening into the intercellular space (Figs. 13 and 31), suggesting that soluble or particulate material is being transported across the cell surface in one or both directions. Similar vesicles are sometimes locally abundant at the inner surface of the basal cells (Fig. 15).

3) Mucous cells:

Mucous cells in larval and adult skin appear morphologically similar (compare Figs. 9 and 12 with 34-36). Immature mucous cells are seen in the basal and deep intermediate cell region. They are typified by a large amount of granular endoplasmic reticulum in parallel array and the presence of mucous droplets within the cytoplasm (Fig. 34). Mucous droplets appear as large membrane-bound, agranular vesicles filled with a fine filamentous substance, and are usually seen associated with the Golgi complex. Mitochondria are also quite numerous within these cells (Fig. 35).

In more superficial regions of the epidermis these cells are larger (Fig. 35). The mucous droplets have increased in size, coalesced, and occupy most of the cytoplasmic space. The other cytoplasmic organelles, which are again primarily granular endoplasmic reticulum, appear more concentrated and electron dense in a ring or sphere around the centrally located mucous droplets.

At the surface of the epidermis the mucous cells sometimes appear to be in the process of releasing their mucus (Figs. 16, 18, and 36). Mucous cells with discharged mucus are not seen in the epidermis.

The cytoplasmic membrane of the mucous cells lacks microvillous convolutions and interdigitations. Previous descriptions of mucous cells in this species (215) and other species (84) describe a lack of desmosome-like structures between the mucous cells and adjacent squamous cells. However, on close inspection, a few desmosomes are seen (Figs. 34 and 35).

4) Other cell types within the epidermis:

Pigment cells--

Melanophores are the only kind of pigment cell observed in the epidermis of the flathead sole (Fig. 16). Electron micrographs of mature epidermal melanophores have not been observed*. However, one cell which is interpreted as an immature melanophore has been seen within the

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*This is probably because epidermal melanophores are rare in the epidermis of the fin web. Most of the electron micrographs of the epidermis were taken from fin web tissue. More recent light micrographs suggest that epidermal melanophores may be common in the epidermis of the pigmented, scale-containing skin.

epidermis (Fig. 63). Melanophores and other pigment cells are discussed in greater detail later (pages 43 to 45).

Lymphocytes--

Cells interpreted as lymphocytes are sometimes located within the intercellular spaces between squamous cells in the basal region of the epidermis (Fig. 22). These cells have a round to oval nucleus with an irregular nuclear membrane. The nuclear content is of a comparatively great electron density and may be coarsely clumped. The cytoplasm of these cells contains numerous membrane-bound vesicles or inclusions of varying sizes, some of which contain electron-dense material. The cytoplasm also contains many fine granules, interpreted as ribosomes, and a few structures resembling mitochondria (Fig. 22).

Mitochondria-rich cells--

Portions of cells containing numerous mitochondria are occasionally observed between epidermal cells of the intermediate layer (Fig. 29). This cell type may be analogous to similar cells described in the skin of the leopard frog, *Rana pipiens* (49). The mitochondria are large, but are otherwise not unusual (141).

Other cell types such as "club cells", "granular cells", and "chloride cells" described by Henrikson and Matoltsy (85) in the goldfish (*Carassius*), catfish (*Corydoras*), and eel (*Anguilla*) have not been seen within the epidermis in numerous skin samples.

c) Dermal-epidermal Junction and Basement Membrane.

The basal cells (Figs. 20, 23-25) are separated from the underlying epithelial basement membrane by a narrow, electron-lucent space measuring

approximately 50 mμ in width. The basement membrane (49) itself is approximately 4 times thicker (approximately 200 mμ) than the width of this space, and lies immediately superficial to dermal collagen.

The material of which the basement membrane is composed is homogeneous and finely granular or filamentous in appearance with a smooth, discrete superficial border (Figs. 23 and 24). Along its basal or dermal border, the basement membrane is more irregular and in some cases strands of the fibrillar material appear to extend down into the underlying dermal collagen (Fig. 24).

The subepidermal space (just superficial to the basement membrane) appears to connect freely with the intercellular spaces (Fig. 23). The subepidermal space, like the intercellular spaces, is relatively electron-lucent, but contains some finely filamentous material similar to that seen in the electron-dense basement membrane proper. Electron-dense granules measuring approximately 200 Å in diameter are also seen within the subepidermal space, but are not seen within the intercellular spaces of the epidermis (Fig. 23).

d) Dermis - Light Microscopy.

In the scale-containing skin (covering most of the body surface), the dermis can be divided into two morphologically distinct layers: 1) an outer, loose region (stratum spongiosum) and 2) a deeper, dense region (stratum compactum) (Fig. 16). The scales are located within the outer loose region. Chromatophores predominate in the zone just below the basement membrane and superficial to the scales, but they are found in all regions of the dermis and within the epidermis. Blood

vessels are seen between the scales in the stratum spongiosum and deep to the scales in the stratum compactum.

In the fin web (Figs. 17 and 18), which is without scales, the dermis appears to lack this stratification. Here the collagen fibers appear rather closely packed except possibly around the blood vessels located in the central region.

e) Dermis - Electron Microscopy.

1) Collagen:

The morphology of dermal collagen of this species is similar to that described in other animal phyla. The collagen fibers have the typical 600 to 700 Å periodicity (Figs. 37 and 38), and are organized into sheets or lamellae, each lamella composed of numerous fibers oriented in parallel. The fiber axes of adjacent lamellae are approximately perpendicular to each other, giving the dermis a structure analogous to plywood. On cross-section the matrix between the collagen fibers appears relatively electron-lucent (compare dermal collagen, Fig. 38, with scale collagen, Fig. 55). In some sections, "microfibrillar" material is seen in the spaces between the collagen fibers (Figs. 38 and 40).

Fibroblasts are typified by the presence of large amounts of granular endoplasmic reticulum within the cytoplasm and by their stellate or spindle shape with long cytoplasmic processes (Figs. 37-39). These cells also have numerous agranular vesicles, mitochondria, and a nucleus that contains homogeneous, pale staining chromatin.

Of interest is the observation that the fibroblasts always appear to be located between the collagen lamellae and not within the lamellae.

This means that the collagen fibers on one side of the cell are running at approximately 90° to those fibers adjacent to the opposing cell wall (Figs. 36 and 37).

2) Scale:

By light microscopy the scales are located in the outer loose connective tissue of the dermis (Figs. 16, 52, and 53). Below this scale-bearing region is a thick zone of dense, compact collagen overlying the muscle. In the flathead sole, layers of loose connective tissue are disposed between overlapping scales*. Melanophores are the predominant pigment cell type and are located mostly in the dermal region superficial to the scales and just beneath the epidermis. Some pigment cells also occur within the deeper region of the dermis and in the epidermis.

Cross-sections of portions of two scales are seen in Figure 16. The caudal, peripheral portion of the scale is represented by the more superficial scale in the illustration, while the deeper scale is sectioned through a more central area. The deeper scale is composed of two parts: a deeper, dark staining layer which by electron microscopy is composed of collagen-like material and a more superficial, paler staining area that appears to be calcified. At the peripheral margins of the scale (upper scale, Fig. 16), the calcified regions appear as discrete "tooth-like" structures which may actually be rings of calcification around the entire structure.

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*In other species the epidermal components extend down between the scales (83, 132, 138).

The bulk of each scale stains red (as does bone) by the Masson trichrome method, while the dermal collagen is blue (Figs. 52 and 53). Blue staining is also observed in a thin layer along the internal surface of each scale. This blue-staining region becomes thicker toward the periphery of the scale and seems to be similar to the collagen-containing portion of the scale seen in Figure 16.

Electron micrographs of the scales reveal multiple layers of collagen throughout. The collagen fibers are embedded in a matrix of moderate electron density (Fig. 55). The collagen fibers in each layer are oriented in parallel and possess the usual longitudinal periodicity with the major period measuring about 600 Å. The diameter of the collagen fibers varies from 300 to 1100 Å. The fibers of each layer, moreover, are oriented at an angle of approximately 90° to the layers above and below. Thus, the structure of the scale also has a striking resemblance to plywood.

The deeper layers of the scale consist only of collagen fibers embedded in the matrix material. The collagen of the scale (Fig. 55) differs from dermal collagen (Fig. 37) in that the fibers are more tightly packed, the matrix between the fibers is more electron dense, and cells or cell processes are not seen between the collagen lamellae.

The outer, superficial portion of the scale contains electron-dense, crystal-like structures which appear identical to hydroxyapatite crystals seen in other calcified tissues (Figs. 55 and 56) (6, 55, 167, 181, 197). The hydroxyapatite crystals are numerous in the matrix between the collagen fibers when the fibers are seen in cross-section (Fig. 55). However, in longitudinal section (Fig. 56), crystals are

also observed within the collagen fibers. The crystals are grouped at 600 Å periods along the length of the collagen fibers, suggesting that the hydroxyapatite crystals may be concentrated within "hole zones" between the tropocollagen molecules as hypothesized by Glimcher, et al., in his work with reconstituted bone collagen (63, 64).

Cells which are apparently responsible for scale formation are located along the deep and superficial surfaces, and the edges of the scale (Fig. 54). These cells contain abundant granular endoplasmic reticulum and are morphologically identical to fibroblasts except that desmosome-like junctions (49) are often observed between them. In a narrow zone between the scale-forming cells on the deep surface of the scale and the scale proper, the collagen fibers are of progressively increasing diameter in the direction of the interior of the scale (Fig. 54). Unlike the other connective tissue structures of the dermis, the scale lacks blood vessels and pigment cells. Scale-forming cells are observed only on the surface of the scale, not in the interior.

At the periphery of the scale the collagen-like fibers lack a typical lamellar pattern (Fig. 57). Small calcified areas are noted within the region of disorganized collagen fibers.

3) Chromatophores:

Melanophores--

Melanophores are by far the most numerous pigment cells in this species (*H. elassodon*). They give the pigmented side of the fish a brown color. Only a few large macromelanophores (182) (approximately 0.40 mm in diameter) are seen among the numerous micromelanophores (approximately 0.07 mm in diameter). Incidental observations indicated

that the color patterns of the fish changed to approximate varying patterns of the bottom within the sea water aquaria in which live fish were maintained.

Ultrastructurally, melanophores are typified by the numerous, homogeneous, well circumscribed, electron dense melanosomes within their cytoplasm (Fig. 60). The melanosomes measure approximately 600 mμ in diameter and can be seen with the light microscope. The cytoplasm also has mitochondria and a large amount of smooth endoplasmic reticulum (Figs. 60-62). Microtubule-like structures which were seen in larval melanophores are not seen in adult melanophores.

A cell interpreted as an immature melanophore is demonstrated within the epidermis (Fig. 63). This cell lacks desmosomal connection with the adjacent epidermal cells. It contains a large Golgi complex and numerous agranular vesicles of varying sizes. Some of these vesicles contain electron-dense material which on close inspection appears to be organized as thin parallel lines or sheets similar to the developing melanin granule or premelanosome described by Fawcett (51). These premelanosome-like structures are similar to some of the granules observed within the larval melanophore (Fig. 14).

Iridophores - guanidophores--

Iridophores (also called guanidophores), like melanophores, predominate in the outer dermal region just below the basement membrane on the pigmented side of flathead sole. In this species iridophores are scarce on the pigmented or eyed side of the fish, but are numerous on the "non-pigmented" or blind side. On the blind side the iridophores appear most numerous deep to the skin in the junction between the muscle

and the dense dermal collagen. Iridophores have not been seen within the epidermis in this species. They appear to be absent in the newly hatched larva.

Ultrastructurally the iridophores are similar to those described in other species (8, 185). They contain iridescent, crystalline material which is arranged in parallel rows within the cytoplasm. During preparation the crystals are leached out of the sections, leaving spaces or clefts oriented in parallel arrays within the cytoplasm. Iridophores possess a Golgi complex, agranular endoplasmic reticulum, and numerous vesicles of varying sizes.

Lipophores--

Lipophores are not definitely recognized in flathead sole by light microscopy. However, with the electron microscope, cells interpreted as lipophores are observed (adult skin, Fig. 60, and newly hatched larva, Fig. 9). These cells are similar to other lipid-containing cells in that they contain numerous, large, agranular vesicles with homogeneous material of varying electron density within these vesicles (51). Like melanophores they predominate in the outer dermis just below the basement membrane. They are often observed adjacent to melanophores (Fig. 60).

At present, further morphological and biochemical studies of the different types of lipophores are needed.

4) Blood vessels and blood cells:

The blood vessels and the blood cells in the fish are similar to those of other vertebrates (14, 96, 99, 144, 167, 210). Endothelial cells (Figs. 65-68) contain numerous agranular vesicles which are

localized adjacent to the cell membranes of both luminal and interstitial surfaces. Cytoplasmic filaments measuring 80 Å in diameter are noted within the cytoplasm (Figs. 67 and 68). Desmosome-like structures are seen between endothelial cells (Fig. 68). These desmosomes are identical to those seen between adjacent epidermal cells (Figs. 28 and 33) and between scale-forming cells (Fig. 54). A thin basement membrane is observed between the endothelial cells and the surrounding dermal structures (Fig. 67).

Nucleated red blood cells and leukocytes are observed within the small blood vessels of the dermis. The cytoplasm of the red cells (Figs. 65, 78, and 68) is homogeneous, finely granular, of low electron density. Nuclei are spherical to ovoid in shape, with coarse chromatin patterns, similar to that seen in developing human red blood cells (normoblasts) in the bone marrow (226).

Only a few leukocytes of uncertain type were observed. Two leukocytes are demonstrated and described in Figure 65.

5) Muscle:

The muscle of the body wall is located just beneath the dermis (Fig. 15, larva, and Fig. 66, adult). The ultrastructure is similar to that described in other species (2, 13, 41, 51, 80, 156, 188). In the adult, as compared to the larva, the myofibrils are more numerous and the mitochondria are smaller and less numerous.

DISCUSSION

The present work is an electron microscopic study of the skin of larval and adult flathead sole, *Hippoglossoides elassodon*. Scale-free adult fin web, which has been studied in various teleost species (33, 83-85, 119, 215), is compared with the scale-bearing skin of the body of the fish (22).

It is clear that teleost skin differs from the skin of amphibians, birds, and mammals in that both morphologically and biochemically it lacks any signs of keratinization (23, 34, 83, 119, 215). However, in other respects teleost skin is very similar to the epidermis of amphibians, described previously by Farquhar and Palade (49). Desmosomes and complex cellular interdigitations between adjacent cells are common to both. The intercellular space appears occluded (terminal bar or zonula occludens [49]) toward the surface of the epidermis, but is otherwise open and continuous with the subepidermal space. Numerous filaments, 80 Å in diameter, are seen within the cytoplasm.

In the present work the squamous cells at all levels in the epidermis are morphologically very similar, although the number of cytoplasmic filaments increases as the cells move from the basal layer toward the surface, where the cells are eventually exfoliated.

There appears also to be some species variation in the morphology of squamous cells in fishes. Cytoplasmic filaments are far more abundant in the fresh-water species described by Henrikson and Matoltsy (83) than the same structures are in the instance of the flathead sole. The question arises as to whether these differences are related to differences in the salinity of the surrounding water in marine versus

fresh-water species. More comparative studies are needed before this question can be answered.

The function of cytoplasmic filaments in epidermal cells is at present unclear. They are associated with desmosomes and perhaps help to increase the strength of intercellular attachments. As stated previously, there is no evidence that cytoplasmic filaments condense to form keratohyaline-like structures typical of mammalian squamous cells (116, 177), nor is there any evidence of granule formation such as that seen in amphibians (49, 143).

Desmosomal attachments between adjacent squamous cells are common in the skin of the flathead sole, in contrast to the findings of Henrikson and Matoltsy (83) in the instance of fresh-water teleost species. These authors described only occasional desmosomes between squamous cells.

The teleost desmosomes in this study appear morphologically identical to those described in other classes of vertebrates: human (88); rat and guinea pig (48); *Rana, Bufo, Xenopus, Amblyostoma* (49); *Taricha torosa* (105). There is no evidence of morphological variation in the desmosomes at different levels in the epidermis such as the "modified" and "composite" desmosomes described by Farquhar and Palade (49) in the frog, *Rana pipiens*. The observations are consistent with the lack of variation in fish skin epithelium and the absence of a cornified layer*.

Hemi-desmosomes, which are located along the cell membranes at the base of the epidermis and adjacent to the subepidermal space in the

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*"Modified" and "composite" desmosomes are located within cornified, superficial layers in frog epidermis.

eel, *Anguilla* sp. (83); frog, *Rana* sp. (49, 189, 204); newt, *Taricha torosa* (105); boa constrictor, *Constrictor constrictor* (178); and guinea pig (190). However, for reasons which are unclear, hemidesmosomes are not observed in fresh-water teleosts (83) or in this marine species.

The comparison of desmosomes in the larva (Figure 11) with those of the adult (Figure 28) suggests that with age the desmosomes increase in diameter (or length on cross-section). However, the distance between the outer leaflets along the course of the desmosome appears to be constant (at approximately 250 Å) (see page 27 in Results). An intermediate line similar to those described by Farquhar and Palade (49) and Kelly (105) in amphibian species is seen in both the larval and adult desmosomes of skin of the present species (Figure 11, larva; Figure 33, adult).

These findings are consistent with the idea that the epidermis of teleosts can act as a permeability barrier, carrying out electron transport, and transporting molecules between adjacent cells within the epidermis, as demonstrated in other phyla (11, 43, 50, 102, 206).

The surface of the epidermis typically possesses regularly spaced, broad-based microvilli, and thin, filamentous strands (measuring 30 to 50 Å in diameter and averaging 125 mμ in length) are often seen attached to the outer surface in extracellular locations. These observations agree with earlier studies of teleost skin (33, 83, 119, 215). Henrikson and Matoltsy (83) suggest that these surface filaments may be specializations of the plasma membrane as suggested by Ito's work on intestinal microvilli (93-95). The diameter of the surface filaments is approximately the same as filaments seen within the mucous cells (Figures 34

and 35) which suggests that they may be constituents of the mucous coat as suggested by Wellings, Chuinard, and Cooper (215).

It is possible that the short, broad-based microvilli are actually a fixation artefact caused by shrinkage of the cells during fixation, or wrinkling of the skin when it is removed from the fish. Observation of skin fixed under tension might answer this question.

Mucous cells in the fish appear to be typical holocrine secretory cells which discharge their mucous contents at the surface, followed by cell exfoliation and death. The ultrastructure of teleost mucous cells has been studied previously and is discussed on page 4 of the Introduction. It is felt that mucous cells are derived from basal cells of the epidermis (138). The most immature fish mucous cells have been described as cells containing a large amount of granular endoplasmic reticulum and a Golgi complex which is apparently responsible for the formation of mucous droplets (84, 138). Cells in transition from basal cells (containing predominantly cytoplasmic filaments) to immature mucous cells (containing predominantly granular endoplasmic reticulum) have not been described in fishes. In the present work, the cells which appear to be the earliest recognizable mucous cells possess abundant mitochondria, as illustrated in Figure 21.

The idea that "mitochondria containing cells" may represent early goblet cells (mucous cells) has been previously suggested by Peachey and Rasmussen (145), based on their studies of the epithelial cells of the urinary bladder of toads. Choi (32), however, interprets the mitochondria-rich cells of toad bladder epithelium as responsible for ionic transport across the bladder wall.

The presence of desmosomes between mucous cells and adjacent squamous epithelial cells supports the suggestion that both of these cell types arise from a common epithelial precursor. In this species desmosomes are also seen between scale-forming cells (Figures 54 and 57) and between endothelial cells (Figure 68), both of mesodermal origin. In other species desmosomes have also been described between cells within bone, tendon, and tooth pulp (45, 73, 7, respectively).

Although the origin of the epithelial basement membrane remains controversial, the bulk of the data suggests that this structure is a product of the epithelial cells (51). The presence in basal cells of vesicles in cytoplasmic regions adjacent to the basement membrane suggests that material may be transported from the epithelial cells by a pinocytotic process. A similar process may occur along the cell membranes between adjacent epithelial cells with the basement membrane-forming material being deposited into the intercellular space and then moving to the subepithelial space and to the basement membrane.

Observations on the newly hatched larva show the basement membrane to be fairly well developed with only minimal development of the adjacent dermal collagen. This suggests that the basement membrane is formed prior to the collagen and may be of epidermal origin. Studies on wound healing in the frog (212) also suggest that the epidermis and basement membrane form before the organized collagen lamellae.

The presence of large amounts of granular endoplasmic reticulum in larval epidermal cells suggests that these cells are actively producing some type of proteinaceous material for export from the cell. Because the basement membrane is forming at this time it seems possible that

protein produced in the granular endoplasmic reticulum is being transported to the cell surface via membrane-bound vesicles, crosses the cell membrane by a pinocytotic process, and there forms the basement membrane. The apparent reduction of granular endoplasmic reticulum in adult epidermal cells adds strength to this hypothesis, but is in no way confirmatory.

Electron-dense granules of unknown nature are located in the subepidermal space, between the basal cells and the basement membrane. These granules are most prominent in adult skin (Figure 23), but are also present in the larva (Figure 13). An adepidermal globular layer is described by Kelly (105) in the larval newt, *Taricha torosa*. This author states that these granules are lipid-mucopolysaccharide complexes of obscure function that disappear in the adult newt. In this teleost species the granules are small in the larva and large in the adult, suggesting that they may differ from similar structures in the newt.

Morphologically, dermal collagen in the fish is similar to collagen in other phyla (from sponges to mammals [75]). The collagen fibers have the typical major periods with 600 to 700 Å spacing, and are grouped into multilamellar arrangements. The fibers within a particular lamella are parallel, and the fiber axis of each lamella is approximately 90° out of phase with the lamella immediately above and below it, thus giving the dermal collagen a structure very similar to plywood.

The fibroblasts contain a large amount of dilated granular endoplasmic reticulum and numerous smooth-walled vesicles. This is in agreement with theories that tropocollagen molecules are produced in the granular endoplasmic reticulum and are transported to the cell surface

via vesicular elements (see page 10 of the Introduction).

Perhaps the most interesting observation is that the fibroblasts seem to be located between the dermal lamellae rather than within the lamellae (Figures 37-39). It follows that the collagen fibers adjacent to one surface of the fibroblast are oriented at 90° to the collagen fibers arising from the opposite cell surface. The fibroblasts may thus be thought of as polarized, so that collagen fibers deposited on one side of the cell are 90° out of phase with respect to the fibers being deposited on the other side of the cell. Scales are exceptional in that fibroblasts are not seen between the collagen lamellae. In the scale the fibroblast-like scale-forming cells are only observed along the edges, not within the scale.

The matrix between the collagen bundles is relatively electron lucent, but at higher magnifications contains finely filamentous material. The filaments appear to be attached to the collagen fibers, and in some cases join one collagen fiber to another (Figure 40). It is suggested that the function of the filaments is to act as attachment sites and thereby maintain the collagen fibers in parallel orientation and preventing them from sliding along one another.

While most authors (92, 131, 132, 138) believe that the scale is of mesodermal origin, and is bone-like in structure, there is some disagreement (124). The present study supports the mesodermal origin and bone-like structure of teleost scales, and suggests that the scale is composed of three components: 1) collagen fibers which are produced by adjacent scale-forming cells previously called scleroblasts or osteoblasts; 2) a matrix between the collagen fibers that may be composed of

the albuminoid material termed "ichthylepidin"; and 3) hydroxyapatite crystals which are deposited in the matrix between the collagen fibers, and also within the collagen fibers themselves.

The increasing diameter of the collagen fibers in the direction of the interior of the scale agrees with hypotheses which propose that collagen fibers grow by the accretion of tropocollagen monomers onto the pre-existing fibers in extracellular locations (154). Such hypotheses are also supported by other observations that collagen fibers form in solutions of tropocollagen in cell-free systems (76).

Desmosome-like structures are common between epidermal cells in this species (215) and are also seen between the fibroblast-like cells that appear to form the scales. Others have observed desmosomes between collagen-producing cells in bone, tendon, and tooth pulp (7, 45, 73). Desmosomes are not observed in any other type of dermal mesenchymal cell, including fibroblasts. The significance of the desmosomes between adjacent scale-forming cells is not clear. They may offer greater cell-to-cell attachment strength as in the epidermis, or they may help maintain a permeability barrier requisite to the deposition of calcium salts.

Studies of the amino acid composition of ichthylepidin by Seshaiya, Ambuhabai, and Kalyani (184) suggest that this substance may actually be related to collagen. Electron microscopy of collagen-extracted scales might be expected to clarify the location of the ichthylepidin.

Determinations of the percentage composition of the mineral residues show that the inorganic portion of teleost scales is principally calcium phosphate and calcium carbonate (138). In this regard, Norris, Chavin, and Lombard (136) observed that scales retain radioactive calcium and

strontium up to 22 days. This evidence shows that the scales do contain calcium, but does not confirm that the electron-dense crystals seen in Figures 55 and 56 are in fact hydroxyapatite. Confirmatory x-ray diffraction studies have not been forthcoming to date. In studies on dentinogenesis, Takuma (197) noted that calcium salts of teeth are soluble in the water which is used to process the sections for electron microscopy. This may account for the low electron density of the central portions of the calcified areas in the scales observed in the present study (Figures 55 and 56).

Scales increase in size by addition of material in two locations. The scales increase in diameter by deposition of material at the periphery and they increase in thickness by addition along the basal and perhaps the superficial surfaces (138). The observation that the collagen in the periphery of the scale is deposited in a random or disorganized fashion suggests that growth in diameter may be accomplished by a different mechanism than the growth in thickness. Except for the extreme periphery there is no evidence of any disorganization of collagen fibers in the scale, suggesting that some type of reorganization has occurred. Similar reorganization in connective tissues has been described following wounding in amphibia (212).

The flathead sole, *Hippoglossoides elassodon*, appears to have two or possibly three pigment cell types: melanophores, iridophores (guanidophores), and possibly lipophores. The melanophores and cells interpreted as lipophores are most numerous in the outer portion of the dermis, superficial to the scales and just deep to the epidermis on the pigmented, eye-containing, dorsal side of the fish. Iridophores are

more numerous on the white, blind, ventral side. Iridophores are located in the outer dermis as are the other chromatophores on both sides of the fish, but on the blind side they appear to be more numerous in a zone just deep to the dermis between the muscle and the lower dermis. They are also occasionally seen in the fibrous connective tissue between muscle bundles.

At present there are at least two hypotheses for the mechanism of melanosome aggregation and dispersion in teleost fishes. One hypothesis is based on the presence of a basket-like meshwork of 80 Å diameter fibrils in the melanophores of *Lebistes reticulatus* (47). Another hypothesis is based on the presence of microtubules in *Fundulus heteroclitus* (18). The apparent lack of cytoplasmic filaments in the peripheral parts of both larval and adult melanophores suggests that the first mechanism is not operating in *Hippoglossoides elassodon*. The second mechanism is perhaps a possibility because microtubules are seen within larval melanophores (Figure 14)*.

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*Microtubules have been described as a constant finding in non-dividing cells of many different tissues in various species (10, 18, 25, 42, 146, 180). However, in the present work they have only been observed so far in the glutaraldehyde-osmium fixed larval cells (dividing epidermal cells and melanophores). Microtubules have not been seen in adult tissues, unless they are fixed in glutaraldehyde, suggesting that the microtubules are not preserved by the other fixation methods used in this study.

SUMMARY

The normal larval and adult skin of the marine teleost, *Hippoglossoides elassodon* (Pleuronectidae), are described by light and electron microscopy. The adult epidermis consists of 5 to 9 layers of cells, the majority of which are squamous cells and the minority are mucous cells. The squamous cells are characterized by their morphological similarity at all cell levels within the epidermis and lack of keratinization. Numerous desmosomes and associated cytoplasmic filaments are present. The mucous cells accumulate mucous droplets in vacuoles of Golgi origin and are observed apparently in the process of releasing their contents at the surface. The larval epidermis is similar to the adult except that it is generally only two cells in thickness and contains a large amount of granular endoplasmic reticulum.

The dermis consists of alternating lamellae composed of typical collagen fibers. Scales are located in the superficial loose region of the dermis and appear to be entirely of mesodermal origin. Morphologically, scales are similar to bone and teeth in that a cellular layer apparently deposits collagen fibers which increase in diameter in extracellular locations. The evidence suggests that the matrix between the fibers becomes more concentrated, finally crystallizing and depositing material resembling hydroxyapatite between and within the collagen fibers. Pigment cells are of three types: melanophores, iridophores (guanophores), and lipophores.

LIST OF ABBREVIATIONS

A----Anal opening

B----Brain

BC---Basal cells

BM---Basement membrane

CAL--Calcified portion of scale

Cap--Capillary

CF---Cytoplasmic filaments

Ch---Chromosomes

CM---Cell membrane

Co---Collagen

COL--Collagenous portion of scale

D----Desmosome

DC---Dense connective tissue

DF---Dorsal finfold

E----Epidermis

EC---Endothelial cell

ER---Endoplasmic reticulum

F----Fibroblasts

G----Golgi complex

GC---Guanin clefts

H----Heart

HAC--Hydroxyapatite crystals

i----Intermediate line

I----Inclusion

IC---Intermediate cells

IEM--Intraepidermal melanophore

IS---Intercellular space

L----Lymphocyte

LC---Loose connective tissue

Li---Lipophore

m----Mitochondria

M----Melanophore

MC---Mucous cell

MD---Mucous droplets

Mf---Myofibrils

Mt---Microtubules

Mv---Microvilli

My---Myomeres

N----Nucleus

Nc---Notocord

NM---Nuclear membrane

NP---Nuclear pore

NT---Neural tube

Nu---Nucleolus

OC---Otic capsule

OD---Outer dermis

P----Cytoplasmic plaque

PF---Pectoral fin

R----Ribosome

RBC--Erythrocyte

S----Scale

SC---Superficial cells

SS---Subepidermal space

v----Blood vessel

V----Vesicle

VF---Ventral finfold

WBC--Leukocyte

YS---Yolk sac

ZO---Zonula occludens

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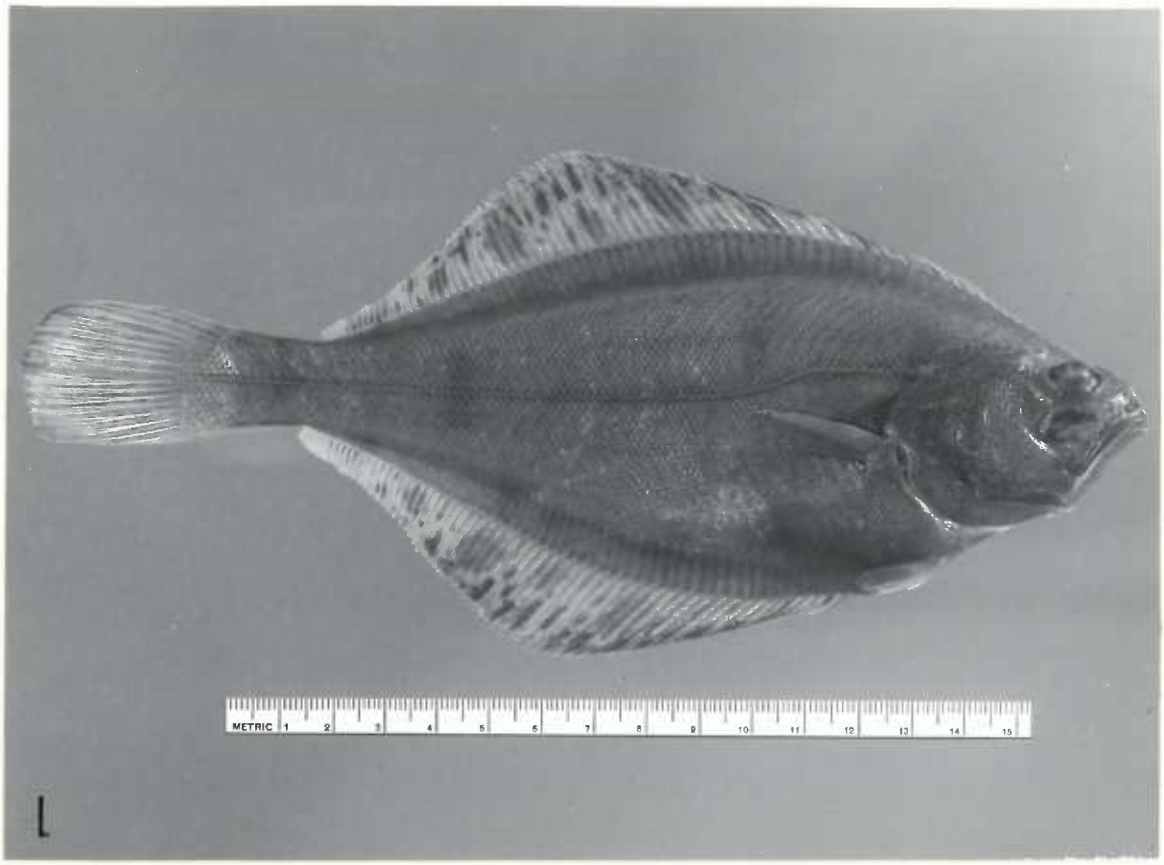
Figure 1:

Right, eyed or pigmented side of a 2-year-old adult female flathead sole, *Hippoglossoides elassodon*.

Figure 2:

Left, blind or non-pigmented side of same fish.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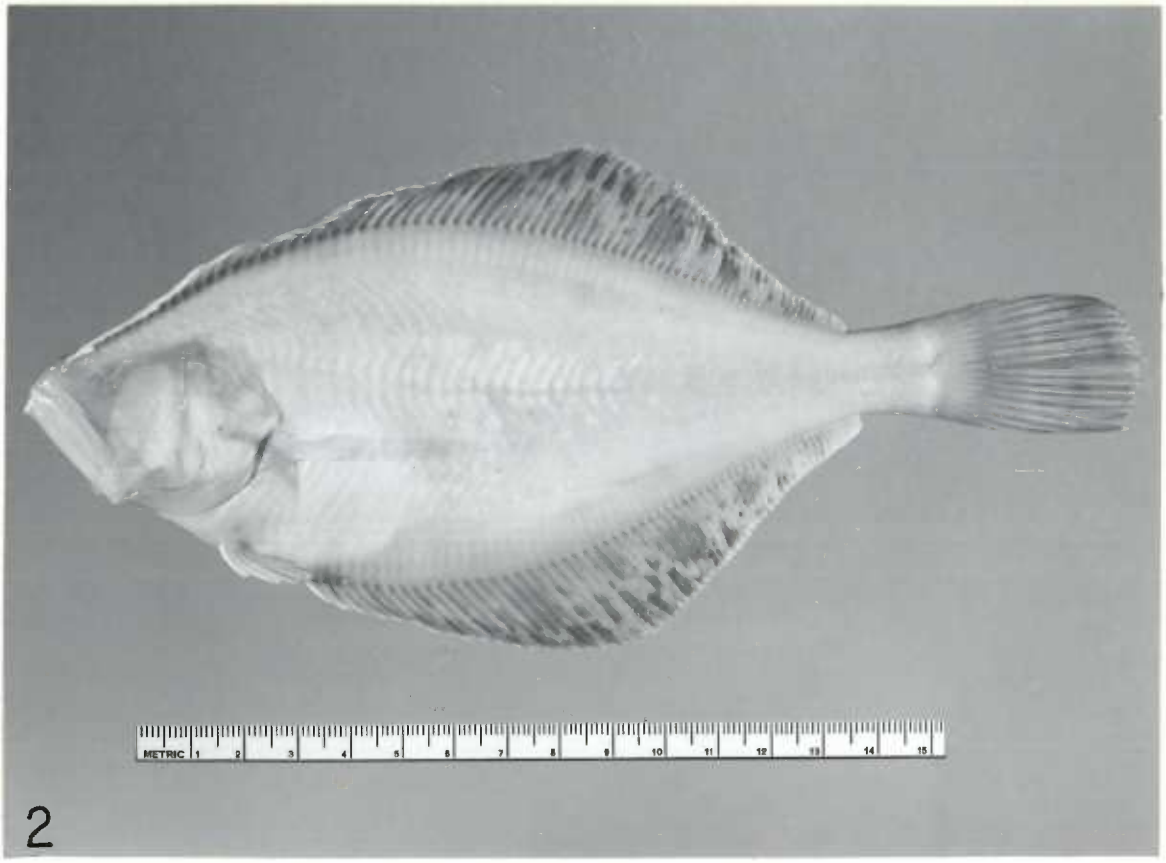
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Figure 3:

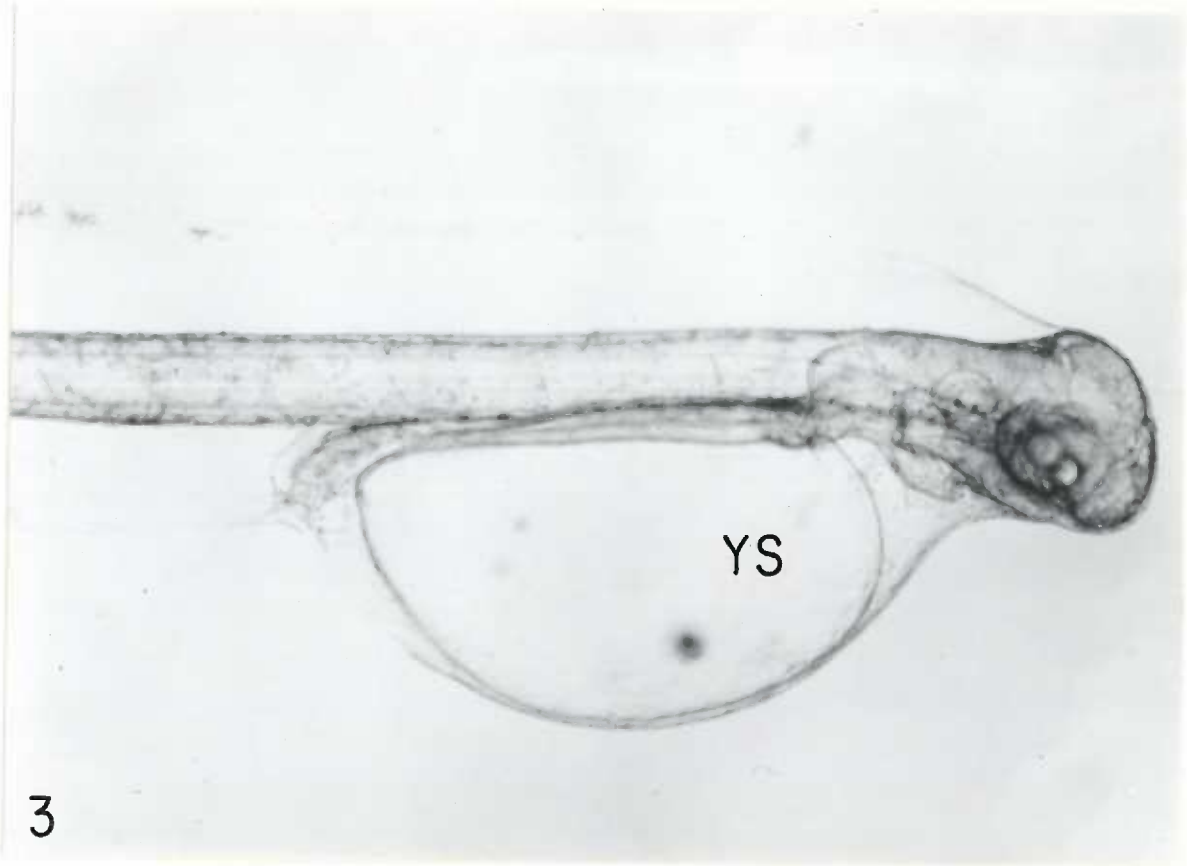
Photomicrographs of living, newly hatched larva of *Hippoglossoides elassodon*. Yolk sac (YS).

Living larva in sea water, 51X.

Figure 4:

Dorsal view of the head of a living, *H. elassodon* larva, 7 days after hatching. Note the marked increase in amount of retinal pigment as compared with the larva in Figure 1. Otic capsule (OC) contains three ossicles (arrows). Pectoral fin (PF).

Living larva in sea water, 138X.



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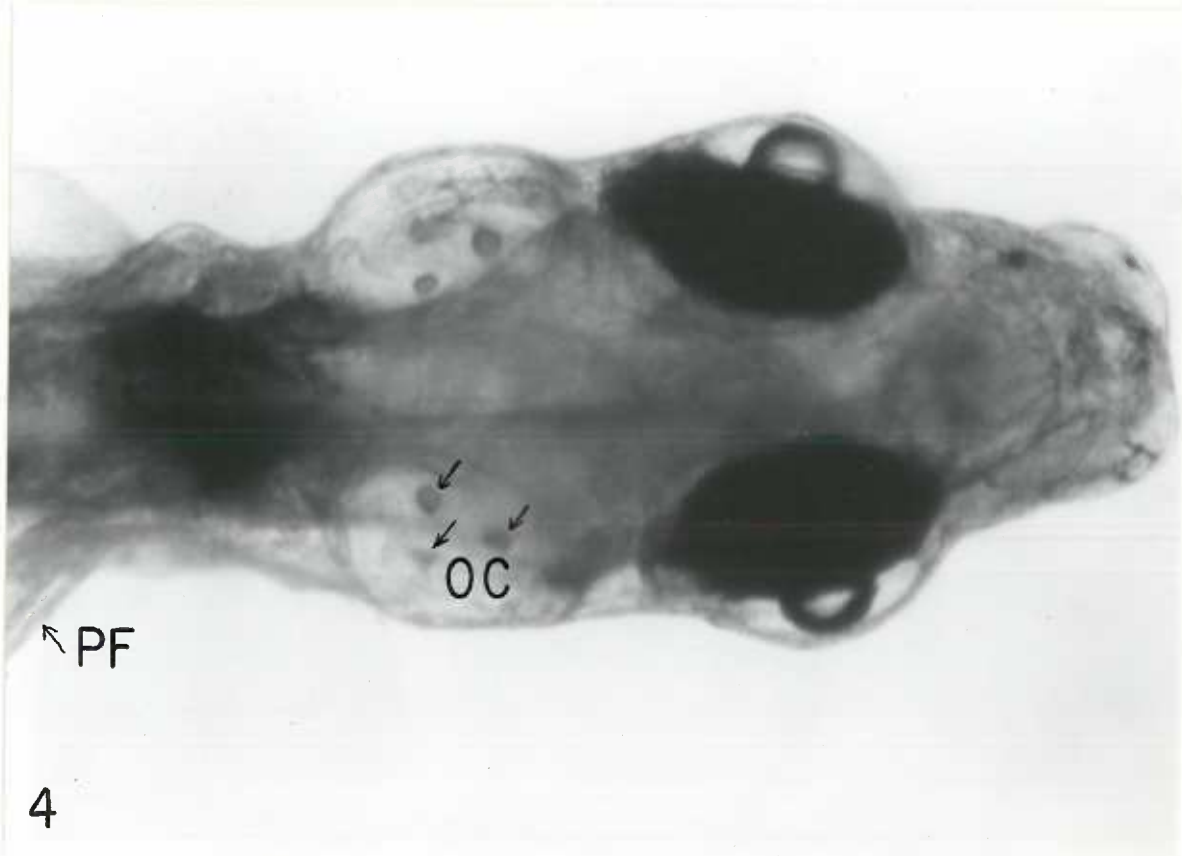


4

FIGURES 5, 6, AND 7

Photomicrographs of newly hatched larva as seen in Figure 3. (138X)

Figure 5:

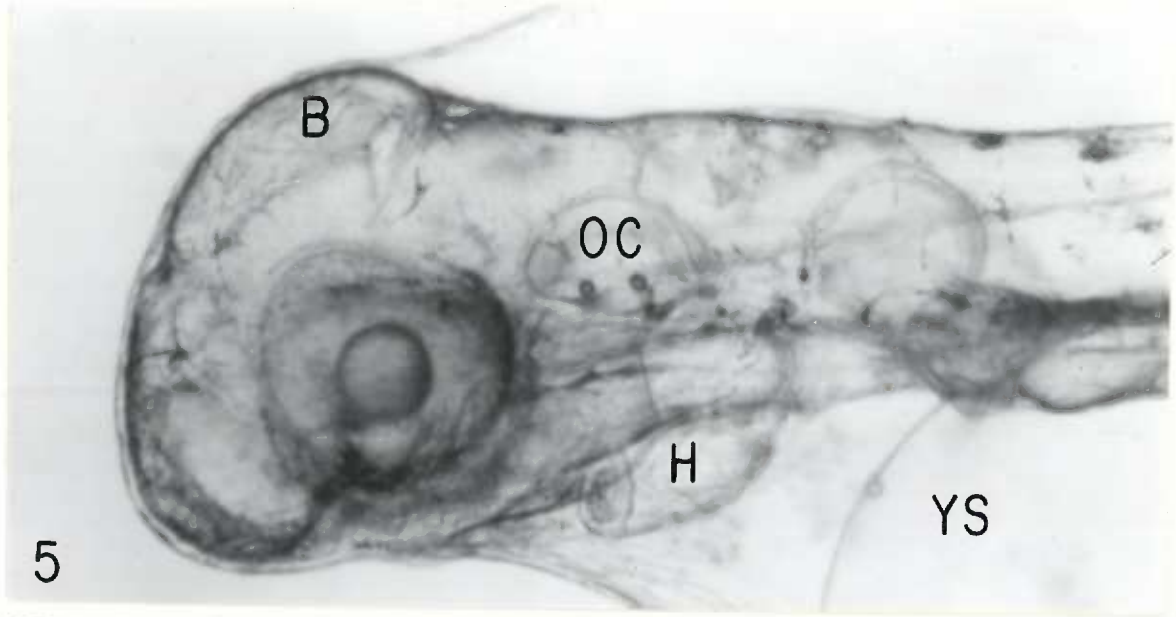
Head region with the brain (B), eye, heart (H), otic capsule (OC) with ossicles, yolk sac (YS).

Figure 6:

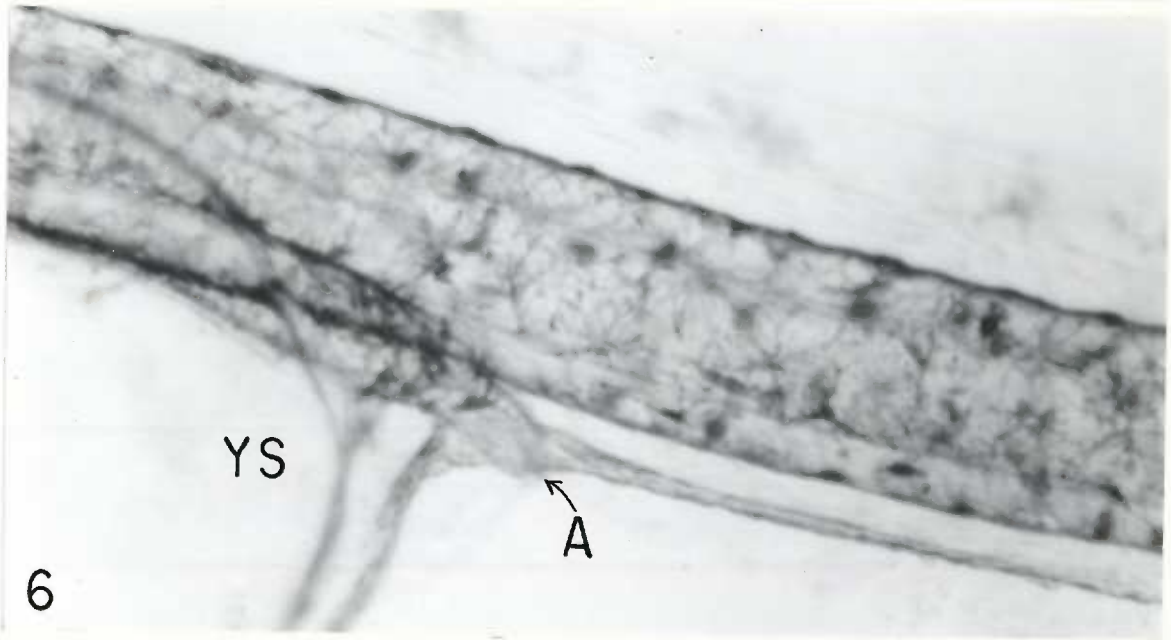
Caudal portion of the gastrointestinal tract and the anal opening (A). Yolk sac (YS).

Figure 7:

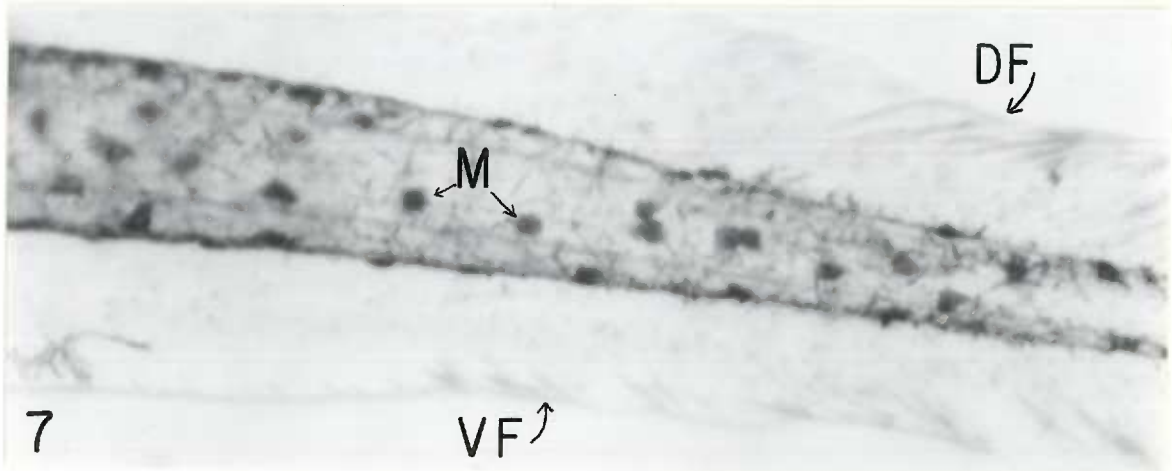
Dorsal (DF) and ventral (VF) finfolds. Stellate melanophores (M) measure approximately 0.07 mm in diameter.



5



6

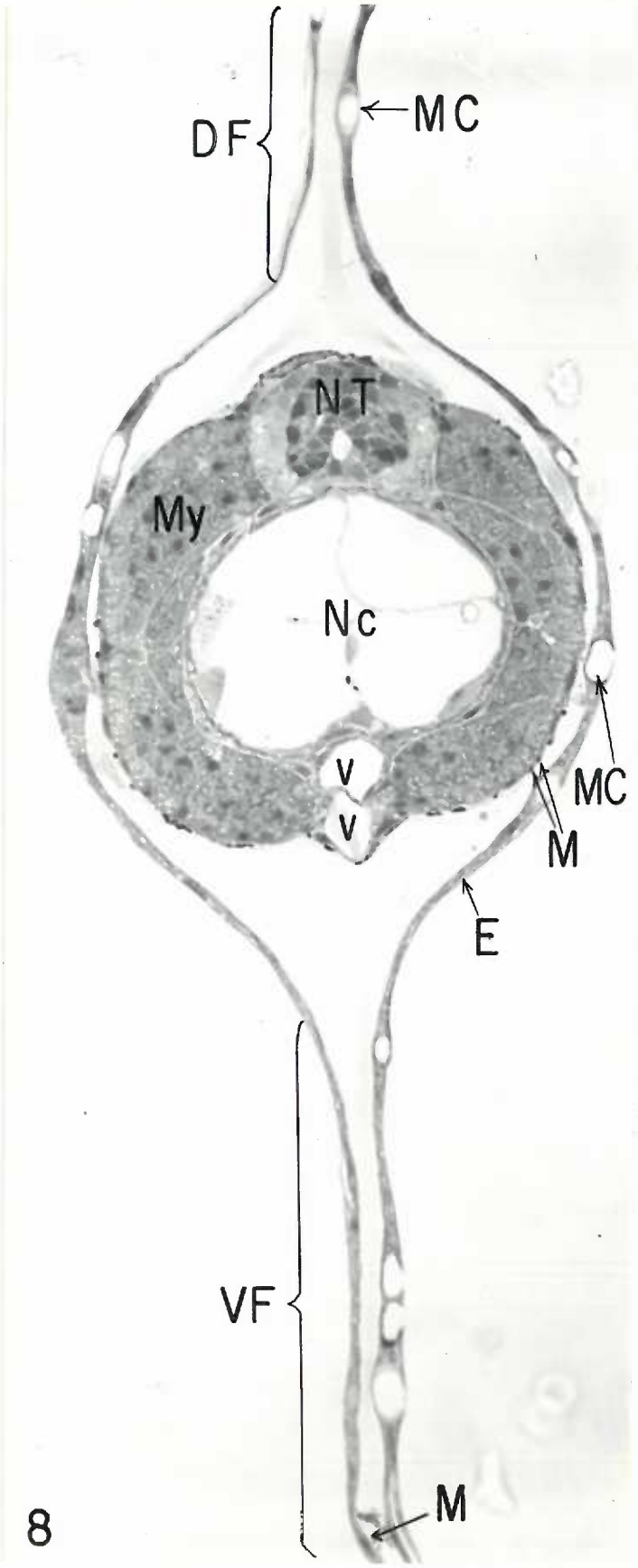


7

Figure 8:

Light micrograph of cross section through the tail region of a newly hatched (1 day) larva. Electron micrographs of larva which follow are based on this and are similar sections through the tail or trunk regions. Epidermis (E), mucous cells (MC), neural tube (NT), notocord (Nc), blood vessels (v), myomeres (My), dorsal (DF) and ventral (VF) finfolds. Melanophores (M).

Lentz fixative, araldite embedded, 1 μ section stained with Mallory's azur II and methylene blue (171). 550X.



8

Figure 9:

Electron micrograph of the full thickness of body wall in the tail region of a 1-day-old *H. elassodon* larva. The epithelium (top of picture) is from 5 to 8 μ in thickness and usually consists of two cell layers with a thin basement membrane (BM) adjacent to the inner cell membrane. Granular endoplasmic reticulum (ER) and large mitochondria (m) are prominent features of these cells.

An early mucous cell with two mucous droplets (MD) is seen within the thicker portion of the epidermis.

Fibrillar and granular profiles in the space beneath the basement membrane may represent early collagen formation (compare with Figure 13).

Mesodermal structures, predominantly muscle cells, are located in the center of the picture.

A cell interpreted as a lipophore (Li) is seen in the space deep to the basement membrane.

Lentz and Trinkaus' fixative, lead citrate and uranyl acetate stain. 8340X.

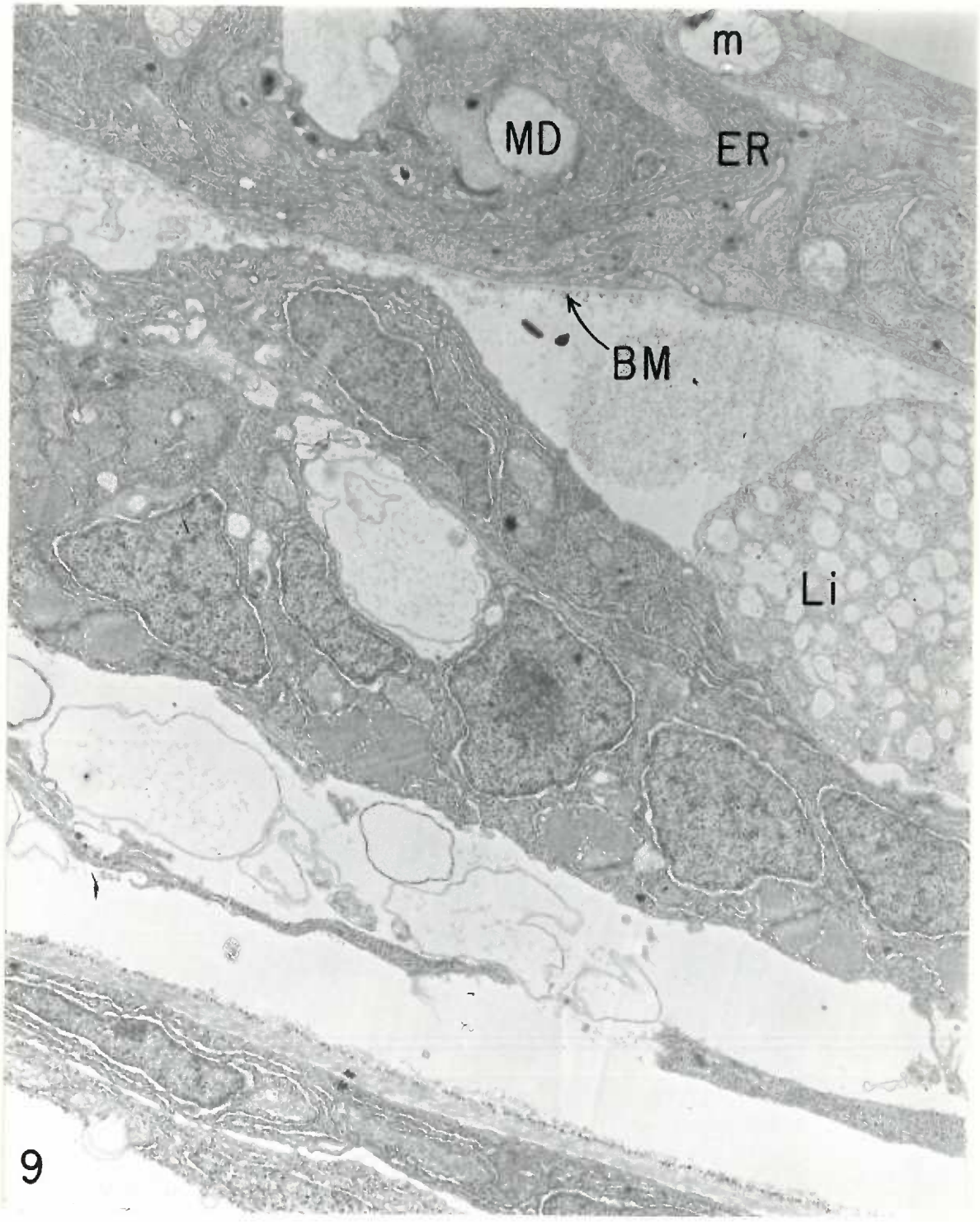

m
MD
ER
BM
Li
9

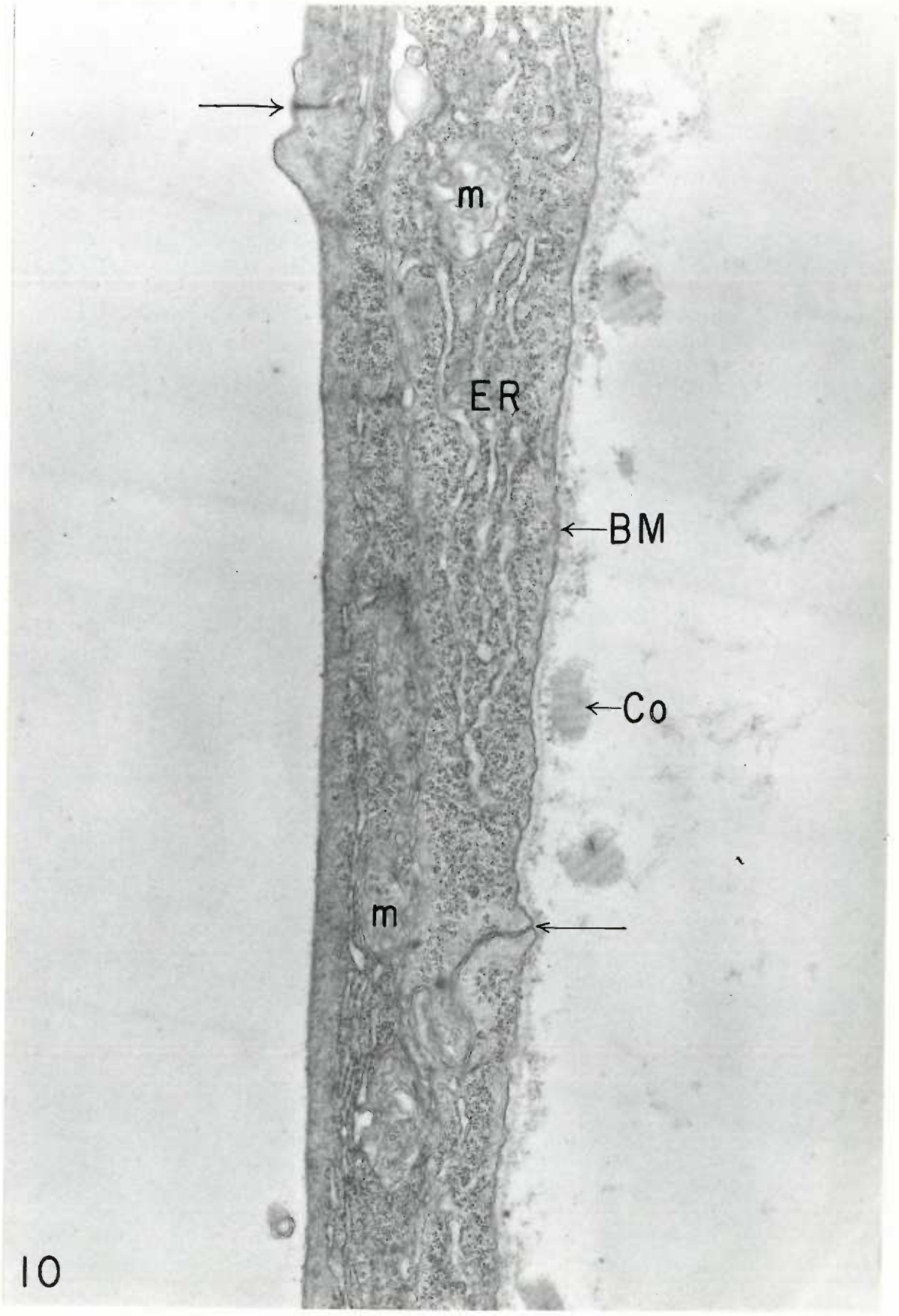
Figure 10:

Electron micrograph of isolated portion of 1-day-old larval epidermis. The epidermis is two cell layers thick. The epidermal cells are broad and flat with marked interdigitation of the membranes between adjacent cells. A tight junction (zonula occludens [48]) occurs at the junction of the intercellular space with the external surface (arrow, upper left). The intercellular space appears open toward the subepithelial space (arrow, lower right).

The surface of the epithelium (right) lacks microvilli (compare with Figures 30 to 32).

Basement membrane (BM), early collagen formation (Co), granular endoplasmic reticulum (ER), mitochondria (m).

Lentz and Trinkaus' fixative, lead citrate and uranyl acetate stain. 28,400X.



10

Figure 11:

Electron micrograph of newly hatched larva epidermis. The epidermis is again two cell layers in thickness with the intercellular space (IS) located just deep to the nucleus. Cytoplasmic filaments (CF), approximately 80 Å in diameter, are most numerous just beneath the epidermal surface (upper left), but they are also seen in the deeper cytoplasm (lower right).

Granular endoplasmic reticulum (ER) and granules interpreted as ribosomes are prominent. A desmosome-like structure (D) is seen between adjacent epidermal cells (upper right).

The nucleus (N) has an oval or flattened appearance. It has a smooth nuclear membrane and rather homogeneous nuclear material.

Lentz and Trinkaus' fixative, lead citrate and uranyl acetate stain. 69,000X.

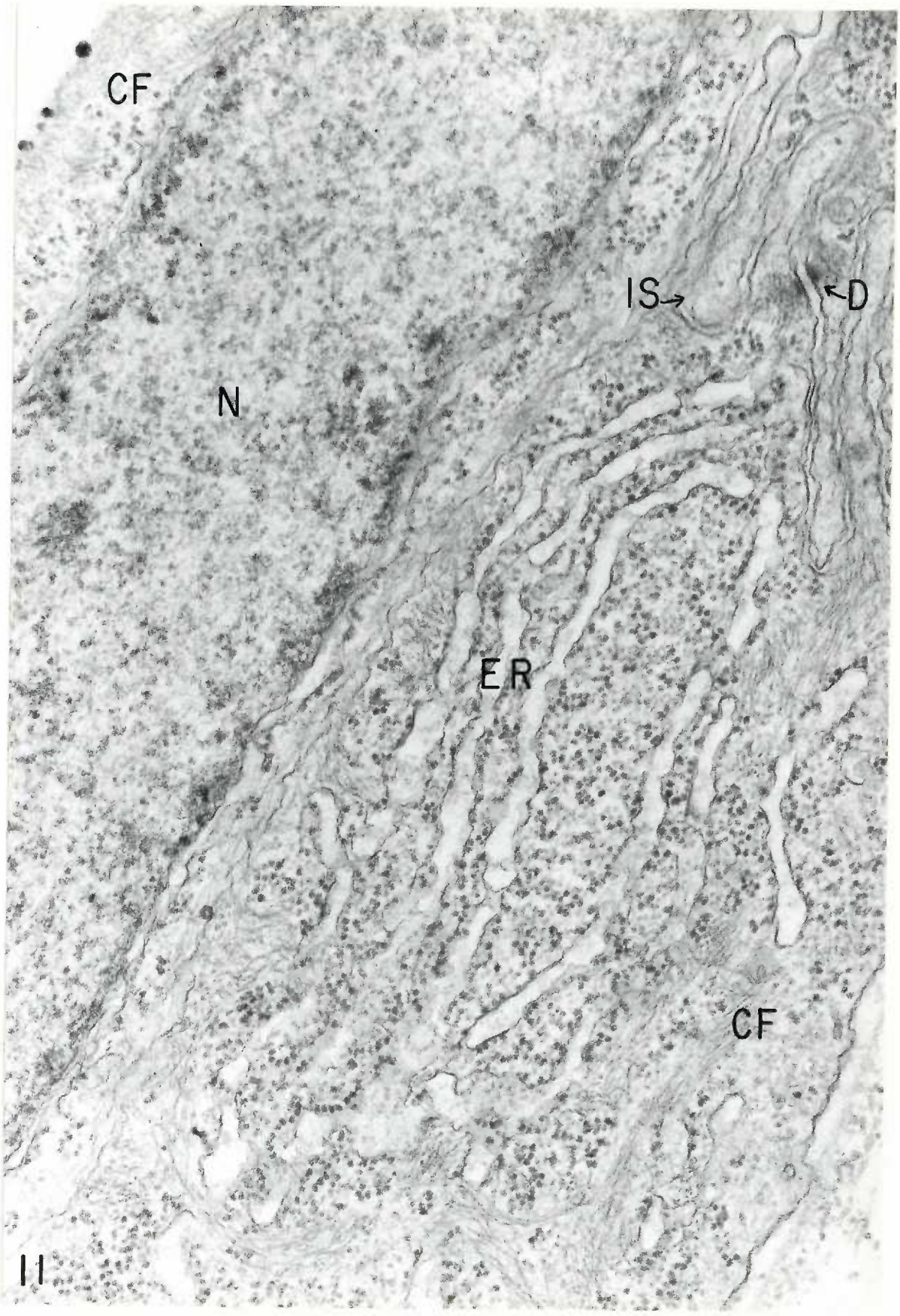

CF
IS
D
N
ER
CF
11

Figure 12:

Electron micrograph of newly hatched larval epidermis. The outer surface is seen to the upper left and the basement membrane is to the right. The basal cell is in mitosis. Chromosomes (Ch). The nuclear membrane (NM) is incomplete. Microtubules (Mt) which measure 250 Å in diameter and over 250 mμ in length are seen within the nucleus. These microtubules are probably spindle fibers. Cytoplasmic filaments are seen in the dividing basal cell and in the surface, epithelial cell.

Part of a mucous cell containing mucous droplets (MD) and a portion of nucleus (N) is seen in the lower left. Note the ribosomes and mitochondrion (m) within the cytoplasm of the mucous cell.

The basement membrane (BM) is approximately 500 Å in thickness and is less electron dense than in the adult (compare with Figure 23). Small collagen fibers (Co), approximately 150 Å in diameter, are located in the space beneath the basement membrane.

Lentz and Trinkaus' fixative, lead citrate and uranyl acetate stain. 36,100X.

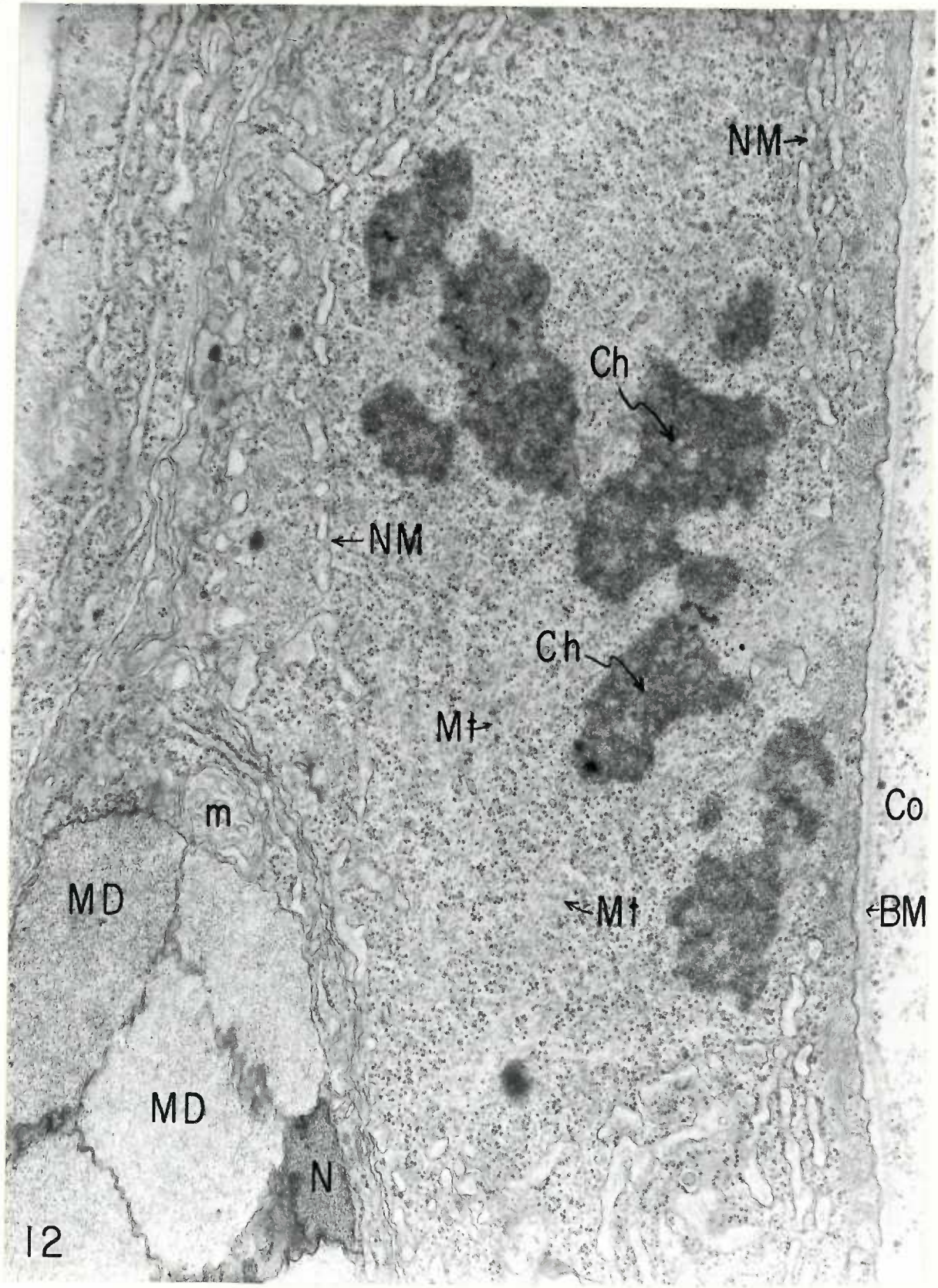

NM
Ch
NM
Ch
Mt
m
Co
MD
Mt
BM
MD
N
12

Figure 13:

Electron micrograph of two adjoining cells in the basal portion of the epidermis of a newly hatched larva.

The basement membrane (BM) appears composed of fine filaments and is approximately 250 Å thick. The basement membrane is separated from the cell membranes by a rather constant space ranging from 200 to 300 Å in width. Within this "subepidermal space" there is a suggestion of the presence of electron-dense granules.

Structures interpreted as collagen fibers (Co), about 150 Å in diameter are forming adjacent to and deep to the basement membrane. In some areas the collagen fibers appear organized into structures with orderly bands. The distance between these bands is approximately 600 Å (double arrows).

Marked interdigitation is seen between the epidermal cells. An agranular vesicle (V) is seen opening into the intercellular space. Desmosome-like structure (D); large mitochondria (m), granular endoplasmic reticulum (ER) and ribosomes (R).

Lentz and Trinkaus' fixative, lead citrate and uranyl acetate stain. 69,000X.

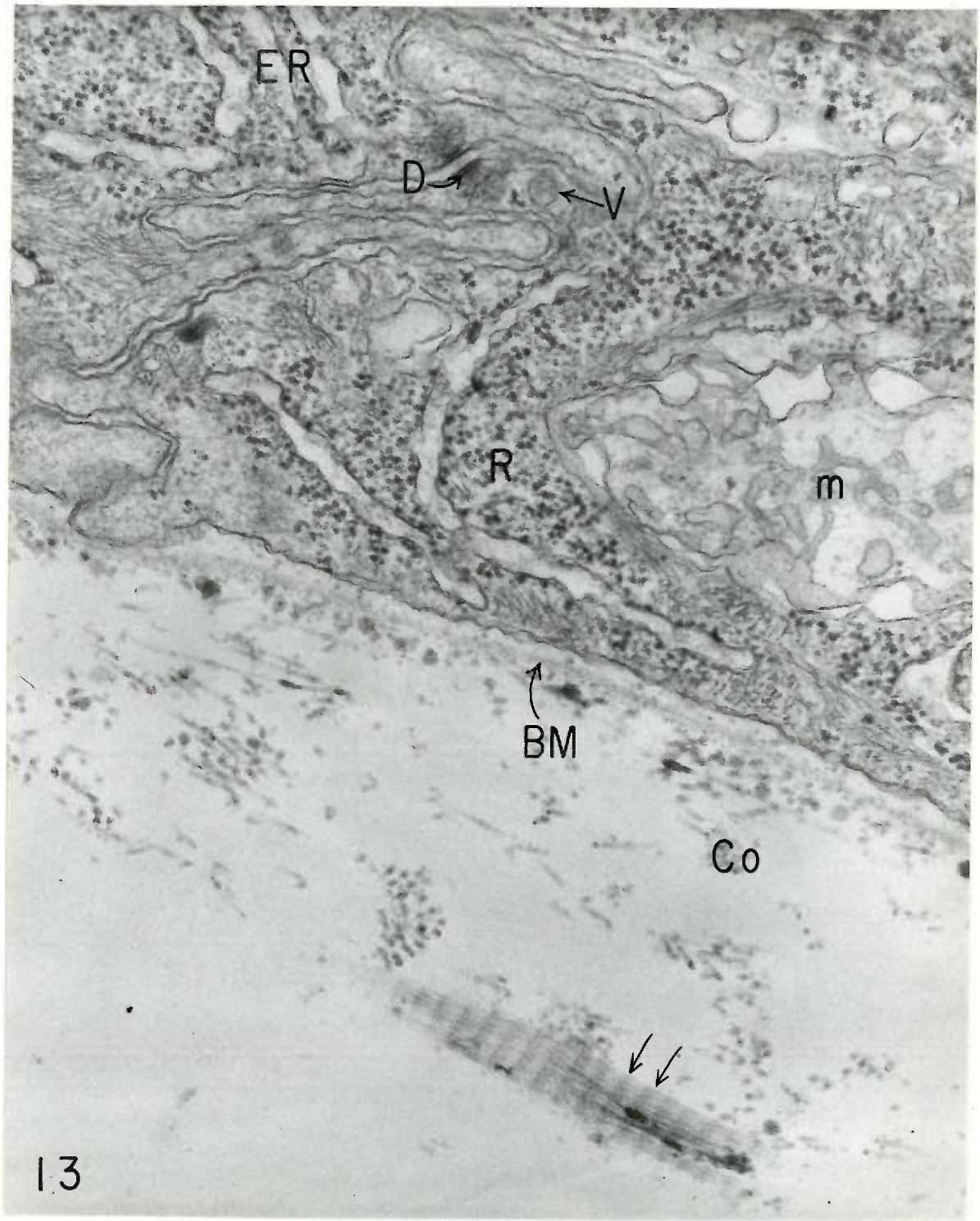
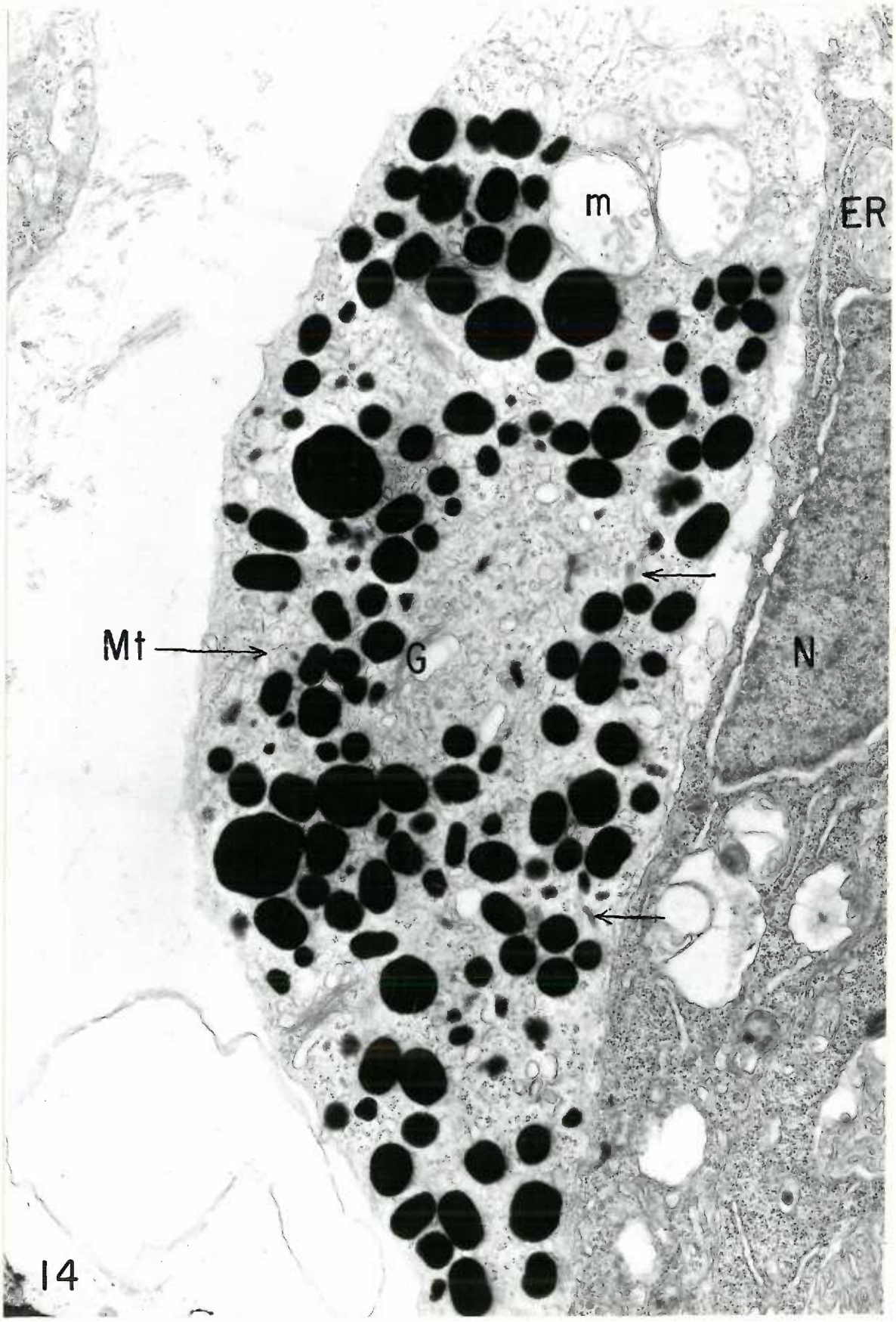

ER
D
V
R
m
BM
Co
13

Figure 14:

Electron micrograph of a micromelanophore in the tail region of a newly hatched larva. The pigment cells are located in the space between the epidermis (left) and mesenchymal cells (right). The melanophore is typified by its dark melanin granules of varying size (the largest in this field measures 650 mμ in diameter). Many smaller granules have a lamellar appearance (arrow). The melanophore also contains small Golgi complexes (G), large mitochondria (m), numerous smooth-surfaced vesicles of variable size, and many ribosomes. There is a suggestion of microtubular structures (Mt) within the cytoplasm.

The mesenchymal cell (right) may be an early fibroblast. The nucleus (N) appears rather homogeneous. The cytoplasm has numerous granules resembling ribosomes and may have a small amount of granular endoplasmic reticulum (ER).

Lentz and Trinkaus' fixative, lead citrate and uranyl acetate stain. 24,400X.



14

Figure 15:

Electron micrograph of the muscle layer in a newly hatched larva. The muscle cells appear to have straight boundaries with a narrow intercellular space (IS). The muscle cell contains numerous large mitochondria (m) and ribosomes. A nucleus (N) and a small Golgi complex (G, upper right) are seen. Myofibrils (MF) are located within the cytoplasm.

The myofilaments (approximately 100 Å in diameter) make up the fine structure of the myofibrils.

Lentz and Trinkaus' fixative, lead citrate and uranyl acetate stain. 24,400X.

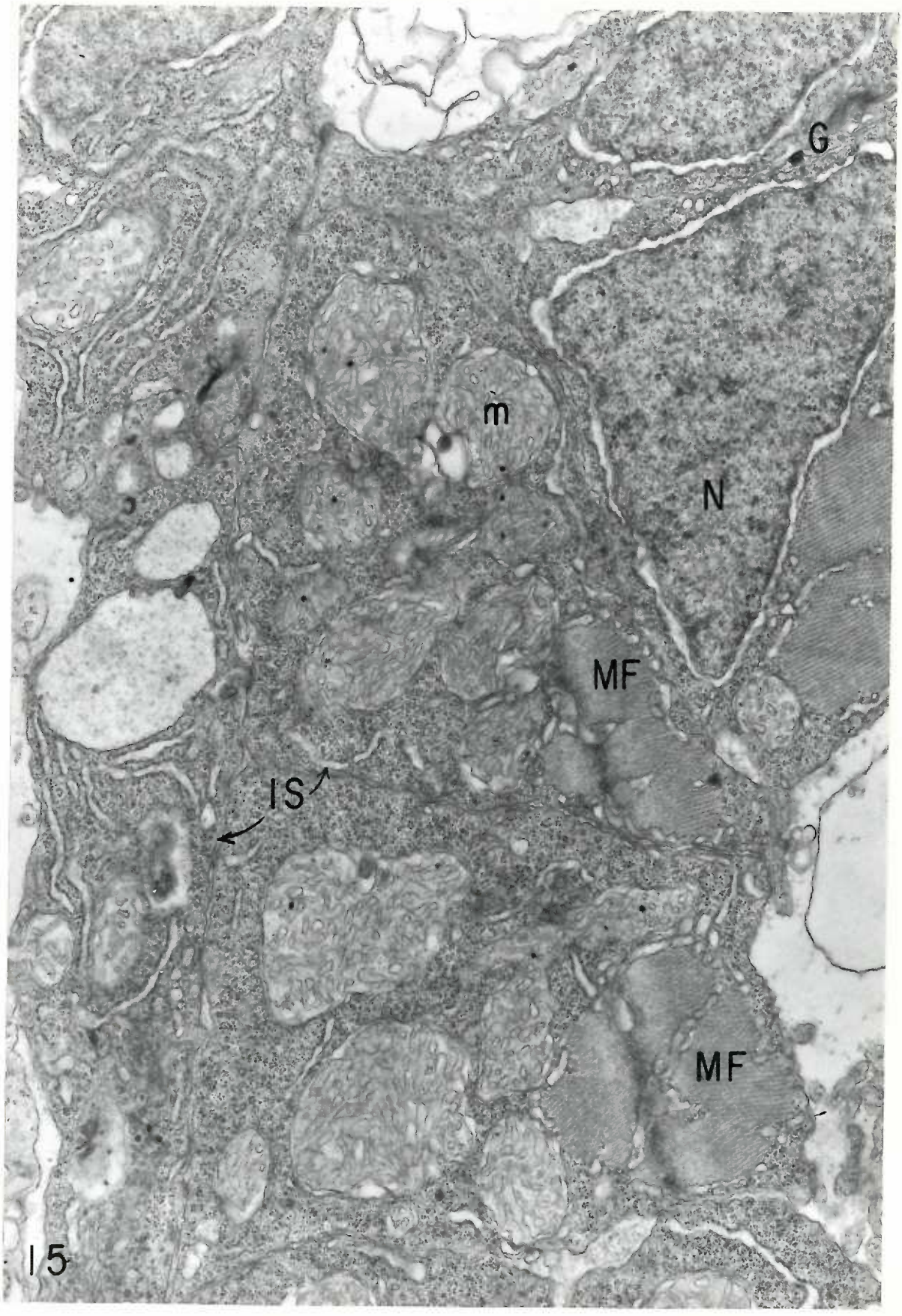

G
m
N
MF
IS
MF
15

Figure 16:

Photomicrograph of araldite-embedded 1 μ section of scale-bearing skin from the pigmented (eyed) side of a 2-year-old (adult) flathead sole, *Hippoglossoides elassodon*. The skin surface is to the left. Starting from the surface and proceeding inward (top to bottom), the structures seen are as follows: epidermis (E), outer dermis (OD) containing melanophores (M), scale (S), loose connective tissue (LC) between scales, scale (S), and dense connective tissue (DC) deep to the scale-containing region.

Boxes numbered 38, 54, 55, and 56 represent areas similar to those shown in corresponding Figures 38, 54, 55, and 56.

Mucous cells (MC), dermal melanophores (M), intraepidermal melanophores (IEM), calcified portions of scale (CAL), uncalcified collagenous portion of scale (COL).

Mallory's azur II and methylene blue stain. 550X.

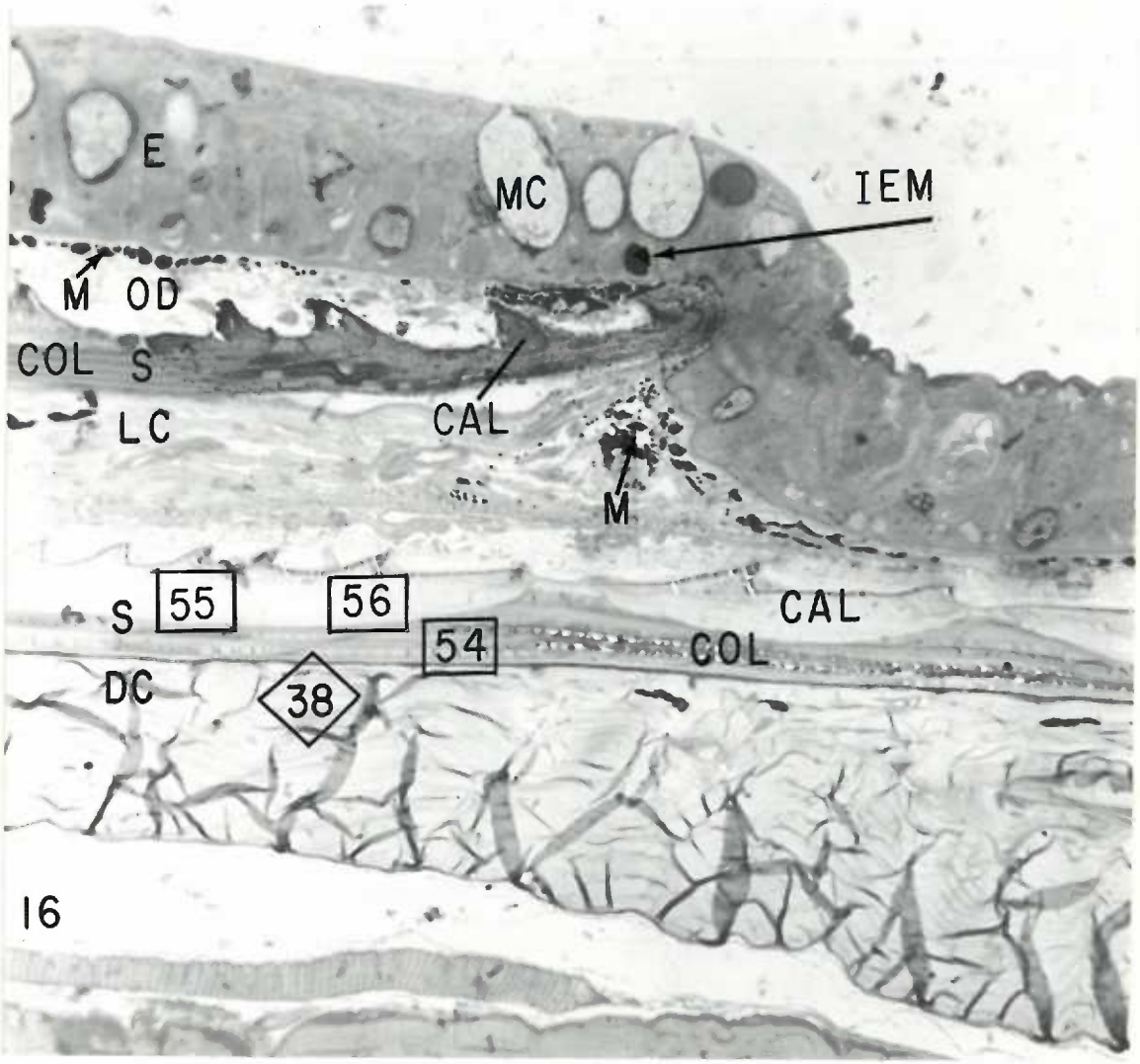

E
MC
IEM
M
OD
COL
S
LC
CAL
M
S
55
56
54
CAL
COL
DC
38
16

Figure 17:

Light micrograph of the skin from the finweb (between the fin rays) of adult *H. elasscdon*. The epidermis (light staining tissue) is approximately 6 cell layers thick and contains one mucous cell (MC).

The dermis (dark staining tissue) is composed of dense collagen with centrally located blood vessels (v).

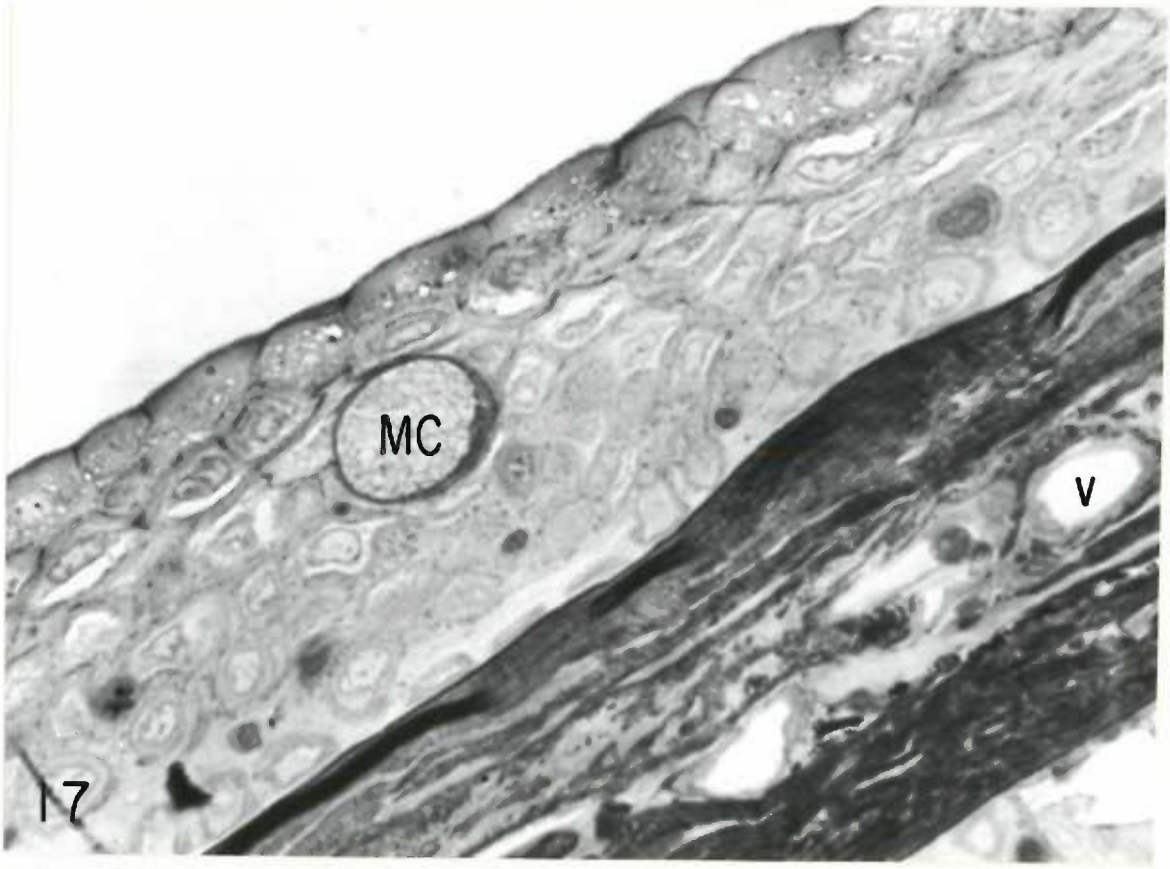
In the lower right hand corner are a few cells from the basal epidermis on the other side of the fin web.

1.33% OsO_4-S-collidine fixed, araldite embedded, 1 μ section, Mallory's azur II and methylene blue stain. 1380X.

Figure 18:

Same as Figure 17 except that mucous cells are more numerous. One is seen discharging its contents at the surface (arrow). Small microvilli (Mv) are seen along the epithelial surface.

1380X.


MC
V
17

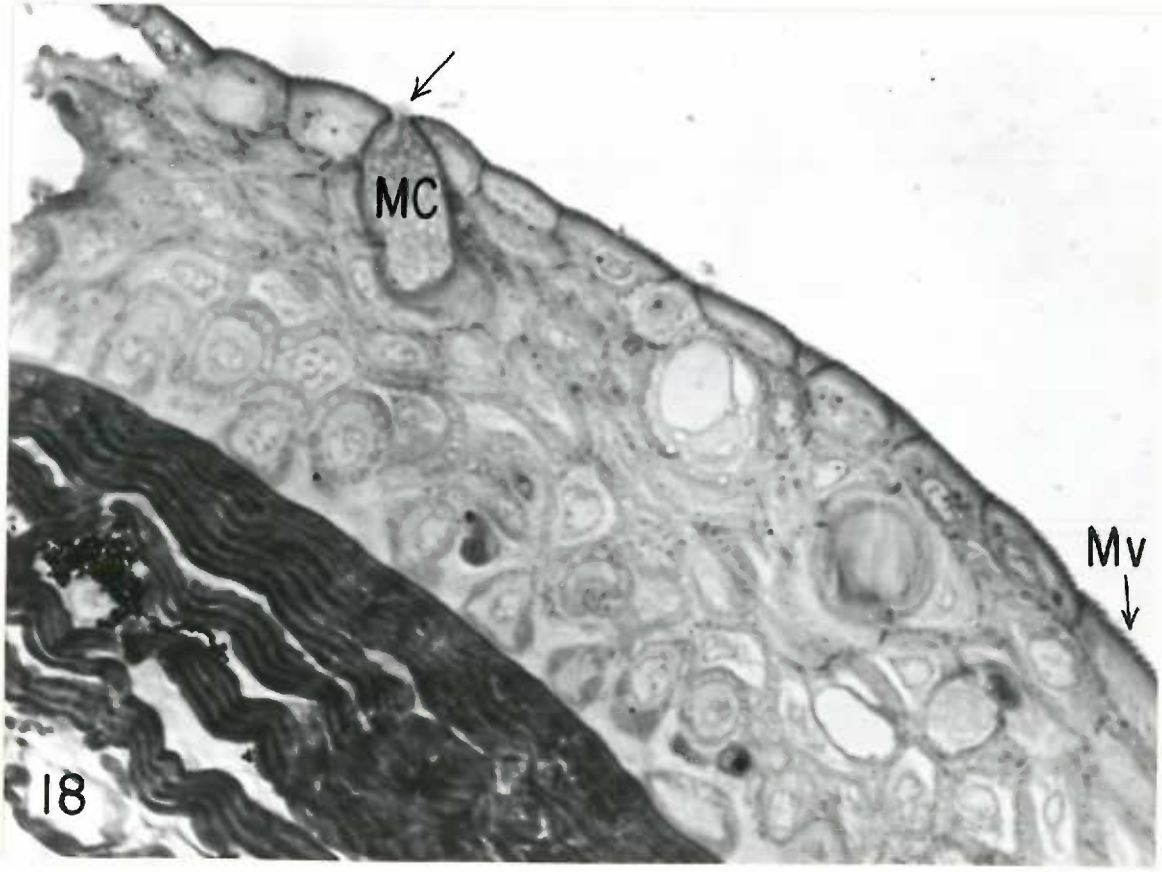

MC
Mv
18

Figure 19:

An electron micrograph of the full thickness of epidermis from the fin web of an adult *H. elassodon*. The basement membrane (BM) and dermal collagen fibers are seen in the lower right of the picture. Basal cells (BC), intermediate cells (IC), and superficial cells (SC) are above and to the left respectively. A mucous cell (MC) is located in the intermediate cell layer.

Note the decrease in the amount of interdigitation and the increase in the number of desmosomes between adjacent cells as the surface is approached. Intercellular spaces (IS) are prominent in the regions of marked interdigitation.

1.33% OsO_4-collidine fixed, lead citrate and uranyl acetate stain. 5470X.

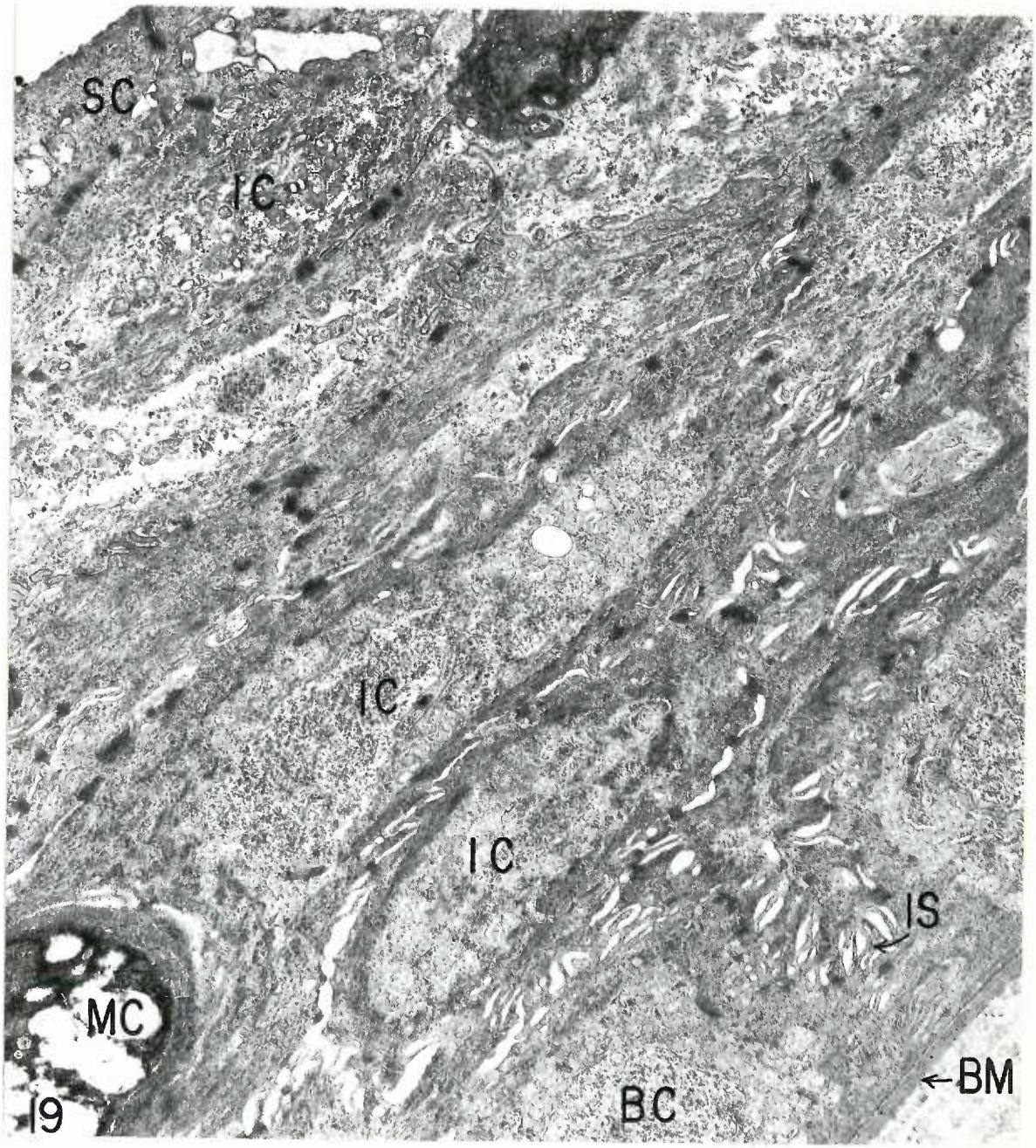

SC
IC
IC
IC
IS
MC
BM
BC
19

Figure 20:

Electron micrograph of a basal cell or cell from the stratum germinativum. The cell membrane (CM) has numerous microvillous convolutions which interdigitate with adjacent cells, and a few small desmosomes (D). The nucleus (N) has an irregular outline. The perinuclear zone of cytoplasm possesses a Golgi complex (G), numerous mitochondria (m), smooth-surfaced endoplasmic reticulum, and ribosomes.

The peripheral zone of cytoplasm contains predominantly cytoplasmic filaments (CF) and ribosomes with a few vesicles. Basement membrane (BM), collagen (Co), intercellular space (IS).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 17,800X.

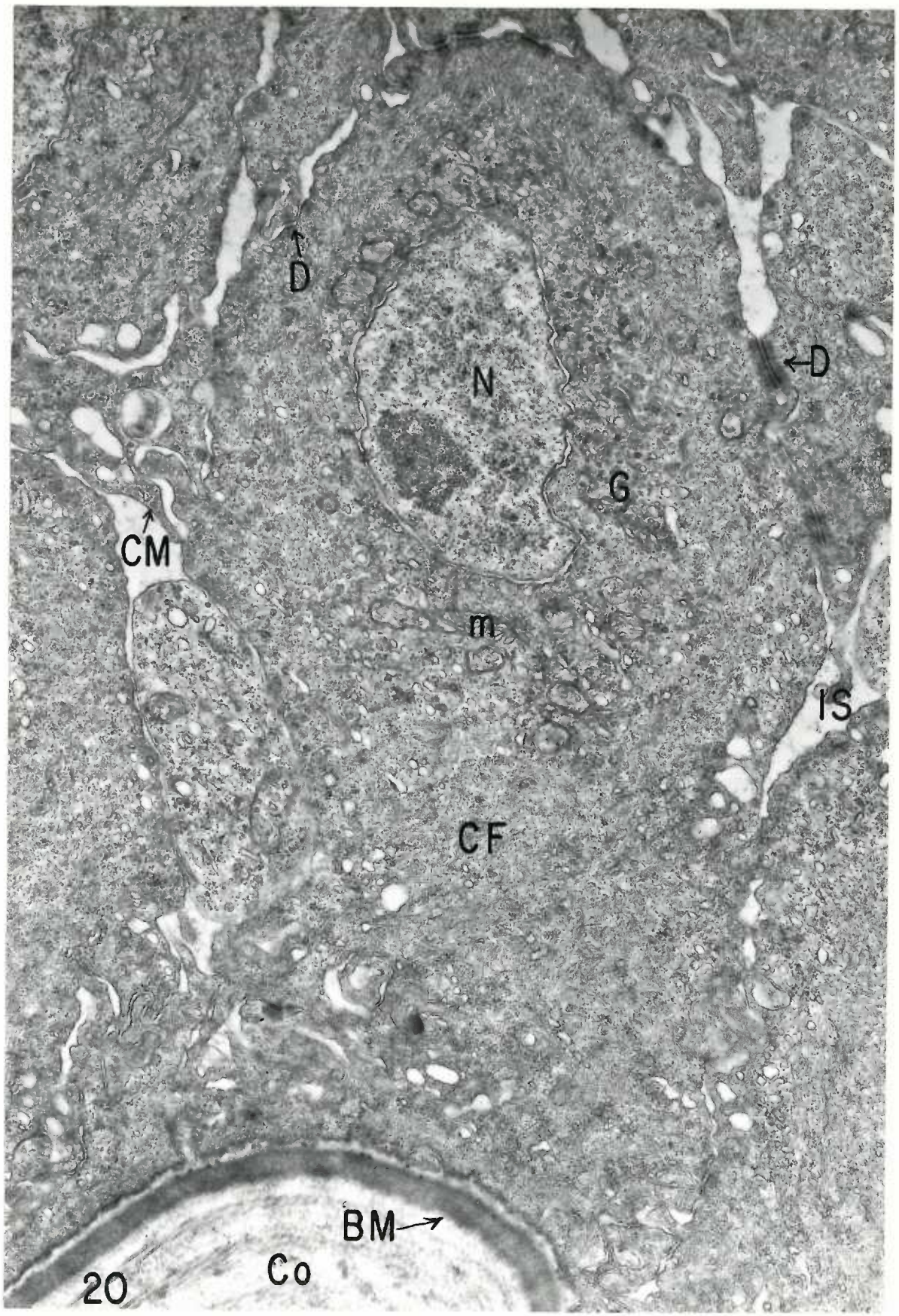
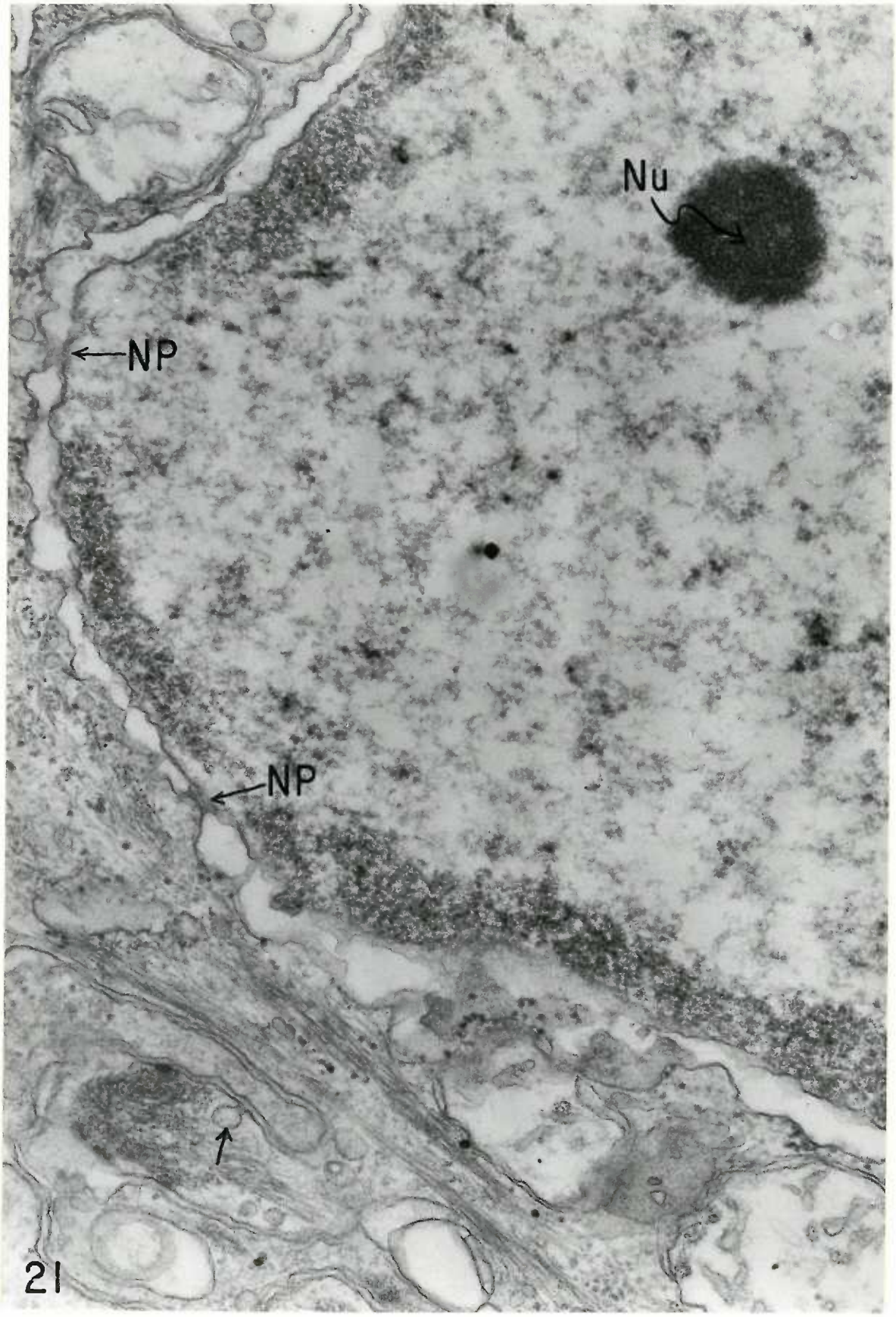

D
D
N
G
CM
m
IS
CF
BM
Co
20

Figure 21:

Electron micrograph of the nucleus of a basal cell. The nucleus contains a nucleolus (Nu) located in the less dense central portion. Nuclear material appears more concentrated around the periphery of the nucleus. There is a prominent space between the nucleus and the cytoplasm. Nuclear pores (NP).

The cytoplasmic membrane is seen in the lower left and a vesicle opens into the intercellular space (arrow).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.

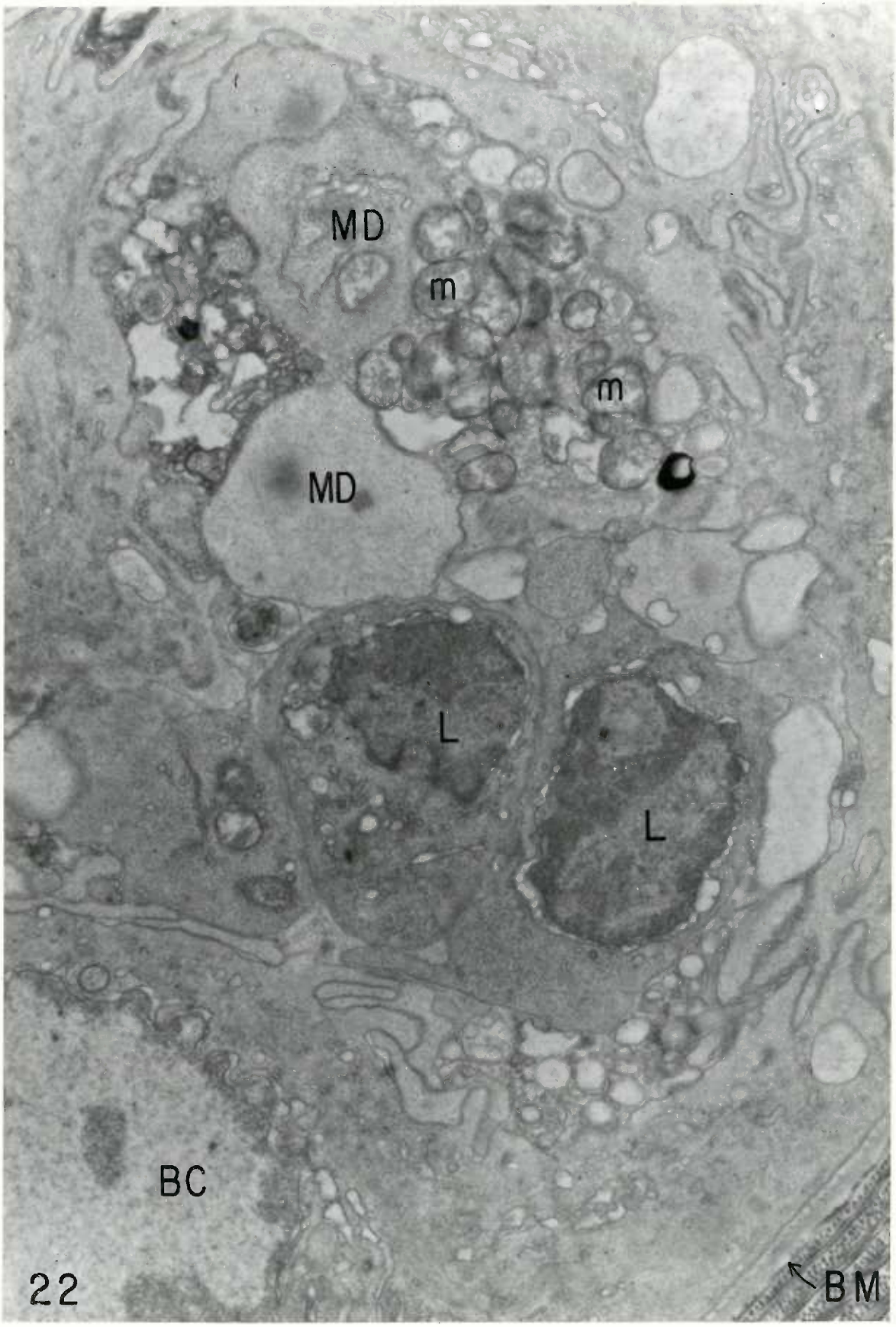


21

Figure 22:

Electron micrograph of the basal region of the epidermis. Two adjacent basal cells (BC) are seen along the basement membrane (BM). Cells interpreted as lymphocytes (L) are located in the intercellular space between these basal cells. An immature mucous cell with mucous droplets (MD) is located above the lymphocytes. Mitochondria (m) are numerous within this cell.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 17,300X.



22

Figure 23:

Electron micrograph of the dermal-epidermal junction. The basement membrane (BM) separates the collagen fibers (Co) in the dermis from the basal epithelial cells (upper left). The basement membrane is from 400 to 600 mμ thick and is separated from the cell membrane of the basal cells by a rather constant space of approximately 500 Å. Within this space are electron-dense granules measuring approximately 200 Å in diameter (arrows). The identity of these granules is unknown. The basement membrane is in close association with the collagen fibers.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.

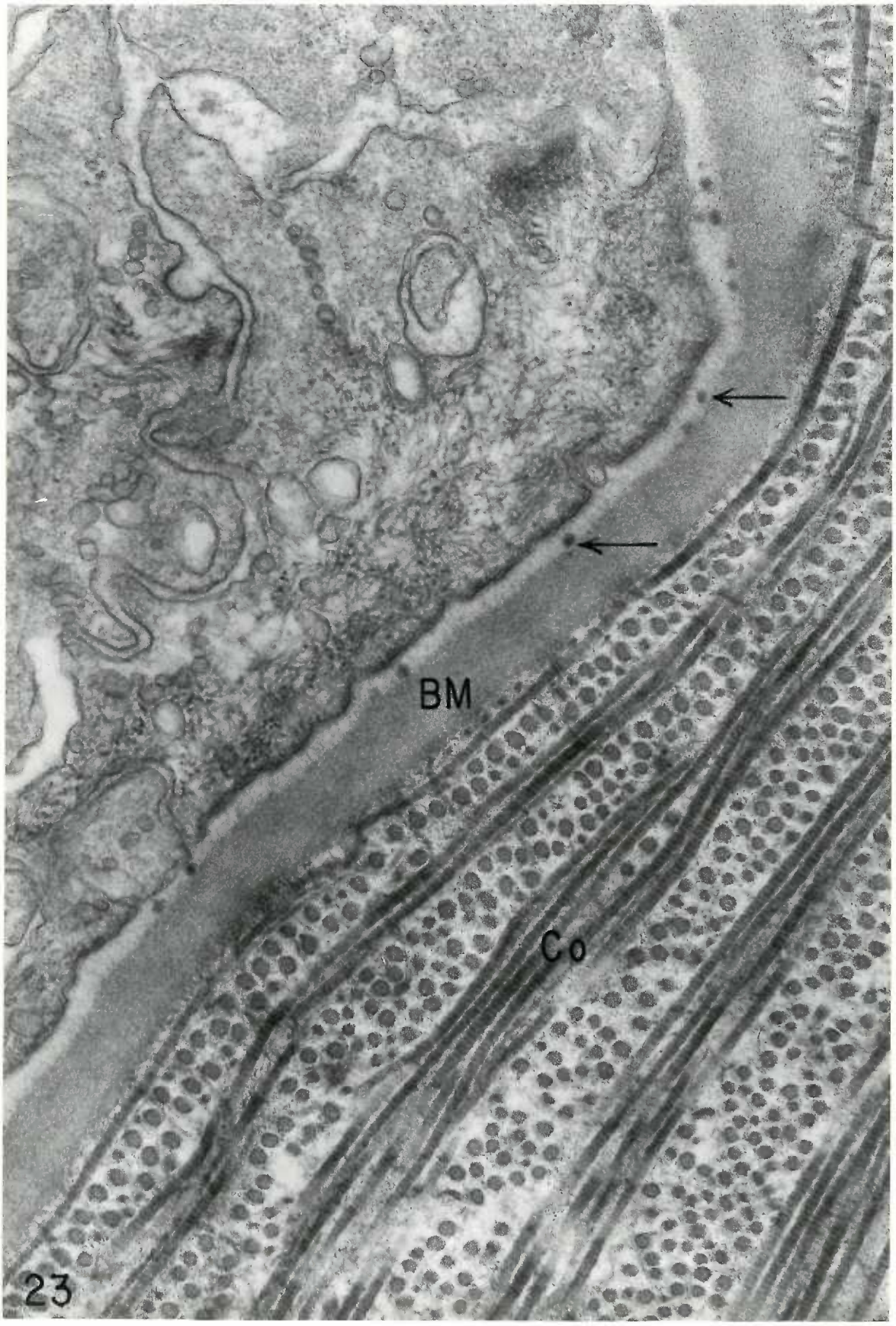

BM
Co
23

Figure 24:

Electron micrograph of the dermal-epidermal junction. Note the association of the basement membrane (BM) with the collagen fibers (Co).

The periodicity of the collagen fibers (lower center) is approximately 600 Å. The cross sectional diameter of the collagen fibers varies between 150 Å and 700 Å.

The cytoplasmic membrane of the basal cell appears to have the typical trilamellar structure of unit membrane in general (arrow).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.

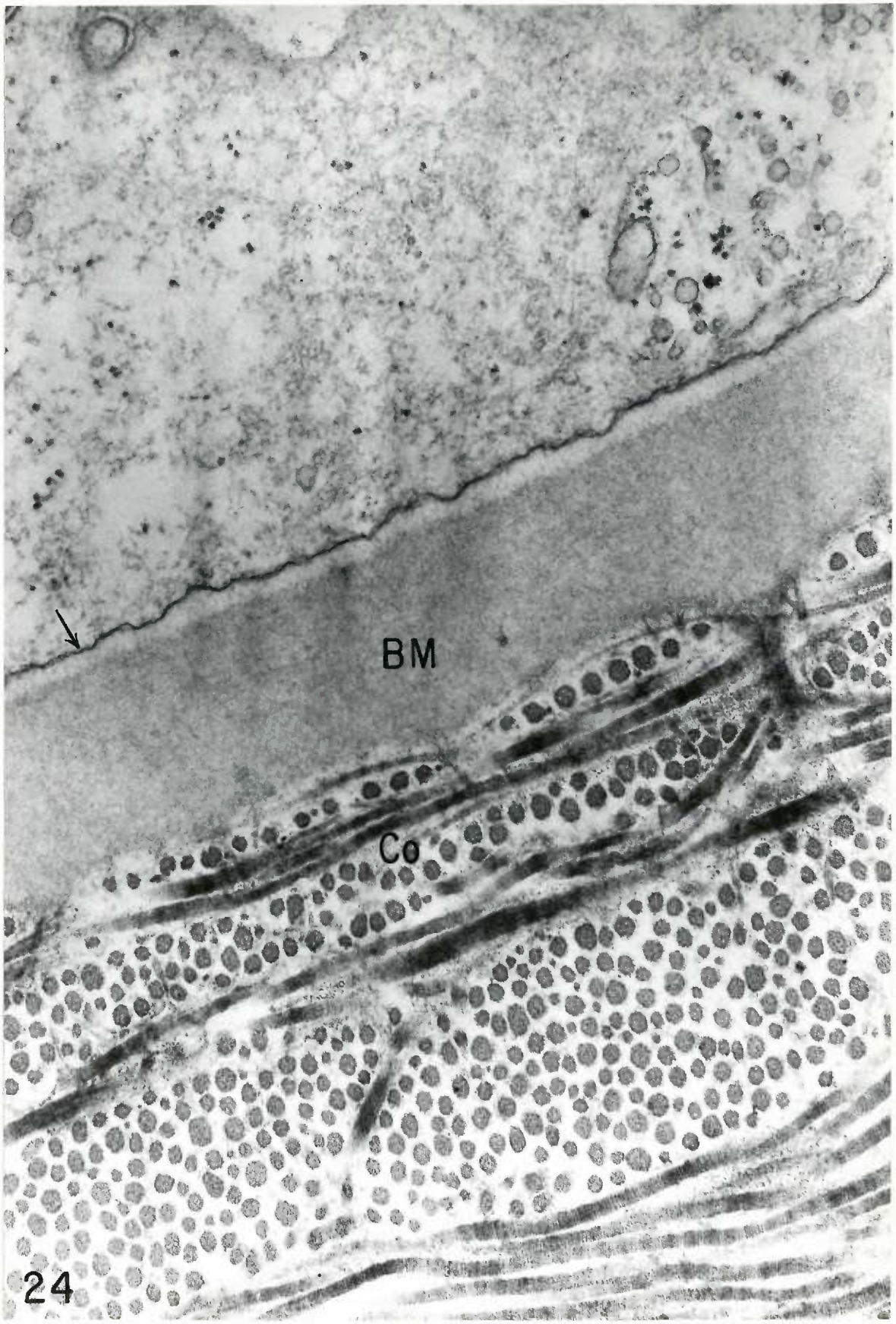

BM
Co
24

Figure 25:

Electron micrograph of the dermal-epidermal junction showing numerous agranular vesicles (V) lined up along the inner surface of the basal cell membrane. None of the vesicles are seen opening into the subepidermal space (SS).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 30,800X.

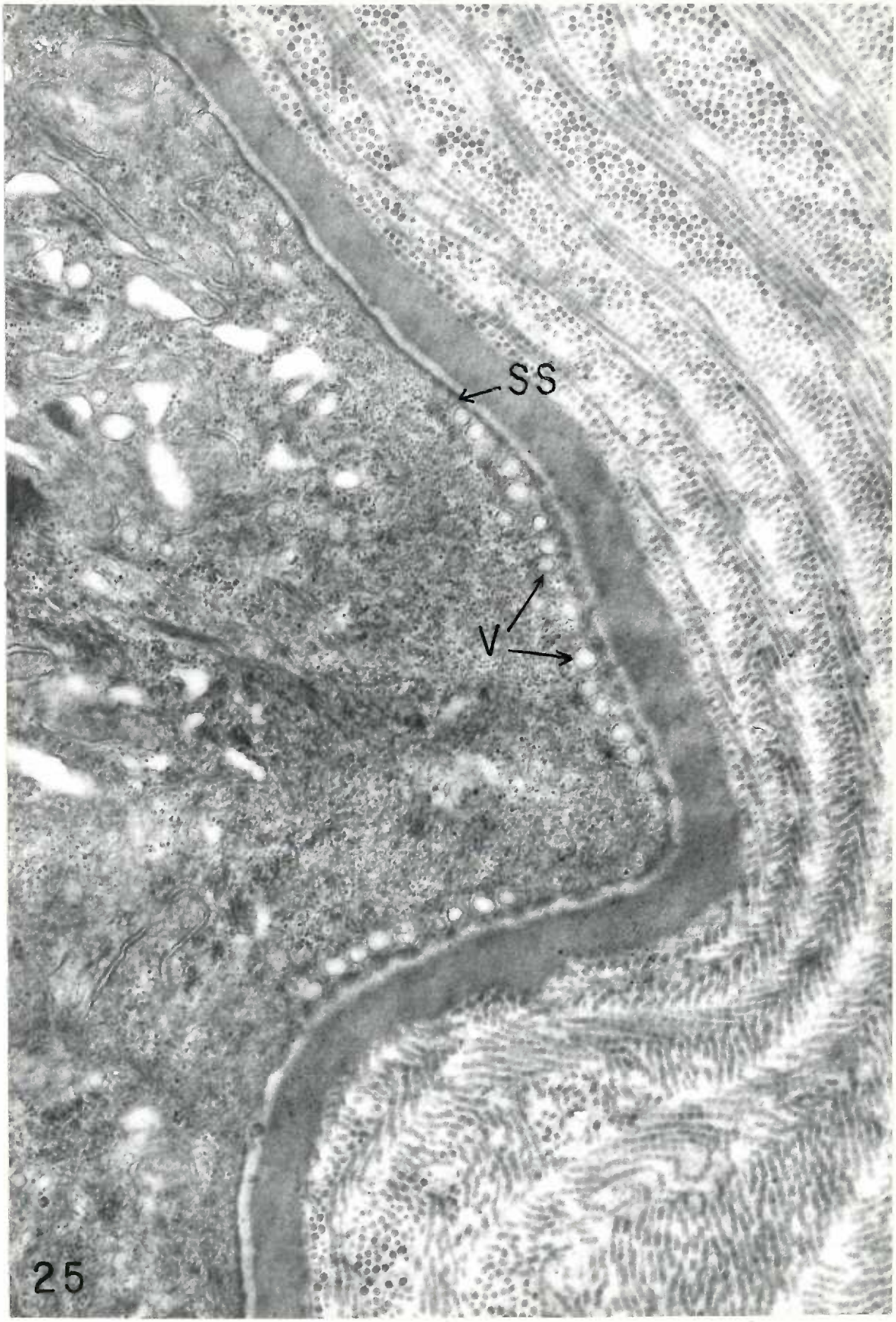

SS
V
25

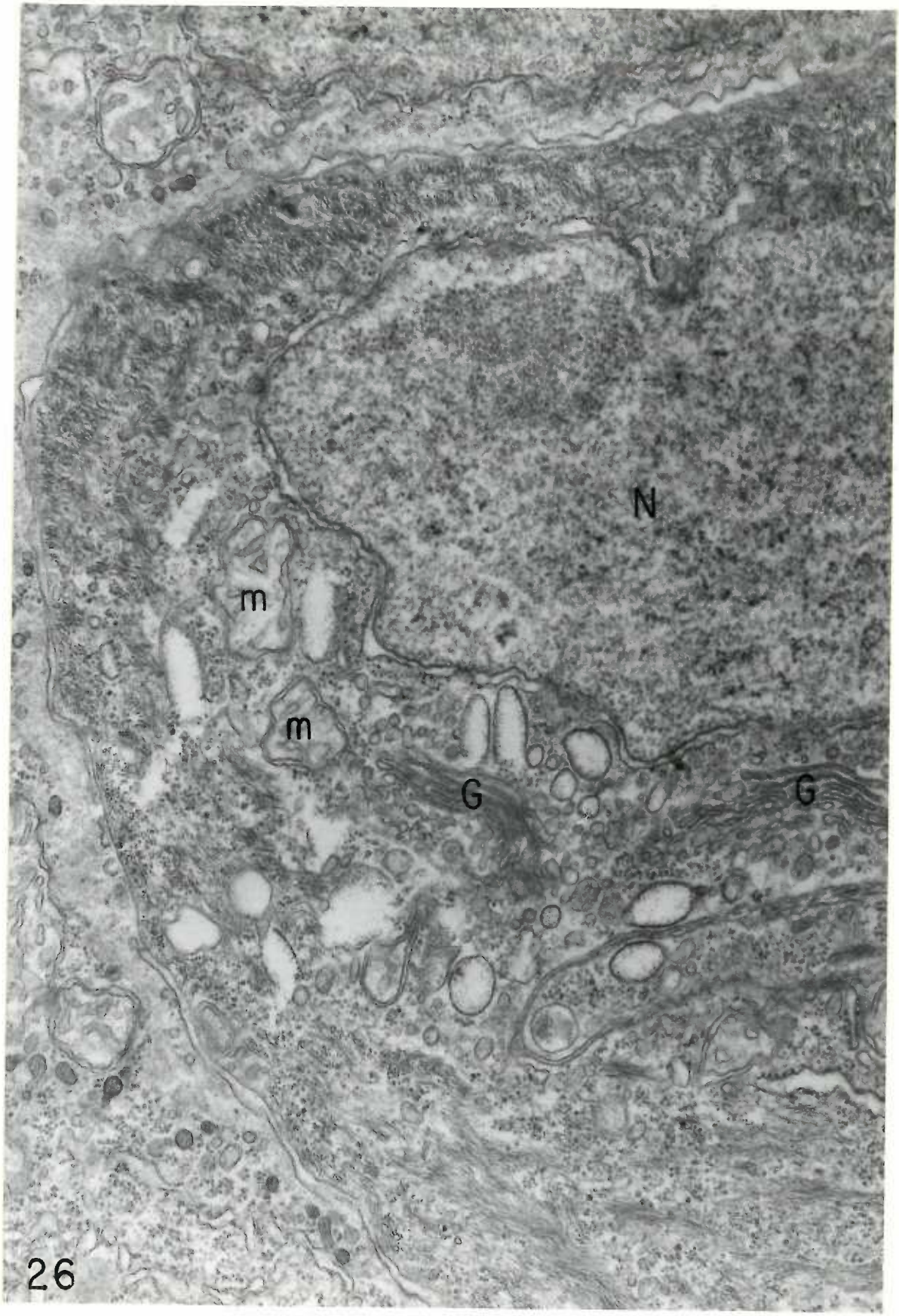
Figure 26:

Electron micrograph of cell from the intermediate region of the epidermis (region between the basal cells and the superficial cells). The cell is very similar to those seen in the surface and basal layers in that the cytoplasmic filaments predominate in the periphery while the Golgi complexes (G), mitochondria (m), and vesicles are in the perinuclear region.

The nucleus (N) has an irregular outline and appears more homogeneous than the basal cell nucleus (Figure 21).

Note the lack of desmosomes along the cell border. This is an uncommon finding for cells in this region.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 39,800X.



26

Figure 27:

Electron micrograph of an intermediate cell similar to Figure 26. Desmosomes (D) are numerous. Interdigitations are present between adjacent cells, but are not as complex as those seen in the deeper layers.

The cytoplasm is again organized into perinuclear and peripheral zones. The nucleus contains a small nucleolus (Nu).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 24,400X.

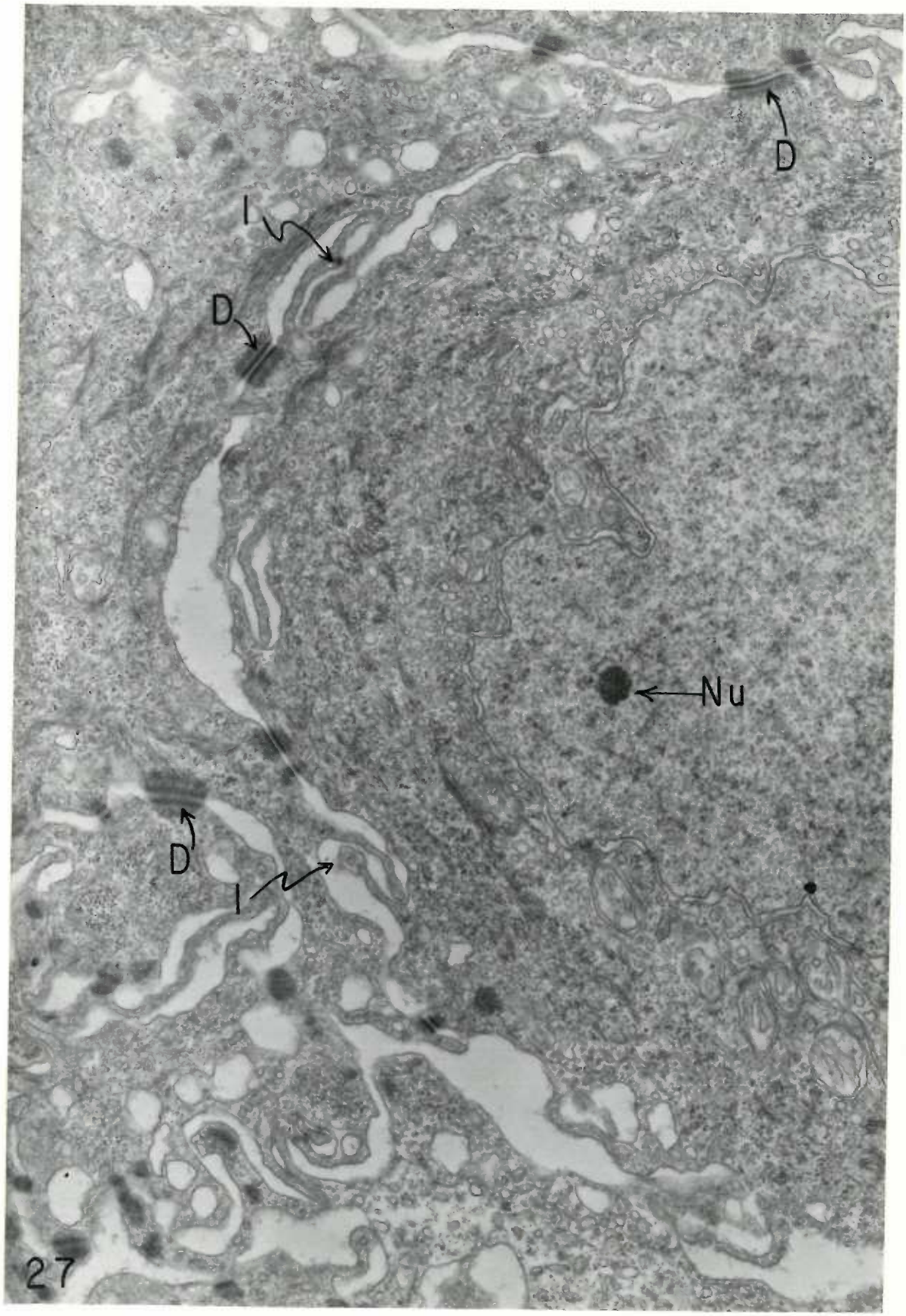

D
I
D
Nu
D
I
27

Figure 28:

Electron micrograph of a desmosome (49) or junction complex between two adjacent cells in the intermediate cell layer.

A "cytoplasmic plaque" (P) (49) is located adjacent to the inner leaflet of the cell membrane just along the course of the desmosome. There is a suggestion that cytoplasmic filaments (CF) enter the "plaque" region, make a 180° turn, and return into the deeper cytoplasm. No filaments are seen crossing the space between the cell membranes.

Intercellular space (IS).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.

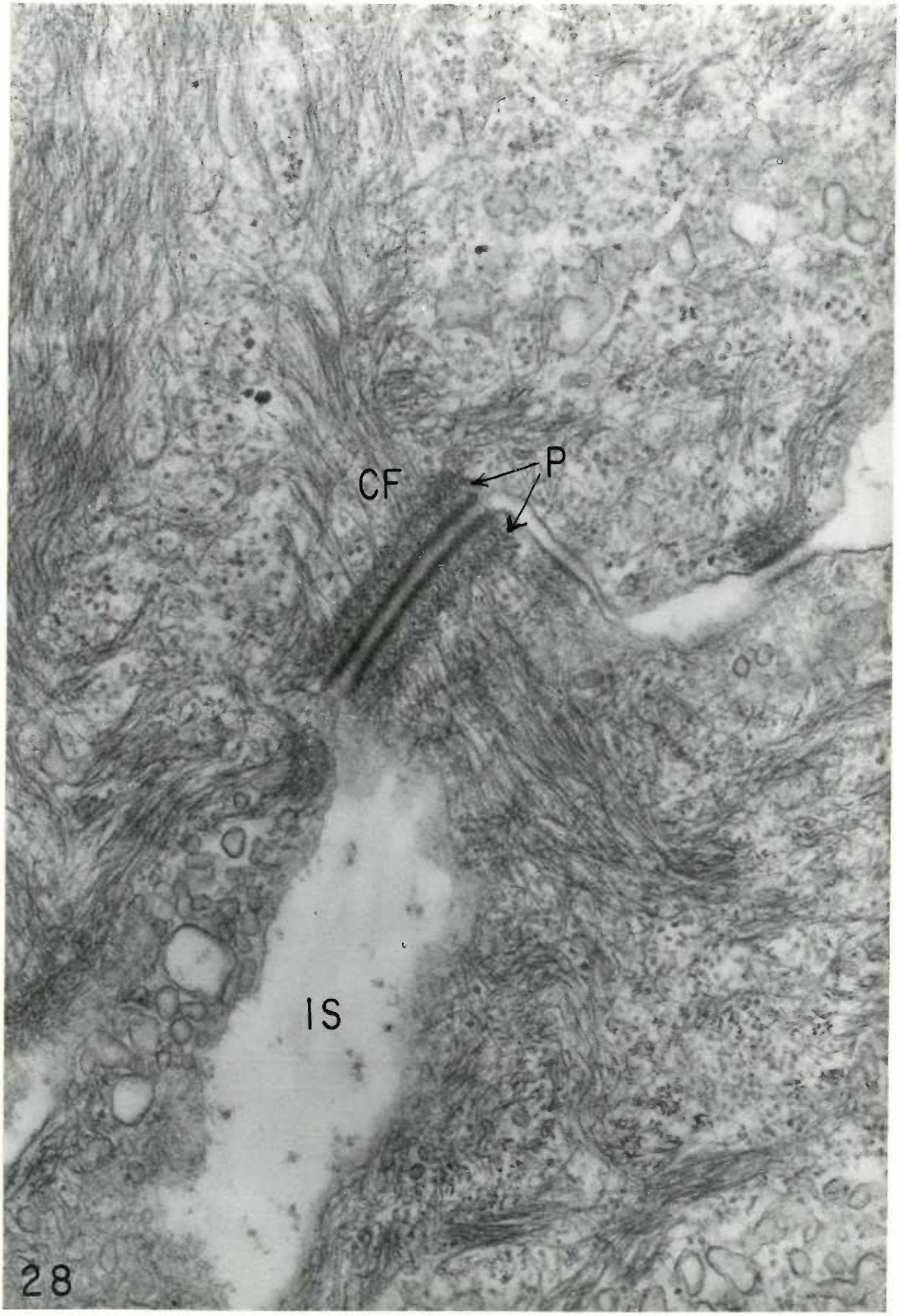
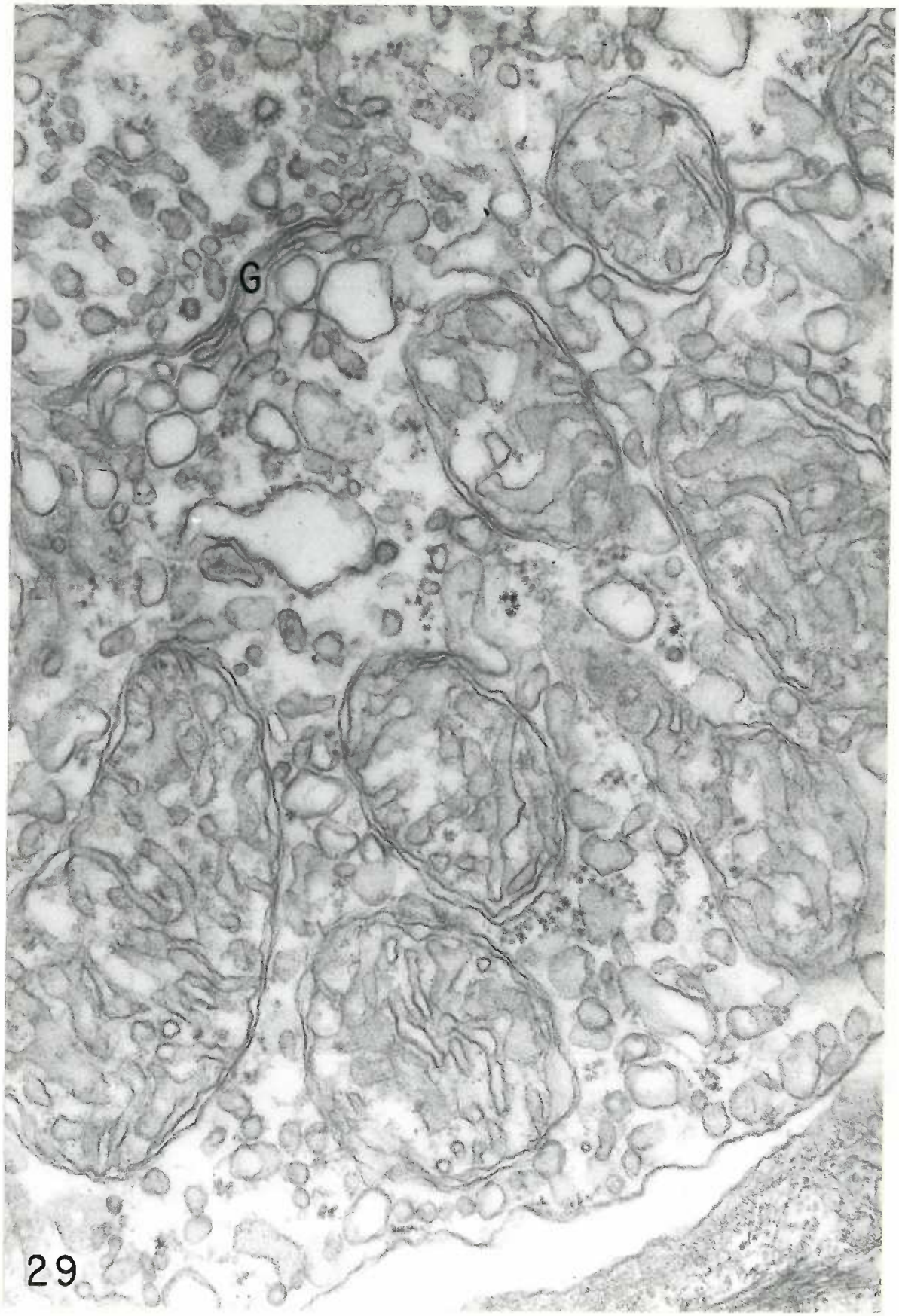

CF
P
IS
28

Figure 29:

Electron micrograph of a "mitochondria rich cell" which is similar to cells described by Choi (32) and Farquhar and Palade (49) in amphibian bladder and skin epithelium respectively. This cell is located in the intermediate cell region of the epidermis. It has a relatively light staining cytoplasmic matrix which contains numerous large mitochondria, a Golgi complex (G), agranular endoplasmic reticulum, and ribosomes.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.



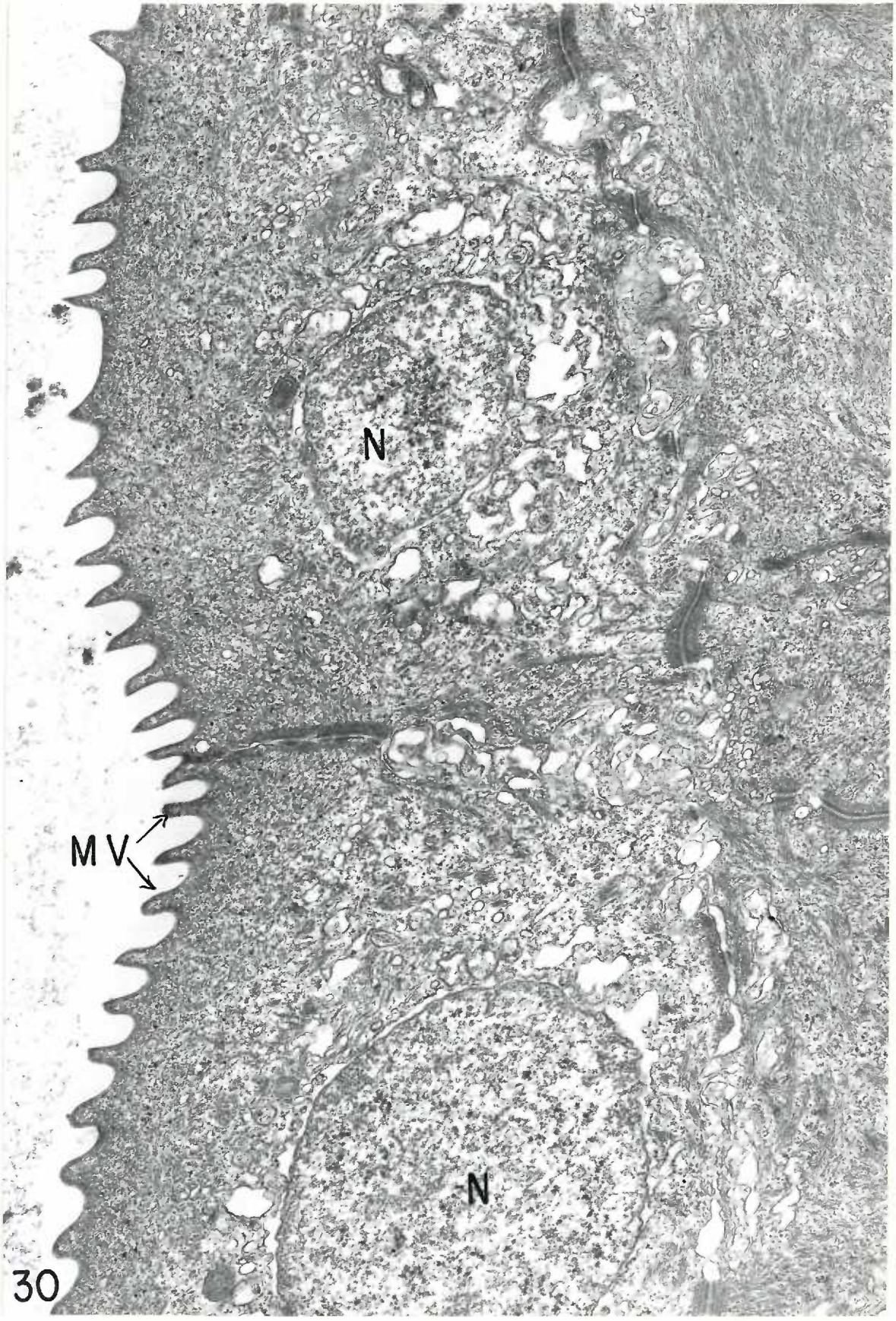
29

Figure 30:

Electron micrograph of two cells in the superficial cell layer. The epithelial surface (right) has short, broad-based microvilli (MV). The nucleus (N) of each cell has a well-defined nuclear membrane and is located close to the basal edge of the cell. The cytoplasm is again divided into a perinuclear region containing most of the organelles (mitochondria, agranular endoplasmic reticulum, and Golgi complex) and a peripheral region containing the cytoplasmic filaments. Most of the cytoplasmic filaments are concentrated above the nuclei of the superficial cells.

Desmosomes (D) are more numerous and the degree of interdigitation between adjacent cells is less than in deeper layers of the epidermis.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 19,300X.



30

Figure 31:

Electron micrograph of two adjacent surface cells. The electron-dense material on the surface of the microvilli may be a mucous coat similar to that seen on the surface of frog epithelia (49) (see page 36 of text).

The Golgi complex (G) is large and appears active. The distance between the membranes of the Golgi cisternae (flattened sacs) appears to increase toward the surface and break up as smooth, membrane-bound vesicles (V) are formed.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 53,800X.

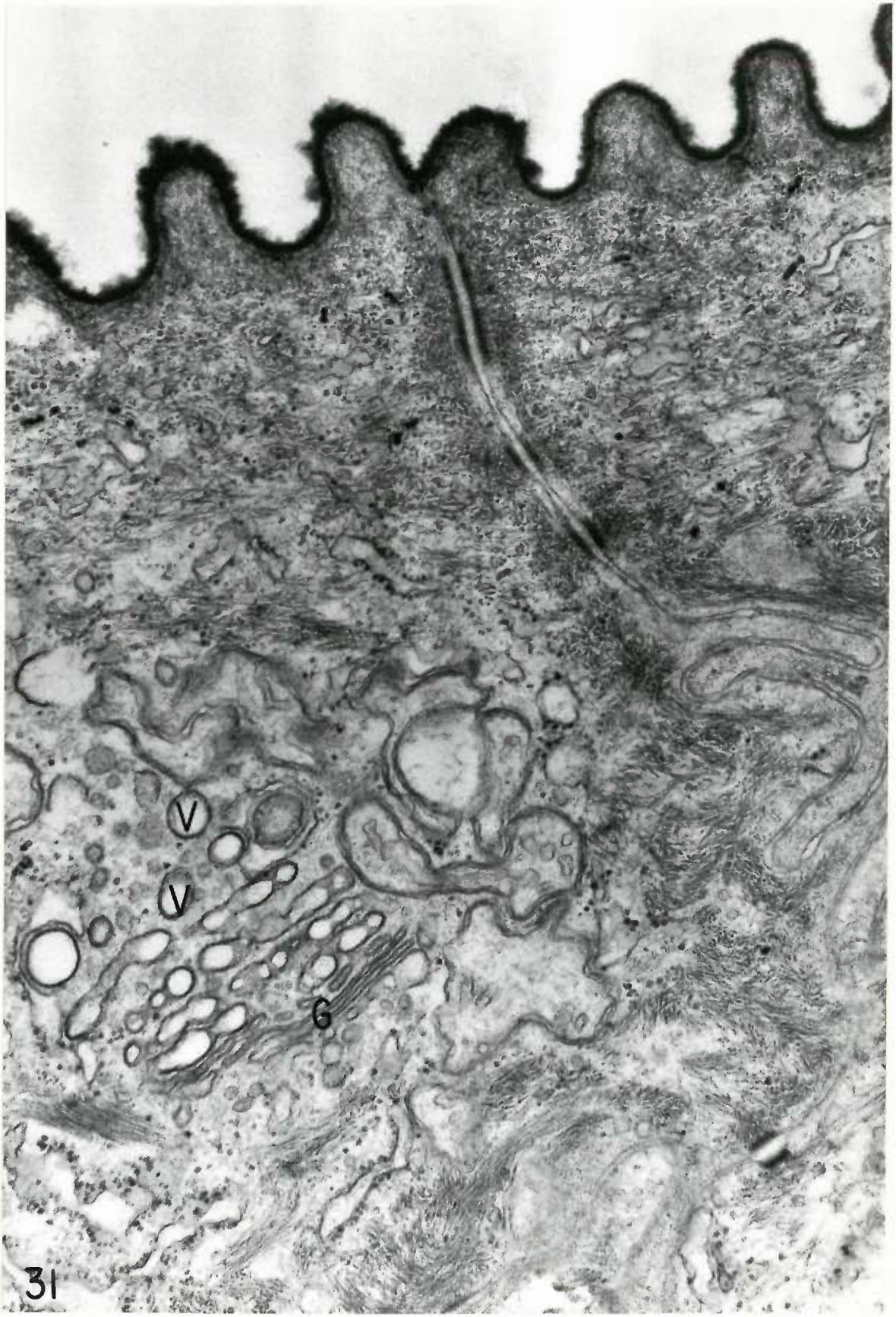
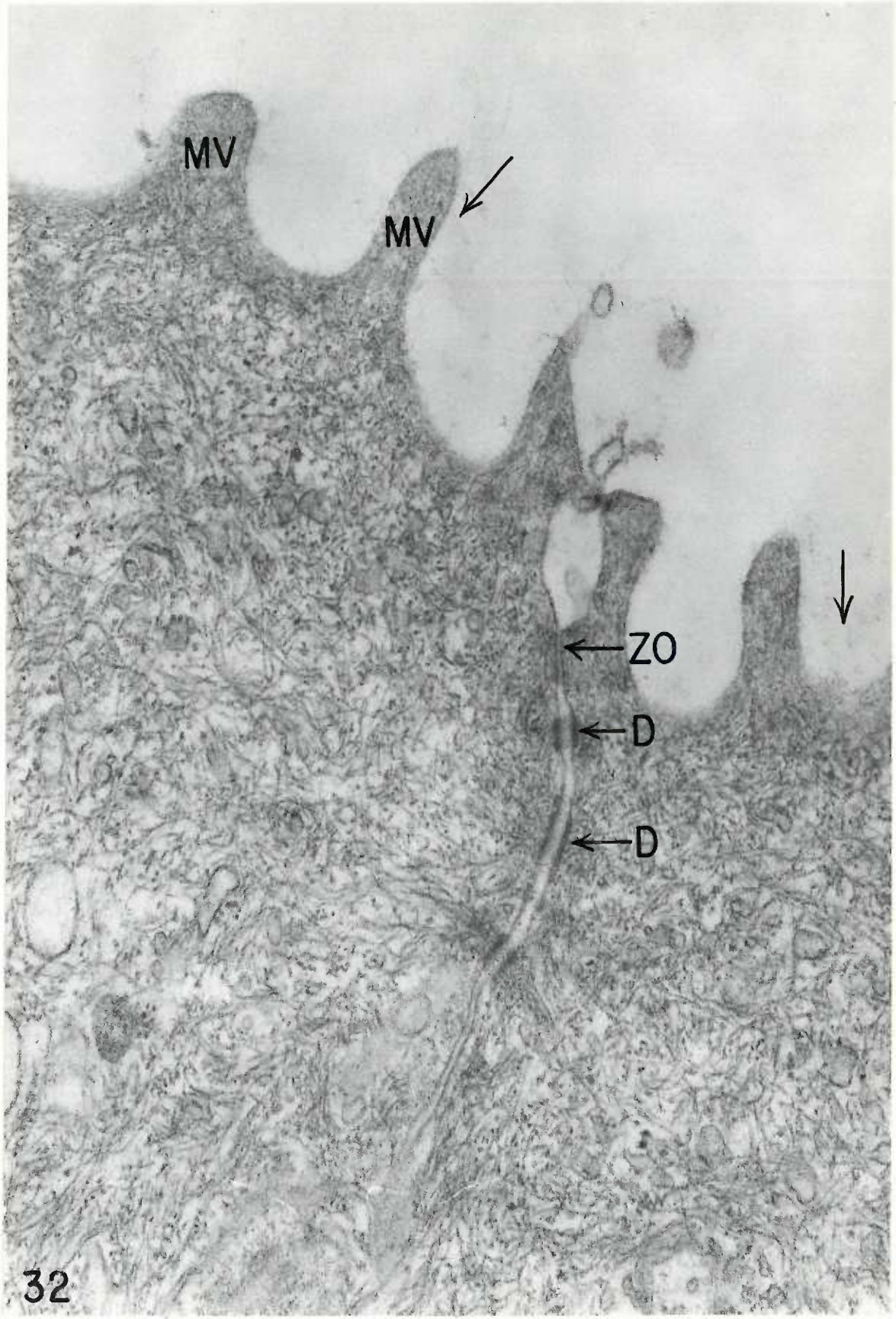

V
V
G
31

Figure 32:

Electron micrograph of the junction between two superficial cells. A typical arrangement of a tight junction or *zonula occludens* (ZO) and desmosomes (D) along the intercellular space of adjacent surface cells (48).

Cytoplasmic filaments measuring approximately 80 Å in diameter are seen within the cytoplasm and appear more concentrated within the microvilli (MV). Note finely filamentous material (measuring approximately 30 Å in diameter) attached to the outer surface of the microvilli (arrows).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.



32

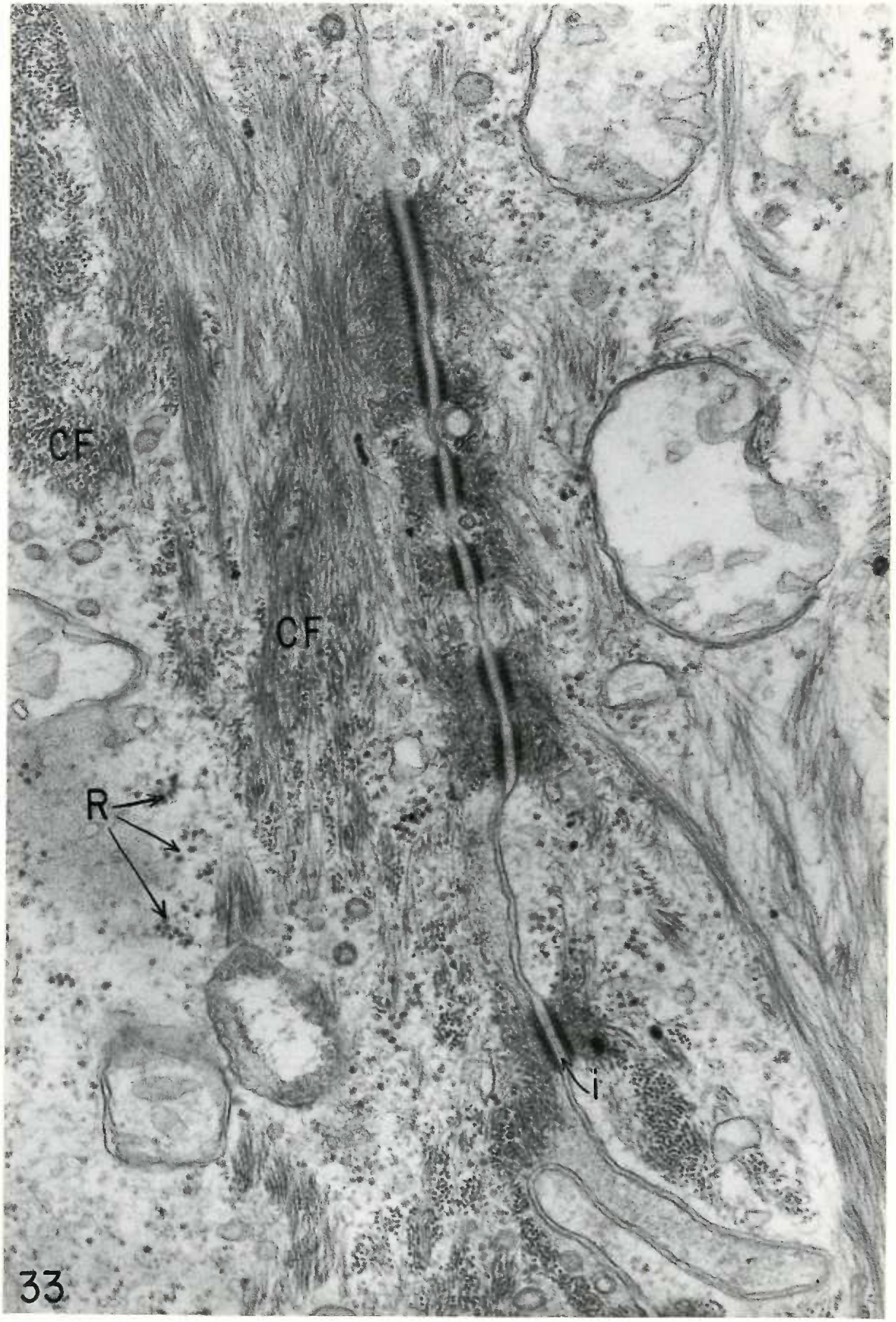

CF
CF
R
i
33

Figure 34:

Electron micrograph of an immature mucous cell in the basal region of the epidermis. Abundant dilated granular endoplasmic reticulum (ER) is present within the cytoplasm. Mucous droplets (MD) are enclosed in smooth-surfaced membranes of probable Golgi origin. A Golgi complex (G) is in close association with the mucous droplets.

Note desmosome-like structure (D) between mucous cell and adjacent epithelial cell. Cytoplasmic fibrils are not seen within the mucous cell. Artefact (arrow).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 22,000X.

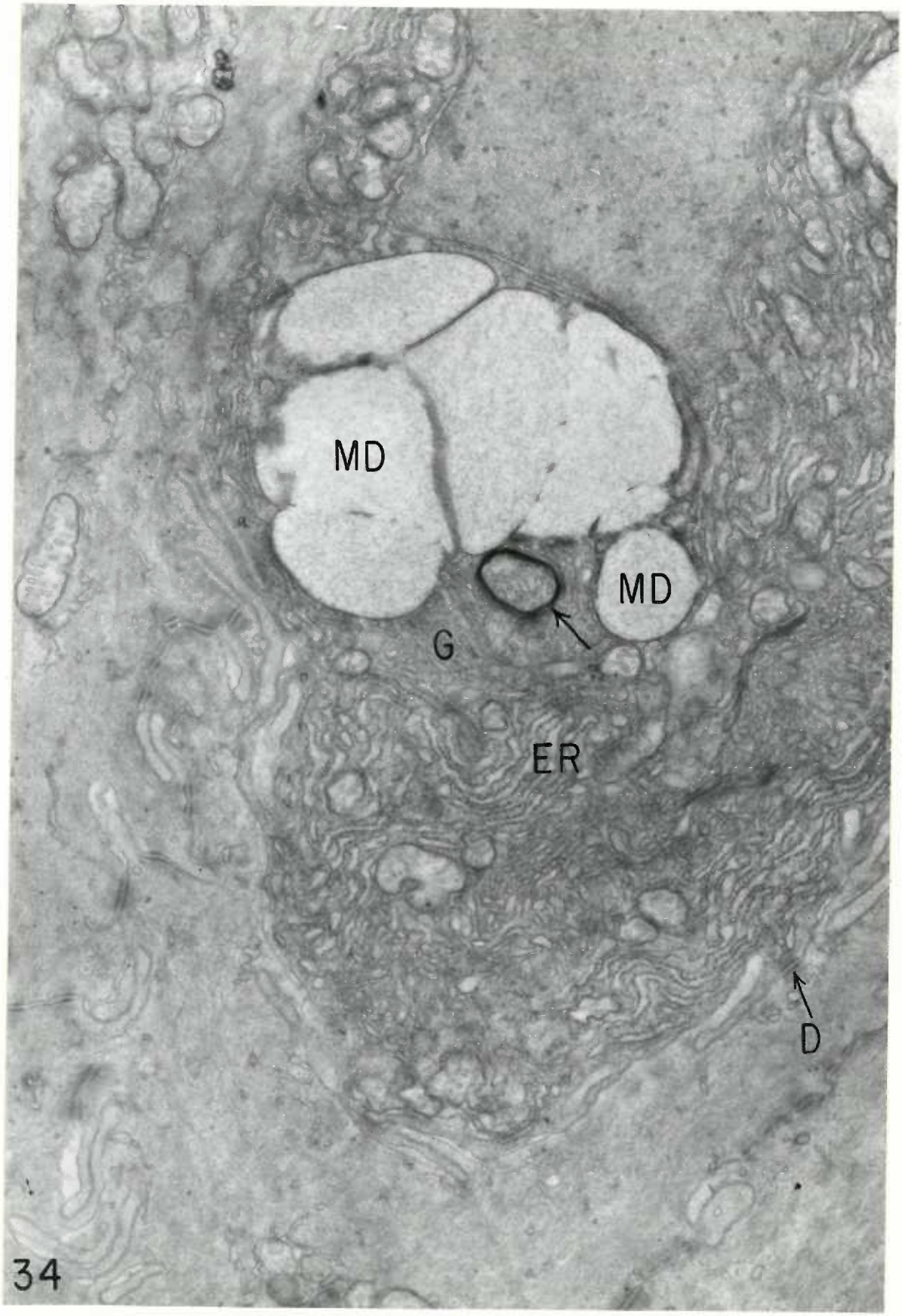

MD
MD
G
ER
D
34

Figure 35:

Electron micrograph of a mature mucous cell in the intermediate cell region. The cytoplasmic organelles (primarily flattened granular endoplasmic reticulum) form a dark ring around the centrally located mucous droplets.

Occasionally desmosome-like structures (D) appear between mucous cells and adjacent epithelial cells. There is very little interdigitation between mucous cells and adjacent cells.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 28,400X.

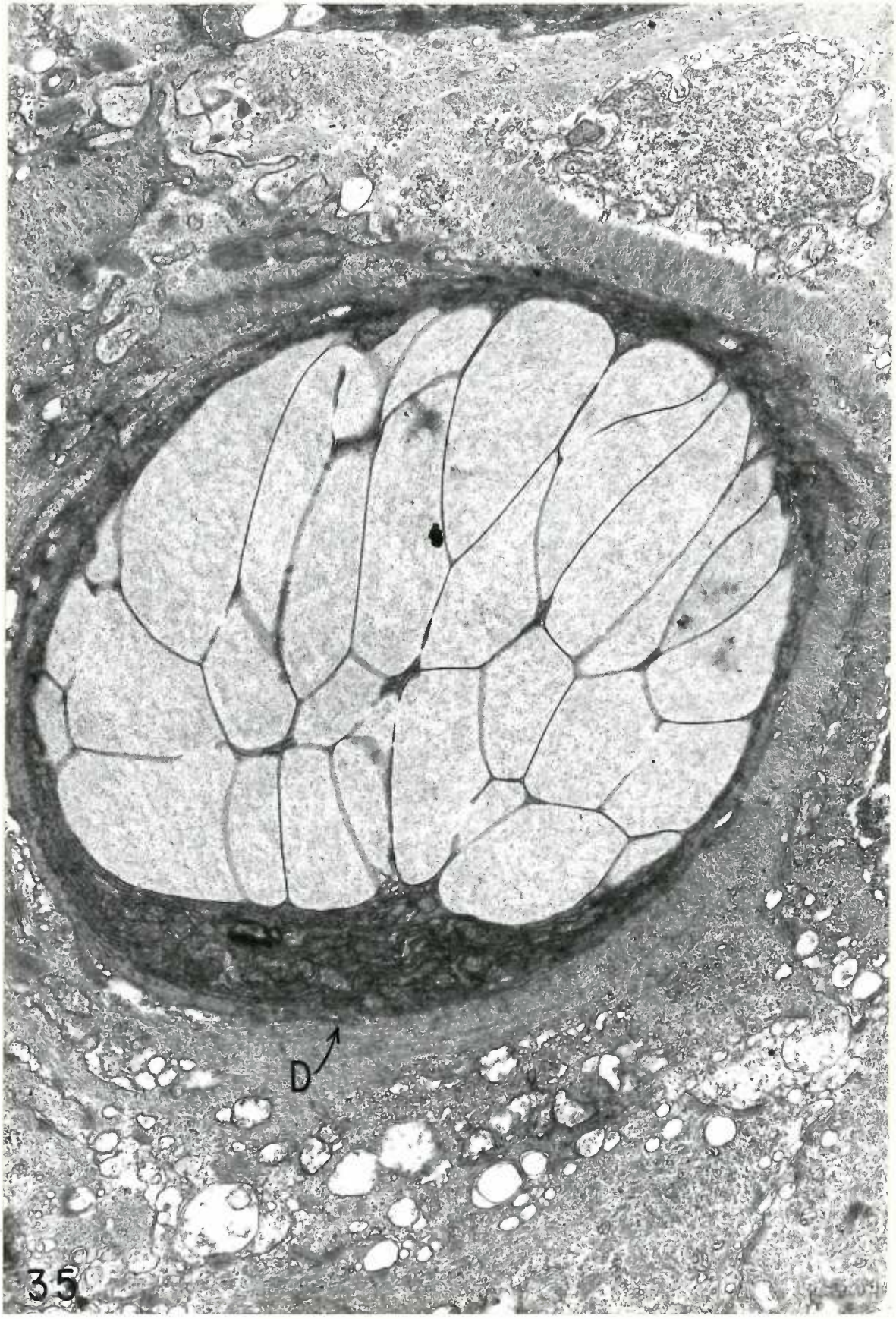
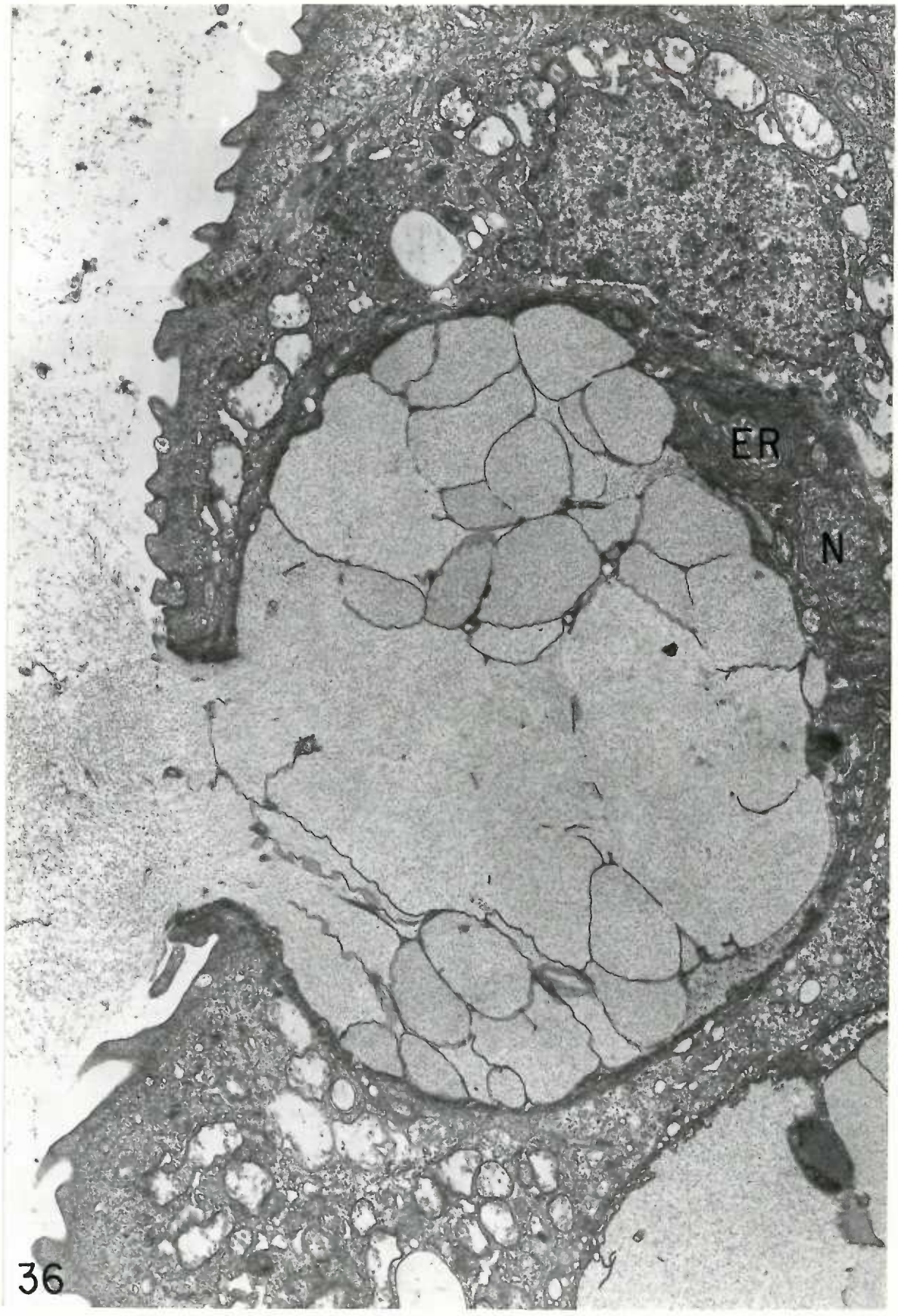

D
35

Figure 36:

Electron micrograph of a mucous cell opening onto the surface and discharging its contents. The membranes between the mucous droplets are partly disrupted. The mucus has a fine fibrillar appearance and on close examination the fibrils seem to branch.

The nucleus (N), granular endoplasmic reticulum (ER) and other cytoplasmic organelles form a crescent in the periphery of the cell.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 14,400X.



36

Figure 37:

Electron micrograph of the dense dermal collagen deep to the scale-containing layer (stratum compactum). This region is composed of collagen lamellae containing many collagen fibers oriented in parallel. The fiber axes of adjacent lamellae are approximately perpendicular to each other. Fibroblasts (F) are located between the collagen lamellae, not within the lamellae. Fibroblasts are typified by their abundant granular endoplasmic reticulum. Mitochondria and agranular vesicles are also seen within the cytoplasm.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 28,900X.

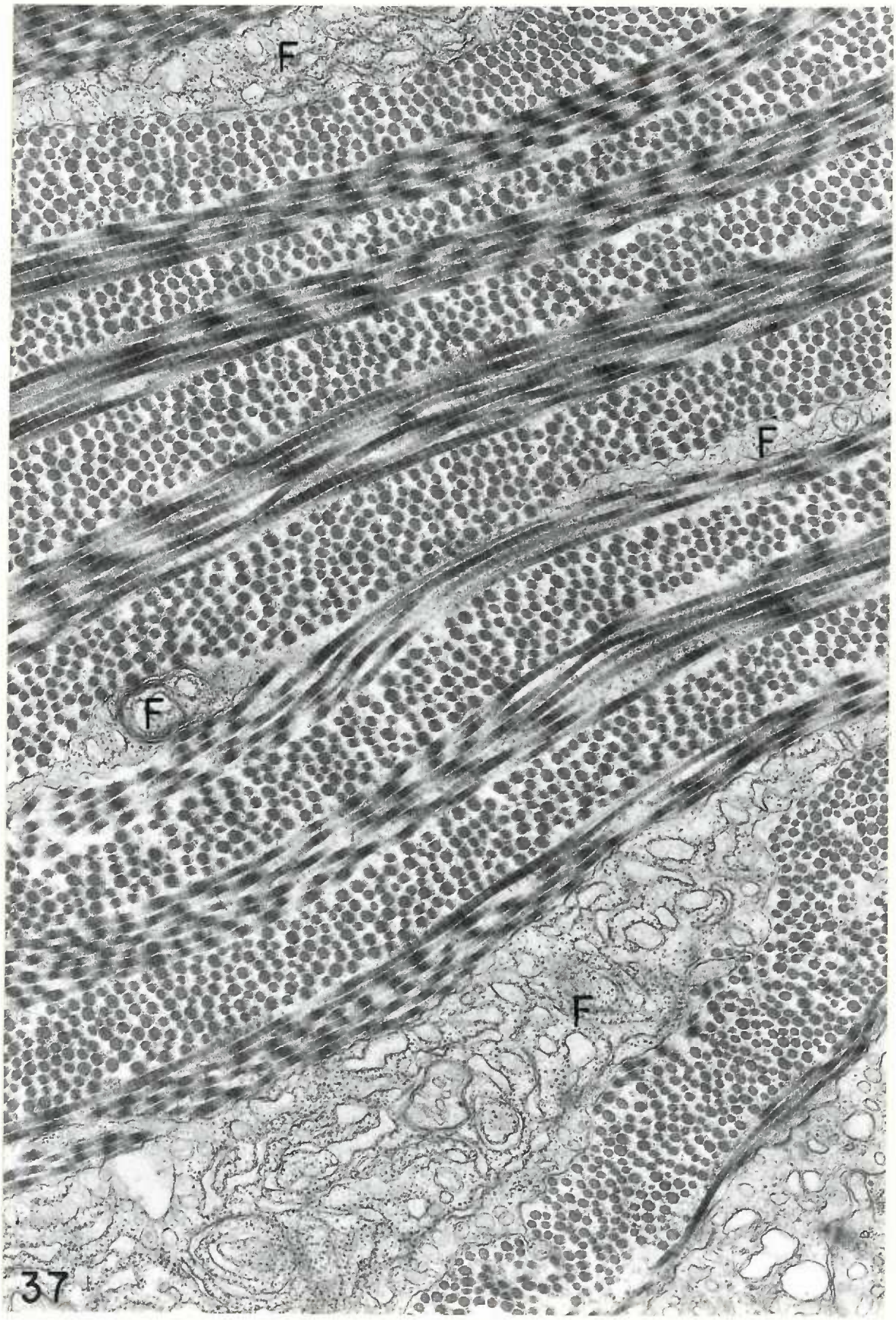
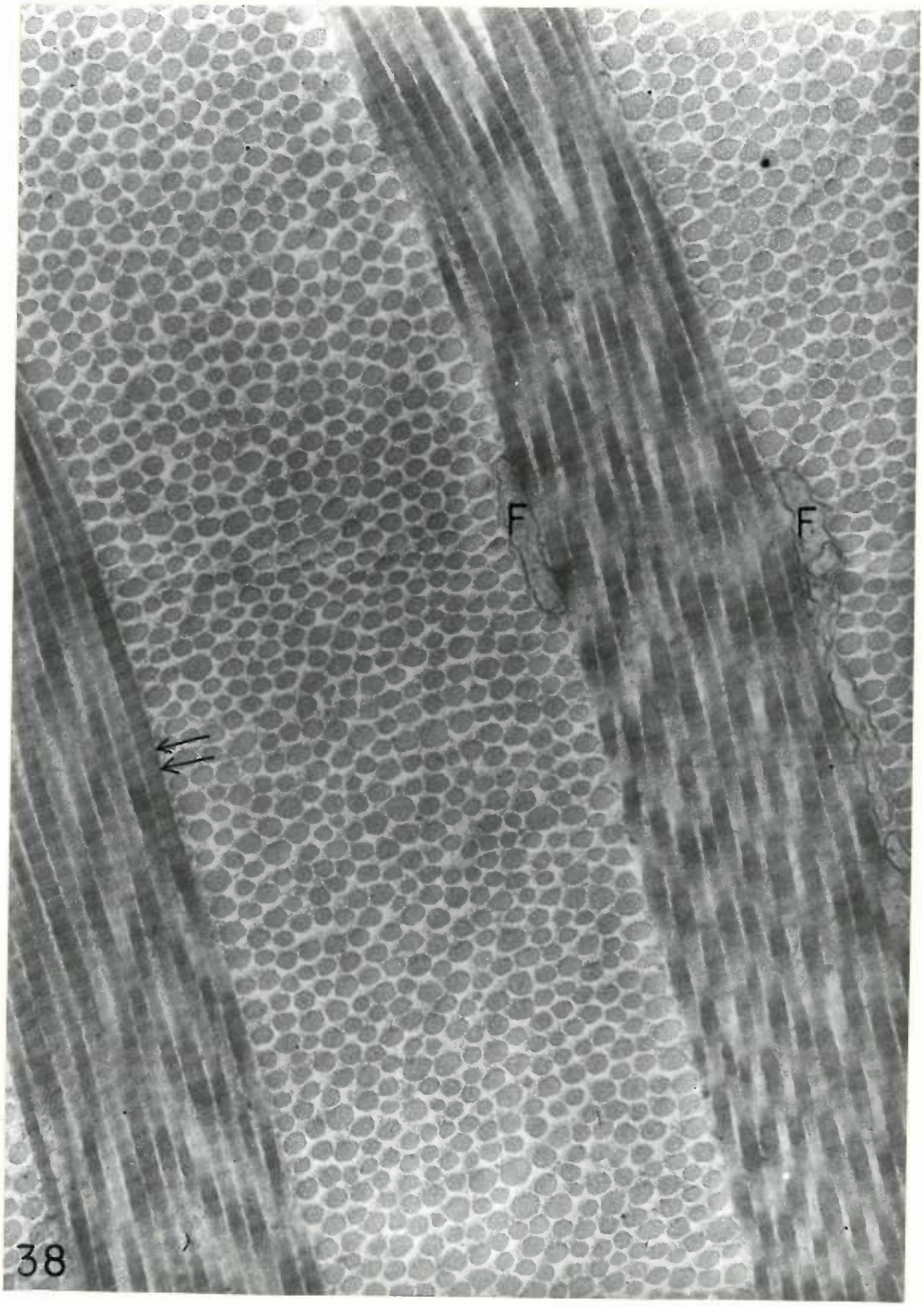

F
F
F
F
37

Figure 38:

Electron micrograph of collagen lamellae from the dermis beneath the scale-containing layer (see Figure 16 for localization). Each lamella contains many collagen fibers oriented in parallel. The fiber axes of adjacent lamellae are approximately perpendicular to each other. Collagen fibers in cross-section vary from 400 to 900 Å in diameter. In longitudinal section there is typical collagen periodicity with an interval of approximately 600 Å (arrows). Note the cell process from a nearby fibroblast (F). Fibroblasts lie between the collagen lamellae, not within the lamellae as seen in Figure 37.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.

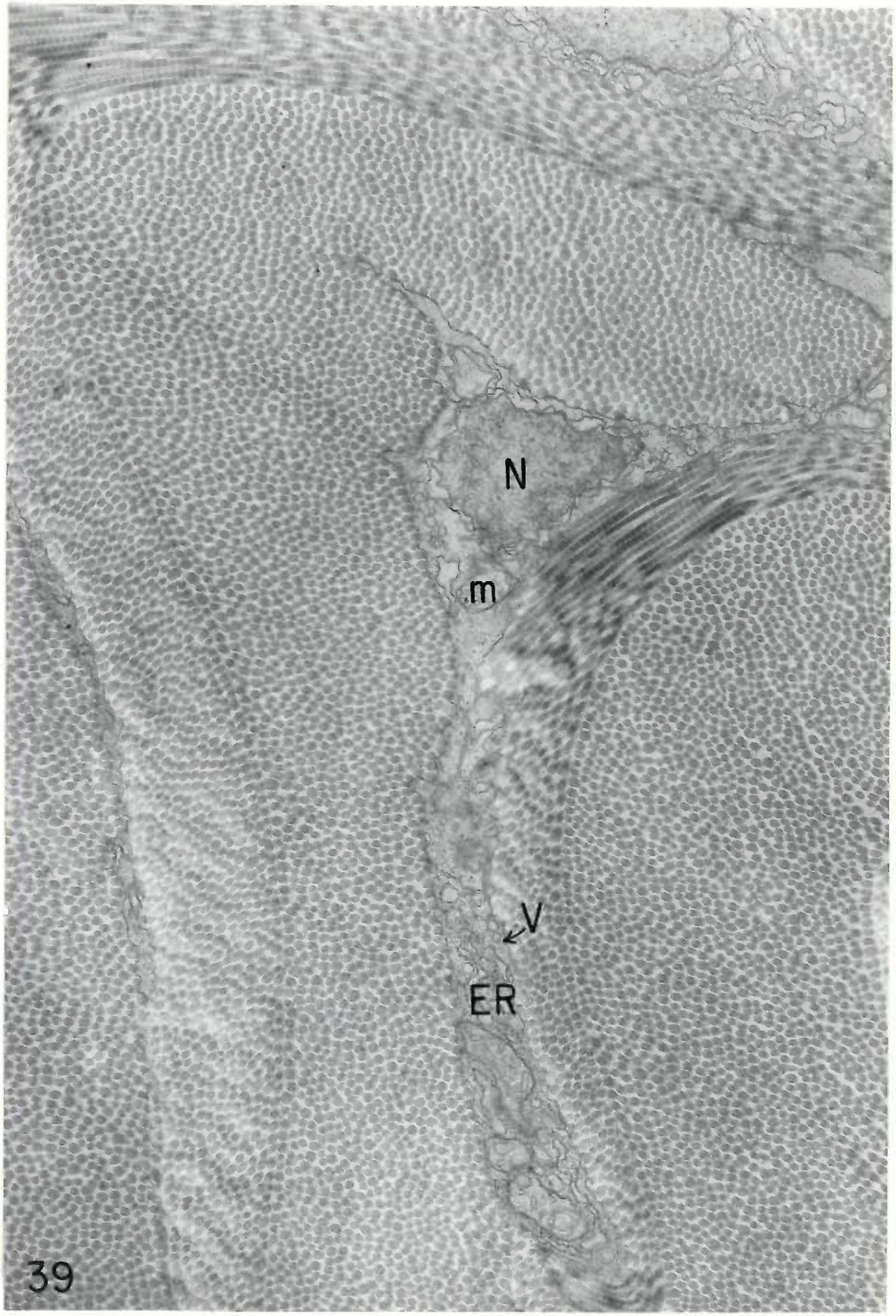


38

Figure 39:

Electron micrograph of the dermis near a scale. In this region the lamellae are not so well organized. Note the organization of fibers around the fibroblast (center). Fibroblast nucleus (N), mitochondria (m), granular endoplasmic reticulum (ER), and vesicles (V).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 23,400X.

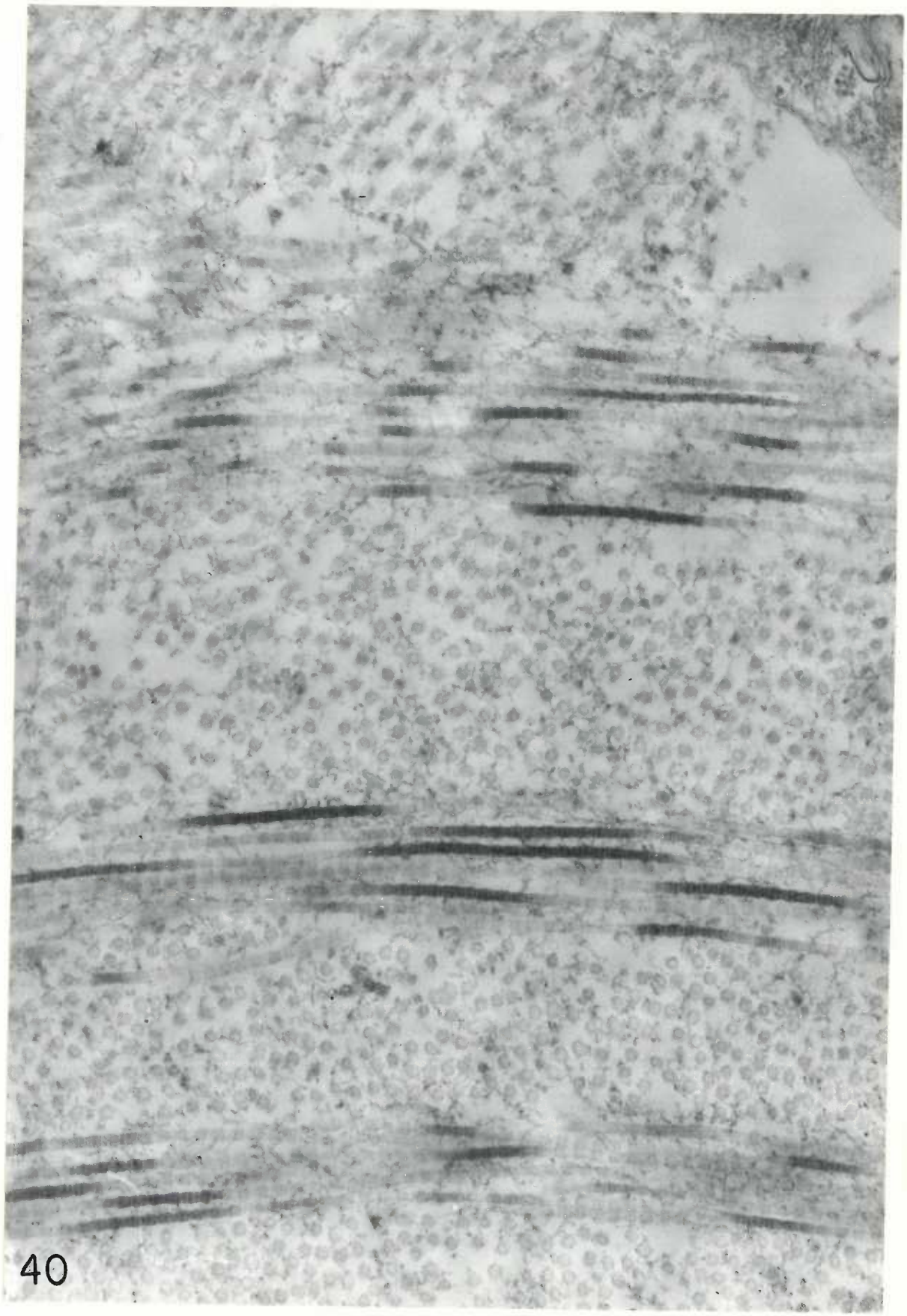


39

Figure 40:

Electron micrograph of loosely arranged collagen in the central region of a fin web. The lamellar pattern is present, but there is a greater distance between the fibers within the lamellae. "Filamentous" material is seen in the matrix between the fibers. The diameter of the collagen fibers measures approximately 400 Å, while the major period is again 600 Å (compare with Figure 38).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.



40

Figure 41:

Photomicrograph of scale-bearing skin from the pigmented (eyed) side of a 2-year-old *H. elassodon*. The scales are shown in their imbricate (overlapping) arrangement with teeth (ctenii) along the caudal or exposed portions (ctenoid scales). The small black densities are melanophores (arrow).

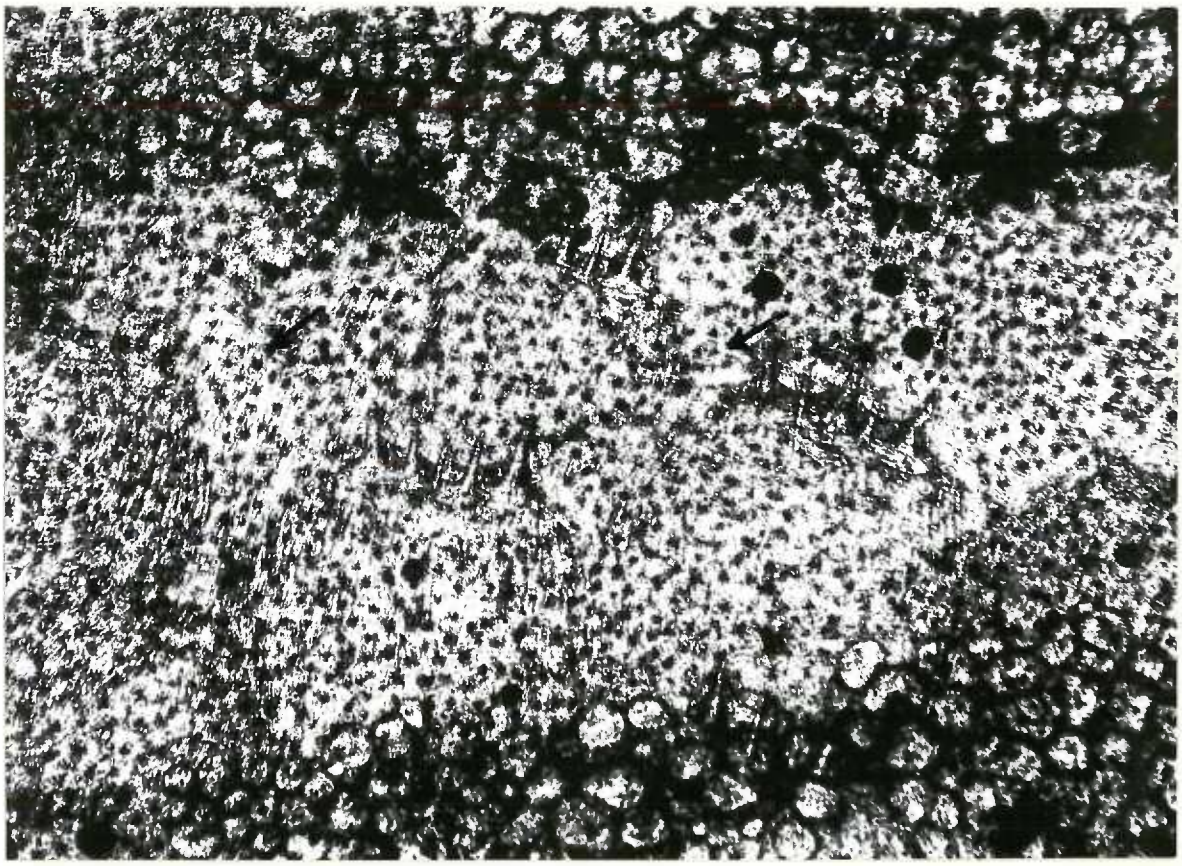
Fat cells located beneath the scales in the subcutaneous layer are seen as small circles along the top and bottom of the picture.

Fixed in 10% formalin, unstained, mounted in glycerine. 50X.

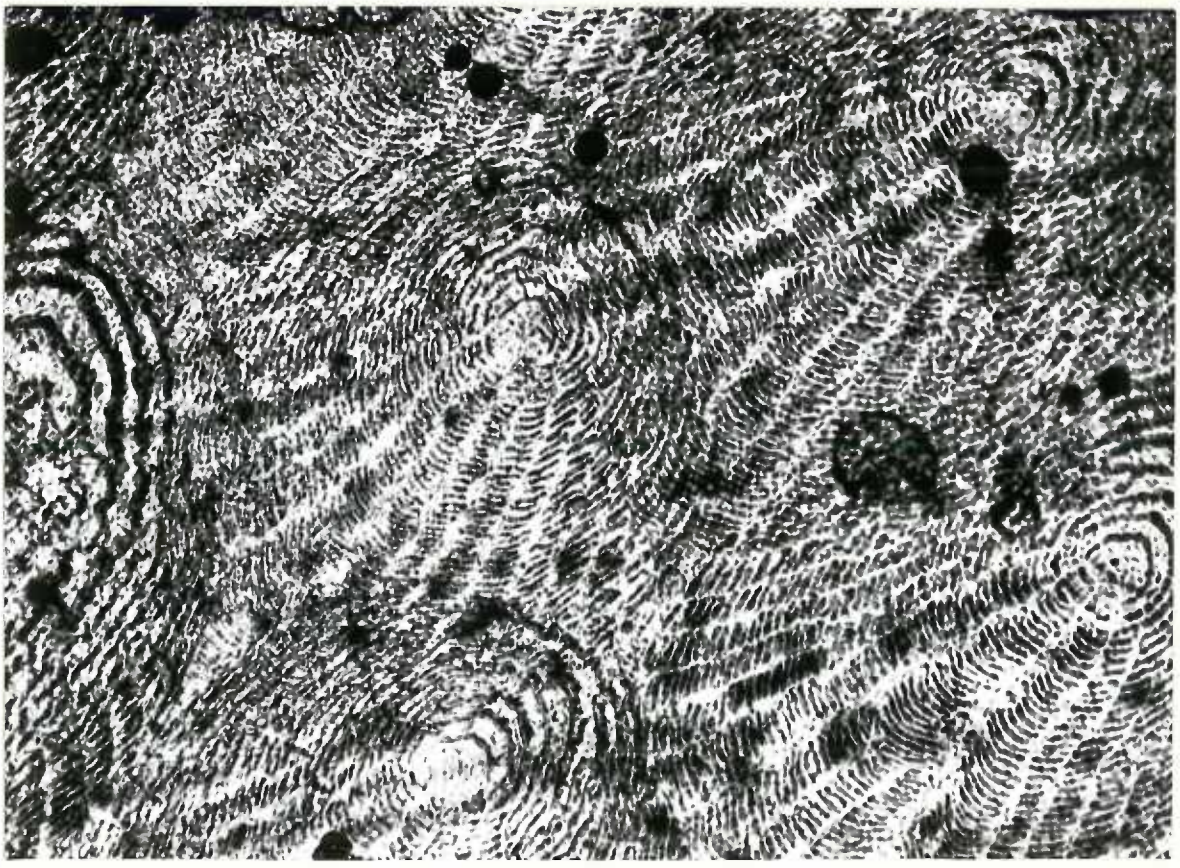
Figure 42:

Photomicrograph of the scale-bearing skin from the non-pigmented (blind) side of a 2-year-old *H. elassodon*. These scales lack the teeth and are therefore termed cycloid scales. Melanophores appear absent on this side.

Fixed in 10% formalin, unstained, mounted in glycerine. 80X.



41



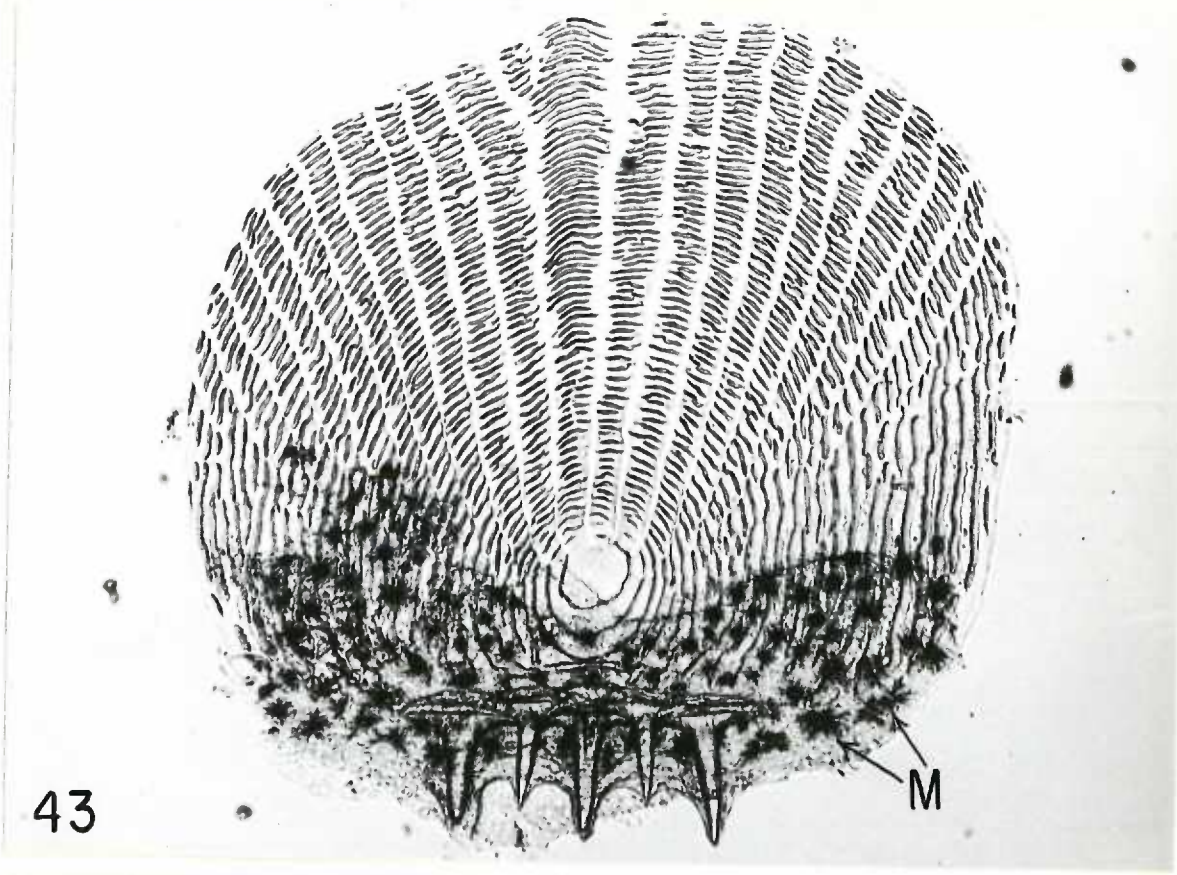
42

Figures 43 and 44:

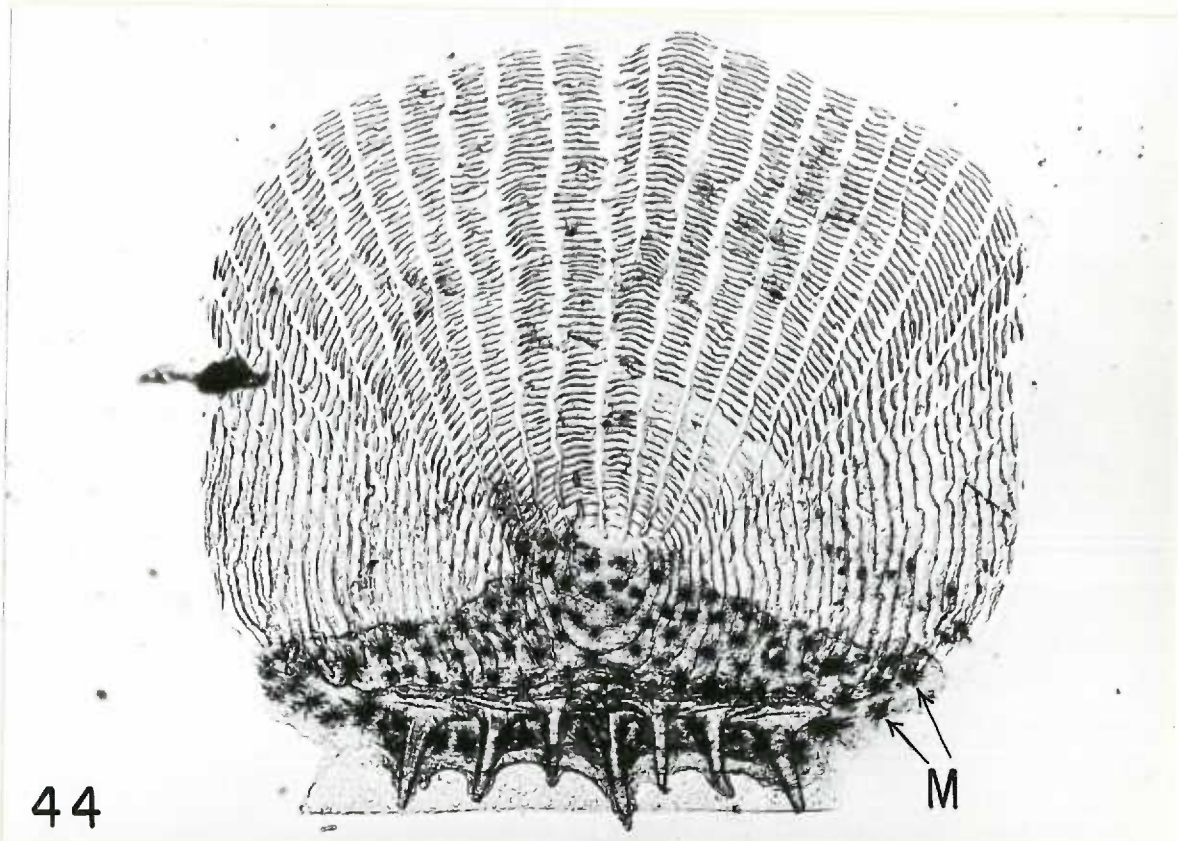
Low power photomicrographs of ctenoid scales removed from pigmented (eyed) side of a 2-year-old flathead sole, *Hippoglossoides elassodon*. The caudal or exposed portion of the scale is to the bottom and contains five ctenii or "tooth-like" structures. Melanophores (dark, stellate, pigment-containing cells [M]) are seen through the unstained epithelial layer. The larger, cephalad portion of the scale (top) was beneath another scale and therefore has no covering of epithelial cells or melanophores.

Concentric rings are centered closer to the caudal, ctenoid edge of the scale (bottom) and appear approximately equal distance apart.

Fixed in 10% formalin, unstained, mounted in glycerin. 77X.



43



44

Figure 45:

Photomicrograph of a single cycloid scale scraped from the non-pigmented (blind) side of a 2-year-old fish. Note the similarity in shape and organization of the concentric rings as compared with scales on the pigmented side (Figures 43 and 44). Teeth are lacking on the caudal exposed edge (right). The epidermis covering this portion of the scale is not obvious because it lacks subepithelial melanophores.

Fixed in 10% formalin, unstained, mounted in glycerine. 45X.

Figure 46:

Same as Figure 45 except that one tooth is present on the caudal margin of the scale. This is therefore a ctenoid scale. Note the portion of epidermis visible above the tooth (arrow).

Fixed in 10% formalin, unstained, mounted in glycerine. 45X.

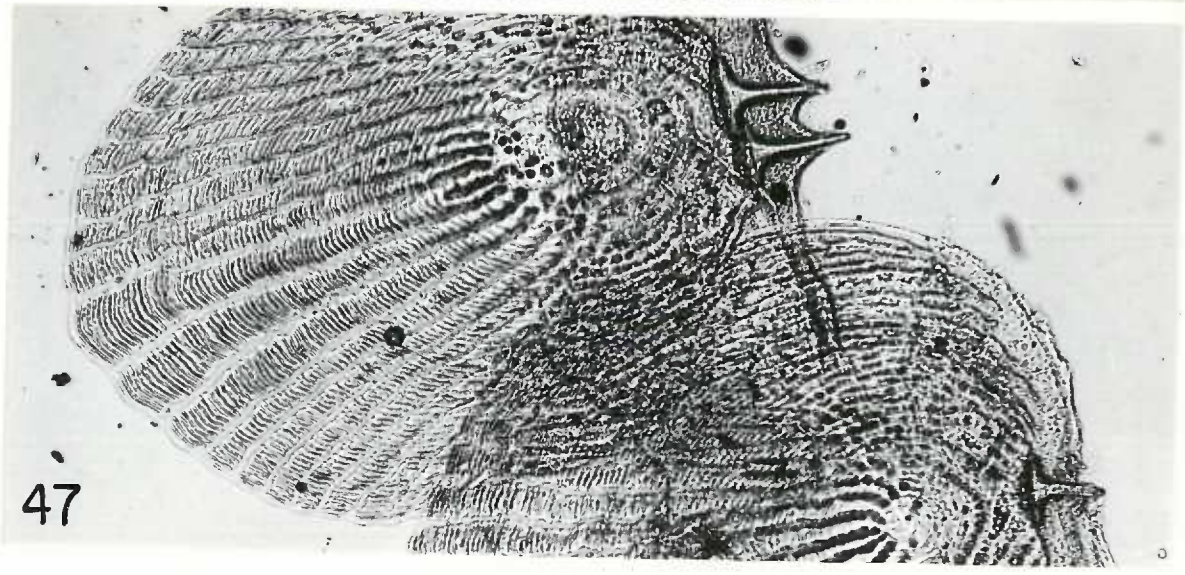
Figure 47:

Same as Figure 46 only one scale has two teeth or ctenii.

Fixed in 10% formalin, unstained, mounted in glycerine. 45X.

45

46



47

Figure 48:

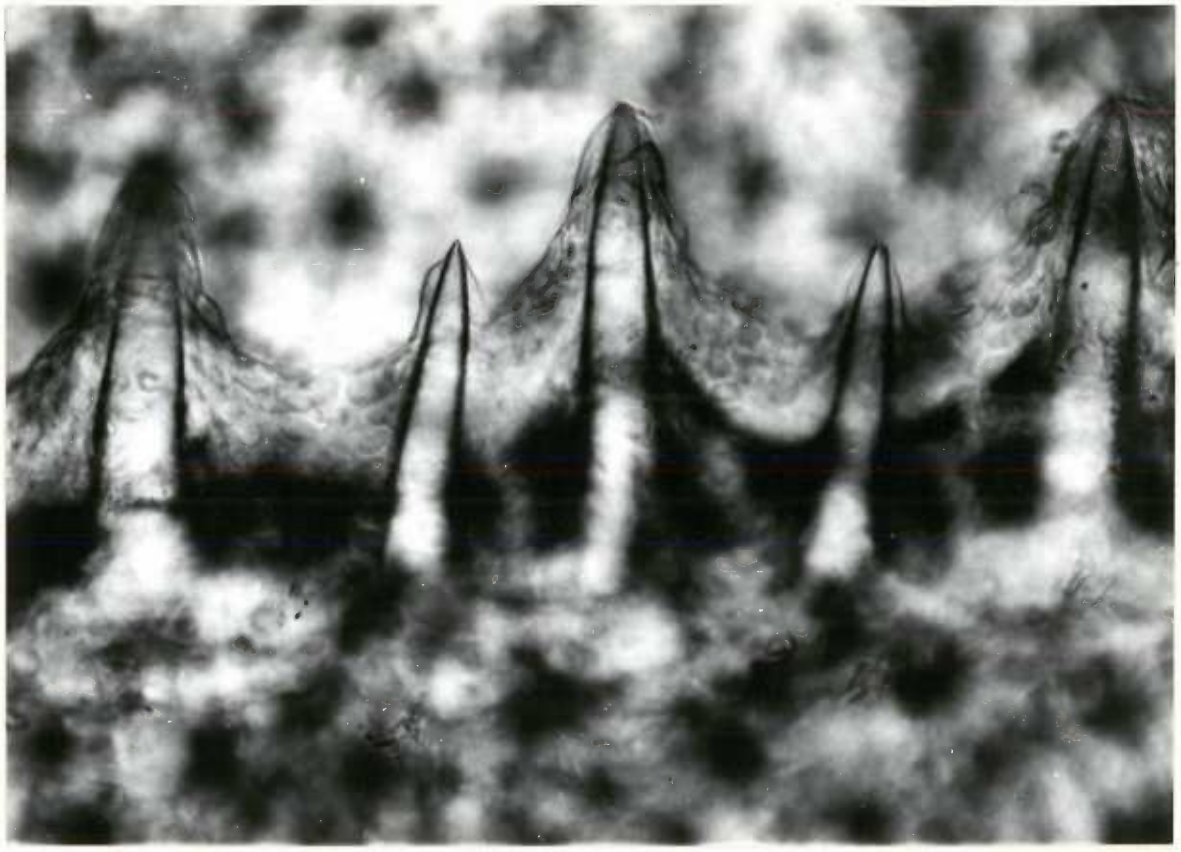
Photomicrograph of teeth (ctenii) on the caudal edge of a ctenoid scale. A sheet of epidermal cells covers the teeth in a tent-like fashion. Subepidermal melanophores are visible as black spots.

Fixed in 10% formalin, without staining, mounted in glycerine. 320X.

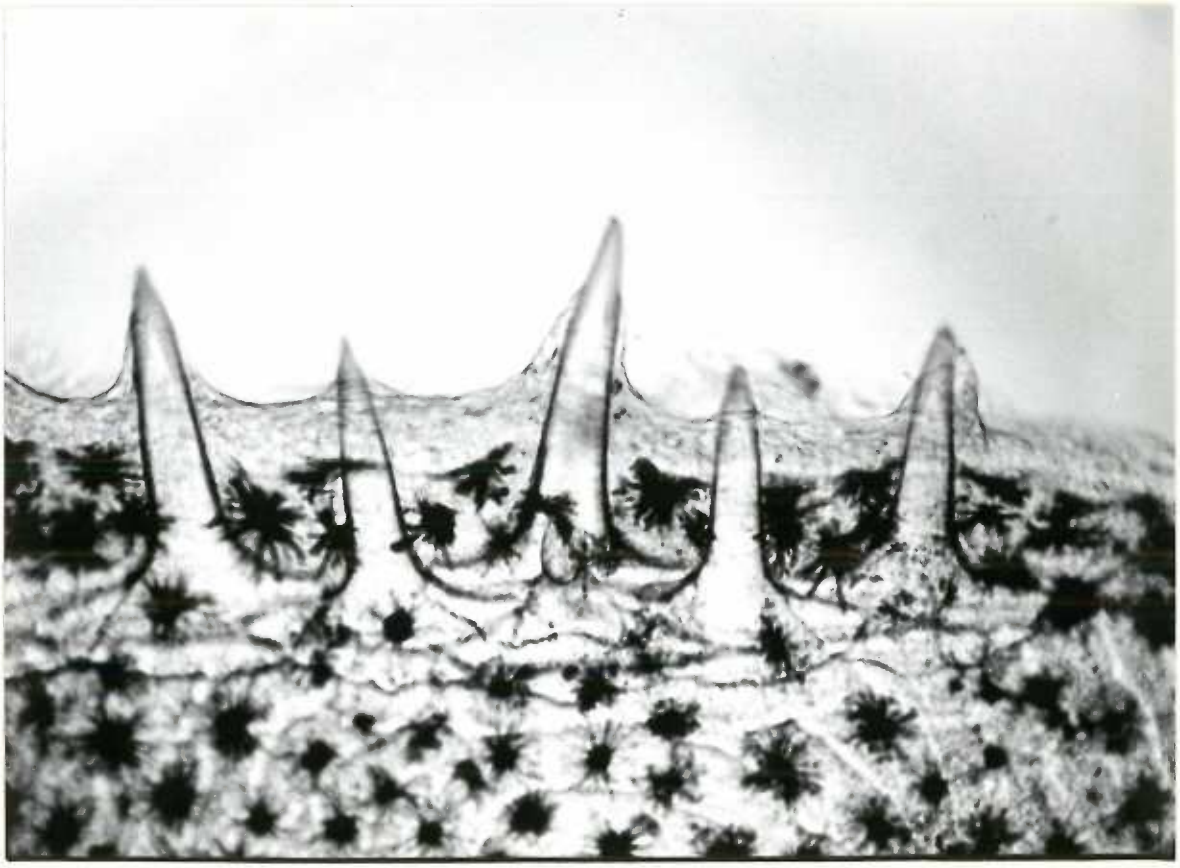
Figure 49:

Lower magnification photomicrograph of the caudal edge of a ctenoid scale (compare with Figure 48). Subepidermal melanophores are in focus.

Fixed in 10% formalin, unstained, mounted in glycerine. 200X.



48



49

Figure 50:

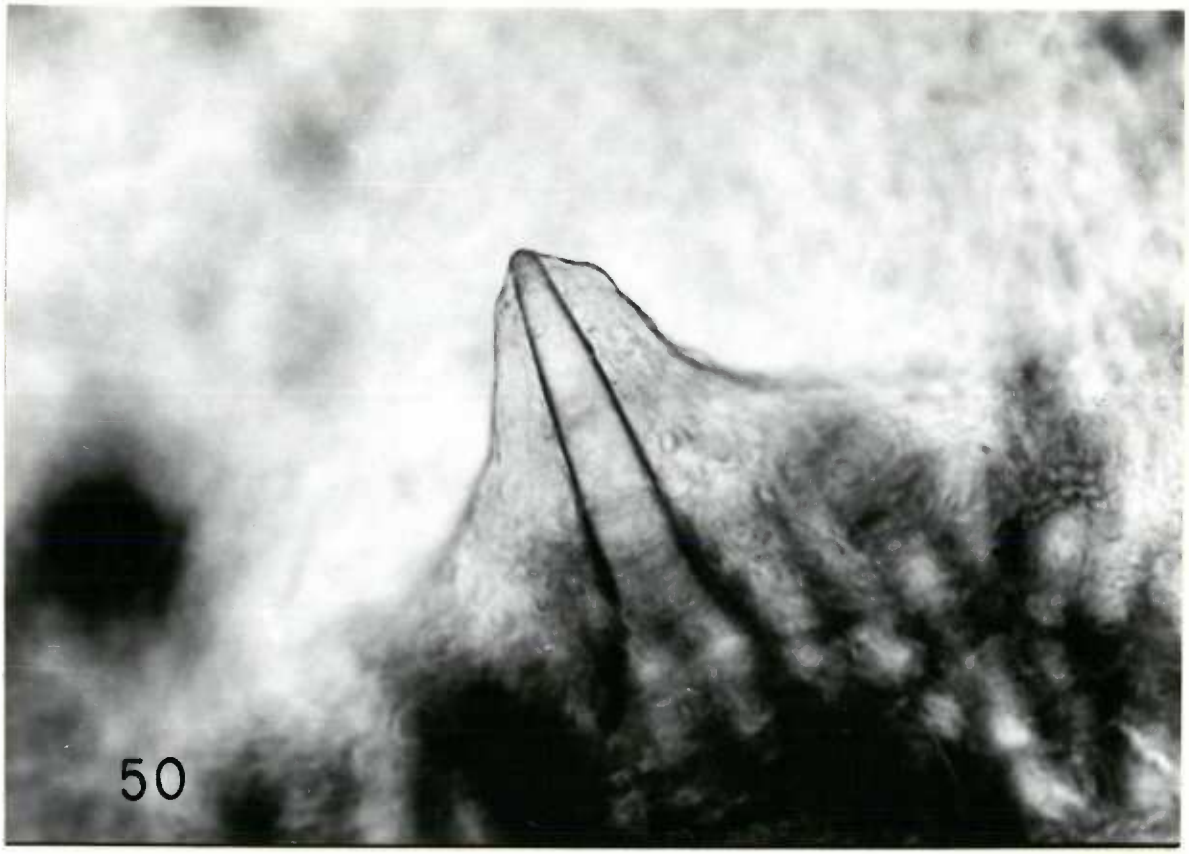
Photomicrograph of a single "tooth" or prong on a ctenoid scale. Note the layer of epidermal cells covering the prong.

Fixed in 10% formalin, unstained, mounted in glycerine. 320X.

Figure 51:

Photomicrograph of the caudal edge of a ctenoid scale on the non-pigmented (blind) side. (Compare with Figures 45-47.) The base or attachment site of the prong is seen. Note the lack of melanophores.

Fixed in 10% formalin, unstained, mounted in glycerine. 320X.


50

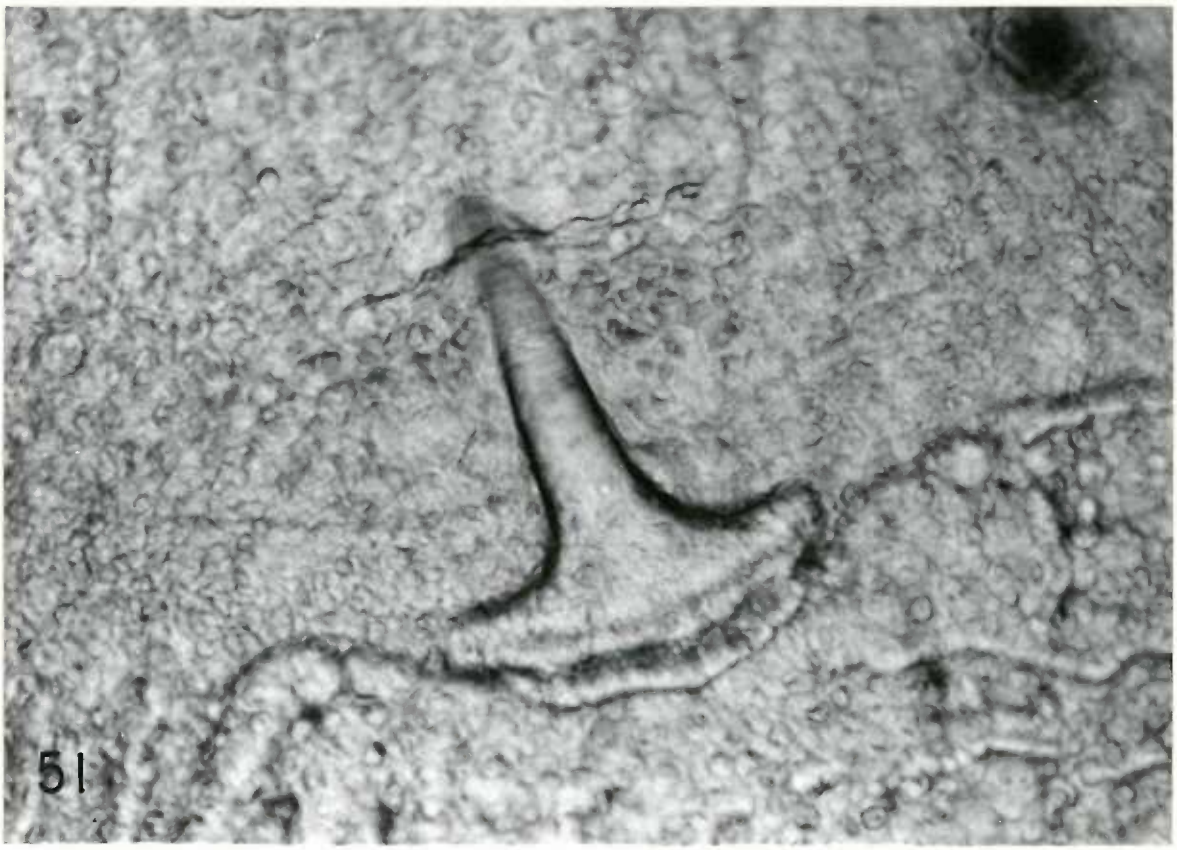

51

Figure 52:

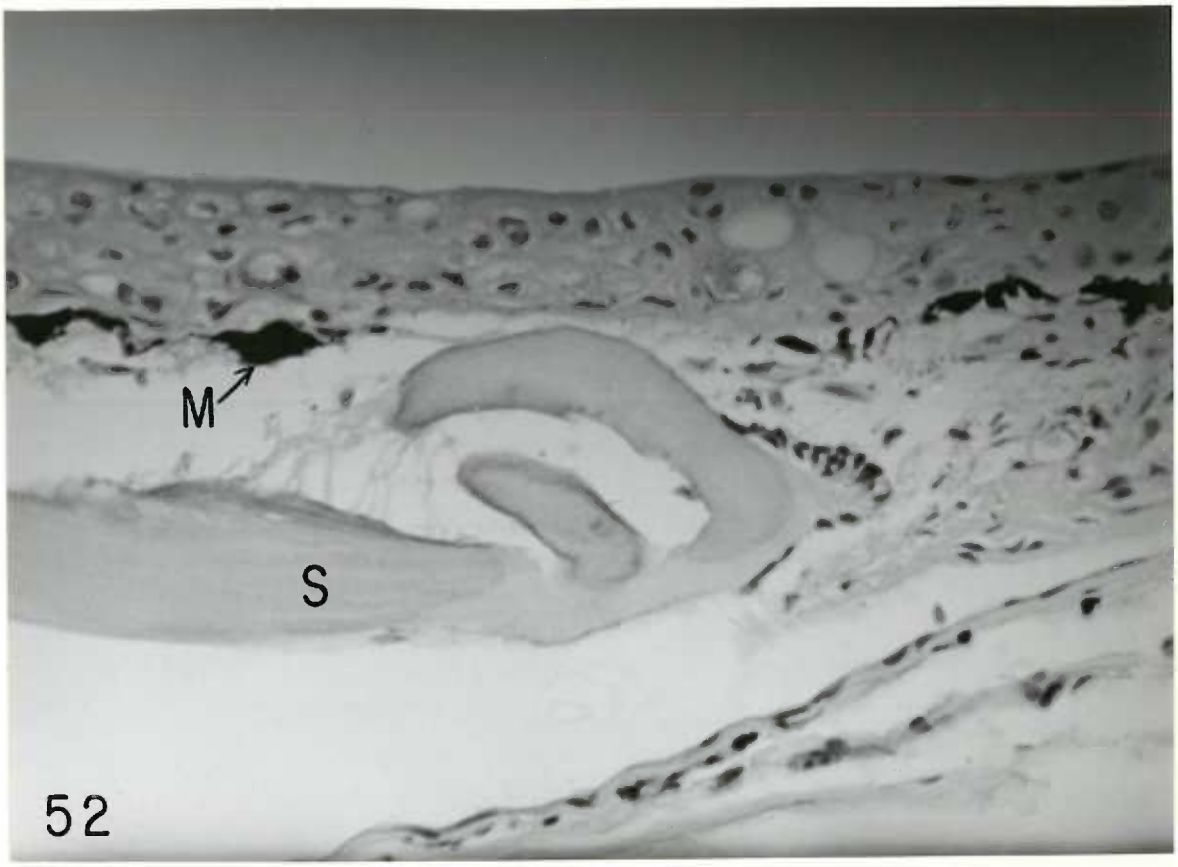
Photomicrograph of the epidermis and caudal edge of an underlying scale (S) on the pigmented or eyed side. Parts of two teeth are seen. The melanophores (pigment cells) (M) are located between the epidermis and the scale.

Bouin's fixed, hematoxylin and eosin stain. 655X.

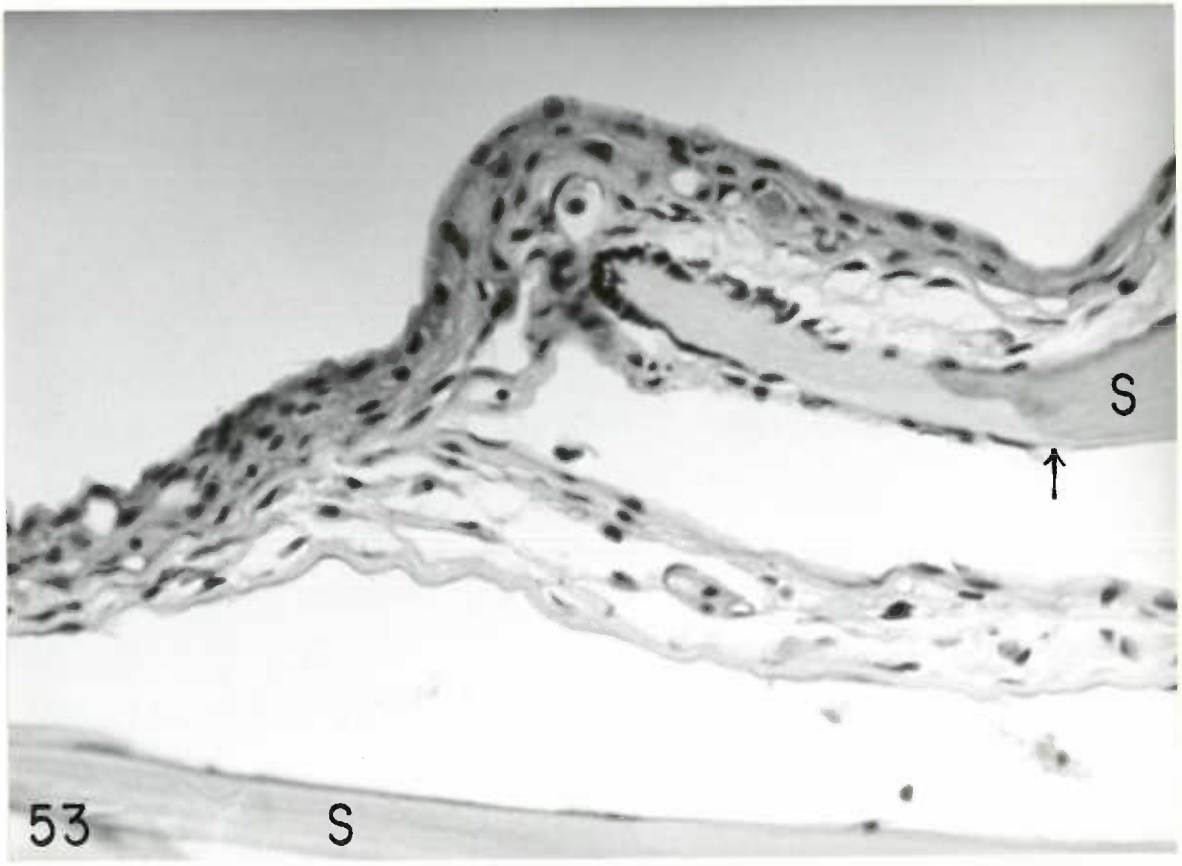
Figure 53:

Photomicrograph of the epidermis and caudal edge of underlying scales (S) on the non-pigmented or blind side. The staining characteristics of the scale change along the periphery (change occurs at arrow). The tissue between the artefactually separated scales consists of connective tissue elements (stains blue with Masson's trichrome).

Bouin's fixed, hematoxylin and eosin stain. 655X.



52



53

Figure 54:

Inner edge of a scale (upper half of picture) and part of two scale-forming cells (lower half) (see Figure 16 for localization). The cells are identical to fibroblasts except that desmosome-like junctions (D) are noted between the cells. Extracellularly (center), collagen fibers cut in cross-section are clearly seen to increase in diameter in the direction of the scale. The bulk of the scale is composed of closely approximated collagen fibers organized into lamellae. The fiber axes of adjacent lamellae are approximately perpendicular to each other, as in the dermal collagen (Figure 38). Cell membrane (CM), granular endoplasmic reticulum (ER).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 54,000X.

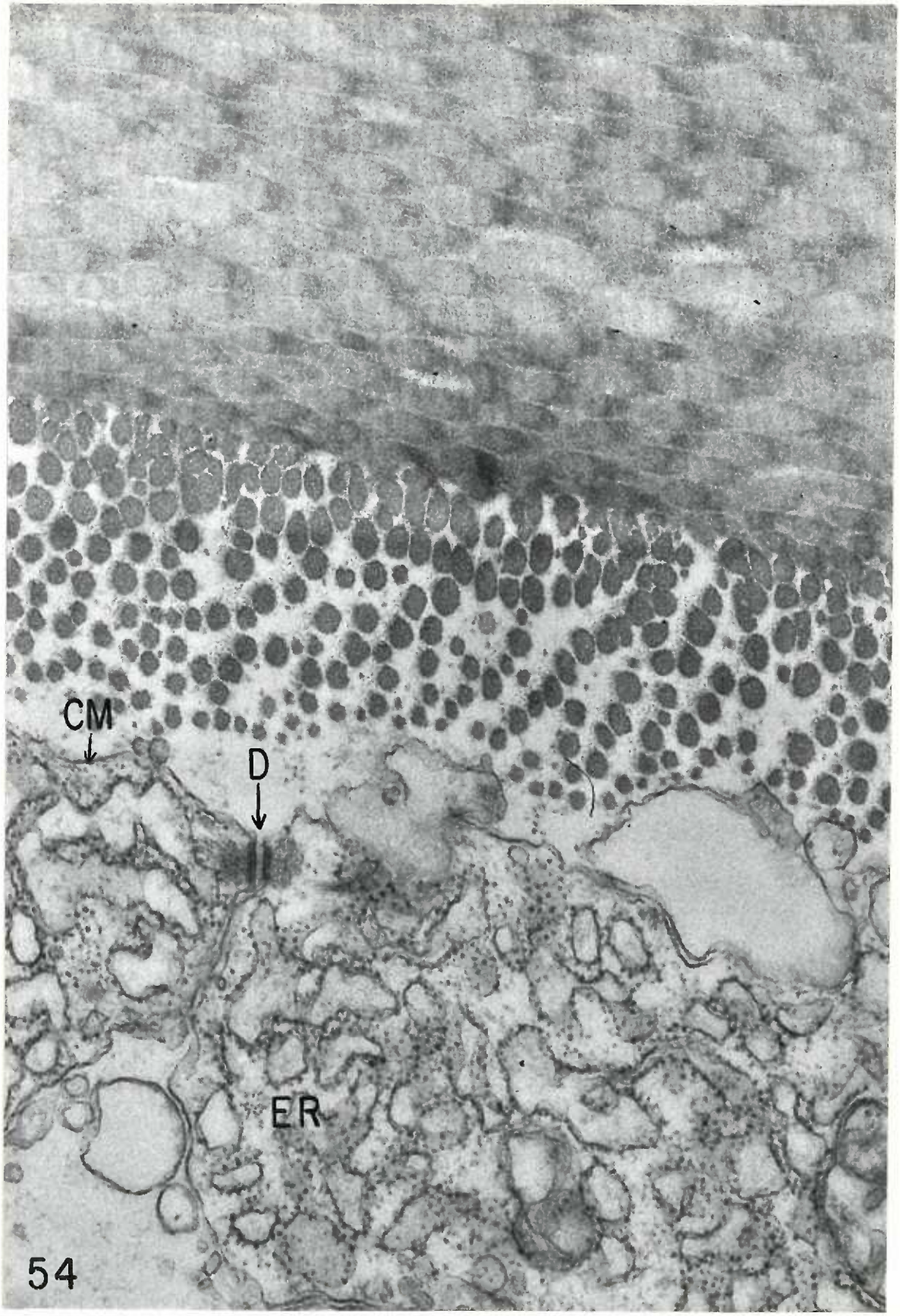

CM
D
ER
54

Figure 55:

Electron micrograph of calcified portion of scale (upper half of picture) and non-calcified portion (lower half) (see Figure 16 for localization). The calcified regions are generally located in the more superficial portion of the scale. The collagen-like fibers (below) show typical collagen banding at about 600 Å and a cross-sectional diameter ranging from 300 to 1100 Å. The matrix between the collagen fibers is more electron dense than the matrix between the dermal collagen fibers (Figure 38) and may represent ichthylepidin. Crystal-like needles of high electron density, visible just above the center of the micrograph, are interpreted as hydroxyapatite. The crystals and the collagen fibers appear to be partly leached out at the top of the photograph.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 107,000X.

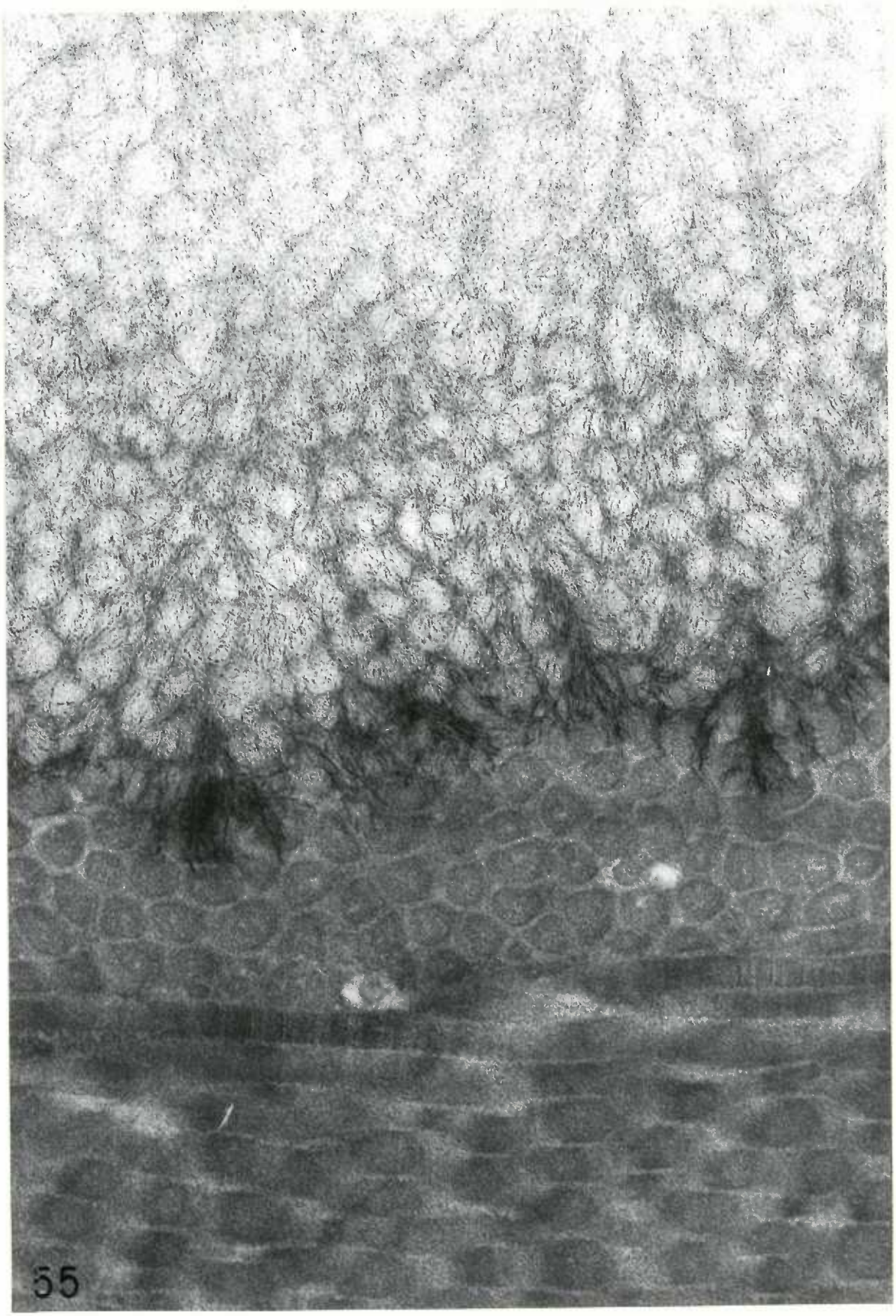

55

Figure 56:

Electron micrograph of the calcified part of a scale (see Figure 16 for localization). Collagen-like fibers are cut longitudinally (upper half of picture). The fibers have bands with a periodicity of approximately 600 Å. The bands may be composed of hydroxyapatite crystals laid down in the "hole zones" within the collagen fibers as described by Glimcher and Krane (64).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.


56

Figure 57:

Electron micrograph of the inner edge of a scale and its adjacent scalablast layer near the peripheral margin of the scale. In this region the collagen-like fibers (Co) lack a typical lamellar pattern. An electron dense border of hydroxyapatite crystals (HAC) is surrounding the calcified, central region of the scale. This central region appears to retain the disorganized pattern of the surrounding collagen.

Scalablast nucleus (N), granular endoplasmic reticulum (ER), desmosome (D), vesicles (V).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 69,000X.

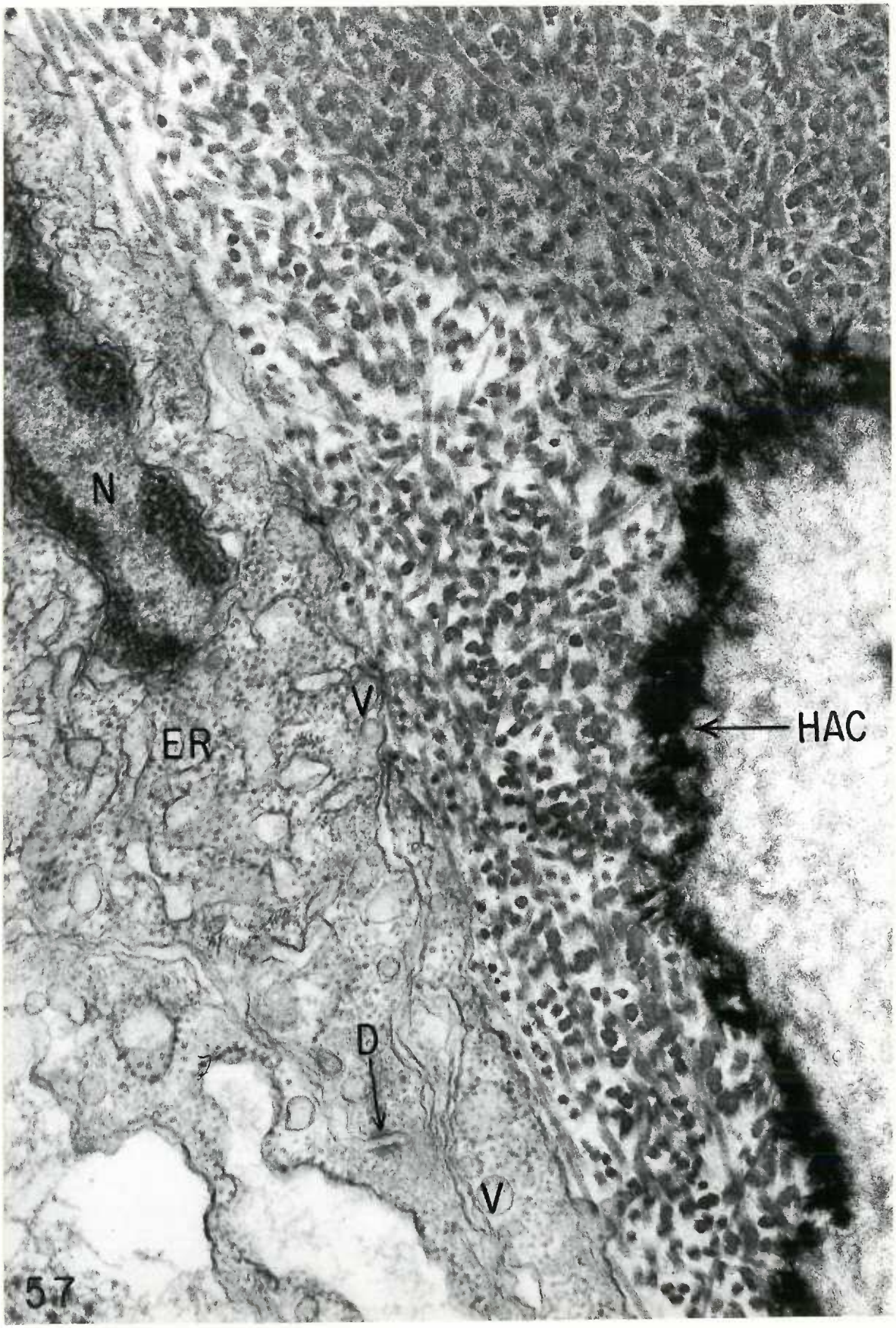

N
V
ER
HAC
D
V
57

Figure 58:

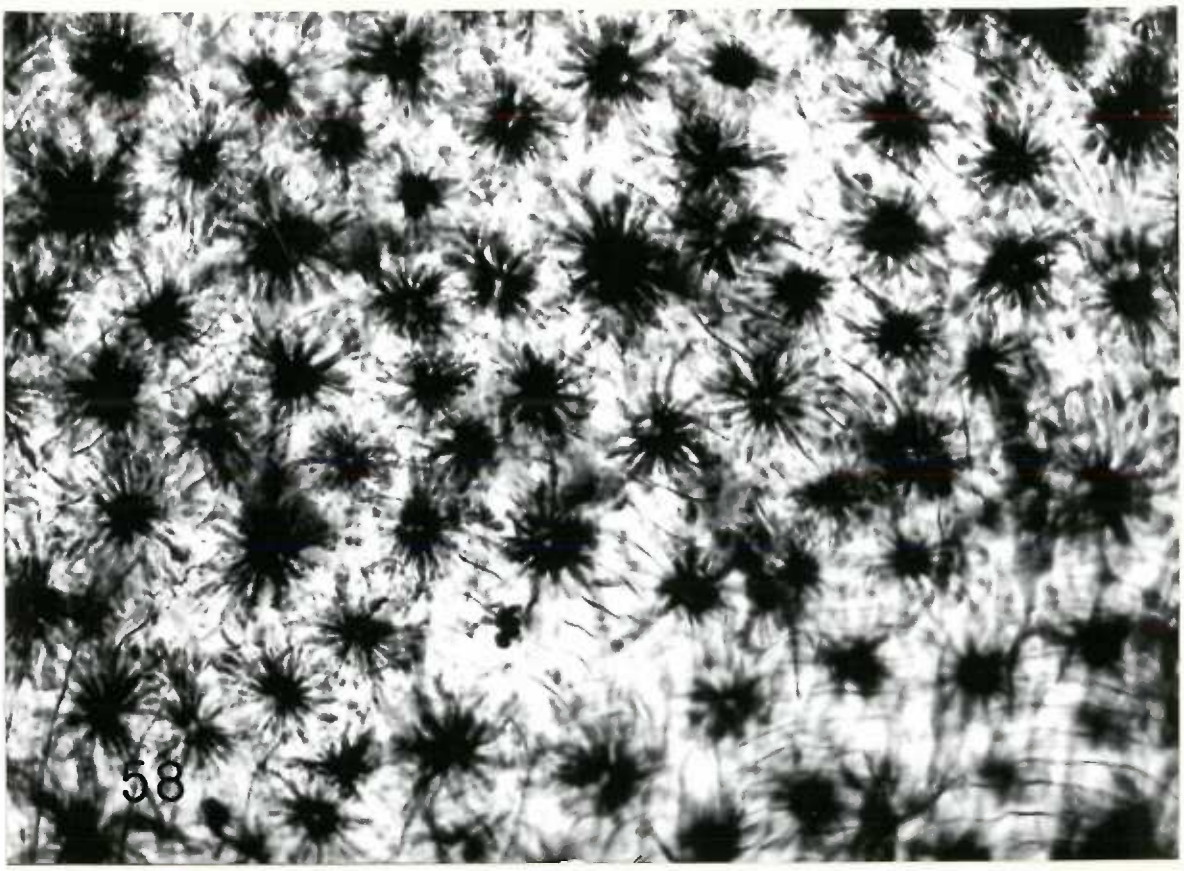
Photomicrograph of scale-containing skin on the pigmented side of a 2-year-old *H. elassodon*. Note the numerous stellate pigment cells or micromelanophores which measure approximately 0.07 mm in diameter.

Fixed in 10% formalin, unstained, mounted in glycerine. 320X.

Figure 59:

Same as Figure 59 except that both macro- and micromelanophores are seen. The macromelanophore measures approximately 0.40 mm in diameter while the micromelanophore (lower right) measures 0.07 mm in diameter. Micromelanophores are the predominant pigment cell in *H. elassodon*.

Fixed in 10% formalin, unstained, mounted in glycerine. 320X.


58


59

Figure 60:

Electron micrograph of a melanophore located in the outer dermis just deep to the basement membrane (BM). Melanosomes (melanin granules) appear as electron-dense granules. Nucleus (N), agranular endoplasmic reticulum (ER).

A cell interpreted as a lipophore (Li) is seen adjacent to the melanophore. This cell contains numerous cytoplasmic inclusions of varying size and electron density.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 16,600X.

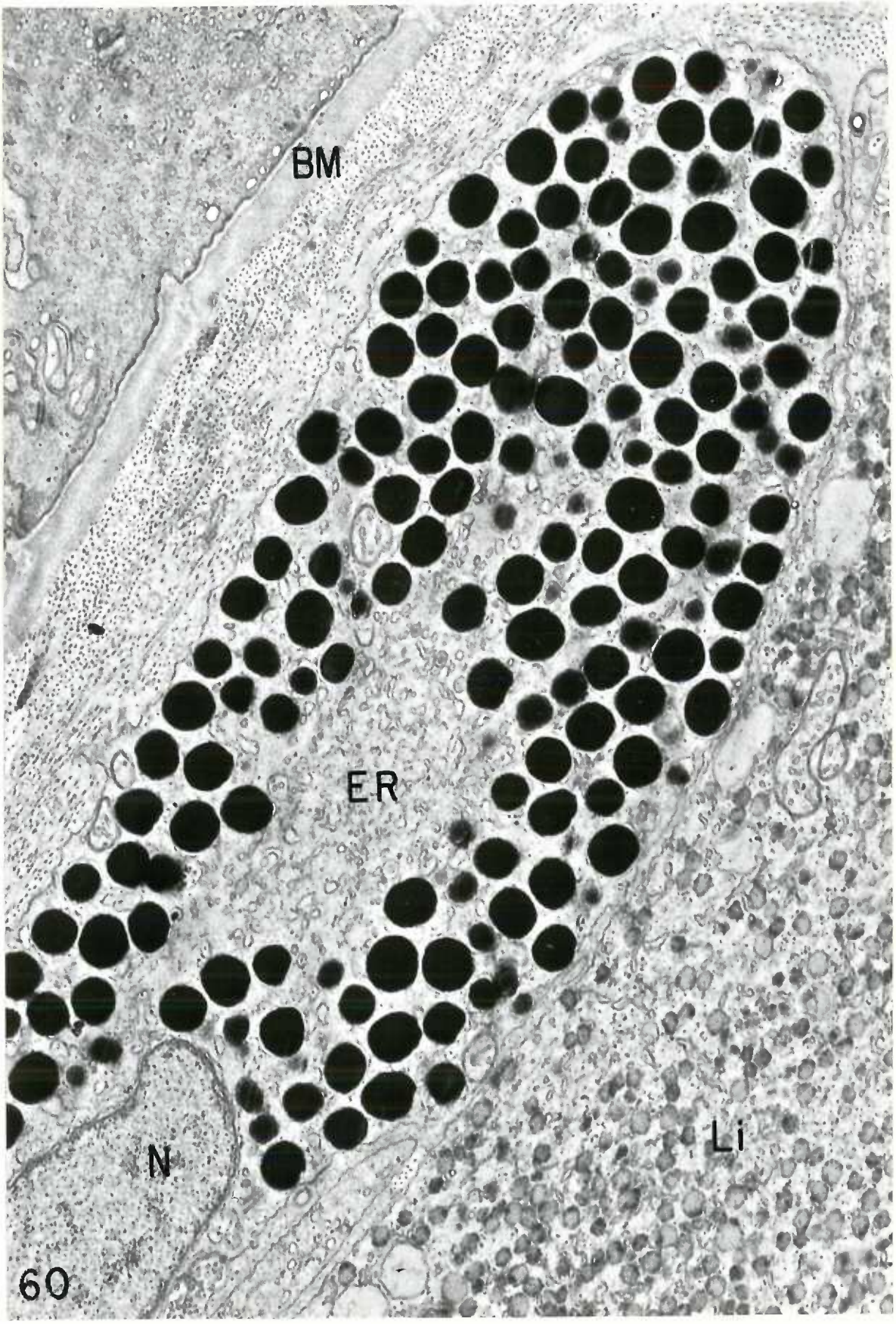

BM
ER
N
Li
60

Figure 61:

Electron micrograph of melanophore processes (containing melanosomes) within the outer, loose region of the dermis (stratum spongiosum).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 8,600X.

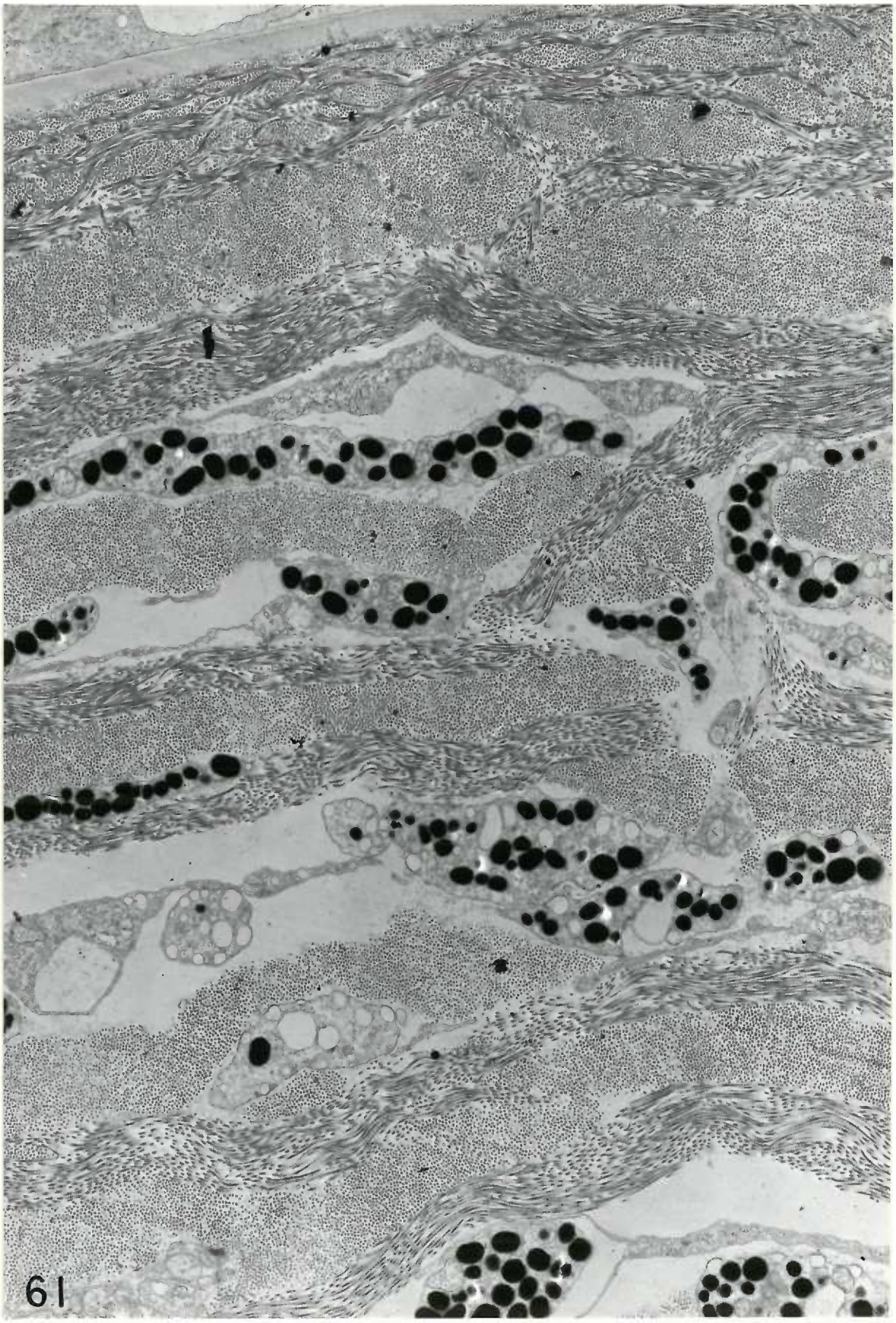
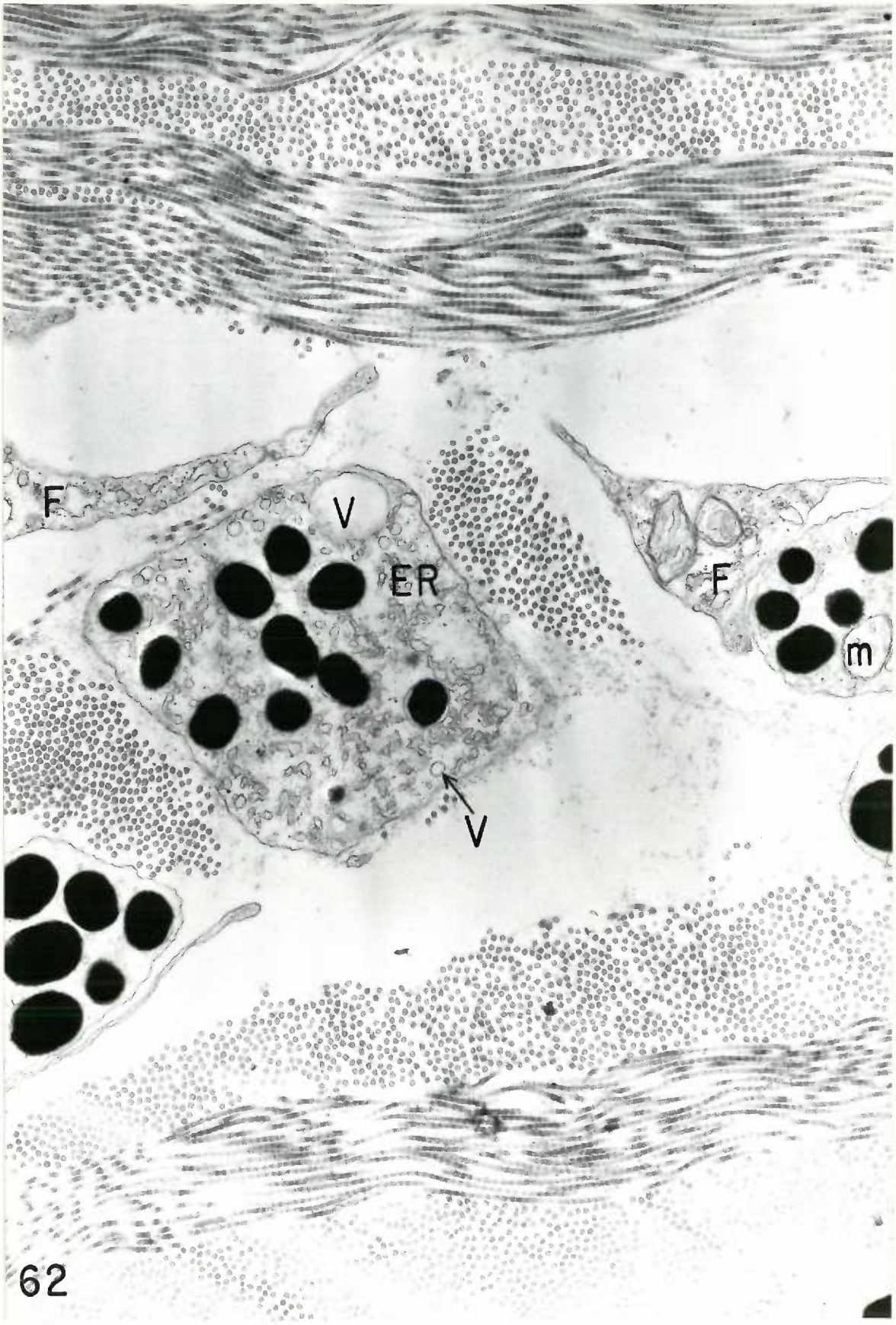

61

Figure 62:

Higher power electron micrograph of melanophore processes in the loose region of the dermis. The intracytoplasmic melanosomes appear homogeneous and very electron dense. The melanophore cytoplasm contains numerous small vesicles (V) and a few large ones (V), abundant granular endoplasmic reticulum (ER) and mitochondria (m). Portions of fibroblasts (F) are also observed.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 23,400X.



62

Figure 63:

Electron micrograph of cell interpreted as an immature melanophore within the intermediate cell region of the epidermis. The fine structure of overlying melanin granules (premelanosomes) can be faintly seen as thin, parallel, closely apposed lines of electron-dense material (arrows). A Golgi complex (G), mitochondria (m), and many agranular vesicles are also present within the cytoplasm. Nucleus (N).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 39,800X.

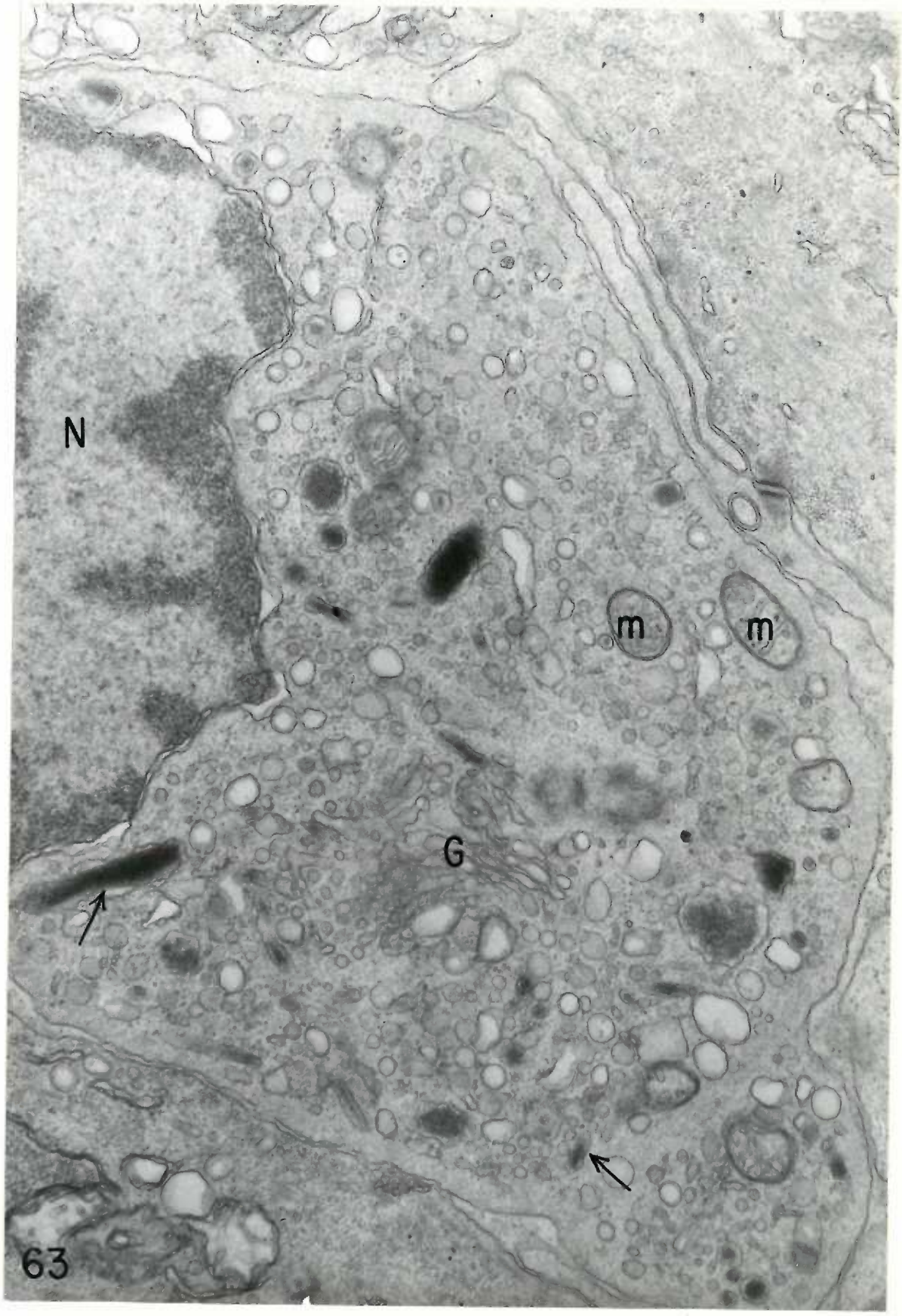

N
m
m
G
63

Figure 64:

Electron micrograph of an iridophore, a pigment cell which contains refractile crystals of predominantly guanin. The guanin crystals are pushed out during sectioning or dissolved, leaving empty spaces or clefts (183). Numerous smooth-walled vesicles (V) are located around the periphery of the cell.

The nucleus (N) displays an irregular outline. Golgi complex (G), mitochondria (m), guanin clefts (GC). This cell was located just below the basement membrane on the non-pigmented (blind) side.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 24,400X.

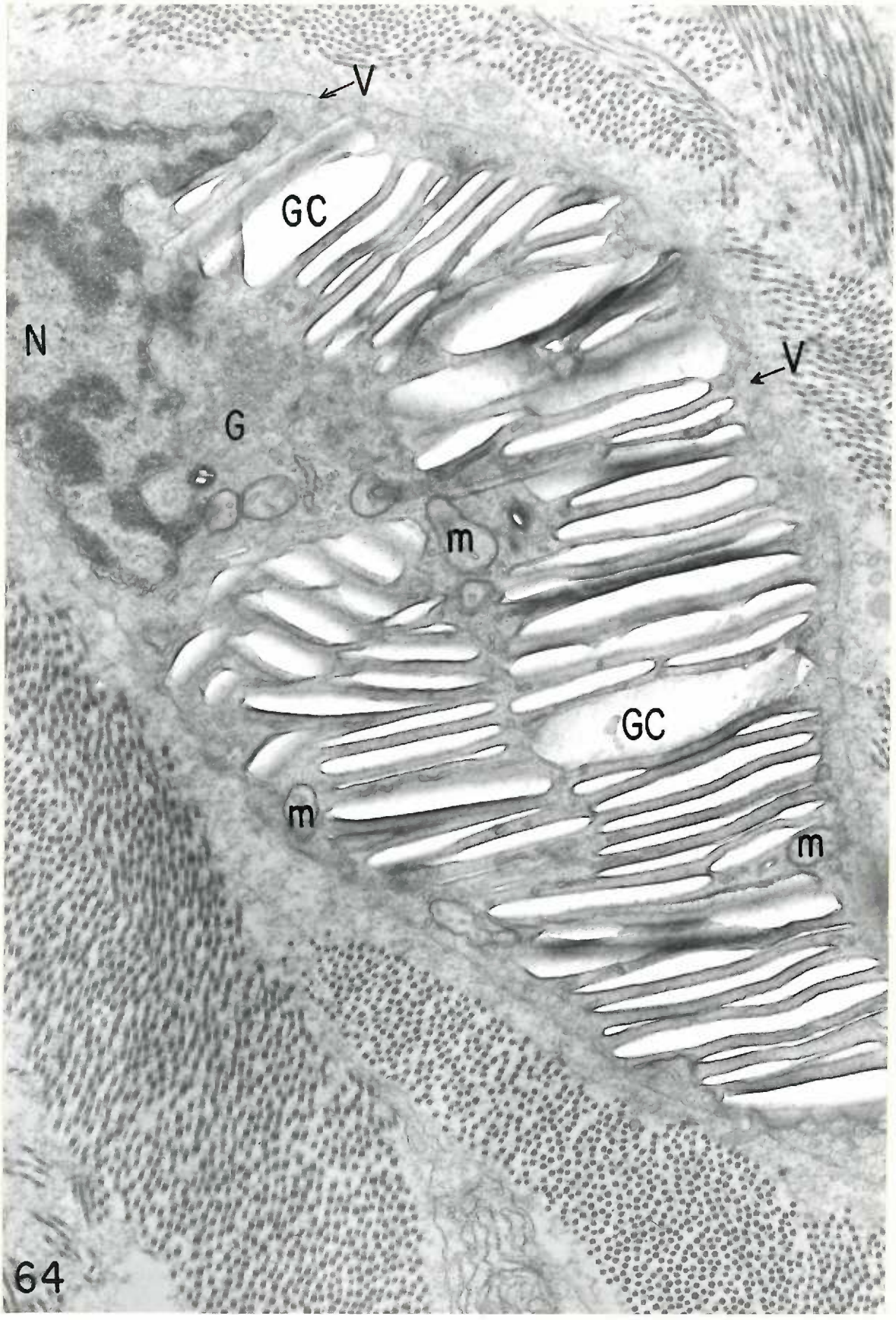

V
GC
N
V
G
m
GC
m
m
64

Figure 65:

Electron micrograph of a small blood vessel located within the dermis and containing one erythrocyte (RBC) and two leukocytes (WBC). The endothelial cells (EC) which line the vascular space contain many agranular vesicles.

The erythrocyte (RBC) has a homogeneous, finely filamentous pale cytoplasm and lacks other cytoplasmic organelles (a small Golgi complex was seen in one cell). The nucleus is circular and contains a large amount of electron-dense material, giving it a pyknotic appearance.

The leukocytes, of uncertain type (WBC), have numerous smooth-walled vesicles, mitochondria, and small granules interpreted as ribosomes. Some structures interpreted as inclusions (I) are also present. The nuclei have irregular outlines and the one in the lower left has an appearance that suggests it might be multilobulated.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 24,400X.

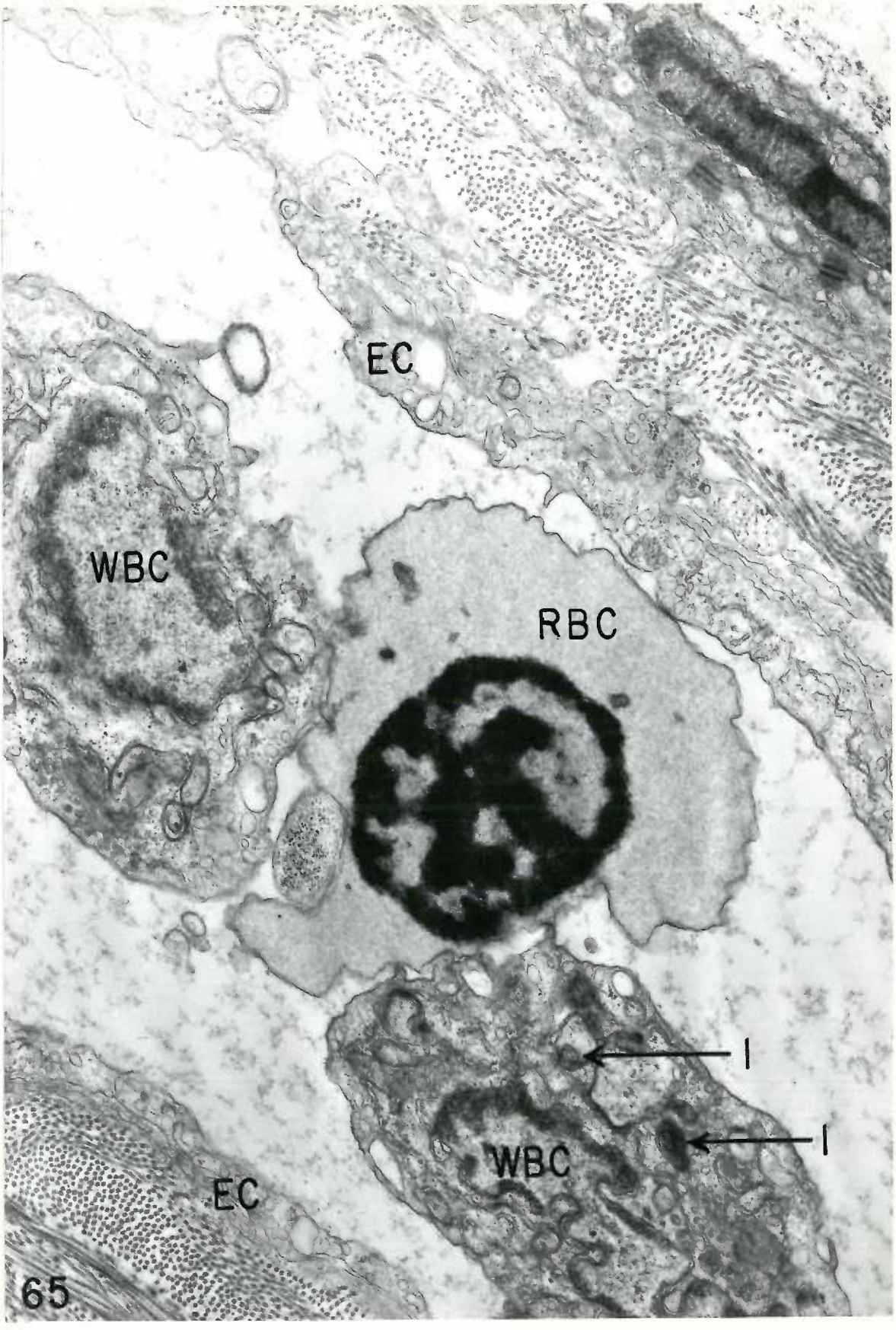

EC
WBC
RBC
WBC
EC
65

Figure 66:

Electron micrograph of a capillary (Cap) within the dermis adjacent to muscle (lower half of picture). An endothelial cell lining the capillary contains a nucleus (N) and numerous agranular vesicles (V).

The muscle layer contains many closely approximated myofibrils (Mf). These myofibrils contain numerous myofilaments (approximately 100 Å in diameter). Mitochondria (m) and many electron-dense granules (measuring approximately 210 Å in diameter) interpreted as ribosomes (R) are located between the myofibrils.

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 23,400X.

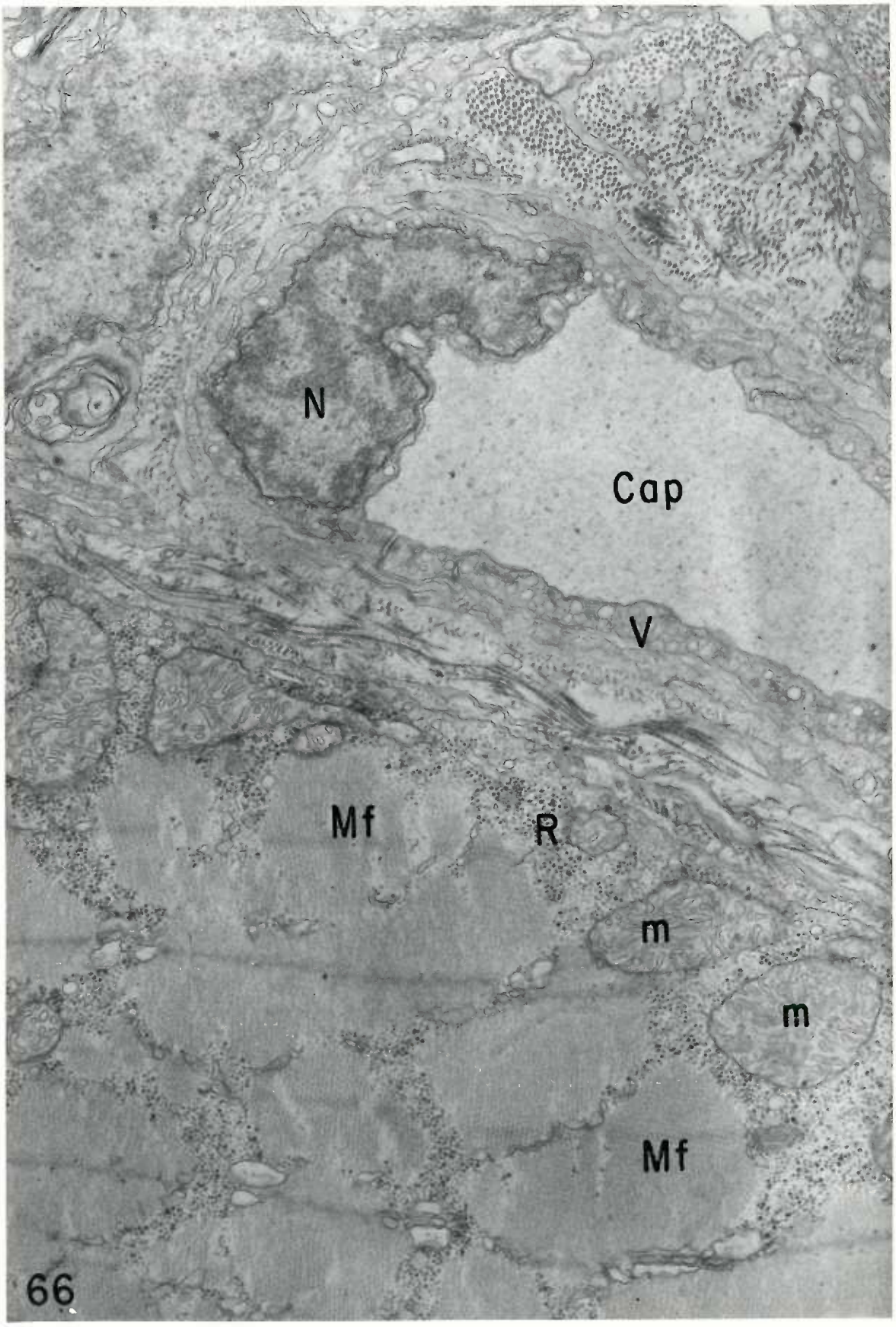
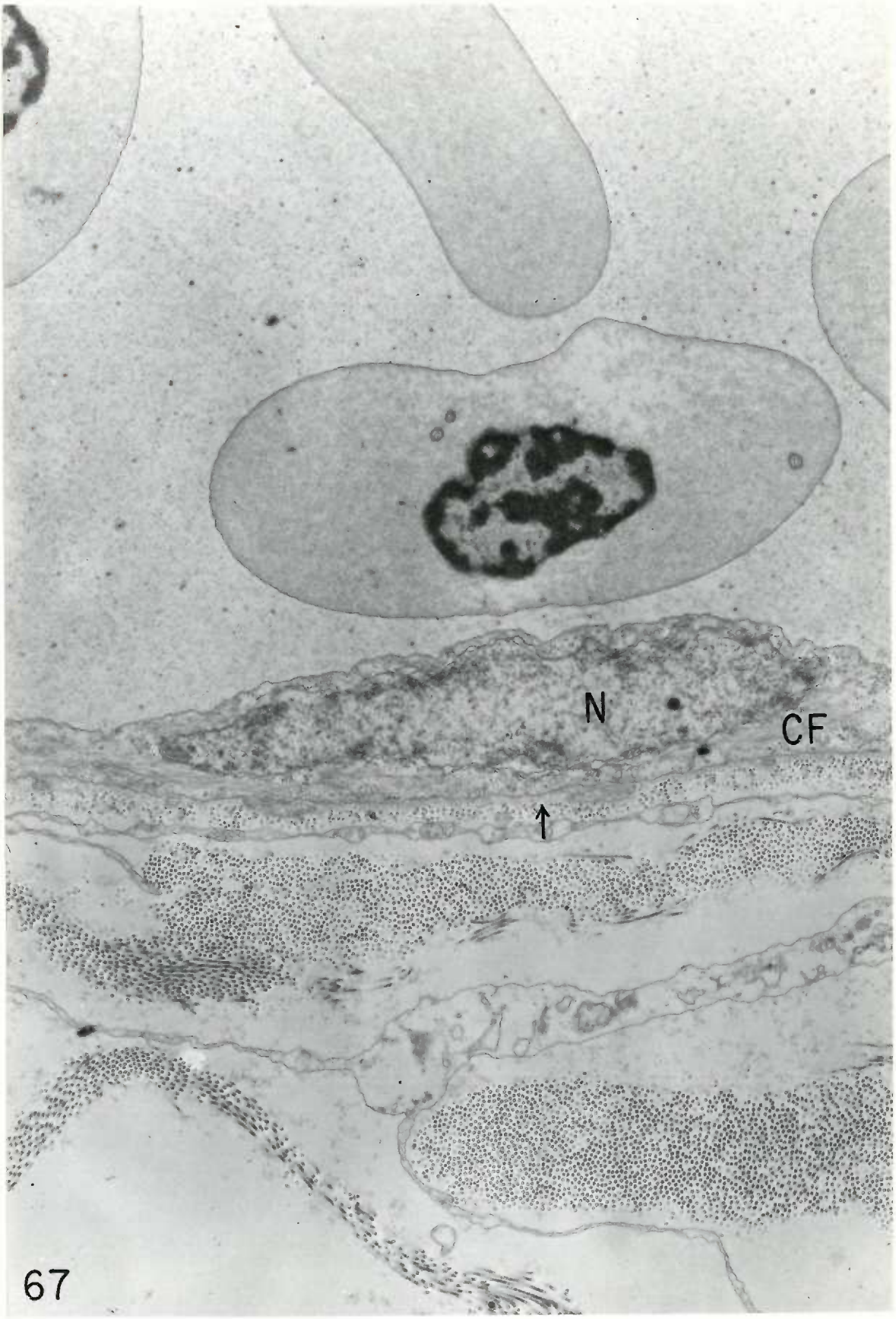

N
Cap
V
Mf
R
m
m
Mf
66

Figure 67:

Electron micrograph of a dermal blood vessel containing one whole erythrocyte and parts of two others. A thin basement membrane is present between the endothelial cell and the dermal collagen (arrow). Cytoplasmic filaments (CF) are seen within the endothelial cell cytoplasm. Nucleus (N).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 14,100X.



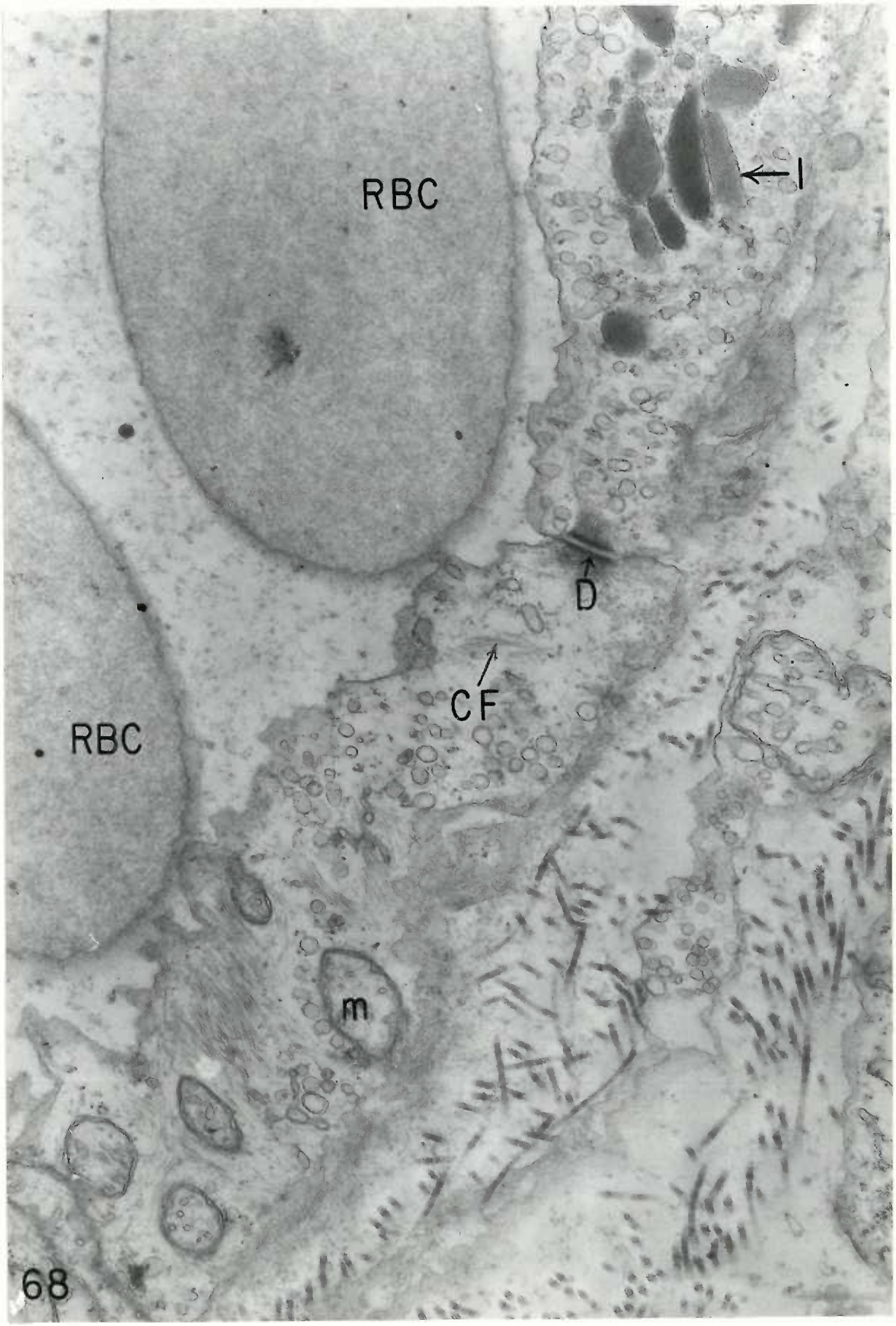
67

Figure 68:

Electron micrograph of the junction of two endothelial cells. A desmosome-like structure (D) modifies the space between the two cells. Membrane-bound structures containing electron-dense material are seen within the cell at the upper right (I). This probably represents phagocytized material within vesicles (inclusions).

80 Å cytoplasmic filaments (CF), mitochondria (m), erythrocytes (RBC).

1.33% OsO_4-S-collidine fixed, lead citrate and uranyl acetate stain. 39,800X.


RBC
I
D
CF
RBC
m
68