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AN ELECTRON MICROSCOPE STUDY OF THE COLUMNAR EPITHELIAL CELL IN THE INTESTINE OF FRESH WATER TELEOSTS: GOLDFISH *(CARASSIUS AURATUS)* AND RAINBOW TROUT *(SALMO IRIDEUS)*

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Summary. The columnar epithelial cells of the intestine in goldfish and rainbow trout were studied by electron microscopy. The most striking feature of the columnar cells, which was common in both fishes, was the extensive formation of lamellar structures in the cytoplasm. These were actually ribbon-like sheets which were bounded by two regular parallel membranes, and were found mainly in the basal half of the cytoplasm. In profile, these lamellar sheets were similar to the basal infoldings in the distal convoluted tubules of kidney, but were independent of the basal plasma membrane. The function of these lamellae is not known; however, these are presumably the structure involved in transport of water or nutrients. The remarkable difference between the goldfish and trout intestine was the occurrence of the invaginations of luminal surface between microvilli, and a variety of vesicles and vacuoles in the apical cytoplasm, observed exclusively in the posterior intestine of goldfish. In the present paper, it is suggested that there are differences in kind or degree of absorption between the goldfish and the rainbow trout, probably between stomachless fish and stomach-possessing fish in general, and that in the former food materials are ingested into the cell of posterior intestine by vigorous pinocytosis.

It is well known that Cyprinid fishes, such as goldfish or carp, have one of the simplest types of digestive tract among vertebrates. The goldfish does not possess a true stomach, and even in the intestine there is no differentiation of the intestinal glands nor crypts of Lieberkühn (McVAY and KAAN, 1940; SARBAHI, 1951). The apparent lack of complexity in structural pattern of the intestinal mucosa has attracted the attention of many morphologists in studying the structure and function of the digestive tract of such stomachless fish.

The most recent study dealing with Cyprinid intestine was made by AL-HUSSAINI (1949a, 1949b) who reviewed the histological literature and examined the intestines of three Cyprinids *(Gobio, Cyprinus, Rutilus)* cytologically and histochemically, and conducted biochemical studies as well. He concluded that their intestinal epithelium was composed principally of columnar epithelial cells and goblet cells, the former being far more numerous. He also reported that the columnar epithelial cell was an absorptive cell and at the same time functioned as a secreting cell which elaborated the enzymes involved in intestinal digestion.

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However, in his light microscopy, he could not show morphological evidence of secretion. McVAY and KAAN (1940), and SARBAHI (1951) studied the histology of the digestive tube in goldfish, and their observations were also in general agreement with those of earlier investigators who had studied the intestine of other stomachless fishes (BABKIN, 1928; ROGICK, 1931; CURRY, 1963).

Electron microscopy has contributed a great deal to our knowledge of the structure and function of mammalian intestine (WEISS, 1955; ZETTERQVIST, 1956; PALAY and KARLIN, 1959a, 1959b; CLARK, 1959; RUSKA, 1960; others), but very little is known about the fine structure of the intestinal mucosa in these interesting fishes. Only it has been recently reported that the columnar epithelial cells of goldfish are provided by special lamellar structures in the cytoplasm (YAMAMOTO, 1961). The present study has been undertaken with a view to extending the earlier observation of light microscopy, and to reveal the fine structure of the intestinal mucosa of goldfish by means of electron microscopy. Particular attention was paid to the detailed internal organization of the columnar epithelial cell which constitutes the bulk of the mucosa throughout the entire intestine. Furthermore the attempt was made to examining the fine structure of the intestinal columnar epithelium of rainbow trout which possesses a stomach, in comparison with that of the goldfish.

Material and Methods

Materials used for this study were 20 young goldfishes ca. 10 cm in length, and 5 rainbow trouts obtained from the aquarium of the Fisheries Center at the University of Washington. Small pieces from the intestinal bulb, and from the middle and posterior parts of the intestine were removed from unanesthetized goldfishes into drops of fixative. Short lengths of the intestine of rainbow trout were also removed in the same way as in the goldfish. After cutting into small bits, the specimens were fixed for two hours in fresh and cold 2.5% osmium tetroxide buffered at pH 7.4 with s-Collidine (BENNETT and LUFT, 1959), dehydrated in graded ethanol and then embedded in Epon epoxy resin (LUFT, 1961).

Thick sections for light microscopy were cut on a Porter-Blum microtome, and stained with 0.5% toluidine blue in phosphate buffer at neutrality. The photomicrographs were made using a Zeiss Planachromat objective and a green interference filter. Thin sections for electron microscopy were made in the same way as above, but stained with lead acetate method (DALTON and ZEIGEL, 1960) or with alkaline lead tartrate method (MILLONIG, 1961). These sections were examined with an RCA EMU 2A or 2C electron microscope equipped with a special stabilized power supply.

Observations

Light microscopy

For better understanding of the epithelial cells of the intestinal tract of the goldfish and rainbow trout, it is necessary to describe briefly the general structure of the mucosa. Viewed externally, the intestine of goldfish may conveniently be divided into two parts; the intestinal bulb and the intestine proper (McVAY and KAAN, 1940). The former joins the oesophagus directly, occupying the place of the stomach in the mammalian digestive tract, and is wider than the remainder of the intestine. The intestine proper is the narrow long winding portion.

On the other hand, the intestine of rainbow trout, succeeding to a stomach, is shorter straight tube and shows almost the same diameter everywhere in the intestine.

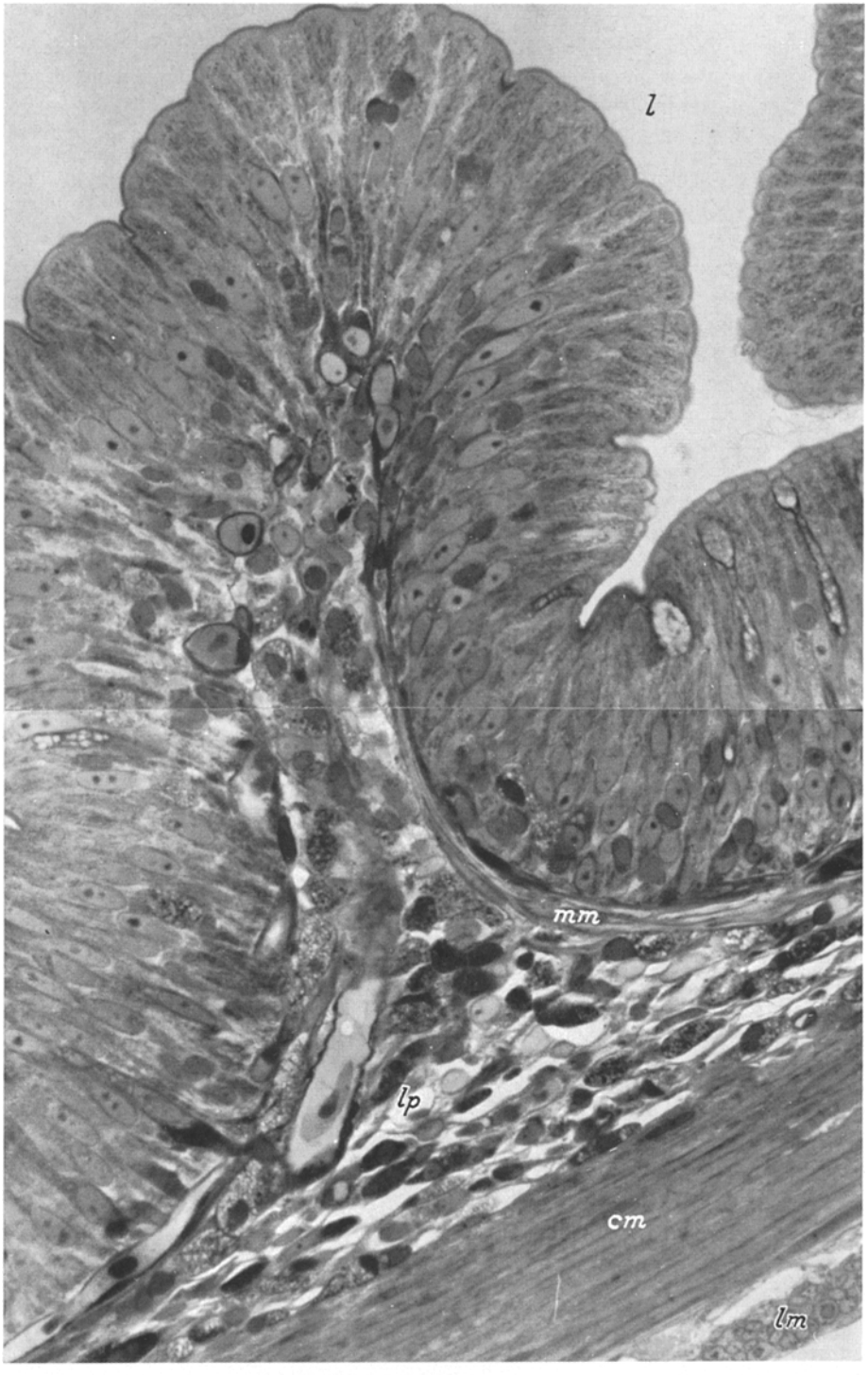

Fig. 1

In both fishes the principal structure of the mucous membrane is the same throughout the entire intestine, though there are some minor differences in its architecture in the goldfish. Mucosal folds can be seen everywhere in the intestine, though they are more complex in the intestinal bulb of goldfish (Fig. 1). There is no differentiation to form multicellular glands nor crypts of Lieberkühn. The lining cells covering the folds are of two types, the most numerous of which is simple columnar cells (Figs. 1, 4, 8); the remainder is the goblet cells. Besides these two types of cells, a few migratory cells are present in the epithelial layer.

The columnar epithelial cells are long and narrow in appearance (approximately 70 μ in length, 10 μ in width), and their long nuclei are located just below the middle of the cell. The free surface of these cells is covered with a distinct striated border. In the cytoplasm immediately beneath the striated border occurs a prominent clear zone which corresponds to the terminal web (Figs. 1, 4, 8).

The cytoplasm except in this clear zone, is characterized by two regions of concentrated granulation which are due presumably to mitochondria. The first region occurs just below the clear zone in the apical cytoplasm, and the second appears in the basal area. Besides these granulation zones, a few mitochondria are also distributed around the nuclei.

In the posterior half of the intestine of regularly-fed goldfish the columnar epithelial cells contain a variety of vacuoles in the apical cytoplasm (Fig. 4), and it appears that the mitochondria are reduced in number when vacuoles occur. These vacuoles, however, can never be observed in the intestinal bulb of goldfish (Fig. 1) and the intestine of rainbow trout (Fig. 8).

In the cell boundaries beneath the striated border, typical terminal bars are present at the level of the clear zone. In the rainbow trout other attachments can sometimes be seen as minute dense spots along the cell boundaries below the terminal bar, whereas in the goldfish these are not prominent. This second type of attachment is seen sporadically on the cell boundaries between adjacent cells from the top to the base of the cell.

In goldfish intestine, subjacent to the epithelium a well developed framework of blood capillaries can be seen surrounding the epithelial layer (Figs. 1, 4). At the base of the folds, an irregular layer of modified smooth muscle lies immediately beneath the blood capillary layer and extends into core of the mucosal folds (Fig. 1). This muscle layer becomes more sparse toward the posterior intestine, althouth a few smooth muscle cells can still be seen scattered in the lamina propria.

In rainbow trout intestine, the epithelium rests on a distinct basement membrane (Fig. 8). The blood capillaries are sparse in the core of the intestinal folds, and, the morphological relationship between the intestinal epithelial cell and the blood capillary is not as close as in goldfish intestine where a well-developed blood capillary bed underlies the basement membrane.

Fig. 1. A light micrograph showing a part of cross section of the intestinal bulb in goldfish. A mucosal fold protruding into the lumen (*l*) is shown in the center. The principal cells composing epithelium are columnar epithelial cells and goblet cells; the former is most numerous. Some granular cells and small round cells also are seen in the basal half of the epithelial layer. These are regarded as migratory cells from the lamina propria (*lp*). In the columnar epithelial cells, numerous mitochondria are found in granular or filamentous form at the supra-nuclear region. At the base of fold, the thin smooth muscle layer (*mm*) is seen surrounding the epithelium. Lamina propria (*lp*), circular muscle layer (*cm*), longitudinal muscle layer (*lm*), and serosa also can be seen in this picture. × 600

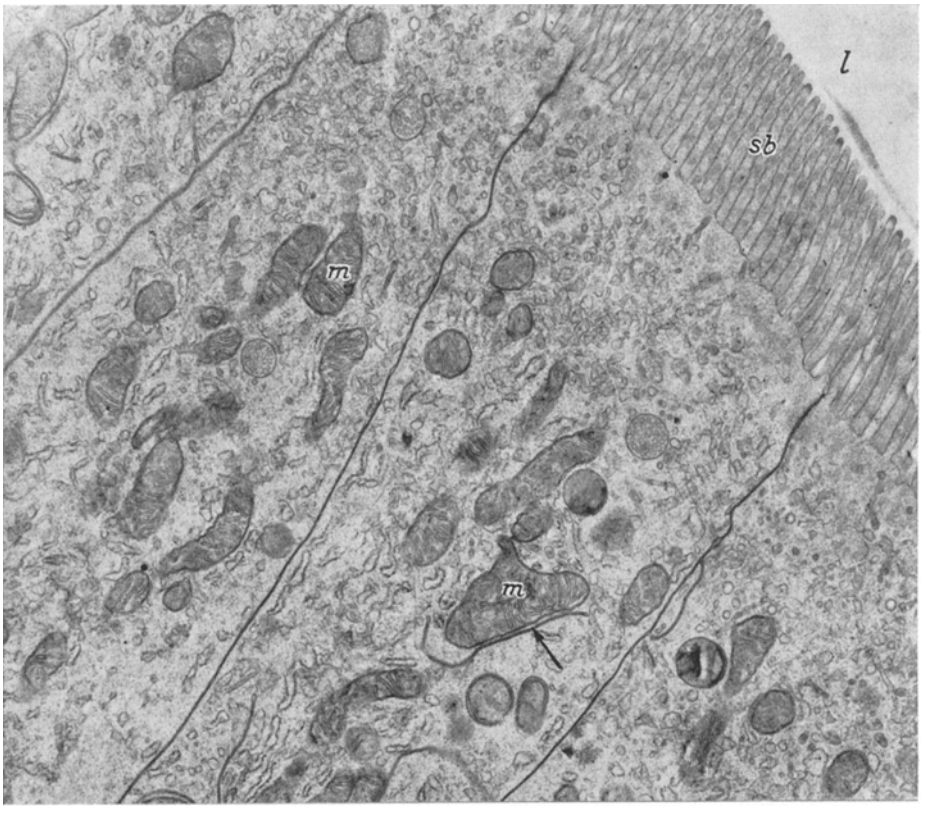

Fig. 2. An electron micrograph of the apical portion of columnar epithelial cells in the intestinal bulb of goldfish. The striated border (*sb*) facing the intestinal lumen (*l*) is tightly packed with narrow long microvilli. Beneath the microvilli is seen the terminal web in which cell organelles are absent, except for a few small tubules and vesicles which are more frequent in the cytoplasm below the terminal web. Multivesicular bodies also are seen confined to this region. A lamellar structure (arrow) is seen closely associated with a mitochondrion (*m*). × 20,000

Electron microscopy

1. Goldfish

a) The columnar epithelium of intestinal bulb. The columnar epithelial cells in this portion are long narrow cells with large oval nuclei located below the middle of the cell. These cells tend to taper toward the cell base, though there is some variation in size from place to place.

The luminal surfaces of these cells are densely covered with microvilli (Fig. 2). These microvilli appear to be the same as those of the intestinal epithelial cells in mammals which have been reported so far (WEISS, 1955; ZETTERQVIST, 1956; PALAY and KARLIN, 1959; RUSKA, 1960; and others). The lateral surfaces of the cells make smooth contact with neighboring cells, and the elaborate interdigitations between adjacent cells which have been found in mammalian intestine are not encountered here (Fig. 2). Beneath their free surfaces, the neighboring cells are attached to each other by a junctional complex similar to that described by FARQUHAR and PALADE (1963). Instead of the interdigitations, desmosomes occur irregularly distributed between adjacent cells.

The cytoplasmic matrix beneath the microvilli is free of cell organelles, except for a very few vesicles and small tubules (Fig. 2). Instead, this area reveals a fine filamentous complex called the terminal web, corresponding to the level of the terminal bar. However, the terminal web is not as prominent as in the case of mammalian intestine.

Numerous mitochondria are scattered in the supra and infra-nuclear cytoplasm, and are irregular in shape and orientation. The Golgi apparatus, with its stacked lamellae and vesicles, is well developed and is found just above the nucleus. The granular or agranular endoplasmic reticulum in irregular form is distributed throughout the cytoplasm. Multivesicular bodies are seen frequently in the apical cytoplasm (Fig. 2). Other small accumulations of vesicles, some of them partially bounded by membranes, are present as well, and these appear to be in some way related to formation or breakdown of multivesicular bodies.

The most striking feature of the columnar epithelial cells is the extensive formation of single lamellar structures (Figs. 2, 3). These structures, which have not been found in mammalian intestine, are best developed in the basal half of the cell, but a few of these lamellae also can be seen above the Golgi region (Fig. 2). Most of them appears to be arranged parallel to the long axis of the cell. In cross sections, these lamellar structures are identifiable as flattened sacs which are bounded by two very regular parallel membranes separated by about 250 Å (peak-to-peak distance). The membranes comprising the lamellae appear to be thicker and more dense than the membranes of endoplasmic reticulum. It is therefore easy to distinguish these lamellae from other endoplasmic components. They are never associated with RNP particles, but their cavities frequently appear to be denser than the cytoplasmic matrix. Sometimes these lamellae are located near the lateral surface of the cell. Occasionally these peripheral lamellae can be seen to open into the intercellular spaces, in which case the membranes of lamellae are continuous with the lateral plasma membrane (Fig. 3). In cross sections, it is not unusual to encounter lamellae giving the appearance of infoldings of the lateral plasma membranes (Fig. 3). From serial micrographs it is apparent that these lamellar structures are ribbon-like sheets, in which multiple fenestrations occur, and at the periphery of which are seen many finger-like projections associated with small vesicles. In many sections a close morphological relationship can be seen between the mitochondria and the lamellae, similar to that found between the mitochondria and the basal infoldings in the epithelium of the distal convoluted tubule in kidney.

Among these lamellae one can see small membrane-bounded bodies containing a light staining granular substance which bear some resemblance to secretory granules (Fig. 3). However, this type of structure is not present in the apical region, but is confined solely to the basal half of the cell. Therefore, the presence of such a structure does not seem to furnish morphological evidence that the epithelial cells are secretory in function.

b) The columnar epithelium of the posterior intestine. The columnar epithelial cells in this portion resemble in appearance and arrangement those in the intestinal bulb, though differing considerably in some aspects of their internal structure.

The microvilli on the luminal surfaces are slightly lower, and somewhat more loosely arranged than in the columnar epithelial cells of the intestinal bulb. A

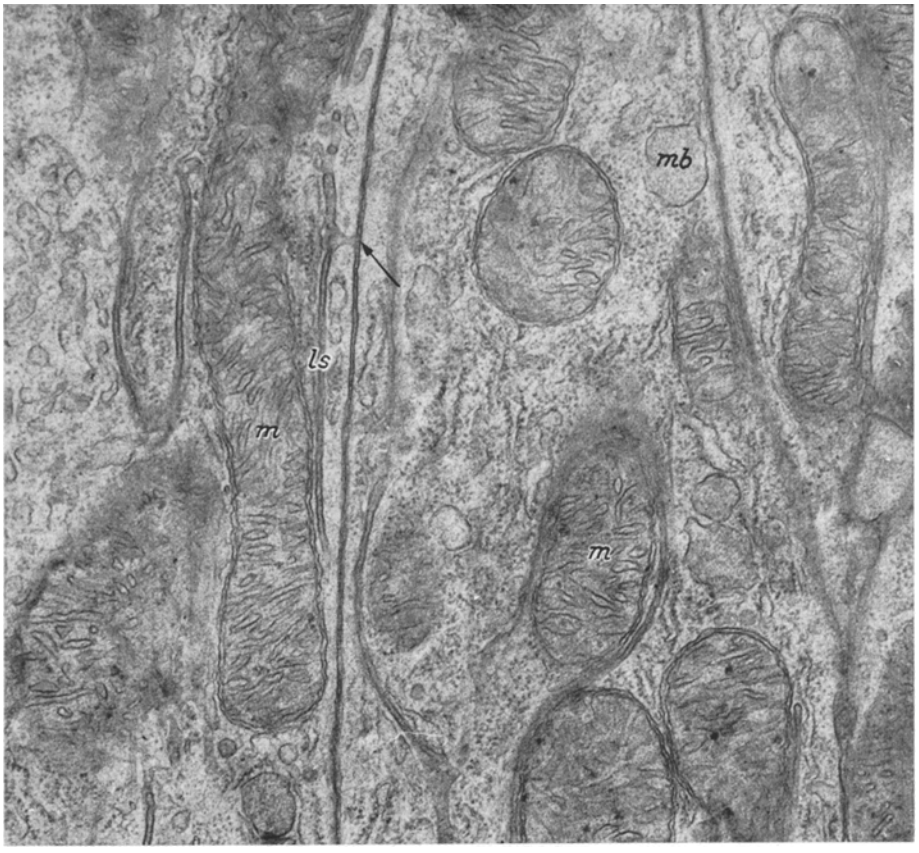

Fig. 3. A longitudinal section of the infra-nuclear cytoplasm of the columnar epithelial cells in the intestinal bulb of goldfish. The close association between the lamellar structures (*ls*) and a mitochondrion (*m*) can be seen. One of these lamellar structures shows continuity with the intercellular space by the small short tube (at the point indicated by arrow). Less dense bodies (*mb*) bounded by a single membrane are seen in the cytoplasm. × 35,000

distinctive feature of the luminal surface of these cells is the conspicuous occurrence of invaginations of the intermicrovillous plasma membrane (Fig. 5). Such invaginations appear to be confined to the cells of the posterior intestine, and in the form of flattened tubules, occasionally show indications of dense materials attached to their walls. The cytoplasmic surfaces of these invaginations do not appear to possess filaments or rodlets (ROTH and PORTER, 1964). The lateral surfaces of the cells are identical in structure with those in the intestinal bulb.

In the apical cytoplasm a variety of vesicles and tubules can be seen among and below the invaginations of the intermicrovillous plasma membrane (Fig. 5). These vesicles and tubules appear to be closely related to such invaginations. Among the varying vesicles and tubules, rather thicker filamentous structures oriented parallel to the long axis of the cell, are scattered sparsely (Fig. 5). These thick filaments are apparently composed of bundles of finer filaments, sometimes branched, which extend through the terminal web. Below the zone of vesicles the cytoplasm usually shows a few large vacuoles containing materials of varying density (Fig. 5). This zone seems to correspond to the single vacuole zone reported by MCVAY and KAAN

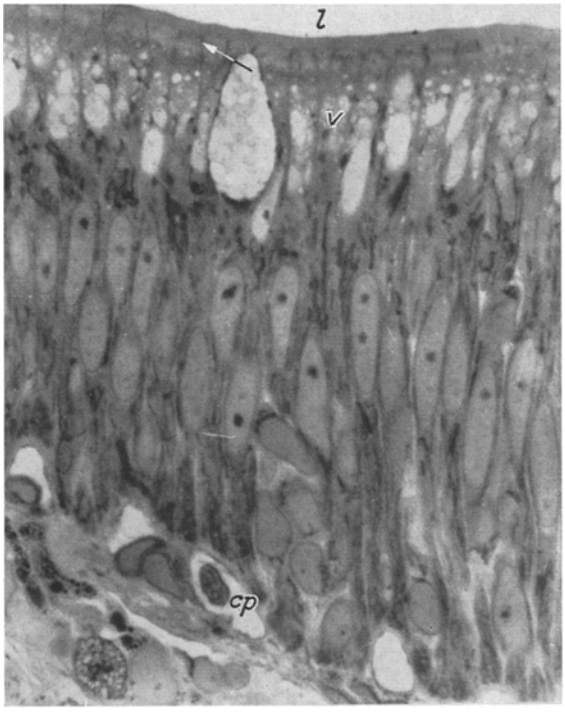

Fig. 4. A light micrograph showing the epithelium of the posterior intestine of goldfish. The intestinal lumen (*l*) is seen at the top. The cells constituting the epithelium are mainly columnar epithelial cells, among which one goblet cell can be seen. The homogeneous striated border is seen on the free surface of the columnar epithelium. The terminal bars (arrow) present between adjacent cells are seen clearly beneath the striated border. The apical cytoplasm of the columnar epithelium is particularly characterized by a variety of vacuoles (*v*) which are found solely in the posterior intestine (compare with Fig. 1). Mitochondria in filamentous form are found concentrated both in the supranuclear region and the base of cells. Blood capillaries (*cp*) are seen in close contact with the base of epithelium. The section from Epoxy resin embedded tissue was stained by toluidine blue. × 960

(1940). The granular endoplasmic reticulum is very infrequent in the apical cytoplasm, although RNP particles are freely scattered here (Fig. 5). Mitochondria also are few in this region. The mutlivesicular bodies frequently found in the apical region of the epithelial cell in the intestinal bulb appear to be virtually absent in the lower intestine. The typical Golgi apparatus is found above the nucleus, although it is not so well developed as in the epithelial cells of the intestinal bulb. From the supranuclear region to the base of the cell, the granular endoplasmic reticulum is conspicuously arranged parallel to the long axis of the cell (Fig. 6). Numerous RNP particles also are freely scattered throughout the cytoplasm.

The lamellar structures present in the columnar epithelial cells of the intestinal bulb are also encountered in the basal half of the columnar cells of lower intestine. Although the lamellar profiles present the same appearance, they are greatly reduced in number in the lower intestine (Fig. 6). In electron micrographs, it is rare to see continuity of the lamellar membranes with the lateral plasma membranes. In addition, the lamellae here do not show such close relationship with the mitochondria as they did in the case of the intestinal bulb. Usually both apical and basal ends of the lamellae are accompanied by vesicles and short tubules of approximately the same dimensions, aligned in one row. These may be artefacts

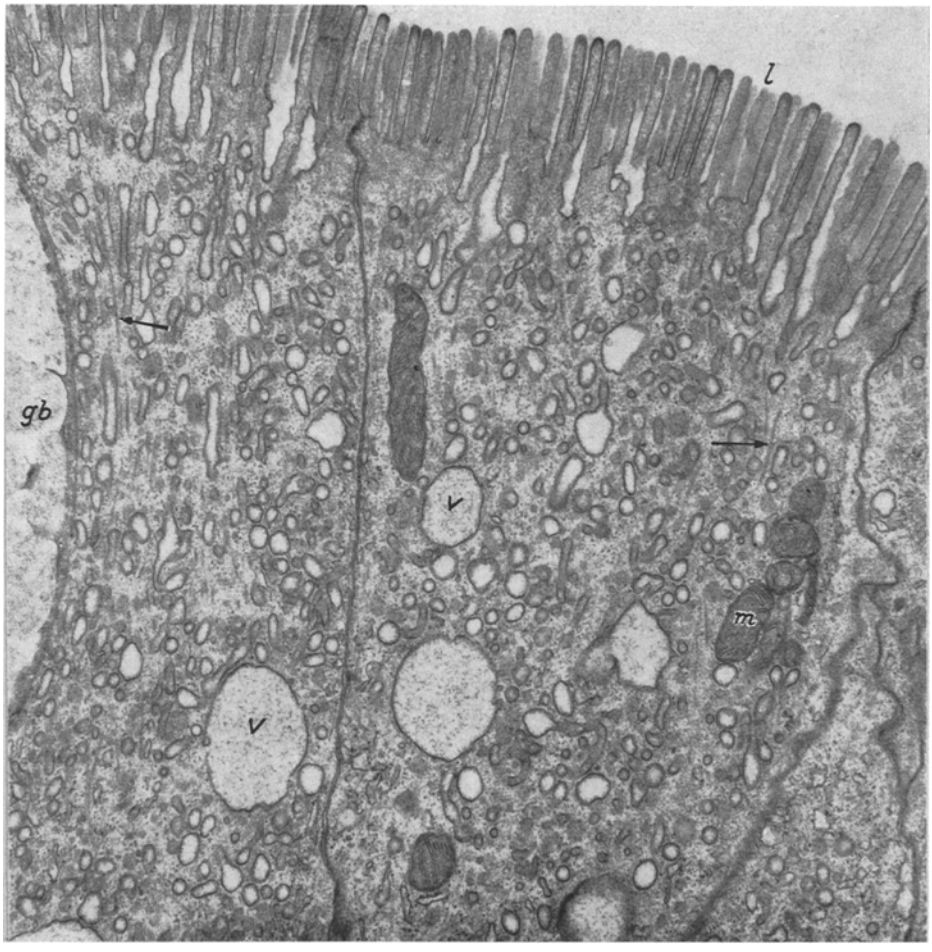

Fig. 5. An electron micrograph showing the apical portion of the columnar epithelial cells in the posterior intestine of goldfish. The intestinal lumen (*l*) is shown at the upper right. The striated border is composed of numerous regular microvilli. There are remarkable invaginations of the intermicrovillous plasma membrane, pushing deeply into the apical cytoplasm through the terminal web. Besides these invaginations, small vesicles and tubules are seen here. Vacuoles (*v*) containing less dense materials are also found among vesicles and tubules. Mitochondria (*m*) are sparse in this region. The fine filamentous structures (arrows) running parallel to the long cell axis are seen among vesicular components. A part of a goblet cell (*gb*) is shown at the left. × 20,000

resulted from breaking of the lamellae into rows of vesicles when fixed in osmic acid fixative. The mitochondria are concentrated in the basal cytoplasm displacing the lamellar structure (Fig. 7).

As noted by PALAY and KARLIN (1959) in the columnar epithelial cells of the rat intestine, fine filamentous materials are distributed throughout the cytoplasmic matrix, but here their arrangement is mostly parallel to the long axis of the cell and they tend to be more or less grouped. Some of them converge on the desmosomes which occur irregularly between the adjoining cells.

c) Relations of the epithelium to the underlying tissues. The epithelial cells rest on the basement membrane (Fig. 7). Usually blood capillaries are found immediately subjacent to this membrane; in such cases, the blood capillary wall facing the

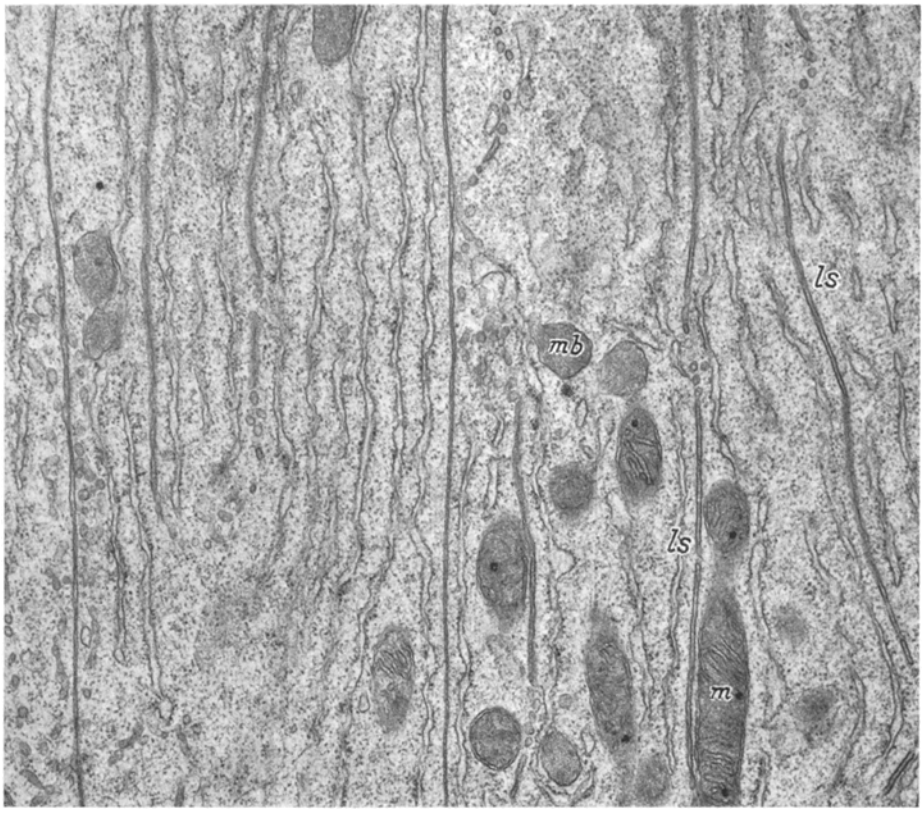

Fig. 6. The longitudinal section of the infra-nuclear part of the columnar epithelium in the posterior intestine of goldfish. Granular endoplasmic reticulum is arrayed parallel to the long cell axis. Lamellar structures (*ls*) are seen running parallel to the endoplasmic reticulum, and distinguished easily from the latter. Small vesicles and tubules are also found aligned in one row. Numerous RNP particles are found scattered freely in the cytoplasm. Less dense bodies (*mb*) bounded by a single membrane also are seen among mitochondria (*m*). × 25,000

intestinal epithelium is attenuated, and sometimes fenestrated. In electron micrographs the smooth muscle layer can be seen immediately surrounding the blood capillary layer. This muscle layer is particularly prominent at the base of the mucosal folds in the intestinal bulb, and is composed of small thin smooth-muscle cells (Fig. 1). Although McVAY and KAAN (1940) noted the absence of muscularis mucosa in goldfish intestine, it is suggested that such muscularis mucosa is in fact present, though of an unusualy type.

2. Rainbow trout

a) The columnar epithelium of intestine. The luminal surface of the columnar epithelial cell carries regularly arranged cytoplasmic projections, the microvilli, a standard structure in intestinal epithelium (Fig. 9). On the surface membrane there are no invaginations of the intermicrovillous plasma membrane such as those in the columnar epithelium of the posterior intestine in goldfish.

The lateral surfaces of the epithelial cell show both similarities and differences in comparison with intestinal epithelia which have been studied so far. The terminal bar occurs between adjacent cells at the level of the terminal web (Fig. 9) and its

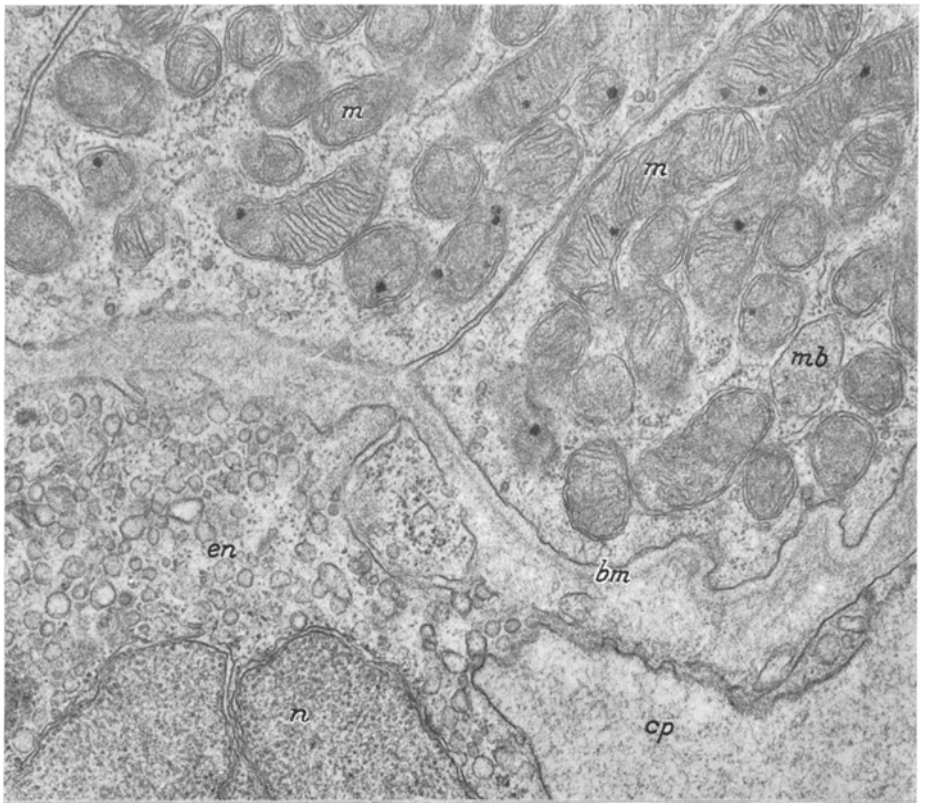

Fig. 7. An electron micrograph representing the base of the columnar epithelial cells in the posterior intestine of goldfish and the underlying blood capillary. The basal cytoplasm of the epithelial cells is devoid of the lamellar structure, but numerous mitochondria can be seen concentrated here. Among mitochondria (*m*), less dense bodies (*mb*) also are seen. The thin basement membrane (*bm*) is visible along the basal plasma membrane of the epithelium, but absent on the side of the endothelium (*en*) of blood capillary (*cp*). The endothelium facing the epithelium is attenuated, except for the vicinity of nucleus (*n*) where the cytoplasm is characterized by numerous vesicles. × 31,000

structure is similar to that described in various epithelia (FAWCETT, 1958; FARQUHAR and PALADE, 1963) by many electron microscopists. The outer, luminal portion of the terminal bar reveals the opposed plasma membranes of neighboring cells, arrayed parallel to each other, and separated by about 120—150 Å (peak-to-peak distance). It appears to be typical tight junction (FARQUHAR and PALADE, 1963) although it does not have a central density, presumably due to different preparation and staining methods. The lower, desmosomal portion of this terminal bar (corresponding in position to the lower boundary of the terminal web) is densely supplied with fine filaments. The intermediate junction (FARQUHAR and PALADE, 1963) appears to be absent or greatly attenuated in this epithelium. Besides the normally found terminal bar type of cell attachment, there are other attachments occurring between adjacent cells below and apart from the terminal bar. These attachments are very similar to the desmosome previously described (ODLAND, 1958; FAWCETT, 1958; FARQUHAR and PALADE, 1963). These attachment areas seem to be composed of three or more of the same type of desmosome, in locations corresponding to the level of the coarse filament layer immediately

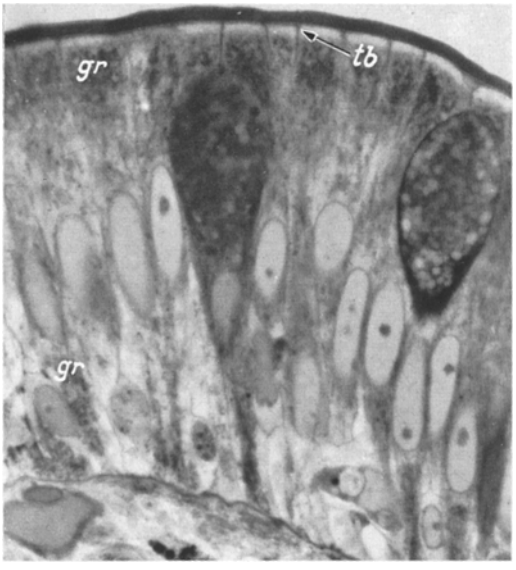

Fig. 8. A light micrograph of the intestinal epithelium of rainbow trout. Two cell types can be identified; numerous columnar epithelial cells and sporadic goblet cells. The luminal surface of the epithelium is covered with a thick striated border. The cytoplasm below the striated border shows a light zone, representing the terminal web. In this zone, the terminal bars (*tb*) can also be seen at the cell border. In the cytoplasm two areas of granulation (*gr*) can be found at the apical and basal regions. The dense, prominent basement membrane can be seen between the epithelium and the lamina propria. The blood capillary in the core of fold is located a short distance from the basement membrane. × 1100

beneath the terminal web in the cytoplasm (Fig. 9). The appearance of such groups of desmosomes will be referred to in this paper as "multidesmosomal attachments". The plasma membranes of the two adjoining cells in the desmosome are opposite and parallel, maintaining a constant distance of about 500 Å apart. Each of these plasma membranes is accompanied by a second dense line within the cytoplasm (at a gap of about 100 Å from the first) toward which the coarse filaments converge (Fig. 9). Fine filamentous structures are faintly visible crossing the space bounded by the two outer membranes of the desmosomes. Desmosomes of this type do not appear to encircle the cell continuously but occur irregularly as isolated discs, and are encountered frequently in other regions of the lateral plasma membranes. An electron micrograph of a cross section of an intestinal epithelial cell in the infranuclear zone shows this multiplicity of isolated desmosomes clearly (Fig. 10).

The lateral plasma membranes do not show complicated interdigitations between the adjoining cells, but generally make a smooth contact with them. However, some slight interlocking can be seen sometimes in cross sections.

The basal surface of these cells is, in general, smooth, sometimes wavy, and is in contact with the conspicuous basement membrane. Occasionally a few parallel coarse filaments (about 50 Å in diameter) appear to be attached by their ends to the basal plasma membrane (Fig. 13). These are probably tonofilaments and are frequently seen in the cytoplasmic matrix, where they are oriented parallel to the long axis of the cell. Such filaments show a tendency to group when going deeper in the cytoplasm. At times, the intercellular spaces are dilated, especially at the basal region of the epithelium, and dense small particles can often be seen in these intercellular spaces (Fig. 13).

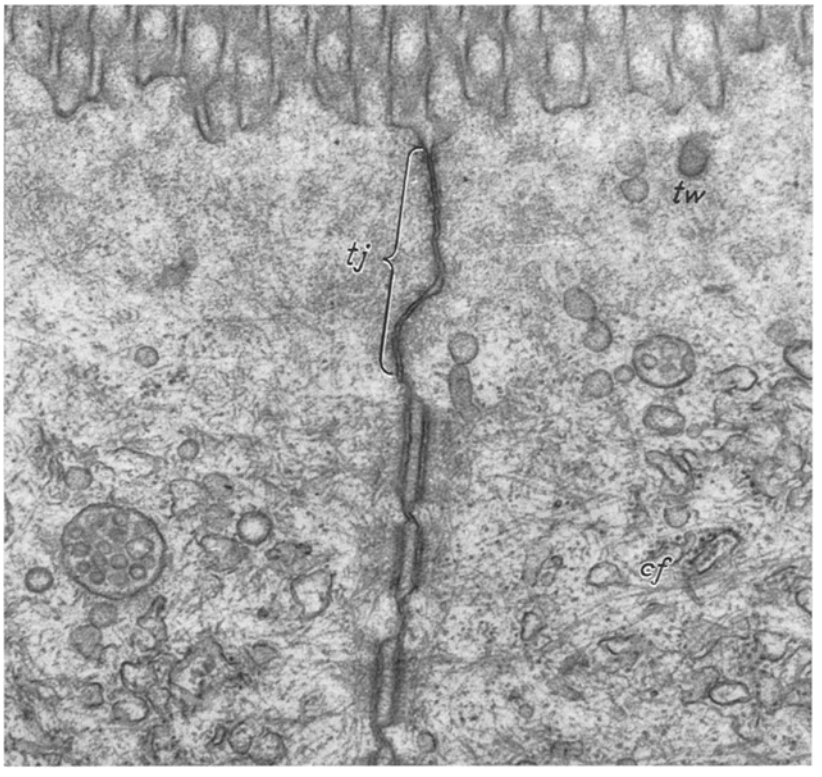

Fig. 9. A high magnification of electron micrograph of the apical cell boundary between two adjoining epithelial cells in rainbow trout intestine. This picture demonstrates the fine details of the tight junction (*tj*), the terminal web (*tw*), the coarse filament layer (*cf*), and the multi-desmosomal attachments. × 35,000

The cytoplasm immediately beneath the striated border consists of a layer, the terminal web composed of extremely fine filaments (Fig. 9). This web is also present in other vertebrates. From this layer, the mitochondria and the endoplasmic reticulum are largely excluded, though a few vesicles can be seen which are considered to be pinocytotic in origin.

Just below the terminal web there is another layer of coarse filaments (Fig. 9). The filaments comprising this layer are larger in diameter (about 70—100 Å), coarser than those in the terminal web, very similar to the tonofilaments in epidermis, and are interlaced in a direction parallel to the free surface of the cell. It is thus easy to distinguish this layer by virtue of these characteristic features. In the margins of this layer some filaments converge to the desmosomal attachments, as mentioned before (Fig. 9). Packed among these filaments is a variety of granular or agranular endoplasmic reticulum dispersed throughout the layer, together with a few mitochondria and multi-vesicular bodies.

The endoplasmic reticulum, granular and agranular, is equally well distributed throughout the cytoplasm except at the terminal web. In the electron micrograps most of the granular endoplasmic reticulum is cisternal in profile, whereas the agranular element is vesicular in form. Such a granular endoplasmic reticulum is particularly abundant in the region of supra-nuclear granulation visible in the light micrograph (Fig. 8) and it is here that the mitochondria are mainly accumula-

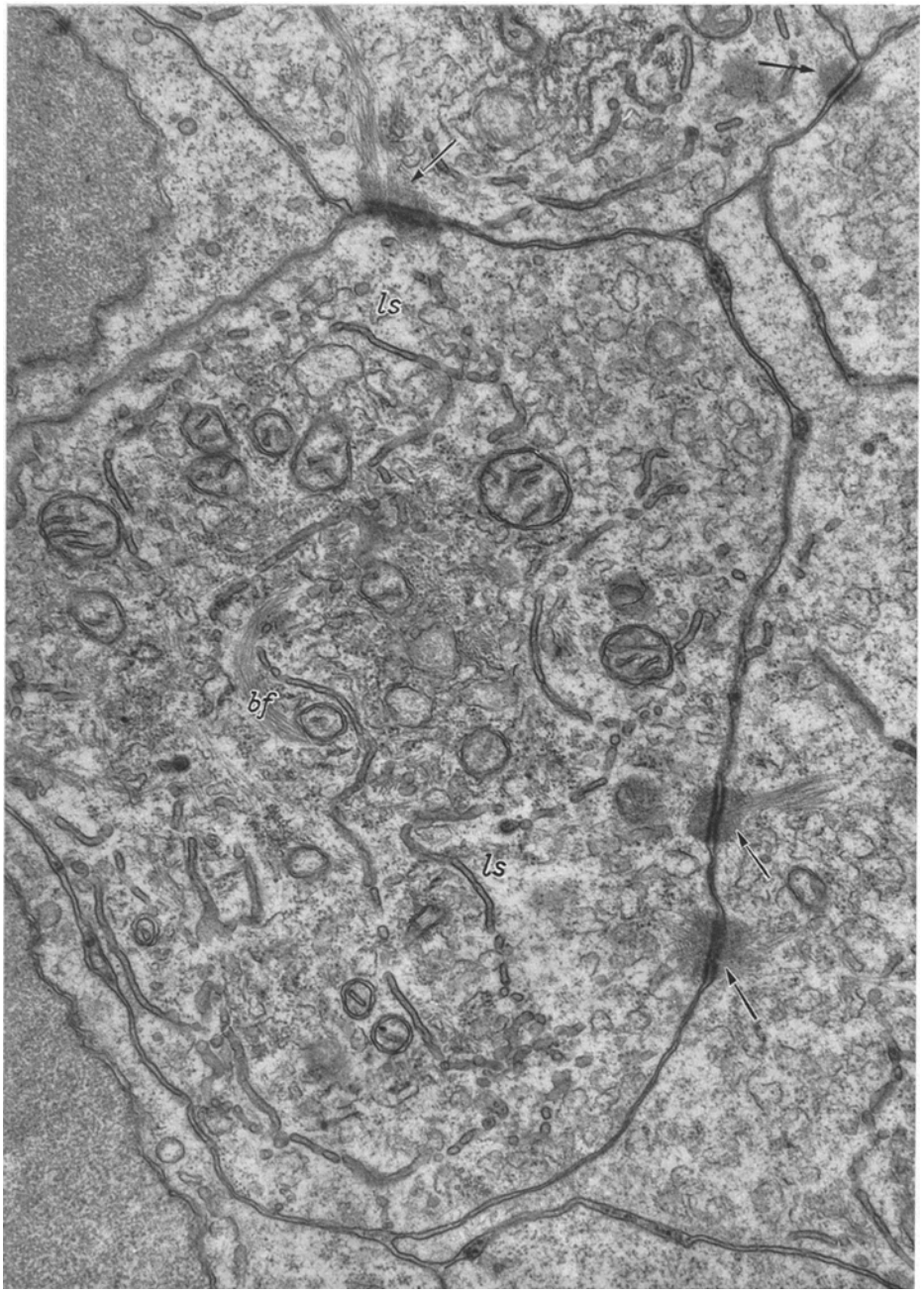

Fig. 10. A cross section of the infra-nuclear region of the columnar epithelial cells in rainbow trout intestine. On the smooth lateral cell boundaries, typical desmosomes can be seen distributed sporadically between adjacent cells (as indicated by arrows). In the cytoplasm the lamellar structures (*ls*) can be readily distinguished by their regular profile and higher density from the usual endoplasmic reticulum. Bundles of coarse filaments (*bf*) or small groups of filaments are also seen sectioned in various directions. × 19,000

ted. Here also the agranular endoplasmic reticulum appears to be in close association with the mitochondria. Besides the RNP particles attached to the

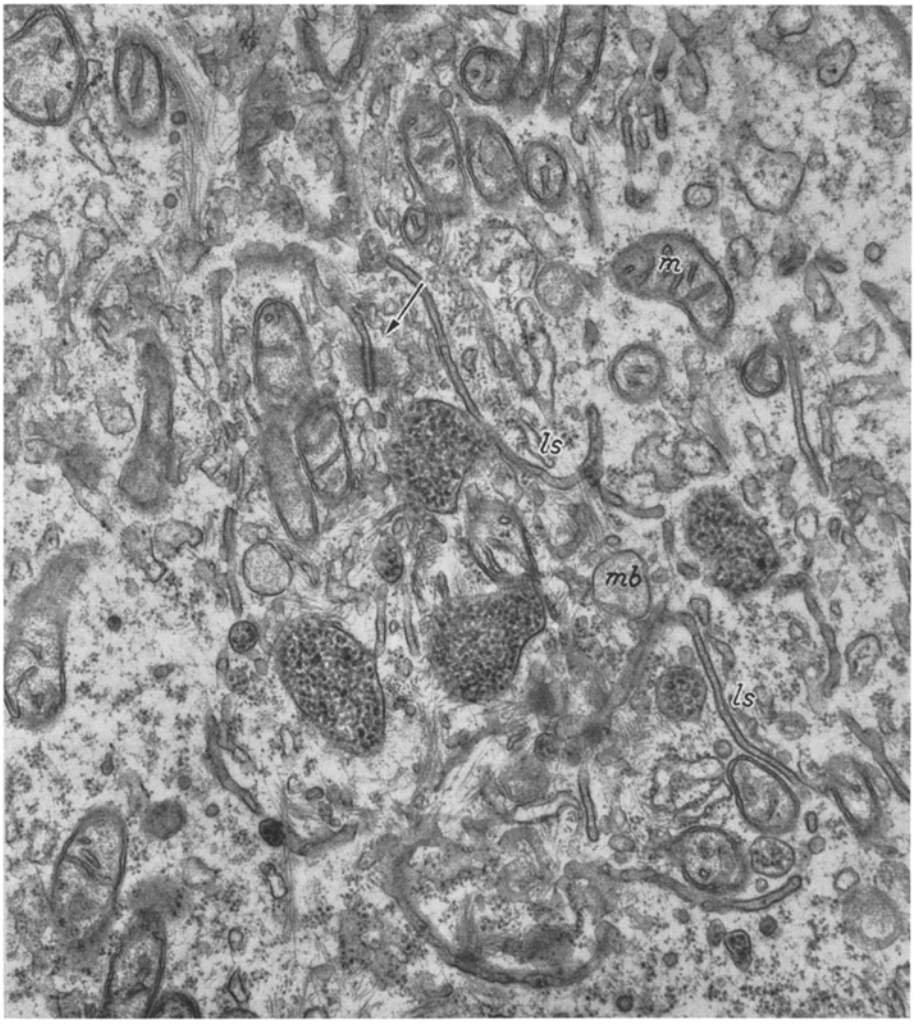

Fig. 11. An electron micrograph showing an area of the basal cytoplasm of the columnar epithelial cell in rainbow trout intestine. Cytoplasmic filaments are seen near the lamellar structures (*ls*). The end of one lamellar structure indicated by arrow shows a structure similar to a desmosome, but occurring across a lamella within a single cell. Large cisternae containing numerous dense particles, and round membrane-bounded structures (*mb*) containing low density materials are also seen between mitochondria (*m*). × 22,500

endoplasmic reticulum, numerous RNP particles are present freely dispersed in the cytoplasmic matrix.

An unusual variety of endoplasmic membranous system in the cytoplasm has been encountered in trout (Figs. 10, 11, 12), similar to that seen in the columnar epithelium of goldfish intestine. These membranous structures form single lamellae about 350 Å in width and are confined in general to the basal half of the cell. By serial reconstruction it appears that the tri-dimensional structure of these lamellae is a ribbon-like sheet, in which multiple fenestrations occur. Finger-like projections extend from the peripheral edges of these lamellar sheets, often accompanied by vesicles located near their tips. These special lamellae are, in general, oriented

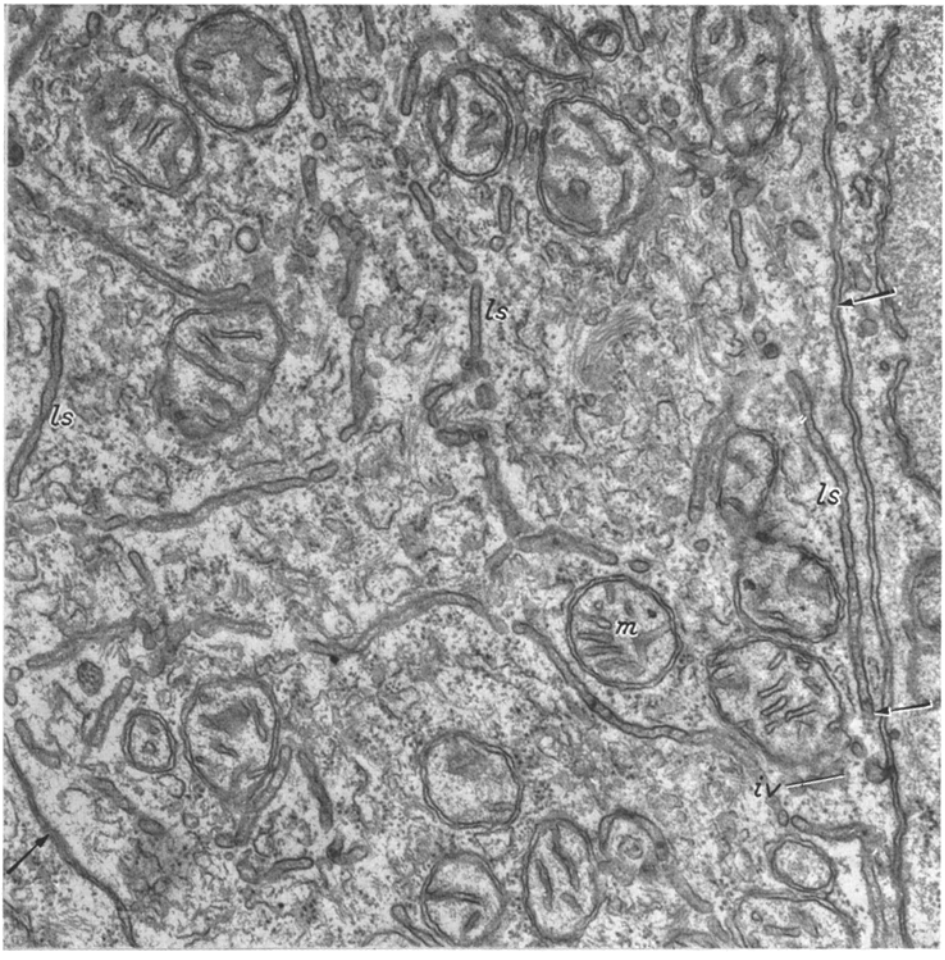

Fig. 12. A cross section of the infra-nuclear region of the columnar epithelial cell in rainbow trout intestine. Numerous lamellar structures (*ls*) can be readily distinguished from the usual endoplasmic reticulum by their linearity and higher density. The cavities of these lamellar structures are filled with finely granular materials, and some lamellae contain small dense particles. Many filamentous structures, mitochondria (*m*), and free RNP particles are also seen among lamellae. On the smooth border of cell (arrows) small, short invagination of the lateral plasma membrane (*iv*) can be seen. × 29,000

parallel to the long axis of the cell, and usually do not show anastomosis with other endoplasmic reticulum, or with the other special lamellae. Nor are RNP particles ever attached to these lamellae. Instead, some filaments occasionally converge to them. In such places, the cross section of lamellae has shown an appearance similar to desmosomes occurring between adjacent cells (Fig. 11). The lamellae in trout are not seen in as intimate relationship with mitochondria as they are in goldfish. Also whereas in goldfish there is, in places, a continuity of the membranes of the special lamellae with the lateral plasma membranes (giving the impression that the lamellae are arising from a lateral infolding of the cell membrane), it was not possible to find such infolding in trout. In some cases, however, short tubular invaginations of the lateral plasma membrane can be observed (Fig. 10, 12). The

cavities of these lamellae occasionally contain less dense, more or less homogeneous materials or dense fine particles (Fig. 12).

Among the endoplasmic reticulum and the lamellar structures there are membran-bounded bodies (Figs. 10, 11), nearly round in shape, containing low density granular materials, which show a similarity in appearance to the secretory granules in glandular cells.

As noted by light microscopy, mitochondria are particularly abundant in the supranuclear and basal regions. They are relatively small in size, irregular in form and their cristae mitochondriales are few in number.

The Golgi apparatus in the columnar epithelium, consisting of a few stacked lamellae and vesicles, is located not only above the nucleus, but is also frequently visible dispersed around the nucleus.

The filamentous structure found by PALAY and KARLIN (1959) in the cytoplasmic matrix of the columnar epithelium of the rat intestine can be also seen spreading throughout the cytoplasm in the columnar epithelium of trout intestine. There are, however, differences in size and orientation between them. The filaments in the cytoplasmic matrix seem to be substantially the same as those in the coarse filament zone just below the terminal web (Fig. 9). Although the filaments are oriented in various directions, the majority of them tend to be parallel to the long axis of the cell, especially in the basal half of the cell. An exception occurs at the desmosomes where the filaments converge horizontally (Fig. 10). Often these filaments group to form small bunches among the mitochondria and other cell organelles (Fig. 10). Such grouped filaments are mainly located in the central column of the cell, and not in the periphery.

Occasionally filaments course so close to the nuclear envelope as to suggest attachment to it. In the cell base, the filaments often appear to be attached to the basal plasma membrane (Fig. 13) without, however, showing the definite form of attachment which occurs with the hemi-desmosomes described in the epidermal basal cell.

b) Relations of the columnar epithelium to the underlying tissues. The epithelial cells lie with their basal plasma membranes facing toward the thick basement membrane (Fig. 13). Usually, there is a thin clear layer interposed between the basement membrane and the epithelium (Fig. 13). This clear layer, however, is missing in some pictures. In the low magnification micrographs, the basement membrane appears to be composed of dense homogeneous materials. However, the high magnification micrographs, after lead staining, reveal that it is, in fact, not homogeneous but filamentous in nature. Within the basement membrane small dense particles are frequently embedded (possibly fat droplets), which appear similar to those found in the cavities of special lamellae (Fig. 12) or in the intercellular spaces of the intestinal epithelium (Fig. 13).

In goldfish intestine, a well-developed blood capillary bed is seen in immediate contact with the basement membrane, surrounded by a smooth muscle layer; in trout intestine, on the contrary, the infrequent blood capillaries are usually separated by intervening thick connective tissue, in which the majority of cell components appear to be smooth muscle cells oriented parallel to the basement membrane (Fig. 12). A few unidentified cells, probably fibroblasts, are found in this connective tissue and abundant collagen fibrils fill the spaces between smooth muscle cells.

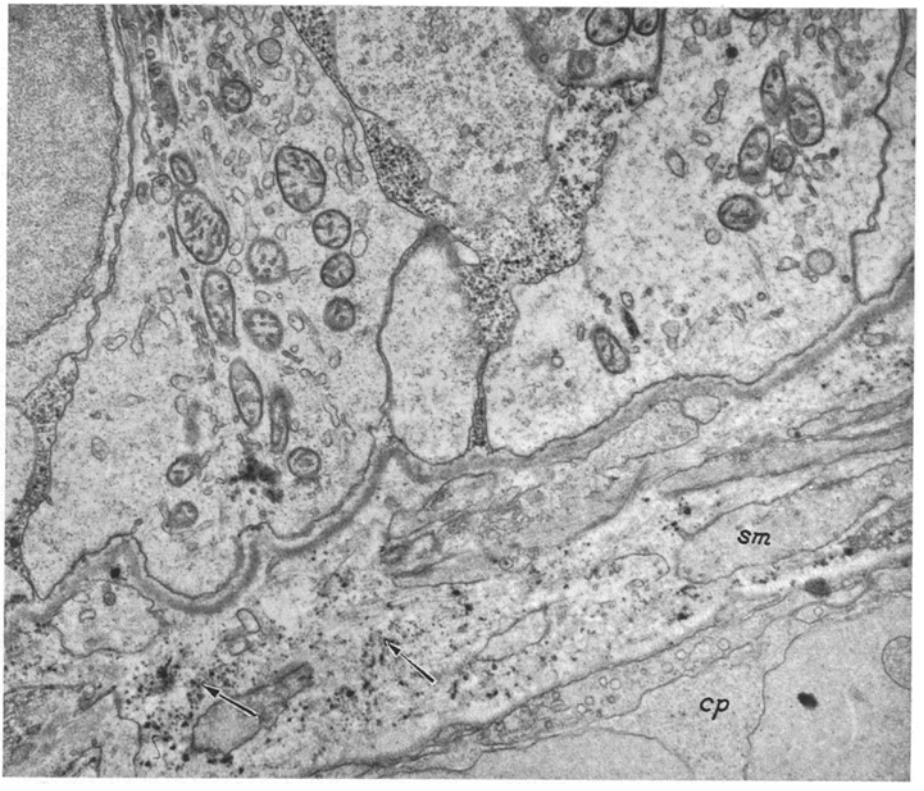

Fig. 13. The basal portion of the epithelium in the caudal intestine, and the underlying tissue components in rainbow trout. The intercellular spaces between adjacent epithelial cells are dilated and filled with dense small particles. The prominent basement membrane is seen along the basal plasma membrane of the epithelium. The connective tissue present between the epithelium and the blood capillary (*cp*) contains a few smooth muscle cells (*sm*) and collagen fibrils. Small dense particles are also found between them (arrow). × 10,000

Discussion

a) Goldfish intestine. AL-HUSSAINI (1949) reported that the striated border covering the luminal surface of the columnar epithelium in intestine of Cyprinid fishes appears as a layer composed of numerous canals. The results of the present study, however, do not support his interpretation. The fine structure of the striated border in goldfish is the same as that found in mammalian intestine, which is composed of regularly-arranged cytoplasmic projections, the microvilli. This is also the case in the intestine of rainbow trout.

Electron micrographs presented here show that there are differences in the configuration of the apical cell surfaces between the intestinal bulb and the posterior intestine in goldfish. Intermicrovillous invaginations of the free surface membrane which were predominant in the columnar epithelium of the posterior intestine suggest vigorous pinocytotic activity. The presence of such invaginations only in the posterior intestine further suggests that there are differences in kind or mechanism of absorption between the intestinal bulb and the posterior intestine. The invaginations of free surface membrane quite similar to those found in goldfish were observed by CLARK (1959) in the columnar epithelial cells of intestine of the suckling rats and mice after administration of the bovine gamma globulin and ovalbumin, but never in the adult animals. On the basis of his experiments,

CLARK (1959) suggested that cellular ingestion by invagination of the apical cell membrane is part of the mechanism of absorption of intact proteins in suckling animals.

There are some data on the distribution of enzymes in the intestine of Cyprinids which are based on the results from biochemical assay; both lipolytic and amylolytic enzymes occur in the intestinal bulb but amylolytic enzymes are maximally concentrated in the intestinal bulb and decrease gradually towards the caudal end of the gut: On the other hand, proteolytic enzymes are more concentrated in the posterior intestine than in the anterior region (AL-HUSSAINI, 1949; SARBAHI, 1951). Judging from similar observations in the suckling rats and mice, the biochemical data above mentioned, and the fact that the goldfish is devoid of a true stomach and, therefore, lacks peptic digestion, such invaginations of the free surface membrane in goldfish intestine may be related to the absorption of proteins. The present study does not furnish any evidence to support or deny such relationship; however, the work of ROTH and PORTER (1964) on mosquito oocyte supports this conclusion. The vesicles which, they suggest, are involved in protein uptake show short filaments on the inside (extracellular) surface similar to the filamentous thickening seen on the invaginations at the luminal surface of the posterior intestine. However, the rodlets on the cytoplasmic surfaces of the vesicles described by ROTH and PORTER (1964) have not been seen in the goldfish material.

The numerous vesicles in the apical cytoplasm of the columnar epithelium in the posterior intestine apparently are related to the invaginations mentioned above. In this study, both light and electron micrographs revealed the presence of large vacuoles in the apical cytoplasm of the columnar epithelium in the posterior intestine of goldfish. Similar vacuoles also have been described by earlier light microscopists in some fish intestine (DAWES, 1929; ROGICK, 1931; MCVAY and KAAN, 1940; AL-HUSSAINI, 1949). DAWES (1929), and MCVAY and KAAN (1940) suggested that such vacuoles appear in direct relation to the presence of food in the intestinal lumen. However they did not discuss the nature of the vacuoles. On the other hand, ROGICK (1931) considered such vacuoles as the early stage in mucous formation, and AL-HUSSAINI (1949) thought that the vacuoles seemed to represent the loci occupied by fat globules. As MCVAY and KAAN (1940) had noticed in goldfish, the vacuoles were found mainly confined to the posterior intestine of goldfish in the present study.

From the topographical association of invaginations with vesicles and vacuoles in the apical portion of the same cell, it seems possible that all these structures are linked to each other, each of them representing a stage of pinocytosis by means of which food materials are ingested into the cell in bulk.

Of particular interest in this study is the presence of numerous lamellar sheets in the columnar epithelium, which are more concentrated in the intestinal bulb, gradually decreasing in frequency towards the posterior intestine. Similar structures have been found in the distal convoluted tubule of kidney (PEASE, 1955, 1956), or in the choroid plexus (MAXWELL and PEASE, 1956; PEASE, 1956), or in the striated duct of the parotid gland (SCOTT and PEASE, 1959) where they have been described as basal infoldings, and have been generally interpreted as structure related to water transport (PEASE, 1959). The lamellar sheets described in this paper are, however, not basal infoldings but are independent of the basal plasma

membrane. Some of them show continuity with the lateral plasma membrane of the epithelial cell.

The similarity between these lamellar sheets and the basal infoldings of the distal convoluted tubule of the kidney, their physical relationship to mitochondria, coupled with the fact that goldfish live in fresh water and appear to drink large quantities of water when eating (ALLEE and FRANK, 1948), together tend to suggest some correlation of these lamellar sheets with water transport.

In the relationship of the columnar epithelium to the underlying tissues, it should be noticed that the blood capillaries are located immediately subjacent to the basement membrane of the epithelium and frequently show fenestrations in their endothelial walls. Such arrangement and structure of blood capillaries are similar to that in many endocrine organs. This might reflect the absorptive function associated with intestinal physiology. The lamellar sheets found in the cytoplasm of the columnar epithelial cells may likewise be associated somehow with absorption of the products of digestion, or water.

b) Rainbow trout intestine. The fine structure of the columnar epithelial cells in rainbow trout intestine is similar to those in the goldfish intestine, and some differences also.

Probably the most significant finding in the epithelial cells of the trout intestine is the presence in their cytoplasm of many endoplasmic lamellae. These lamellae appear to be ribbon-like sheets with multiple fenestration, and after osmium fixation, with vesicles associated with their margins. Such sheets occur in the goldfish intestine as well as in the trout, and it is possible that they may be a common feature of fish intestine, althought, of course, this cannot be established until other species have been examined. Here it is considered that these lamellar sheets may be a specialized form of agranular endoplasmic reticulum. This interpretation is based on their structure, since it appears that they are a reticulum in their continuity over a considerable extent, and also the fact they are located in the endoplasm of the cell. On the other hand, it is possible to consider them as deep infoldings of the lateral plasma membrane of the epithelial cell. The evidence for this in the goldfish is better than for the trout since in the goldfish membrane continuity between the sheet-like lamellae and the lateral plasma membrane is directly traceable occasionally across narrow tubular connections. These direct connections have not been in the trout, but only potential connections detected in terms of occasional short, apparently blind, invaginations from the lateral plasma membrane. In favor of potential connection is the observation that material of similar density and granularity appears both within the cavity of the lamellae and in the extracellular space between epithelial cells. It is possible that connections existing in reality are disorganized during osmium fixation along the lines suggested by TORMEY (1964) and others. The function of these lamellar sheets is actually not known at present. However, the finding of similar contents both within the cavity of the lamellae and in the extracellular space, and the similarity to the basal infoldings of distal convoluted tubules of kidney as discussed in goldfish, suggest that the lamellae may be involved in the transport of water or nutrients, or both.

A second similarity to goldfish intestine is the presence of numerous small membrane-bounded bodies in the basal part of the epithelial cells of the trout.

These bodies are circular in profile about 0.3 μ in diameter, contain a finely granular material of low density under the preparation methods used, and are dispersed in the cytoplasm among the various membranes of the cell. Although these structures are similar in appearance to secretion granules, there is no evidence that they are involved in secretion.

There are several points of difference between the trout and goldfish intestine, and the possibility exists that there may be an underlying mechanical explanation for these differences. First, in the intestinal epithelium of the trout, the lateral cell surfaces are distinguished by the occurrence of multidesmosomal attachments below the terminal bar, but these groups of desmosomes are not found in the goldfish. Secondly, although both trout and goldfish epithelia have a well developed terminal web composed of a feltwork of very fine filaments, only the trout possesses a layer of larger filaments beneath the terminal web. These thick filaments course throughout the cytoplasm of trout intestinal epithelial cells to insert into the multidesmosomal attachments and into the basal regions of the cells. Thirdly, the goldfish has a layer of blood capillaries disposed between the basement membrane of the intestinal epithelium and the smooth muscle of intestinal wall, whereas the trout epithelium sits on a thick basement membrane which is in direct contact with the muscularis of the intestine, within which are found the absorptive capillaries. Finally, the epithelial cells of the intestine proper of goldfish show at their apices numerous pits and invaginations between the microvilli with a variety of vesicles and vacuoles in the apical cytoplasm. This morphological complex was interpreted as indicative of vigorous pinocytotic activity. Conversely, the intestinal epithelial cells of trout show few if any such features anywhere within the cell. This remarkable difference in structural feature of cell surface appears to be greatly related to the fact that the goldfish does not possess a stomach and lacks peptic digestion, whereas the trout does.

It is further suggested that all these differences might be accounted for by the assumption that intestinal absorption in the goldfish is a mechanically gentle event carried out primarily by pinocytosis at the epithelial cell surface in a relatively quiescent viscus. In contrast, digestion in the trout may be carried out by a different process, accompanied by vigorous mixing of intestinal contents, and necessitating a mechanically resilient epithelium and a firmly supported capillary bed. However, no specific evidence is presented to support the speculations incorporated in this suggestion.

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