

MORPHOLOGY OF THE INTESTINE OF THE GOLDFISH (*CARASSIUS AURATUS*)

by

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ABSTRACT

A survey of the structures of the intestine of the Goldfish is given. Several morphological techniques have been used. Special attention has been paid to those characteristics of this organ that may be of importance to physiologists working with the same material, with emphasis on cell-to-cell contact in the mucosal epithelium, which appeared to be quite "tight".

I. INTRODUCTION

During the past two decades, studies concerning biological membranes have grown in number. Particularly the phenomena of active and passive transport of molecules across membranes have been investigated, and as these processes are far from elucidated, many workers still concentrate their efforts upon them.

The experimenter may choose between the use of single membranes, like the cellular envelope of erythrocytes or giant cells, or complex tissues such as epithelia. Gallbladder and gut epithelium are among the most popular tissues used by physiologists.

Hollands & Smith (1964) and Smith (1966), were among the first authors to make use of the intestine of the Goldfish for physiological experiments. They made everted sacks out of segments of gut, and studied transport phenomena across this tissue. More recently, Albus & Siegenbeek van Heukelom (1976) made also use of the Goldfish gut for their physiological studies,

using a perfusion-type measuring chamber with conditions more similar to the in-vivo situation than the experimental setup used by the previous authors.

For interpretation of their results, however, the physiologists must acquire some information on the morphological properties of the tissues they use. Excellent papers on the morphology of the Goldfish gut were presented by McVay & Kaan (1940), Yamamoto (1966) and Gauthier & Landis (1972). The first authors gave a description of the gross anatomy and histology of this organ. The last authors paid special attention to the uptake of particles in the epithelial cells by the phenomenon of pinocytosis. Yamamoto (1966) compared the ultrastructure of the intestine of the Goldfish with that of the Rainbow Trout, thus revealing some interesting features.

Some questions remained, however, one of the most important ones being whether on morphological data the epithelium of the Goldfish gut might be called a "tight" or a "leaky" one. The transport of water and solutes not only follows the active, cellular route, but also to some extent bypasses the cells through the pericellular space. The zonulae occludentes (tight junctions) play an important role in this respect, as has been pointed out by Claude & Goodenough (1973).

The dimensions and patterns of the intestinal folds throughout the Goldfish gut have also been incompletely described.

The present author therefore undertook to investigate the structural aspects of the Goldfish gut, with emphasis on morphometry and on the occurrence of tight junctions.

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II. MATERIAL AND METHODS

Male and female specimens of the Goldfish, *Carassius auratus* (Linnaeus, 1758), were used, their size ranging from 8-12 cm. The animals were killed by incision of the spine just posterior of the gills, followed by destruction of the brain. Immediately thereafter, the gut was excised, and brought in a bicarbonate saline containing 27.8 mM glucose (Krebs & Henseleit, 1932). The gut then underwent preparation for one of the following techniques: light microscopy, transmission electron microscopy or scanning electron microscopy.

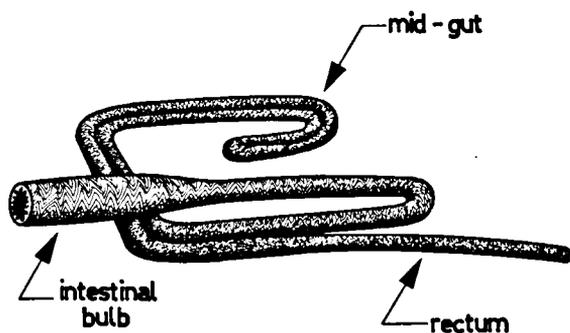


Fig. 1. Schematic view of the anatomy of the Goldfish intestine.

Light microscopy

The guts were fixed in Zenker-formaldehyde-trichloroacetic acid fixative (Veldman, 1970). Subsequently, the gut was divided in 16 segments of equal length, which were embedded in paraffin. From these numbered blocks, longitudinal sections were cut with a thickness of 4 μm . These sections were mounted in series and stained with hematoxylin-eosin. In this way a survey of the whole gut was ensured.

Transmission electron microscopy

Fixation was carried out with a formaldehyde-glutaraldehyde fixative (Karnovsky, 1965). This is a simultaneous fixation with 4% formaldehyde and 5% glutaraldehyde in a 0.2 M phosphate buffer solution. The advantage of this fixative is the fast penetration of the smaller formaldehyde

molecules, resulting in a rapid prefixation of the tissue, followed by a fixation by glutaraldehyde. Fixation was done by injecting the fixative with a syringe through the gut, and leaving the organ to soak in fixative for 30 minutes at room temperature. Thereafter, the gut was cut into pieces which were put into small jars according to their origin (intestinal bulb, mid-gut or rectum; see § III), and fresh formaldehyde-glutaraldehyde fixative was added, the fixation being continued for 3 hours at 20°C. Washing occurred in 0.1 M phosphate buffer during 8 hours, and postfixation was done with 1.33% S-collidine buffered osmium tetroxide during 2 hours. Both washing and postfixation were carried out at 4°C. After a second postfixation in 1% aqueous uranyl acetate for 30 minutes at room temperature, the fragments of gut were dehydrated in ethanol, carried through propylene oxide, and embedded in Epon 812 (Luft, 1961). Sections with a thickness of 400 to 700 Å were cut with glass knives on a LKB Ultratome. Some sections were stained with uranyl acetate and/or lead citrate.

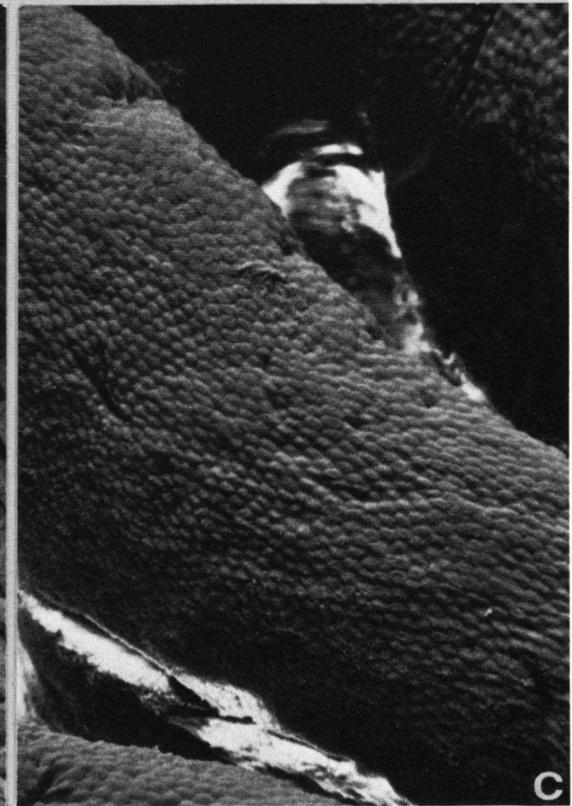
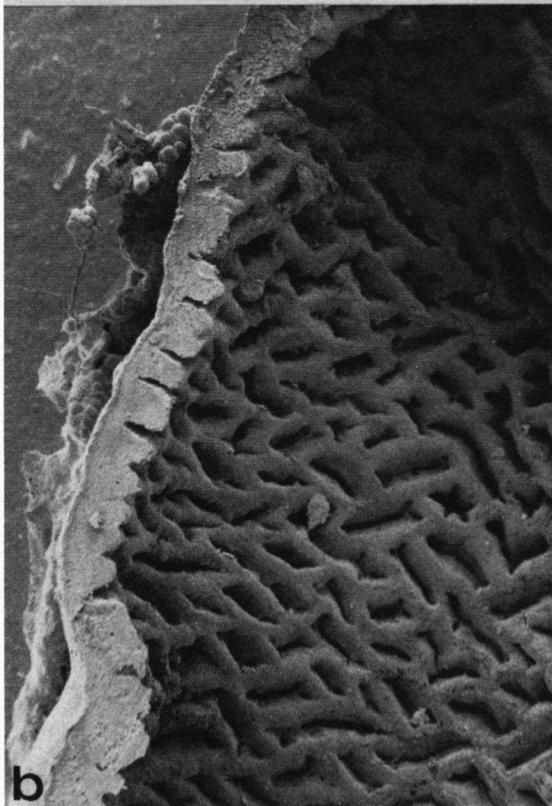
One intestine was used for a freeze-etching experiment. For this purpose a freshly excised gut was soaked during one hour at 4°C in a mixture consisting of two parts phosphate buffered saline and one part 87% glycerol. Subsequently, fragments were frozen on solid nitrogen (-210°C). The samples were then fractured with a cold knife in a Balzers freeze-etching apparatus, followed by 90 seconds of etching. A platinum-and-carbon film was deposited by evaporation under an angle of 45°.

Both sections and Pt-C replicas were examined with Philips EM200 and EM300 electron microscopes, with an accelerating voltage of 60 kV.

Scanning electron microscopy (S.E.M.)

Prior to fixation, the gut was washed thoroughly several times with saline, in order to remove the mucus clinging to the epithelial cells. A syringe was used for this purpose. Fixation occurred in 2% glutaraldehyde for two hours; washing in 0.3 M sucrose phosphate buffer for 8 hours. The gut was then cut into segments of about 1 cm length, and postfixation was carried out in 1% osmium

Fig. 2. S.E.M. photographs of the inner folds of the Goldfish intestine: a, Zigzag pattern of the folds in the intestinal bulb (30 \times). b, Reticulate pattern of the folds in the rectum (50 \times). c, Detail of a fold in the mid-gut. The tightly packed epithelial cells are clearly distinguished (530 \times).



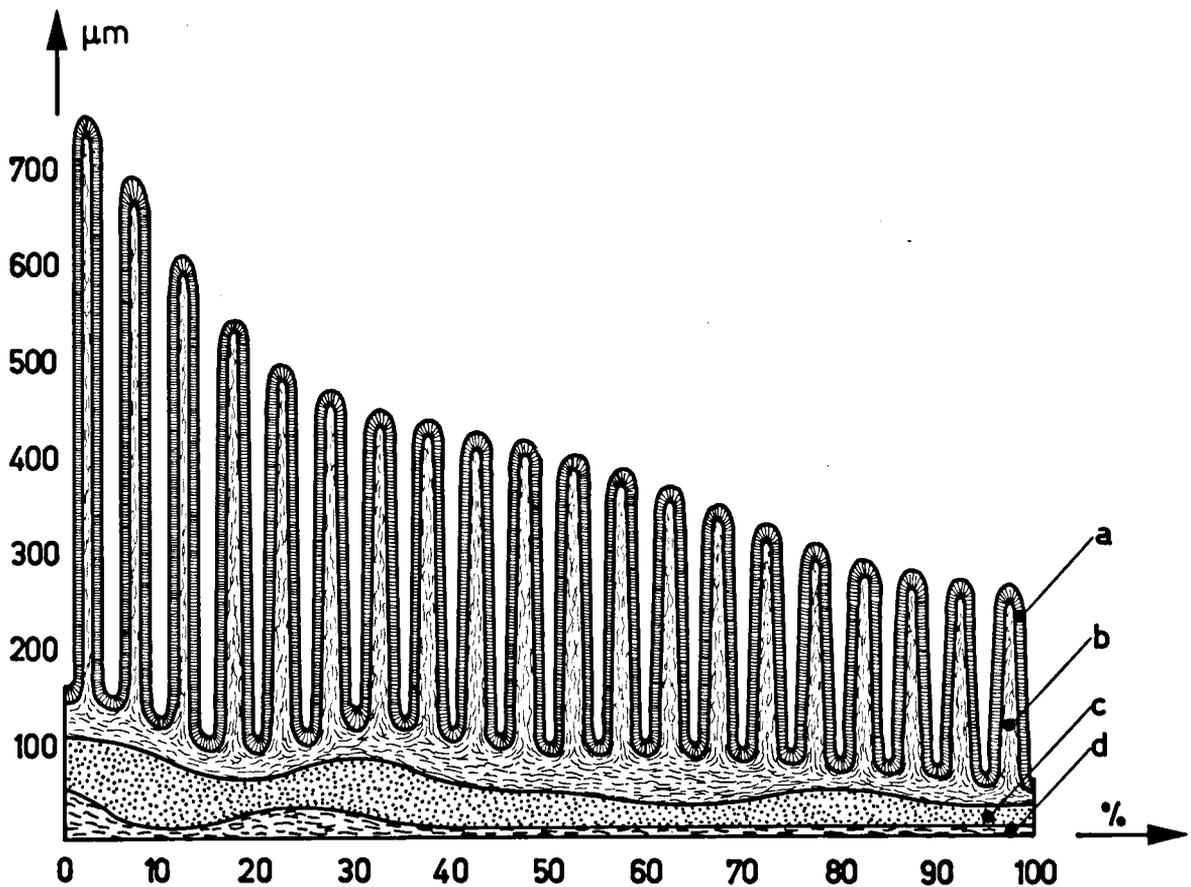


Fig. 4. Schematic longitudinal section of the Goldfish intestine. The height of the mucosal ridges and the thickness of the different layers are given in μm . The epithelial cells are not drawn to scale: their height is about $60 \mu\text{m}$. The length of a whole gut is given in %. The intestinal bulb occupies about the first 20%, followed by the mid-gut. The rectum forms the last 10% of the intestine.

a = epithelium; b = lamina propria; c = circular muscle layer; d = longitudinal muscle layer.

Fig. 3. a, Histology of the rectum of the Goldfish intestine, with numerous goblet cells amidst the epithelial cells ($200 \times$). b, Detail of a fold in the intestinal bulb ($200 \times$). c, Detail of epithelium in the mid-gut; the cells are very elongated ($4\text{--}8 \mu\text{m} \times 60 \mu\text{m}$), small lymphocytes are present as well as goblet cells. The striated border is also to be seen ($1000 \times$). d, Electron micrograph of the apical part of an epithelial cell in the intestinal bulb. A mucopolysaccharide coating can be observed over the tips of the microvilli. The terminal web is clearly to be seen. Deeper in the cell mitochondria and lysosomal structures are found. In the supranuclear cytoplasm lies an intricate pattern of smooth and rough endoplasmic reticulum ($6500 \times$). e, Electron micrograph of the apical part of rectal epithelium. Pinocytotic vesicles and large vacuoles mark the difference with the previous picture. The oval nuclei lie deep in the cells ($2850 \times$).

cm = circular muscle layer; ep = epithelium; er = endoplasmic reticulum; gc = goblet cell; lc = lymphocyte; lm = longitudinal muscle layer; lp = lamina propria; lu = lumen; ly = lysosome; mv = microvilli; n = nucleus; sb = striated border; tw = terminal web; v = vacuole.

tetroxyde for one hour. The sections were dehydrated in ethanol, and were allowed to dry in air, with a glass rod pushed into the lumen to prevent artifacts caused by excessive shrinking. The sections were broken into two longitudinal halves with a razor blade. A thin layer of gold was deposited by evaporation. The samples were examined with a Cambridge Stereoscan Mk2A scanning electron microscope.

III. GENERAL DESCRIPTION

A very short oesophagus leads via a sphincter into the rest of the digestive tract of the Goldfish, which contains no stomach. Therefore, the intestine is quite uniform in appearance. Such stomach-less intestines are proper to all carps and many other teleosts.

The tubular organ lies more or less coiled in the coelom, with this distinction, however, that halfway along its length, it bends backwards upon itself, following the preceding coils, with exception of the last portion, which points straight backwards, leading to the anus. A schematic drawing of the intestine is given in fig. 1. The gut is surrounded by more or less diffuse liver-, spleen- and pancreatic-tissue, and gonads. Immediately behind the oesophageal sphincter, the gut reaches its largest diameter (up to 5 mm), which may even increase when the fish has just been fed. This part is called the intestinal bulb, and covers about one fifth of the gut.

The intestinal wall being extremely thin (about 0.1 mm), the intestinal folds can be seen by transparency. The inner surface of the Goldfish intestine is increased not by fingerlike villi, but by folds or ridges, which lie in a zigzag pattern (fig. 2a). The pattern is quite regular in the intestinal bulb and in the largest part of the intestine proper or mid-gut. In the posterior part of the mid-gut the folds tend to anastomose, and in the rectum (about the last 10% of the intestine)

the ridges form a reticulate pattern (fig. 2b). Both these pictures are S.E.M.-photographs, as is fig. 2c, which shows the detail of one fold. The epithelial cells are clearly distinguished here, lying tightly packed against each other.

As compared to the mammalian intestine, the Goldfish gut shows a rather simple histology. A longitudinal and a circular muscle coat surround a lamina propria (connective tissue) and the mucosal epithelium (figs. 3a & b). No submucosa nor muscularis mucosae are found, neither are there any glands of Brunner, crypts of Lieberkühn or lacteals. The epithelium is of the columnar type.

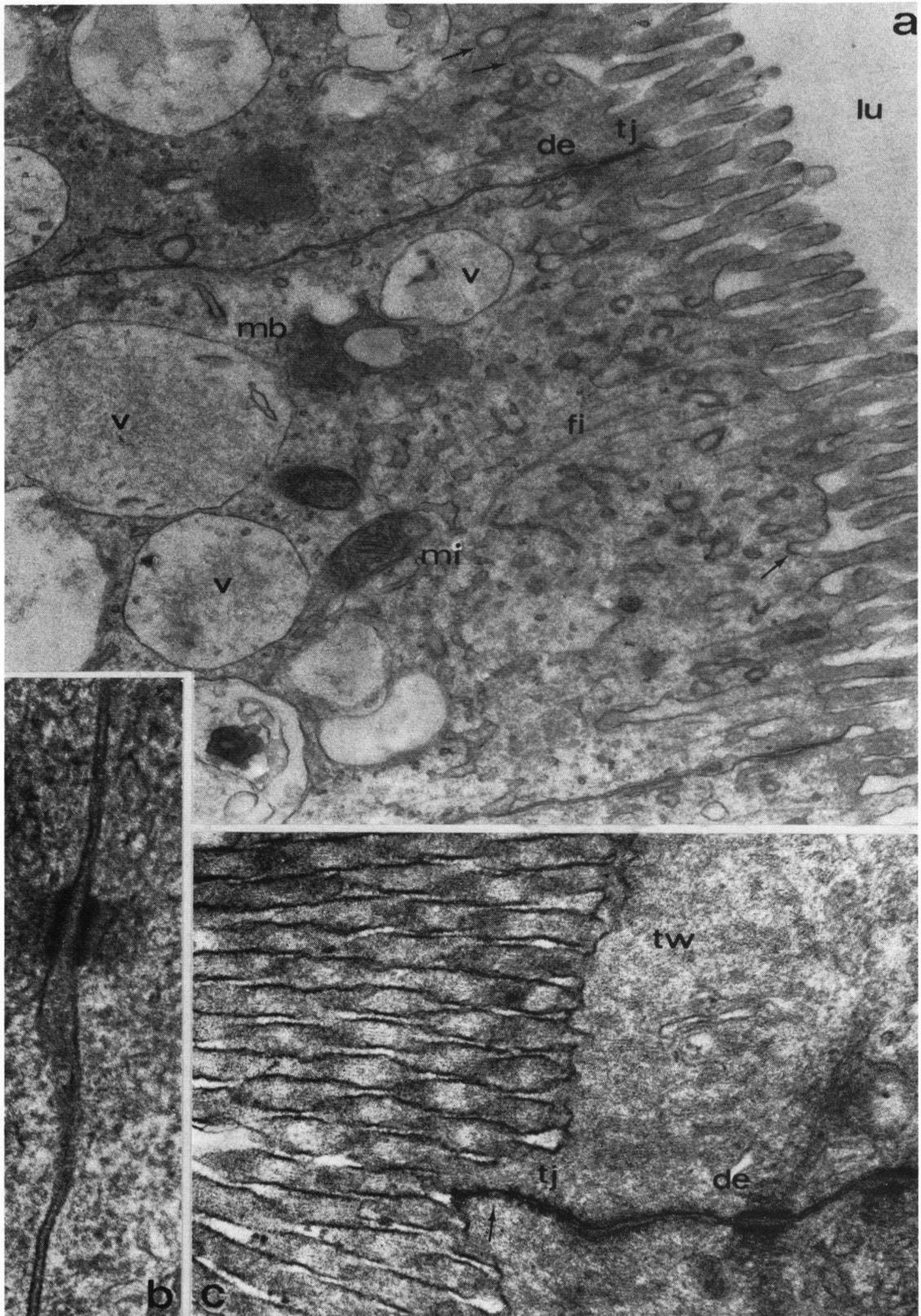
The height of the ridges decreases from the intestinal bulb (0.6 mm) to the rectum (0.16 mm), as does the thickness of the other layers. This might be explained by the decreasing size of food particles on their way through the intestine. Fig. 4 shows the schematic longitudinal profile of the Goldfish intestine. The length being quite variable, it has been given in percentages rather than in absolute units. In the fishes we used, however, the average length of the intestine was about 20 cm.

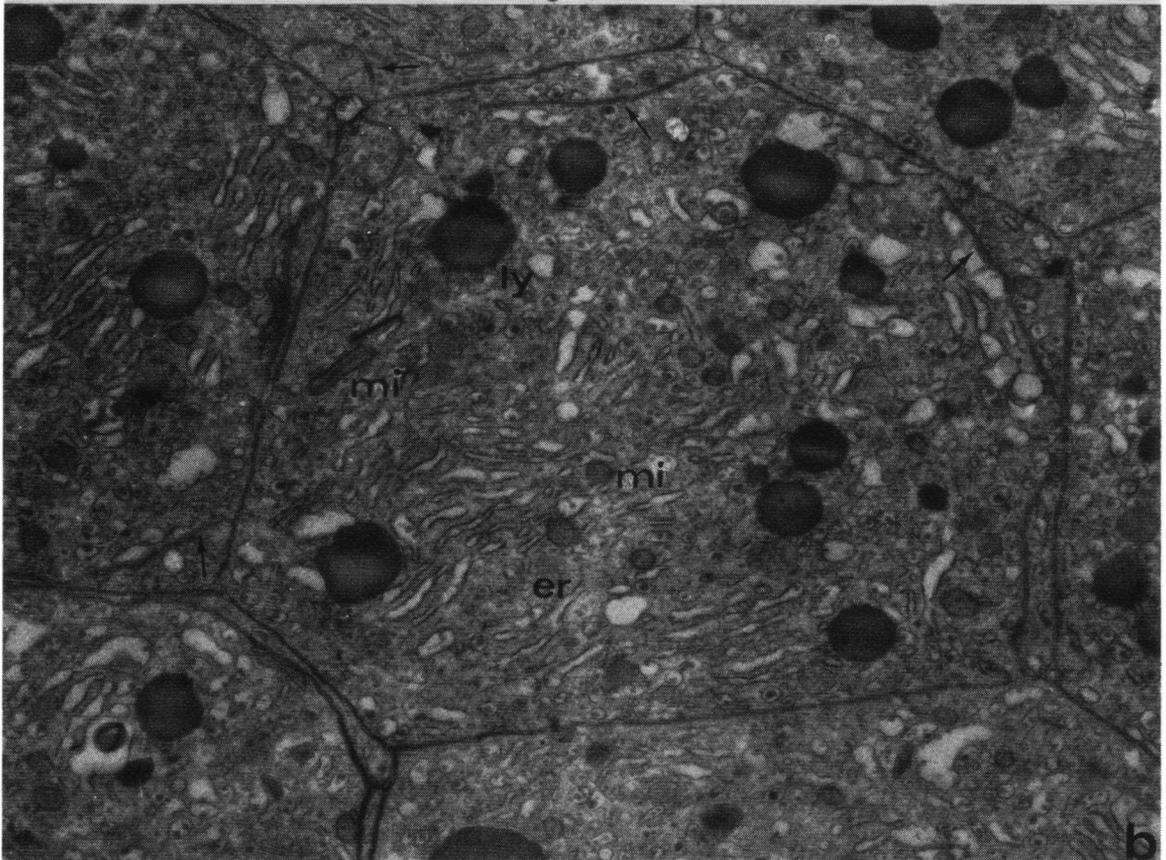
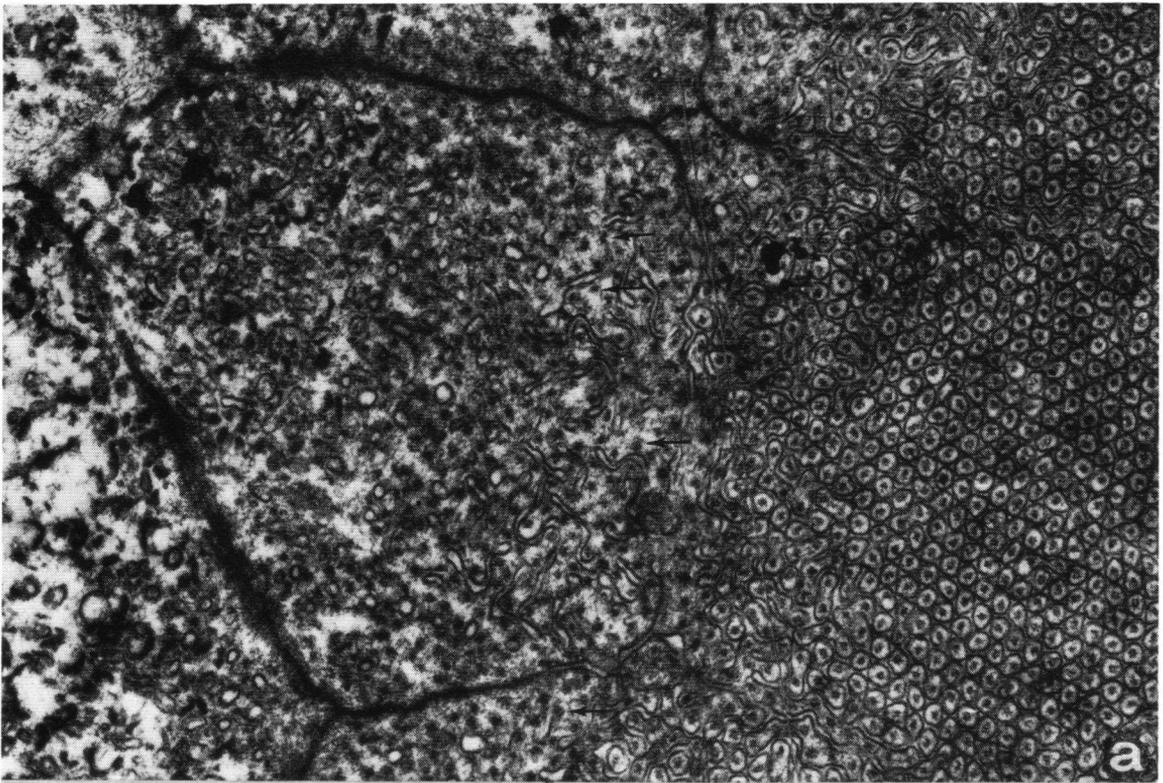
IV. THE EPITHELIUM

The epithelial cells are extremely elongated (fig. 3c). Their height is about 60 μm , their width varies from 4 to 8 μm . As has already been pointed out by Smith (1966), the mucosal epithelium occupies some 40% of the gut, making this tissue very suitable for physiological experiments. The present study confirms this percentage. As it is possible to strip off the muscular coat, the epithelial percentage may even be increased by another 10-20%.

Between the epithelial cells small motile cells with very dark nuclei are found. These cells, already mentioned by McVay & Kaan (1940) and Yamamoto (1966) are small lymphocytes (Wein-

Fig. 5. a, Apical part of epithelial cell in the rectum. The junctional complex contains a tight junction and a desmosome. Pinocytotic activity is clearly to be seen (arrows). The cytoplasm contains many unstaining vacuoles, some mitochondria and multivesicular bodies. Filamentous structures extend into the cytoplasm, their direction parallel to the longitudinal axis of the cells (29500 \times). b, Detail of a desmosome. The distance between the plasma membranes increases here to some 200-250 Å (89000 \times). c, Detail of a junctional complex. The packing of the plasma membranes at the tight junction is very close (arrow) and pericellular space is reduced to about 20 Å. The tonofilaments of the microvilli may be faintly observed, extending into the terminal web (55000 \times).
de = desmosome; fi = filamentous structure; lu = lumen; mb = multivesicular body; mi = mitochondrion; tj = tight junction; tw = terminal web; v = vacuole.





berg, 1975). Numerous goblet cells are also present, their number increasing towards the distal part of the mid-gut, and becoming a little less at the rectum.

Although Yamamoto (1966) found no morphological evidence of a secretory function in the epithelial cells of the intestinal bulb, during the present study osmiophilic vacuoles are found (fig. 3d), which may contain some digestive enzyme. Gauthier & Landis (1972) demonstrated the presence of lysosomal enzymes in the apical part of epithelial cells in the intestinal bulb, with acid phosphatase as an indicator. However, these authors failed to obtain ultrastructural confirmation of this fact. Our photographs may confirm the possible (but certainly not permanent) presence of lysosomes in this part of the intestine.

In the distal part of the intestine we found the epithelial cells to contain the large, unstaining vacuoles that have already been mentioned by McVay & Kaan (1940), Yamamoto (1966) and Gauthier & Landis (1972) (fig. 3e). These may be the result of pinocytosis, as supposed by Gauthier & Landis (1972), but they may also be the morphological expression of water secretion that has been measured by Smith (1966). The event of pinocytosis and/or water secretion may also be observed in fig. 5a. It is also to be seen that in both parts of the gut only small vacuoles are present in the apical part of the cells. This is due to the "terminal web", consisting of tonofilaments protruding from the microvilli into the cellular cytoplasm, and preventing larger organelles from reaching the luminal side of the cells. These structures may be observed very faintly in fig. 5c.

In their apical part, the epithelial cells exhibit distinct junctional complexes. Fig. 5a shows the detail of this part of the cells, in a longitudinal section. Pinocytotic vesicles are present (arrows), as well as the filamentous structures that have previously been described by Yamamoto (1966). Tight junctions and desmosomes are also to be observed, as well as in the more detailed picture

of fig. 5c. The detail of a desmosome (fig. 5b) shows that a pericellular "bottleneck" is not to be found in these structures, with a membrane-to-membrane distance of about 200-250 Å. The average width of the pericellular space seems to be about 100 Å here.

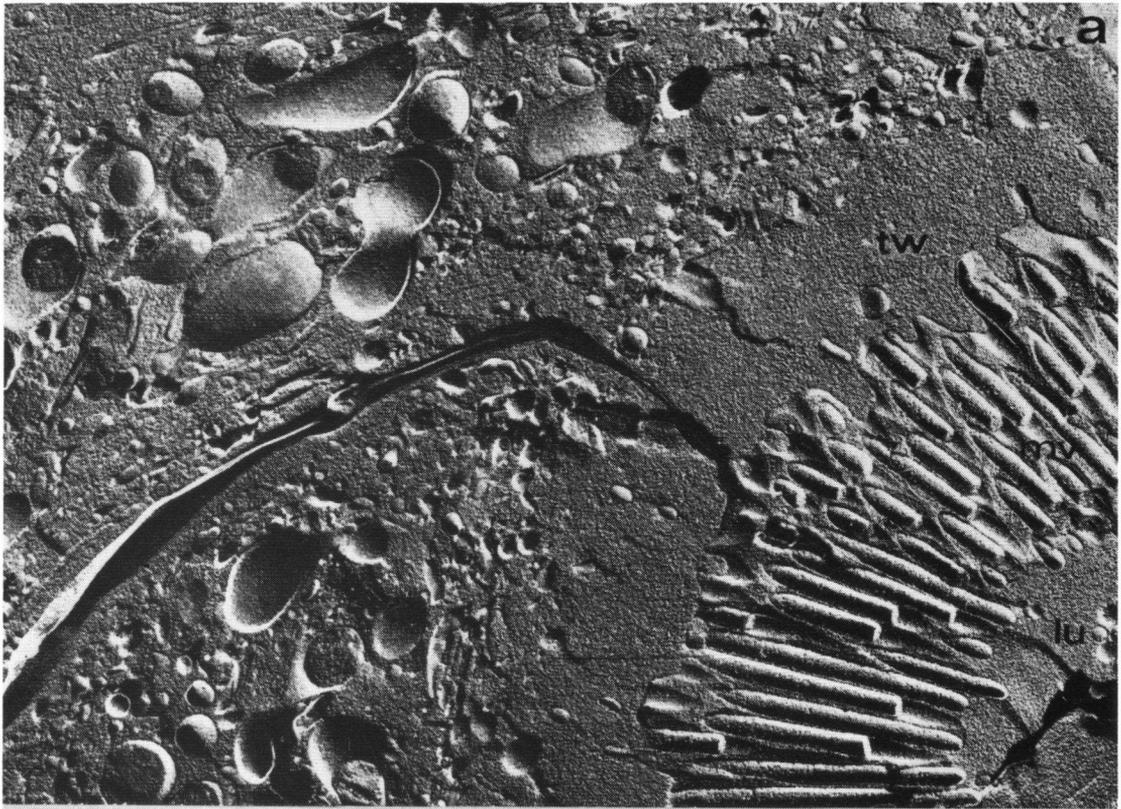
At the site of the tight junction in fig. 5c, no light band is to be observed between the plasma membranes of two adjacent cells, so it is here that the pericellular pathway reaches its minimal width (< 20 Å). These junctions may surround the cell completely, as a belt (zonula occludens) but they may also occur only in patches (fascia occludens or macula occludens).

In order to investigate this feature, which is of prime importance for physiologists, tangential sections were cut. Fig. 6a shows a micrograph of a nearly tangentially cut epithelial cell. Towards the right hand side of the picture, the section goes through the microvilli, whereas the left half of the section goes right through that part of the cell where the junctions are usually observed. It can be observed that the tight contact between neighbouring cells seems to follow the entire outline of the cell (dark band). According to McNutt & Weinstein (1973), larger molecules (molecular weight >40000) do not pass through such zonulae occludentes, which may even block the bypass diffusion of smaller electrolytes and water. The tonofilaments of the terminal web are clearly seen to extend from the microvilli into the cytoplasm in this picture. Another cross section of an epithelial cell is shown in fig. 6b. This section was made deeper into the cell (cf. fig. 3d) and shows that the cells are tightly packed. The lamellar structures signalled by Yamamoto (1966) are also to be found here.

In fig. 7a the apical part of two longitudinal freeze-fractured cells of the posterior mid-gut are to be observed. Pinocytotic vesicles are seen, as well as the clearly distinguishable terminal web. The cell borders appear as a heavy shadowed band.

In fig. 7b, the detail of some cell-to-cell

Fig. 6. a, Transverse section through an epithelial cell, passing through the microvilli at right, through the terminal web on left. The dark staining tight junction is seen to surround the whole cell (zonula occludens). The tonofilaments of the microvilli extend in a regular pattern into the terminal web (arrows) (23500 ×). b, Transverse section through the supranuclear cytoplasm of an epithelial cell from the intestinal bulb. Note the invaginations of the plasma membranes forming lamellar structures (arrows). The endoplasmic reticulum contains numerous osmiophilic granules (13000 ×).
er = endoplasmic reticulum; ly = lysosome; mi = mitochondrion.



contacts has been photographed by means of the same technique. Plasma membranes diverge from one another in some spots, but the packing is often so tight, that the space between membranes of neighbouring cells seems inferior to the width of the cell membrane itself, and is estimated at some 50-70 Å. The epithelial cells would then be in so close a contact over nearly their whole surface, that the term "zonula adhaerens" would seem appropriate in this case for the overall cell contact. On account of this feature, and the already described tight junctions, the epithelium of the Goldfish intestine may be expected to be rather impermeable for passive diffusion, at least of bigger molecules.

Also to be observed in the last picture is a Golgi complex, with secretory granules stocked in one of its saccules.

V. CONCLUSION

The intestine of the Goldfish is very well suited to

physiological experiments, thanks to its simple structure and the large proportion of epithelium. Although rather monotonous in outward appearance, some differences exist between successive parts of the intestine: intestinal bulb, mid-gut and rectum. The height of the intestinal folds decreases and the ridges tend to anastomose towards the rectum. In the intestinal bulb lysosomal enzymes may be expected, whereas pinocytosis and/or water excretion occurs in the cells of the distal part of the intestine. Therefore, being the least specialized part of the Goldfish intestine, the mid-gut seems most appropriate for physiological experiments.

The epithelial cells are very narrow as compared to their length (4-8 µm versus 60 µm respectively). The pericellular pathways are narrow (50-70 Å) and are obturated at the luminal side by tight junctions with an estimated width of 20 Å at most. It is to be expected, therefore, that only smaller ions are able to bypass the cells through this pericellular shunt.

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Fig. 7. a, Electron micrograph of freeze-etched epithelial cells. The terminal web is clearly seen as an organelle-less zone (28500 ×). b, Detail of cellular contacts. Several plasma membranes are observed (arrows), their trilaminar structure being faintly visible. They join each other over large surfaces, leaving a pericellular space of 50-70 Å. At some spots, however, the membranes part, to form intercellular voids (*) (11500 ×).

go = Golgi apparatus; lu = lumen; mv = microvilli; tw = terminal web.

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