# THE GUT OF THE RAINBOW TROUT (*SALMO GAIRDNERI*): A GROSS, HISTOLOGICAL AND ULTRASTRUCTURAL STUDY WITH SOME ASPECTS OF FUNCTIONAL SIGNIFICANCE.

BY

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VOLUME ONE TEXT AND REFERENCES

DEDICATED TO MY LATE FATHER, AARON OKEKE EZEASOR

## DECLARATION

I hereby declare that this Thesis embodies the results of my own work, and that it has been composed by myself.

Daniel N. Ezeasor

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## SUMMARY

1. The gut of adult rainbow trout, Salmo gairdneri, was studied by dissection, scanning electron microscopy, light microscopy and transmission electron microscopy.

2. The mucosa exhibited the following topographical characteristics: longitudinal ridges in the oesophagus and stomach, villi in the intestine, fine longitudinal ridges in the caeca and annular folds in the rectum, arranged like a stack of caudally-directed funnels starting from the intestino-rectal valve to the vent. Detailed scanning EM studies revealed taste pores in the anterior oesophagus and the sculpting of the luminal surface of the surface cells into microridges with complicated patterns. The surfaces of the posterior oesophagus and stomach were demarcated into polygons by rows of stubby microvilli - each polygon representing the luminal surface of an epithelial cell. Each rectal fold consisted of a smooth, caudally-directed apex and a base which was supported by perpendicular buttress-like secondary folds.

3. The gut wall consisted of mucosa, submucosa, muscularis externa and serosa, showing appropriate regional differences. A muscularis mucosae was present only in the stomach and rectum, a submucosa only in the oesophagus and stomach, while the muscularis externa consisted of a circular layer of striated muscle in the oesophagus, and two coats of smooth muscle in the remainder of the gut.

4. The oesophageal epithelium showed a progressive posterior reduction in height, ranging from an anterior stratified

epithelium to a simple columnar mucous epithelium in the distal third. The epithelium was associated with globular mucous cells and taste buds in the anterior portion, acinar mucous glands in the middle portion, and tubular serous glands in the distal third.

5. Cytological features of the columnar mucous cells lining the surface and pits of the gastric mucosa, oxyntic cells lining the glands and the endocrine cells of the gastric mucosa were studied. Microfilaments were prominent in the basal cytoplasm of the surface cells, where they formed annular bands around the convolutions of the basal lamina complex, which impressed the basal plasma membrane. Release of the mucous vesicles was achieved by exocytosis. The oxyntic cells possessed apical and basal microvillous processes, a well-developed tubulo-vesicular system, zymogen granules, extensive granular endoplasmic reticulum and many large mitochondria. When stimulated by distension of the stomach, the apical cytoplasm was converted into a labyrinth of cytoplasmic processes, while annular lamellae, each of which showed a short peripheral linear density, appeared in the basal cytoplasm. The endocrine cells showed such apical modifications as microvilli, cilia, centrioles, small lucent vesicles, microtubules and a reduced glycocalyx covering. Three types were distinguished on the basis of their granular morphology.

6. The absorptive cells of the intestine, caeca and rectum were columnar, bore apical microvilli and possessed lamellar

structures in their supra-nuclear and basal cytoplasm. Active cells of the intestine contained accumulations of fat particles in the Golgi zone, within the lamellar structures and in the intercellular spaces. Even larger accumulations were observed in the lamellar structures and intercellular spaces of the caecal epithelium. The rectal cells displayed tubular invaginations of the plasma membrane between the microvilli, and many vacuoles in their supra-nuclear cytoplasm.

Other cells present included endocrine cells, of which two types were distinguished, goblet cells, pear-shaped cells and intra-epithelial leucocytes.

7. The stratum compactum was composed of densely-packed collagenous fibrils arranged in layers. The fibrils within each layer were unidirectional and alternated within adjacent layers, resulting in an overall plywood or orthogonal pattern. Highly active fibroblasts were associated with its internal surface. Granule cells were associated generally with the connective tissue of the gut wall, but the majority were arranged in a definitive layer, the stratum granulosum. Whatever location however, they maintained physical contact with fixed connective tissue cell types, referred to here as 'ensheathing' cells. Phospholipid, acid mucopolysaccharides and the activities of alkaline phosphatase, acid phosphatase, arylsulphatase, 5-nucleotidase and proxidase were demonstrated in the granule cells.

8. The oesophageal serosa contained vagal nerve trunks, which became incorporated in the myenteric plexus of the stomach.

Each trunk was an encapsulated unit containing nerve cell bodies, myelinated and unmyelinated axons and supporting cells. The same elements, with the exception of myelinated axons, constituted the myenteric plexus outside the trunks in the stomach, and in the remaining parts of the gut. The submucous plexus consisted of two tiers in the stomach and only one tier in the post-gastric gut. Contrary to reports in the literature, nerve cell bodies were demonstrated in this plexus. Six types of nerve cell bodies and three groups of axon profiles were distinguished on the basis of their ultrastructure. "En-grappe" and "en-plaque" types of motor end-plates were demonstrated in the muscularis externa of the oesophagus. Amongst the smooth muscle generally, that of the stomach was most sparsely innervated, while that of the rectal muscularis mucosae showed the densest innervation of all, at the same time, containing nerve cell bodies.

9. The co-existence of intraluminal and intracellular digestion of proteins is discussed in relation to diet and the presence of the stratum compactum. The stratum compactum, 'ensheathing' cells and granule cells are hypothesized as constituting part of the bodily defence system. The demonstration of nerve cell bodies in the submucous plexus is shown to invalidate the existing schema for teleost gut innervation.

# 1.0 INTRODUCTION

The artificial propagation of fish as an alternative source of protein, has its roots as far back as the 5th century B.C., when the legendary Chinese leader Fan-Li first hand-reared carp in ponds for consumption by his perpetually war-stricken population. The idea of such readily-available supplies of protein rapidly spread to Japan, when it was realised that compared with the farming of domestic animals, the farming of fish could provide far greater yields of high quality protein by the efficient conversion of less costly feeds, combined with factors like overall faster growth and higher reproductive rates.

Even so, it was not till nearly 24 centuries later, when the idea that marine fish might be threatened with irreparable depletion by over-fishing, that the notion of pisciculture began to take root in the West. Even then, this was due in part to the influence of early Russian sources, which, as quoted by Bardach et al (1972), recorded figures of assimilation of food by carp as being 1½ times as rapid as in pigs and poultry, and twice as fast as in cattle and sheep.

Thereafter however, the science of fish-farming became firmly established, particularly in America. Interestingly enough though, it quickly became apparent there, that compared with the carp, the endemic rainbow trout, originally named Salmo gairdneri by Richardson in 1874, proved to be an even more amenable and economic "farm animal", so much so that according to MacCrimmon (1971), its range has now been extended by introduction, to Africa, Australasia, Japan,

Europe and South America. In fact, underlining the importance of the rainbow trout, is the fact that since this project started three years ago, at least three new fish farms have been established in Scotland alone.

It is a curious anomaly therefore, that despite such rapid development of commercial fish-farming, with all its attendant problems of husbandry and preventive medicine, little information on the detailed anatomy of the rainbow trout is available, particularly in regard to the digestive system, an understanding of which is an obvious prerequisite for proper care and management.

Certainly Weinreb and Bilstad made general histological studies in 1955; Bullock (1963) contributed pathological studies of the gut, while relevant histological accounts of the guts of related salmonid species have been given by Gulland (1898), Greene (1912) and Burnstock (1959a). Ultrastructural studies too though, are relatively scant, being limited to regional observations by Yamamoto (1966), Iwai (1968), Bergot & Flechon (1970a;b), Kimura (1973), while the only existing scanning electronmicroscope study,by Sperry and Warrersug in 1976, simply covers the anterior oesophagus.

This general lack of anatomical studies in the literature is also emphasized by the fact that only a few outstanding features have been studied in any detail. The presence of a collagenous sheath, and granule cells in the gut wall of salmonids for instance, has received much attention from workers such as Oppel (1896), Greene (1912), Bolton (1933), Al-Hussaini (1949), Smith (1975) and Kimura and Kudo (1975). Similarly, the histological innervation of the gut of the

brown trout as described by Burnstock (1959b), is a widely cited model for general teleost gut innervation.

However, the very fact that there has been so much study in such relatively restricted fields, has left the literature with contradictory observations and often misleading nomenclature.

It is the intention of this work therefore, to attempt to clarify such confusions in these particular fields and, by review of the gross anatomy, with histochemical studies and light, transmission and scanning microscopy, provide an understanding of the general morphology of the gut of the rainbow trout, for the benefit not only of ichthyologists, but also for that of observers in the field of general comparative anatomy.

A portion of this thesis entitled "Ultrastructural observations on the submucous plexus of the large intestine of the rainbow trout, Salmo gairdneri", has been accepted for publication in Zeitschrift für miroskopisch - Anatomische Forschung, Leipzig.

## 2.0 MATERIALS AND METHODS

Specimens for this work were obtained from College Mill Trout Farm, Perth, and Hopewood Trout Farm, Peebles. In all, roughly 150 adult male and female rainbow trout, aged between 22 and 30 months, and weighing 400-500 grams were used. Unfortunately, early attempts at establishing a colony in the laboratory had to be abandoned because of high mortality rates. Consequently, the fish were either processed immediately on collection, or maintained temporarily in aerated running tap-water for use within 24 hours. The fish were despatched in time-honoured fashion with a sharp blow to the head.

### 2.1 GROSS ANATOMY

Gross features of the gut were studied in both fresh specimens and in ones fixed whole in 10% formalin. At the same time, the distribution of blood vessels and nerves were traced with the aid of a dissecting microscope.

### 2.2 SCANNING ELECTRONMICROSCOPY

Portions of different parts of fresh gut, about 2cm long, were removed immediately after dissection. These were then incised to expose the mucosal surface, spread out and pinned with the mucosal surface uppermost on thin cork sheets. Rinsing in physiological saline followed to remove excess mucus after Reutter, et al. (1974), and thereafter, the portions were fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.3, for 24 - 72 hours. After fixation, the specimens were removed, rinsed in distilled water, trimmed into 9mm squares of no more than 1mm thickness, then dehydrated in increasing concentrations of acetone (30% - 100%). The pieces were critical-point-dried using

carbon dioxide in a Polarion E3000 apparatus. The serosal surface of each specimen was mounted on a metal stub with colloidal silver, coated with a thin conductive film of gold in a sputtering coater, and examined with a Cambridge Stereoscan S4 microscope operated at 18 and 22 kv.

## 2.3 LIGHT MICROSCOPY

Materials for light microscopy were excised immediately after the exposure of the viscera in fresh specimens, cut into small pieces and fixed by immersion in buffered 10% formalin after Lillie (1965), or in modified Bouin's fluid, comprising 300ml picric acid, 100ml formalin, and 20ml 1% tricarboxylic acid according to Harris, et al. (1973). Fixation time was 24 hours. Thereafter, the fixed tissues were dehydrated in a series of ascending ethanol concentrations, and embedded in paraffin. Sections were then cut at 4-6 um in thickness, dewaxed in xylene, hydrated in a series of descending ethanol concentrations, and routinely stained with Haematoxylin & Eosin and Masson's trichrome. Where necessary, paraffin sections were supplemented with thick plastic sections, stained with 1% toluidine blue in 1% borax (see Section 2.4).

The following procedures were employed to determine the histochemical profiles of the mucus-secreting cells: Periodic acid-Schiff (McManus, 1948); Diastase treatment (45 minutes at 37°C) followed by staining with PAS (Gurr, 1962); Southgate's mucicarmine (Culling, 1974); Alcian blue (pH2.5)(Pearse, 1968); Alcian blue (pH2.5)-PAS(Mowry, 1956); Aldehyde fuchsin-Alcian blue (pH2.5)(Spicer & Meyer, 1960); High iron diamine-Alcian blue (pH2.5)(Spicer, 1965); Alcian blue (pH1)(Lev & Spicer, 1964).

Chemical modification procedures involved active methylation (0.1N HCl in methanol at 60°C for 4 hours) after Spicer, (1960), and staining with alcian blue, pH1 and pH2.5. Control sections were incubated in methanol alone under the same conditions. Some sections were saponified (1%KOH in 70% ethanol at 25°C for 20 minutes) again according to Spicer, (1960), and stained once more with alcian blue, pH1 and pH2.5. Control sections were methylated and incubated in 70% ethanol only.

## 2.4 TRANSMISSION ELECTRONMICROSCOPY OF THE GUT EPITHELIUM

Portions of the anterior and posterior oesophagus, fundic and pyloric stomach, caeca, intestine and rectum were excised directly and minced into small pieces, which were fixed for 2 hours in 3% glutaraldehyde in 0.1M cacodylate buffer, pH7.3, at 0°C (Sabatini et al.1963). The fixed tissues were then washed overnight in the same buffer containing 4.5% sucrose, and post-fixed for 1 hour in 1% osmium tetroxide in 0.1M cacodylate buffer, pH7.3, at 0°C. At the same time, as an alternative, some pieces were fixed in ice-cold 1% osmium tetroxide buffered to a pH7.3 with Millonig's phosphate, after which they too were washed overnight in cold buffer.

The tissues, at this stage, were then dehydrated in graded ethanol, cleared in ethoxypropane, or dehydrated in graded acetone, and embedded in Araldite. Thick (2 um) sections were cut with glass knives and stained with 1% toluidine blue in 1% borax. Thereafter a number of these sections were used for light photomicrography.

Appropriate areas for electronmicroscopy were determined, and ultrathin sections cut with diamond knives, stained with saturated

solution of uranyl acetate in 70% ethanol (15'), post-stained with Reynold's (1963) lead citrate (4'), and examined, using an AEI 6B electronmicroscope.

The effect of distension on the gastric oxyntic cells was determined by excising the stomach whole, and washing out the contents with buffer. This was achieved by clamping both ends and injecting fixative into the lumen with a syringe. The distended stomach was then immersed in fixative, and after 1 hour, it was incised, and the wall cut into small pieces which were fixed for a further hour, before processing as above.

2.4.1 ULTRASTRUCTURAL LOCALIZATION OF ALKALINE AND ACID PHOSPHATASES

Portions of the caeca, intestine and rectum, at least 1cm in length, were fixed for 2 hours in 3% glutaraldehyde in 0.1M cacodylate buffer, pH7.3, at $0^{o}C$. They were then quenched in isopentane, precooled with liquid nitrogen and sectioned at 40 - 60 um in a cryostat. Thereafter, the sections were immediately incubated in appropriate media.

Alkaline phosphatase activity was determined using Hugon & Borgers' medium of 1966. It's composition was thus:

2ml of 0.2M Tris-maleate buffer (pH8.2),
4ml of 1.25% sodium B-glycerophosphate,
11.4ml of distilled water,
2.6ml of 1% lead nitrate,
2 drops of freshly-prepared 10% magnesium chloride.

The medium was warmed to 37°C for 5 minutes, allowed to remain at room temperature for 1 hour, and filtered before use. The tissues were then incubated for 30 minutes at room temperature.

Acid phosphatase activity was localized according to the method of Barka & Anderson (1962). Stock solutions comprised:

1.25% sodium B-glycerophosphate, adjusted to pH5.0 with 1N HCl,
0.2M Tris-maleate buffer, pH5.0,
0.2% lead nitrate in distilled water.

The incubating medium was prepared by mixing 10ml substrate with 10ml Tris-maleate buffer, and 10ml distilled water. The solution then had 20ml of lead nitrate added drop by drop, care being taken to stir the mixture at the same time.

Control experiments were carried out on substrate-free media, or on media containing varying inhibitors, such as 0.05M L-phenylalanine for alkaline phosphatase, or 0.01M sodium fluoride for acid phosphatase.

Following incubation, the material was post-fixed in 2% cacodylate buffered osmium tetroxide, dehydrated in acetone and embedded in Araldite. Thin sections were then stained in ethanolic solution of uranyl acetate, and examined without counterstaining.

## 2.5 MAJOR COLLAGEN ELEMENTS AND ASSOCIATED CELLS

### 2.5.1 LIGHT MICROSCOPY

Plastic sections from blocks of tissues processed for electron microscopy proved adequate for histological examination of the stratum

compactum, fibroblasts and granule cells. Staining of the last, however, using Periodic acid-Schiff, alcian blue and aldehyde fuchsin, was carried out on paraffin sections from tissues fixed in 10% buffered formalin. Lillie's (1965) technique, and Baker's acid haematein method after Culling (1974), were used respectively in tests for basic proteins and phospholipids.

### 2.5.2 ELECTRONMICROSCOPY

In this study, the following fixatives were used:

(i) 1% osmium tetroxide in 0.1M phosphate buffer, pH7.3;

(ii) 2.5%, 3% glutaraldehyde in 0.1M cacodylate buffer, pH7.3;

(iii) 6.25% glutaraldehyde in 0.1M cacodylate buffer, pH7.3 (Kempson, 1975) containing 4.5% sucrose.

Tissues fixed in (ii) and (iii) were post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer containing 4.5% sucrose. Generally, the fixation procedure involving (iii) proved to be most suitable. Thereafter, the tissues were processed as described in Section 2.4.

### 2.5.3 ULTRAHISTOCHEMISTRY OF THE GRANULE CELLS

(A) Detection of Carboydrates

Three techniques were used in this work, as follows:

(i) Rambourg's method (Rambourg, et al., 1969)

Ultrathin sections from normally processed tissues (Section 2.5.2, Fixative iii) were collected on gold grids. After double oxidation with 1% periodic acid for 20 minutes, and 10% chromic acid for 15 minutes,

the grids were placed in a solution of silver methenamine for 20 minutes at 60°C in the dark. The grids were then washed in distilled water, counter-stained with ethanolic solution of uranyl acetate and examined.

(ii) Thiery's (1967) modification of the method of Seligman, et al., (1965)

Ultrathin sections of glutaraldehyde-fixed, unosmicated specimens on gold grids were oxidised for 40 minutes with 1% periodic acid, impregnated with 0.2% thiocarbohydrazide for 48 hours, and then treated with silver proteinate in the dark for 30 minutes. Sections were then again counter-stained with an ethanolic solution of uranyl acetate.

(iii) Ruthenium red staining (Jollie & Triche, 1971)

This technique was performed by adding the dye (0.5%) to the fixing fluids, and washing solutions of conventionally processed tissues.

(B) Detection of basic proteins

The ammoniacal silver nitrate reaction of MacRae & Meetz (1970), was used to test for basic proteins. The tissues were fixed in 5% acrolein in 0.1M phosphate buffer, pH7.4, for 2 hours at 4°C. After fixation, the tissues were washed in the same buffer containing 4.5% sucrose where they remained for 24 hours. Thereafter the tissues were washed for 20 minutes in distilled water and immersed in ammoniacal silver nitrate solution for 5 minutes. They were washed again before placing in 3% formaldehyde for 5 minutes. Excess formaldehyde was washed off and the tissues were then dehydrated in graded acetone and

embedded in Araldite. Ultrathin sections were only stained with an ethanolic solution of uranyl acetate.

(C) Localization of Enzymes

Tissues were fixed in 6.25% glutaraldehyde in 0.1M cacodylate buffer, pH7.3, containing 4.5% sucrose, and subsequently processed in the same way as the tissues used in the localization of phosphatase activities in the absorptive epithelium (Secion 2.4.1). The methods of Hugon & Borgers (1966), and Barka & Anderson (1962), were again employed for localizing alkaline phosphatase and acid phosphatase respectively.

5-nucleotidase was localized according to the lead method of Wachstein & Meisel (1957). The medium comprised:

4ml of 1.25% adenosine-5-phosphate;
4ml of 0.2M Tris-maleate buffer, pH7.2;
0.6ml of 2% lead nitrate;
1ml of 0.1M magnesium sulphate;
0.5ml of distilled water.

The sections were incubated for 30 minutes at 37°C. Control sections were incubated in media without adenosine-5-phosphate.

Arylsulphatase was localized using the medium of Goldfischer (1965) comprising:

160mg of nitrocathecol sulphate in 4ml distilled water
12ml of acetate buffer, pH5.5
4ml of 8% lead nitrate.

This solution was adjusted to pH5.5 with 0.2M acetic acid. The sections were incubated for 1 hour at 37°C. Controls consisted of incubations in which nitrocathecol sulphate was omitted.

Peroxidase was localized according to the method of Bainton & Farquhar (1970). The medium consisted of 5mg 3,3'-diaminobenzidine tetrahydrochloride dissolved in 10ml of 0.05M tris-HCl buffer, pH7.6, to which 0.01% hydrogen peroxide had been added. Hydrogen peroxide was omitted in control reactions.

## 2.6 INTRINSIC INNERVATION

The distribution of nervous elements was studied by means of the localization of acetylcholinesterase activity. Tissues were fresh-frozen, sectioned at 18-25 um in a cryostat, air-dried and fixed in ice-cold 4% neutral formalin for 20 minutes. Subsequent steps followed the procedure described by El-Badawi & Schenk (1967).

The incubation medium, which was a modification of that of Karnovsky & Roots (1964), comprised:

| | |
|---|---|
| Acetylthiocholine iodide | 12.5mg |
| 0.06N(0.82%) sodium acetate | 15.8ml |
| 0.1N(0.6%) acetic acid | 0.5ml |
| 0.1M(2.94%) sodium citrate | 1.2ml |
| 30mM(0.75%) cupric sulphate | 2.5ml |
| 4mM(0.137%) iso-OMPA | 0.5ml |
| 5mM(0.165%) potassium ferricyanide | 2.5ml |
| Distilled Water | 2.0ml |

The medium was prepared immediately before use by adding to the substrate (acetylthiocholine iodide) the solutions in the order in which they are listed.

The reaction was sufficiently visible after 60 minutes of incubation.

Both light and electron microscopical features of the nerves and muscles were studied in tissues processed in the same way as those used in the study of the epithelium (Section 2.4).

# 3.0 OBSERVATIONS

## 3.1 GROSS ANATOMY

As described by Weinreb and Bilstad (1955), the gut of the rainbow trout consisted of the following consecutive segments: oesophagus, stomach, intestine and rectum, all of which were suspended from the roof of the coelomic cavity by a broad continuous sheet of mesentery. The intestine bore blind tubular appendages - the intestinal caeca. Accessory organs comprised the liver, gall bladder, and pancreas (Fig.1.1).

### 3.1.1 OESOPHAGUS

The oesophagus was a short median muscular tube, about 3 cm long and 1.7 cm wide. It communicated with the swim-bladder by means of the pneumatic duct which arose at the junction of the middle and distal thirds (Fig.1.2). Posteriorly, the oesophagus dilated to form the stomach without any obvious intervening sphincter. Along its course, it was related dorsally to the swim-bladder, to the gonads dorso-laterally, and to the caeca on the left lateral aspect. The entire right lateral surface, and most of the ventral surface, lay medial to the gall-bladder within a groove on the visceral surface of the liver. The mucosa was thrown into high longitudinal ridges which, in transverse section, gave the lumen a characteristic narrow stellate appearance.

### 3.1.2 STOMACH

The stomach was a large J-shaped sac situated in the mid-line. It comprised a relatively long dorsal expanded body or corpus, a strongly curved middle transitional zone, and a short ventral narrow arm, designated the pyloric part. The corpus was related to the swim-bladder and the gonads dorsally, to the liver on the left lateral side, and to the caeca on the right lateral and ventral sides. The transitional

zone made contact with the anterior border of the spleen, whilst the pyloric part was interposed between the corpus and the ventral body wall. The gastric mucosa also bore longitudinal ridges, most pronounced in the pyloric part. A distinct shelf-like flap of mucosa was noted where the stomach gave way to intestine.

### 3.1.3 INTESTINE

The intestine took the form of a loop comprising an ascending (5cm) and a descending (7cm) portion. The ascending portion arose in the mid-ventral line, and ran forward and to the left to make contact with the posterior edge of the liver. Thereafter, it skirted the comma-shaped ventral border of the liver where its dorsal and anterior surfaces impressed the visceral surface of this organ. It crossed the mid-line in the process, and emerged from the hepatic impression just below the level of the pectoral fins, before continuing as the descending intestine.

The ascending intestine bore numerous caeca. These fingerlike diverticula varied from 42 to 64 in number, from 1.3 to 4.5cm in length, and from 3 to 4mm in diameter. The caeca borne on the proximal segment of the ascending intestine were the longest (3.5-4cm). The first seven to ten of these arose around the entire circumference of the gut, whilst the origin of the subsequent ones was confined to the ventral and lateral aspects. Most caeca were oriented caudally, and were related to the corpus of the stomach. Those borne on the segment related to the liver, arose in two or three lines. They were arranged in descending order of length and were disposed radially, with their blind ends directed towards the mid-line (Fig.1.3).

The descending intestine ran along the right body wall related to the gonads, swim-bladder and caeca. At the level of the pelvic fins, it curved medially to gain the mid-line, where it increased in girth before merging with the rectum. This terminal portion was related to the medial border of the spleen.

Both mucosal surfaces of the ascending and descending portions of the intestine bore villiform projections. The caecal mucosa was characterized by fine longitudinal ridges.

### 3.1.4 RECTUM

The rectum (8cm) continued posteriorly along the mid-line. Dorsally, the first portion was related to the swim-bladder while the terminal portion was related to the urinary bladder. It made a ventral contact with the body wall and was flanked by the gonads. It terminated at the vent which opened forward of the urinogenital opening at the anterior margin of the anal fin (Fig.1.4).

At the junction of the descending intestine and rectum, the mucosa showed a prominent funnel-shaped annular fold, the so-called ileo-rectal valve. Posterior to the valve, and along the entire lumen of the rectum, there were numerous lower, regular mucosal folds which were rather annular than annulo-spiral as described by Burnstock (1959a) in the brown trout. They gave the appearance of a stack of caudally-directed funnels. In some specimens, their free edges overlapped each other resulting in the peripheral segmentation of the lumen, and the formation of a narrow continuous central passage, usually occupied by string-like faecal material.

### 3.1.5 ACCESSORY ORGANS

The liver was a firm, dark red, saddle-shaped structure situated to the left of the median plane in the anterior part of the visceral cavity (Fig.1.3). Its parietal surface was related to the transverse septum anteriorly, and to the body wall on the dorsal and lateral aspects. The visceral surface bore a small blunt process which separated two grooves. The ventral groove transmitted a portion of the ascending intestine, while the gall-bladder lay lateral to the oesophagus in the deeper dorsal groove. The gall-bladder emptied by way of a common bile duct, which opened into the ascending intestine on a level with the anterior caeca.

The pancreas took the form of a diffuse, irregular structure filling the gaps between the caeca.

### 3.1.6 BLOOD SUPPLY

The main arterial supply was from a large coeliaco-mesenteric vessel which originated from the dorsal aorta at the antero-dorsal boundary of the coelom. It descended obliquely, piercing the kidney and the peritoneum. Within the peritoneal cavity, it crossed the right lateral aspect of the oesophagus, and gave rise to three branches on a level with the hilus of the liver. The distribution of these branches was found to agree with those occurring in the brown trout, as described by Burnstock (1959b). The three branches were the gastric, hepato-intestinal, and a small intestinal vessel. The gastric artery supplied the stomach and spleen. The hepato-intestinal artery supplied the liver, caeca, intestine and part of the rectum, while the intestinal artery terminated in the region of the anterior portion of the intestine. Two small additional vessels, not described

by Burnstock (1959b), and probably best termed 'posterior mesenteric' arteries, supplied the rectum. The first of these arose from the aorta on a level with the middle of the dorsal fin; the second at the level of the anterior margin of the urinary bladder, to which it detached a small nutritive twig. Both divided into smaller branches on the dorsal wall of the rectum.

Tributaries of the hepatic portal vein drained the gastro-intestinal tract. The immediate formation of the portal vein came from the union of two main tributaries: a left gastric vein, which arose from the left side of the stomach and the associated caeca, and a common branch to which four lesser veins contributed. These subsidiary veins comprised a right gastric vein, a branch which arose from the spleen and into which smaller veins from the caeca drained, an anterior intestinal vein draining parts of the ascending intestine and caeca, and a large posterior intestinal vein which drained the descending intestine and rectum. The main branch of the last named vessel was attached to the ventral wall of the rectum. It received a smaller dorsal tributary at the junction of the descending intestine and the rectum. Numerous minor transverse branches corresponding to the bases of the mucosal folds drained into these.

### 3.1.7 NERVE SUPPLY

The extrinsic nerve supply came from the vagus and splanchnic nerves, similar in fact to that described by Burnstock (1959b) in the brown trout. The branches of the vagus were readily observed on the external surfaces of the oesophagus and stomach. Branches of the splanchnic nerve were closely associated with the branches of the

coeliaco-mesenteric artery. Two small nerves were observed to accompany the twin posterior mesenteric arteries to the rectum.

## 3.2 GUT SURFACE RELIEF AS SHOWN BY SCANNING ELECTRONMICROSCOPY

### 3.2.1 ANTERIOR OESOPHAGUS

The mucosal surface was thrown into prominent longitudinal folds and it was further subdivided by narrow transverse crevices into a series of irregular, well-circumscribed elevations. These elevations were most prominent in the anterior portion of the oesophagus (Fig.2.1).

Higher magnification of the elevations revealed that their surfaces were further sculpted into a network of well-defined, linear elevations, the so-called microridges, which agreed with the observations of Sperry & Wassersug (1976) (Fig.2.2). The luminal margin of each cell was usually clearly demarcated, and in places characterized by peripheral microridges closely applied to similar ridges on neighbouring cells. The pattern of microridges borne by each cell was distinctive of that cell. Though extensively branched and connected, the microridges appeared to be fairly regularly spaced. For the most part, the arrangement was such that a number of contiguous segments ran parallel to each other forming a unit, which gradually blended with adjacent units where the microridges were oriented differently. Occasional 'pock' marks indicated the luminal surfaces of mucous cells.

A few discrete rounded areas, about 9 um in diameter, marked the pores of the taste buds (Fig.2.3). The pores contained three types of closely-packed projections: long and short filiform projections, interspersed with fewer processes which ended in club-shaped expansions (Fig.2.4).

### 3.2.2 POSTERIOR OESOPHAGUS AND STOMACH

The luminal surfaces of the posterior oesophagus and the entire stomach were divided into squares, pentagons or hexagons (Fig.2.5). Each of these units corresponded to the luminal surface of the epithelial cell, the outlines of which bore about three rows of stubby microvilli. The central enclosed areas were, for the most part, smooth, showing occasional microvilli, pin-point depressions and small mucous globules.

### 3.2.3 INTESTINE

This surface was typified by the presence of numerous blunt, fingerlike projections, which conformed to the villi of mammalian gut (Fig.2.6).

### 3.2.4 CEACA

The caecal mucosal surface bore numerous high longitudinal ridges (Fig.2.7). The ridges showed shallow irregular depressions on their surfaces. At greater magnifications, the faint outlines of the luminal aspects of the epithelial cells could be seen, while the apices of discharging goblet cells appeared as localized excoriations (Fig.2.8).

### 3.2.5 RECTUM

The annular or primary folds appeared as parallel transverse walls separated by fairly regular gaps (Fig.2.9). Each annular fold consisted of basal and apical zones. The basal zones bore numerous regularly-spaced, triangular, secondary folds which gave the appearance of buttresses against both the anterior and posterior surfaces (Figs.2.9, 2.10). The secondary folds were usually corrugated, and often showed serrated free edges. Those associated with the same

primary fold displayed similar architectural features which differed however from those of adjacent folds (Fig.2.10).

The apices of the annular folds were oriented caudally and were very finely striated. Their luminal edges showed straight or serrated outlines, resembling those of the free edges of the secondary folds (Fig.2.10).

## 3.3 LIGHT MICROSCOPY

The gut wall consisted of four coats: mucosa, submucosa, muscularis externa and serosa.

The mucosa of the intestine, caeca and rectum, unlike that of higher vertebrates, lacked crypts. From the stomach to the vent, the deep aspect of the lamina propria contained a dense collagenous sheath or stratum compactum. Its superficial and deep surfaces were related to a stratum granulosum which was constituted by looser collagenous tissue, associated with fibroblasts and granule cells. A muscularis mucosae was only present in the stomach and rectum. A submucosa occurred as a definite layer in the oesophagus and stomach. The muscularis externa consisted of a single coat of striated muscle in the oesophagus, and of two coats of smooth muscle in the remaining parts of the gut. The serosa was particularly prominent in the oesophagus, where it contained vagal nerve trunks.

### 3.3.1 OESOPHAGUS

The epithelium of the anterior third of the oesophagus was stratified cuboidal and contained many mucous cells (Fig.3.1). Occasional taste buds were present.

The basal layer was composed of regularly-arranged columnar cells resting on a basal membrane. The deep-staining nuclei were cigar-shaped, and were surrounded by halos of pale cytoplasm which contrasted with the darker peripheral cytoplasm.

The mid-epithelial zone consisted of about 12 cell layers and contained three cell types: indifferent cells, mucous cells and intra-epithelial leucocytes. The indifferent cells were low columnar in the deep layers, but became progressively reduced in size and more rounded towards the luminal surface. The cytoplasm, like that of the basal cells, contained clear perinuclear and deeper-staining peripheral zones. The mucous cells, distinguished by their clear cytoplasm and basal crescentic nuclei, were interspersed with the indifferent cells. Those in the deeper layers were small and spherical, while larger oval, or sac-like cells occurred towards the luminal surface. Intra-epithelial leucocytes consisting mainly of lymphocytes and neutrophils, occurred in "nests" immediately above the basal cells.

The surface layer comprised cuboidal or round cells with oval, horizontally-oriented nuclei. The cytoplasm again showed clear perinuclear and darker peripheral zones. The layer formed by these cells was interrupted where the sac-like mucous cells reached the surface.

The taste buds were ovoid and usually extended through the whole thickness of the epithelium (Figs.3.2, 3.3). Each measured 54-62 um in height and 25-34 um at its widest. Thick plastic sections stained with 1% toluidine blue in 1% borax, permitted a resolution of cellular detail surpassing that achieved with standard paraffin sections. The variation in staining affinity, and the obvious stratification of the

nuclei in these sections, provided a basis for classifying the component cells into four types: basal, light, dark and intermediate cells.

One or two basal cells lay horizontally on the basal membrane. Their nuclei, which constituted the first nuclear level, stained moderately and showed chromatin clumps.

Most nuclei in the second level belonged to light cells. They were pale-staining, oval and surrounded by pale cytoplasm. The latter extended a short process towards the base of the taste bud, and a much longer and tapering process which reached the apical pore.

The dark fusiform nuclei of the third cell type formed the third nuclear level. The cytoplasm of these cells also extended apical and and basal processes. The nuclei of the intermediate cells were of the same size as those of the light cells but stained more intensely. A few occurred in the second level intermingled with those of the light cells, whilst others comprised the fourth and highest nuclear level, located directly below the pore. The cytoplasm also had both basal and apical processes.

The intragemmal nerve plexus was located between the basal cells and the second nuclear level.

In the middle third of the oesophagus, the stratified cuboidal epithelium was restricted to the tops of the folds, while the sides were organised into simple branched acinar mucous glands (Fig.3.3). The epithelium lining the topmost parts of these glands was two or

three layers thick. The basal cells were short, round or polygonal, and were characterized by darkly-staining nuclei surrounded by pale cytoplasm. Sac-like mucous cells, resting on the basal cells, constituted a continuous layer along the entire lumina of the glands. Each of these mucous cells possessed a flattened basal nucleus and elongate cytoplasm filled with pale mucus. A few surface cells similar to those in the top of the folds were sparsely distributed above the mucous cells.

Towards the end of the middle oesophageal region, and over the entire distal third of the organ, the mucosal ridges bore numerous secondary folds (Fig.3.4). The surfaces were lined by a simple columnar mucous epithelium. Each cell contained a basal oval nucleus and had its apical cytoplasm filled with pale mucus. A similar epithelium lined the lumen of the pneumatic duct (Fig.3.5).

LAMINA PROPRIA

The lamina propria under the stratified cuboidal epithelium was composed of an acellular, homogeneous, collagenous band (Fig.3.1). The more posterior parts of this separated into several plies, interspersed with fibroblasts (Fig.3.3). The lamina propria in the distal third of the oesophagus exhibited tubular serous glands similar to the fundic glands of the stomach. Barrington (1957) termed this portion of the gut the 'oesogaster' (Fig.3.6).

SUBMUCOSA

Because of the absence of a muscularis mucosae, the boundary between the submucosa and the lamina propria was ill-defined. It was composed of sheets of collagen fibres, amongst which were many granule

cells, fibroblasts, and blood vessels (Fig.3.7).

MUSCULARIS EXTERNA

This coat, which was composed of about 25 concentric layers of striated muscle fibres, constituted almost half the thickness of the oesophageal wall (Figs.3.4, 3.8). Radial connective tissue strips penetrated the muscle and transmitted blood vessels and nerves. The circular muscle was interrupted by longitudinal striated muscle at the entry of the pneumatic duct, an arrangement possibly constituting a throttle mechanism (Fig.3.5). There was a gradual replacement of striated by smooth muscle in the oesogastric zone.

SEROSA

This comprised collagenous tissue surrounding vagal trunks and blood vessels (Fig.3.8). The outer surface was lined by a simple squamous mesothelial cells.

3.3.2 STOMACH

Numerous small invaginations of the surface, the gastric pits, were present overall. Based on the differences in the mucous membrane and muscularis externa, the stomach was divided into fundic and pyloric regions. The mucous membrane in the fundic region, which involved the entire corpus and the curved zone, consisted of a simple epithelium, a lamina propria crowded with glands, and a muscularis mucosae. Glands were absent in the pyloric region.

EPITHELIUM

A simple columnar epithelium composed of tall mucous-producing cells lined the surface and the pits in the fundic region (Fig.3.9). The

height of the cells decreased from the surface to the base of the pits (105 um - 60 um). The surface cells, which usually had wider apices than bases, possessed central, cigar-shaped nuclei exhibiting eccentric nucleoli and dark patches of chromatin (Fig.3.10). The apical cytoplasm contained coarse granular mucous vesicles. The pit cells contained basal oval nuclei and lesser amounts of mucus (Fig.3.11). Occasional gastric endocrine cells characterized by very pale cytoplasm were observed amongst the pit cells. Migratory leucocytes were found between the bases of both surface and pit cells. The transition from the pit to the fundic glands was abrupt.

The fundic glands were of simple tubular type which were generally branched and occasionally coiled (Fig.3.12). They were lined by a single cell type, oxyntic or oxyntopeptic cells which were large pyramidal or cuboidal cells with round basal nuclei (Fig.3.13). The nucleoli were central and prominent. The apical cytoplasm contained many dense spherical secretory granules. The basal and paranuclear cytoplasm showed pale, oval granular material. The cells often did not stain uniformly with toluidine blue and the presence of light and dark types most probably reflected their functional states. Cells exhibiting pale, striated, apical borders with a linear arrangement of secretory granules were occasionally observed (Fig.3.14).

In the pyloric region, the pits were particularly deep, and the lining cells resembled those of their counterparts in the fundic region (Fig.3.15). Pale endocrine cells were frequently observed towards the bases of the pits (Fig.3.16). The pyloric valve consisted of a mucosal fold with a core of connective tissue and a covering of columnar mucous cells on both surfaces.

LAMINA PROPRIA

This comprised strips of collagenous connective tissue surrounding the gastric glands and the pits (Fig.3.9, 3.12). These strips ran into a connective tissue layer continuous with the fibrous elements of the stratum granulosum. This coat also contained fibroblasts, occasional smooth muscle cells, granule cells, nerves and blood vessels.

MUSCULARIS MUCOSAE

This was composed of an inner longitudinal and an outer circular layer of smooth muscle cells. The longitudinal component was particularly thick and predominant in the cores of the mucosal folds (Fig.3.17).

SUBMUCOSA

This coat consisted of loose connective tissue containing patches of circular and oblique smooth muscle cells (Fig.3.18). The usual cellular elements, blood vessels and nerves were present.

MUSCULARIS EXTERNA

An inner circular, and a much thinner outer longitudinal layer of smooth muscle cells made up this coat (Fig.3.17). In the pyloric region, the circular muscle coat showed an increase of about six-fold in its thickness (Fig.3.15). Longitudinal strips of loose connective tissue divided the muscle into bundles. The longitudinal muscle coat progressively became thinner and finally ended about half-way along the pyloric part. The myenteric and vascular elements were contained in the loose connective tissue between the two muscle coats. The serosa had the features already described.

### 3.3.3 INTESTINE AND CAECA

The ascending and descending portions of the intestine and the caeca, showed similar histological features. The muscularis mucosae was absent and the submucosa poorly defined.

The epithelium was simple columnar and comprised absorptive cells, goblet cells, endocrine cells, pear-shaped cells between which intra-epithelial leucocytes were dispersed.

The absorptive cells were tall and narrow, and bore distinct striated apical borders (Figs.3.19, 3.20, 3.21). The oval nuclei were mostly smoothly-contoured and contained one or two nucleoli. In plastic sections, it was possible to recognise zonation in the apical cytoplasm (Fig.3.21) which seemed likely to be related to the distribution of the cytoplasmic organelles (Fig.7.1). Deeply-staining discrete granules, very probably representing secondary lysosomes, were observed in the supra-nuclear cytoplasm. The infranuclear cytoplasm displayed no special features.

The goblet cells were characterized by distended oval apices, which contained marbled mucous vesicles (Fig.3.22). The rest of the cytoplasm stained more deeply than that of the absorptive cells. The oval, basally-placed nuclei were situated below the nuclear level of neighbouring absorptive cells.

Very few entero-endocrine cells were encountered (Fig.3.21). In one example, the expanded basal cytoplasm surrounded an oval nucleus and was located well below the nuclear level of the absorptive cells.

The pale cytoplasm had a long tapering process which reached the intestinal lumen.

A few pear-shaped cells were wedged between the apical parts of neighbouring absorptive cells (Fig.3.23). Their apices reached the luminal surface. The nucleus was lobed and basal. Several vertical, dark, rod-like structures were observed in the apical cytoplasm.

Intraepithelial leucocytes situated between the bases of columnar cells were mostly characterized by large, dense nuclei (Fig.3.23).

LAMINA PROPRIA

The lamina propria which formed the apical cores of the villi was particularly loose and contained capillaries, small lymphatic vessels, leucocytes and fibroblasts but no collagen fibres (Fig.3.21). Towards the bases, collagen fibres occurred as dense bands which ran into a loose collagenous network below the villi (Fig.3.24). Larger blood and lymphatic vessels, granule cells, leucocytes and fibroblasts were observed in this connective tissue. The collagenous bands were continuous with those of the stratum granulosum internum.

MUSCULARIS EXTERNA

As in the stomach, this coat comprised a thick inner circular, and a thin outer longitudinal, layer of smooth muscle cells separated by a layer of loose vascular connective tissue, containing elements of the myenteric plexus (Fig.3.25). Radial strips of connective tissue carrying nerves, blood vessels and granular cells often penetrated the circular muscle layer and pierced the stratum compactum to gain the lamina propria. The serosa showed no special features.

### 3.3.4 RECTUM

In addition to the pattern of the mucosal folds, the rectum exhibited such distinctive features as the presence of two types of absorptive cell, a muscularis mucosae, and a conspicuously increased vascularization of the wall. The intestino-rectal valve, and the annular folds appeared as inverted L-shaped forms in longitudinal sections (Figs.3.26, 3.27). Villiform profiles, representing the secondary folds, arose from the primary folds and also from the intervening portions of the wall. The posteriorly-directed apices of the mucosal folds bore no such processes. Transverse sections showed central and peripheral parts of the rectal lumen, the latter being traversed by the villiform profiles (Fig.3.28).

The non-vacuolated absorptive cells were found at both the bases and apices, but not in the intervening portions of the annular folds. These cells showed well-defined, striated, luminal borders and oval central nuclei (Fig.3.29). Some dense granular material was accumulated in the apical cytoplasm and, in lesser amount, scattered within the basal cytoplasm.

The vacuolated cells were characterized by large numbers of spherical vacuoles in the apical cytoplasm. In glutaraldehyde-fixed and osmium tetroxide post-fixed tissue, the vacuoles varied in size and staining density. In some cells, the vacuoles were arranged in linear fashion (Fig.3.30). The more apical vacuoles were smaller, and contained translucent material. The deeper ones were larger, and contained darkly staining material. Occasionally, the vacuoles were arranged at random, and the larger, darker ones were then interposed between the translucent vacuoles (Fig.3.31). In paraffin sections,

and sections of tissues primarily fixed in osmium tetroxide, the vacuoles were generally translucent (Fig.3.32).

The striated borders of the vacuolated cells were less prominent, and the oval nuclei were contained in a usually homogeneous basal cytoplasm.

The entire core of the intestino-rectal valve contained a circularly-arranged smooth muscle bundle directly continuous with the circular muscle coat (Fig.3.33). The stratum compactum extended for varying distances within the core, before breaking up into loose strands which merged with the collagenous fibres in the apex of the valve. This was in contrast to the arrangement in the annular folds, where muscle was confined to the basal and apical parts. The basal, muscular core here, was a triangular extension of the smooth muscle coat, whereas the apical muscle was isolated, and surrounded by a very loose and vascular connective tissue containing fibroblasts and leucocytes in a translucent background material (Fig.3.29).

At the base of each fold, an arteriole, a venule, and a lymphatic vessel were located in the connective tissue between the muscle coats (Fig.3.27). Branches of these vessels pierced the circular muscle coat and ascended the folds (Fig.3.34).

### 3.3.5 HISTOCHEMISTRY OF THE MUCOUS CELLS

**The histochemical reactions of the mucosubstances of the mucous cells in the gut epithelium .**

| REACTIONS | | REGIONS OF THE GUT | | | | |
|---|---|---|---|---|---|---|
| Procedure | Colour | Oesophagus | Stomach | Caeca | Intestine | Rectum |
| Mucicarmine | Red | +++ | +++ | +++ | +++ | +++ |
| PAS | Magenta | +++ | +++ | +++ | +++ | +++ |
| Diastase-PAS | Magenta | +++ | +++ | +++ | +++ | +++ |
| AB pH2.5 | Blue | ++ | ++ | ++ | ++ | ++ |
| PAS-AB pH2.5 | Blue | ++ | ++ | + | + | ++ |
| | Purple | ++ | + | + | + | + |
| | Red | + | + | + | + | ± |
| AB pH2.5-AF | Purple | ++ | - | + | + | ++ |
| | Blue/Purple | + | ± | ++ | ++ | + |
| | Blue | ++ | + | ± | ± | ± |
| HID-AB pH2.5 | Black | ++ | - | - | - | - |
| | Blue | ++ | ++ | ++ | ++ | ++ |
| AB pH1 | Blue | ++ | ± | + | + | + |
| Methyl-AB pH1 | - | - | - | - | - | - |
| Methyl-AB pH2.5 | - | - | - | - | - | - |
| Methyl-SAP-AB pH1 | - | - | - | - | - | - |
| Methyl-SAP-AB pH2.5 | Blue | + | + | + | + | + |

Abbreviations: PAS : periodic acid-Schiff
AB : alcian blue
HID : high iron diamine
AF : aldehyde fuchsin
SAP: saponification
Methyl : active methylation

Reactions: + = intensity of reaction
± = weak or doubtful reaction
- = negative reaction

The mucosubstances were positive to Southgate's mucicarmine, periodate-reactive, diastase-resistant and alcianophilic.

The alcian blue (pH2.5)-PAS sequence demonstrated certain differences in the staining affinities of these mucosubstances contained within the cells. In the stratified epithelium of the anterior oesophagus, there were about equal populations of blue- and purple-staining cells. Red-staining cells were few, and appeared to be confined to the base of the epithelium, although many dark-blue cells were also present in this location (Fig.4.1). The columnar mucous cells lining the glands in the mid-oesophagus, stained purple. The lumina of these glands were filled with a coarse network of blue and purple strands of mucus (Fig.4.2).

The mucosubstances within the cells lining the surface, and the adjacent portions of the crypts, in both the oesogaster and the stomach, stained blue. Those contained in cells lining the middle and bottom of the pits, stained purple and red respectively (Figs.4.3, 4.4). In the caeca, intestine and rectum, most goblet cells stained blue; a few stained purple or red (Figs.4.5, 4.6, 4.7). The brush borders of the absorptive cells stained red, though occasional blue fringes were noted.

The red and blue colourations indicated the presence of neutral and acid mucosubstances respectively, while cells containing mixtures of the two types stained purple (Spicer, et al., 1967).

The aldehyde fuchsin-alcian blue (pH2.5) staining sequence demonstrated the differences in the composition of the acid mucosubstances. These differences were most marked in the anterior

oesophagus, where purple-staining cells were largely located in the higher layers of the epithelium, while the blue-staining ones were concentrated in those parts of the epithelium lining the adjacent shallow crypts (Fig.4.8). Here, these cells intermingled with blue-purple cells. The cells lining the mucous glands contained blue and purple traces (Fig.4.9), whilst the lumina of the glands again contained blue and purple interlacing strands (Fig.4.10).

The epithelial cells of the oesogaster and stomach stained blue. In the caeca, intestine and rectum, there were many purple and blue-purple goblet cells (Figs.4.11, 4.12, 4.13). Very few cells stained blue.

The purple colouration indicated the presence of sulphated mucosubstances: blue colouration - carboxylated mucosubstances (sialomucins); blue-purple - a combination of the two types (Spicer & Meyer, 1960).

Alcian blue (pH1) stained most of the mucous cells indicating the general distribution of the sulphated mucosubstances. However, the colouration observed in the oesogastric and gastric regions was very weak.

The high iron diamine-alcian blue (pH2.5) sequence revealed black-staining cells, which according to Spicer (1965) contained sulphated mucosubstances. They were noted only in the anterior oesophageal epithelium, where they were intermingled with blue-staining cells. The mucous cells of the oesogaster and stomach, and the entire goblet cell population of the caeca, intestine and rectum

stained blue.

Active methylation thoroughly suppressed both the AB(pH1) and AB(pH2.5) reactions. The latter reaction was restored in part by saponification, following active methylation.

## 3.4 TRANSMISSION ELECTRONMICROSCOPY OF THE EPITHELIUM

### 3.4.1 OESOPHAGUS

#### 3.4.1.1 FILAMENT-CONTAINING CELLS

The basal cells, indifferent cells of the mid-epithelial zone, and the surface cells in the stratified cuboidal epithelium of the anterior oesophagus are here designated filament-containing cells, conforming to the term applied to the similar cells of teleost skin (Henrikson & Matoltsy, 1968).

The basal cells rested on a basal complex comprising a lamina lucida and a felt-like lamina densa, about 67nm and 135nm thick respectively (Fig.5.1). Thin anchoring fibrils ran through the sub-epithelial collagenous tissue to attach on the undersurface of the lamina densa. The basal plasma membrane exhibited continuous marginal increased density, and was continuous in places with necks of micro-pinocytic vesicles. Fingerlike processes of the basal complex indented the bases of the cells (Fig.5.2). The highly plicated lateral and apical plasma membranes were involved in extensive microvillous interdigitation, and formed desmosomal attachments with adjacent cells (Figs.5.1, 5.3). The nuclei had irregular outlines due to deep indentations of the nucleolemma. The nucleoplasm showed small irregular patches of heterochromatin.

The zonation of the cytoplasm, visible with the light microscope, was found to correspond to the arrangement of organelles (Fig.5.4). The peripheral cytoplasm was replete with tonofilaments. Each tono-filament was of variable length, and measured about 8nm in diameter. The majority coursed circumferentially, but occasional fascicles departed tangentially to reach the attachment plaques of the desmosomes

(Fig.5.3). Some vertical tonofilaments occurred in the basal cytoplasm where additional annular bands surrounded the indenting processes of the basal complex (Figs.5.1, 5.3). Aggregates of free ribosomes intermingled with the tonofilaments: a few clear vesicles were located in the lateral cytoplasm (Fig.5.4).

The perinuclear cytoplasm contained many mitochondria with prominent tubular cristae and intercristal dense bodies (Figs.5.1, 5.4). The moderately-developed Golgi complex was paranuclear in position (Fig.5.5). Dilated cisternae of granular endoplasmic reticulum and free ribosomes were also present.

The basal cells beneath the columnar mucous cells lining parts of the mucous glands of the mid-oesophagus, were short and triangular (Fig.5.6). Their lateral, apical, and basal plasma membranes were plicated. The nuclei were segmented, and contained one or two nucleoli. Zonation of the cytoplasm was less distinct than in the columnar form. Nevertheless, microfilaments were located in the basal cytoplasm, while a few cisternae of granular endoplasmic reticulum occurred in the perinuclear parts. A moderately-developed Golgi complex, and several small mitochondria were present. The most prominent feature of the cells was the accumulation of numerous, small, spherical, electronlucent vesicles in the apical cytoplasm.

The filament-containing cells of the mid-epithelial zone had their entire plasma membranes thrown into interdigitating villiform processes. Desmosomal contacts were common. The nuclei were mostly lobed. The cells in the lower layers showed a distribution of cytoplasmic organelles similar to that of the columnar basal cells (Fig.5.7). In

the higher layers, the cells possessed numerous small spherical vesicles, containing flocculent material of medium electrondensity throughout their cytoplasm (Fig.5.8). These vesicles appeared to be of Golgi origin, and presumably, younger and paler vesicles of the same size were located on the concave face of the apparatus.

The surface, filament-containing cells had convex luminal borders on which the microridges were represented as stubby projections, 0.4 um high and 0.25 um wide (Fig.5.8). Along the clefts corresponding to the narrow grooves separating circumscribed elevations (see Fig.2.1) the microridge profiles projected like barbs, their hooked apices over-riding those of opposed cells (Fig.5.9). Pale material of parallel fibrillar substructure - most likely streaming mucus - seemed to be suspended by the apices of the microridges. The luminal plasma membrane was covered by a filamentous coat of variable thickness (Fig.5.10). The apical lateral plasma membrane established normal junctional complexes with neighbouring cells, whether of like form or mucous. Elsewhere along the attached borders, the cells bore microvillous processes and desmosomes. The nuclei had deeply-indented outlines.

Zonation of the cytoplasm differed from that of the other filament-containing cells. The luminal plasma membrane was associated with a sublemmal linear density, followed by a layer of dense filamentous network, which formed the cores of the microridges (Figs.5.9, 5.11). The rest of peripheral cytoplasm consisted of a background of looser, interwoven tonofilaments, intermingled with short, dilated cisternae of granular endoplasmic reticulum, free ribosomes, and a few small mitochondria. The small vesicles with

fibrillar, medium-electrondense cores, occurred in much fewer numbers here, and several were observed close to the luminal plasma membrane (Fig.5.8). Occasional particulate dense materials, somewhat larger than the ribosomes, were scattered in this part of the cytoplasm (Fig.5.9).

Annular bands of tonofilaments surrounded the Golgi apparatus, granular endoplasmic reticulum, dense bodies and small mitochondria in the perinuclear cytoplasm (Figs.5.8, 5.11).

3.4.1.2 MUCOUS CELLS

Two morphologically different mucous cell types were distinguished on the bases of shape, mode of discharge, and regional distribution. Type I cells were contained in the stratified cuboidal epithelium in the anterior oesophagus. Their mature forms were globular, and discharged their mucous contents in apocrine fashion. Type II mucous cells were columnar. A few were intermingled with Type I in the stratified epithelium, and they alone comprised the mucous cells lining the glands of the mid-oesophagus. Their mucus was released through the rupture of individual vesicles (merocrine).

(1) TYPE I MUCOUS CELLS

In the initial stages of development, these mucous cells were situated deep in the epithelium, where they were distinguished from the adjacent filament-containing cells by their basal nuclei and more prominent apical Golgi complex (Fig.5.12). Oval electronlucent vacuoles were related to the convex face of the lamellae, while the concave face was related to numerous small vesicles, and multivesicular and dense bodies. Many mitochondria, short cisternae of granular

endoplasmic reticulum, and a few fascicles of tonofilaments were located about the Golgi complex. Elsewhere, there were aggregates of free ribosomes, and many small clear vesicles.

Higher in the epithelium, these cells showed slight indentations of their nuclei and a marked increase in the volumes of the granular endoplasmic reticulum and the Golgi complex (Fig.5.13). The dilated cisternae of the endoplasmic reticulum contained loose flocculent material. Immature mucous vesicles, limited by somewhat scalloped membranes, and containing very loose flocculent material, were observed on the concave face. Later stages were characterized by progressive accumulation of mucous vesicles in the central cytoplasm, peripheral displacement of the nuclei, other cytoplasmic organelles, and eventually by involution of the Golgi complex (Figs.5.14, 5.15). The mucous vesicles also changed in character. The limiting membranes became smoother, and enclosed finely-filamentous materials of varying electrondensity. The vesicular profiles were discoid or oval, and they were usually very closely stacked, sometimes in divergent rouleaux-like patterns (Fig.5.15).

The flask-shaped discharging mucous cells had open apical poles through which amorphous, pale flocculent plugs composed mostly of ruptured vesicles protruded into the oesophageal lumen (Fig.5.16).

(2) TYPE II MUCOUS CELLS

The nuclei of these columnar cells were basal and mostly segmented (Fig.5.6). They contained one or two nucleoli and scanty heterochromatin. An elaborate supranuclear Golgi zone was associated with several dense, and myelin bodies (Fig.5.17). Dilated cisternae

of granular endoplasmic reticulum were abundant; a few mitochondria, microfilaments, and free ribosomes were disposed towards the periphery of the supranuclear cytoplasm. Mucous vesicles filled the central apical cytoplasm. The vesicles appeared smaller and less closely packed than those of the Type I cells. The hyaloplasm between the vesicles contained much smaller, round, clear profiles (Fig.5.18). Where the apices of these cells were flanked by surface filament-containing cells, they extended an apical cytoplasmic process bearing mucous vesicles, which appeared to be released individually (Fig.5.19).

### 3.4.2 GASTRIC EPITHELIUM

The gastric epithelium comprised columnar mucous cells lining the surface and the pits, oxyntic cells within the glands, and scattered endocrine cells. This agrees with the observations of Ling and Tan (1975) on the stomach of Chelmon rostratus, and with those of Noaillac-Depeyre and Gas (1978) on Perca fluviatilis. In the latter species, however, mucous neck cells similar to those described in the mammalian stomach by Ito and Winchester (1963) were observed. Such cells were not observed in this present study.

#### 3.4.2.1 COLUMNAR MUCOUS CELLS

The luminal plasma membranes of these cells presented irregular profiles and occasional short microvilli located towards the lateral borders, conforming to their scanning electronmicroscopic appearance (Fig.6.1 and see Fig.2.5). The lateral membranes showed apical junctional complexes, desmosomes and occasional short, interdigitating villiform processes in the middle portions of the cells. These processes increased in number and complexity towards the base thereby bringing about more extensive interdigitation with adjacent cells

(Fig.6.2). The basal plasma membranes were indented by looped profiles of the much folded basal complexes on which they rested (Fig.6.3). The oval nuclei showed small clumps of heterochromatin and central nucleoli (Fig.6.4).

As in the mucous cells of the oesophageal epithelium, these cells possessed extensive and elaborate Golgi zones (Fig.6.5). Up to eight groups of curved Golgi complexes, each comprising 4 - 5 flattened sacks with associated vesicles, were arranged in rectangular formation in the supranuclear cytoplasm (Fig.6.6). The convex faces of the lamellae were associated with elongate, dilated cisternae of granular endoplasmic reticulum containing a pale flocculent material. At the concave face, larger and possibly immature vesicles were present. These were bounded by scalloped membranes and contained similar pale, loose flocculent material. Denser spots within some vesicles suggested focal condensation of the contents (Fig.6.7). Numerous rod-shaped and oval mature mucous vesicles with finely stippled cores of moderate electrondensity filled the central apical cytoplasm (Fig.6.1). They appeared to be released individually by exocytosis (Fig.6.8). As in the columnar mucous cells of the oesophageal epithelium, small spherical electronlucent vesicles were interposed with the mucous vesicles.

Several oval and spherical membrane-bound structures, interpreted as secondary lysosomes, were observed close to the Golgi lamellae (Figs.6.5, 6.6). Some were multivesicular bodies; others contained coarse filamentous material and dense rectangular components with a compact, rectilinear and regularly-repetitive membraneous substructure. Mitochondria were concentrated in a belt above the Golgi zone (Fig.6.5).

The paranuclear and central infranuclear cytoplasm contained numerous elongate cisternae of granular endoplasmic reticulum (Fig.6.2). The peripheral cytoplasm contained many microfilaments which were especially prominent in the basal region where they formed girdles around the processes of the basal complex (Fig.6.3). The basal cytoplasm also contained numerous free ribosomes and a few mitochondria. The villiform processes were devoid of organelles.

The cells lining the pits showed essentially the same ultrastructure though here, the plasma membrane and the basal lamina were smooth (Fig.6.9). The transition from pit to gland cells was abrupt. The last pit cell retained its typical columnar shape while most of the mucous vesicles in its apical cytoplasm were rod-shaped in profile, and were oriented parallel to the long axis of the cell (Fig.6.10). The intervesicular hyaloplasm also contained increased numbers of small oval or spherical vesicles.

3.4.2.2 OXYNTIC CELLS

The oxyntic cell of the fundic glands was, as mentioned previously, similar to that described in other teleosts. These cells were generally polygonal and possessed round, basal nuclei with prominent nucleoli (Fig.6.11). The luminal plasmalemma was thrown into thick microvilli of variable length with filamentous cores which extended into the apical cytoplasm (Fig.6.12). The lateral plasma membranes showed junctional complexes and slight apical folding while numerous interdigitating processes occurred towards the lateral aspects of the cell base (Fig.6.11). These were compressed between the cell body and a thin, smooth basal lamina.

The apical cytoplasm featured numerous, closely-packed, electron-lucent smooth membrane-bound, round, oval, or tubular profiles (Fig.6.12). The round profiles measured 120-180nm in diameter whilst the tubular profiles, which were mostly oriented along the long axis of the cell, showed slight dilations along their lengths. A number of secretory granules, multivesicular bodies, microtubules, short cisternae of granular endoplasmic reticulum and numerous free ribosomes were located in the intervening hyaloplasm.

The Golgi complex was paranuclear and consisted of series of irregularly-expanded electronlucent sacs associated with membrane-bound spherical secretory granules (Fig.6.13). A few small granules were located on the concave face of the Golgi lamellae whilst denser, and presumably more mature granules (0.3 - 0.7µm in diameter) were scattered at random in the apical cytoplasm.

The entire basal and most of the paranuclear cytoplasm contained numerous long profiles of granular endoplasmic reticulum in parallel arrays (Figs.6.1, 6.14). These were interspersed with many large mitochondria which contained numerous transverse tubular cristae and dense granules in a moderately-dense intercristal matrix. Dense and lamellated bodies were occasionally encountered in the basal cytoplasm.

In preparations of the stomach which had been distended before fixation, the apical cytoplasm resembled that observed in actively secreting oxyntic cells of the amphibian stomach described by Sedar (1965). The tubulo-vesicles had disappeared, and the whole apical cytoplasm was converted into a labyrinth (Fig.6.15). The immediately supranuclear cytoplasm and the apical junctional complexes remained

intact, though the lateral plications of the cell base were reduced (Fig.6.16). The basal cytoplasm contained whorls of concentric, smooth double-membrane lamellae. Each of these showed a short linear density in the cytoplasm between two of the more peripheral lamellae.

3.4.2.3 ENDOCRINE CELLS

These cells, which were more common in the pyloric than in the fundic epithelium, were characterized by pale cytoplasm and abundant intracytoplasmic membrane-bound granules. The apices of most reached the lumen. Three types were distinguished on the basis of variation in shape, size and density of the granules.

Type I These were pear-shaped, located amongst the mucous cells at the bottom of the pit in the fundic epithelium (Fig.6.17). The luminal plasma membrane carried a few microvilli with filamentous cores. The nucleus, contained in the basal expansion, had an irregular contour, and a uniform distribution of heterochromatin patches within the nucleoplasm.

The intracytoplasmic granules were largely supranuclear. They were spherical, (50-125nm in diameter), extremely dense and were usually centrally located within tight investments. Occasional granules were eccentrically-placed within a large vacuolar space, and a sparse number of rod-shaped granules were also observed.

The perinuclear cytoplasm contained dilated granular endoplasmic reticulum, and occasional small mitochondria. Secondary lysosomes, in the form of dense and lamellated bodies, were mainly infranuclear.

Type II These cells were most numerous, occurring in especially large numbers in the pyloric epithelium. They were pear-shaped, spindle-shaped, or columnar and had deeply indented irregular nuclei, which occasionally appeared segmented (Fig.6.18, 6.19). The luminal surface bore microvilli and cilia, and was covered by a loose filamentous material (Fig.6.20). The filamentous cores of the microvilli extended for a considerable distance into the apical cytoplasm. The cilia showed paired hollow fibrils, and a basal body situated deep in the apical cytoplasm where clear vesicles and microtubules were also found.

The Golgi complex was supranuclear and well-developed (Fig.6.21). An expanded sac on the convex surface contained scant, loose, osmiophilic material. The concave surface contained clear vesicles and large, condensing granules with pale cores. The mature granules showed an even distribution within the cytoplasm, and had extremely dense cores, separated from the investing membranes by regular narrow halos. The granules were pleomorphic, also showing a wide variation in size (Fig.6.18). In some cells, granules appeared to be in various stages of depletion with enlarged spaces about them. In extreme cases, the granules were vacuolar and showed only traces of osmiophilic material in their cores (Fig.6.22). Some granules were located very close to the basal plasma membrane, and granular material, which appeared to have been exocytosed, was observed above the lamina densa (Fig.6.23).

Arrays of cisternae of granular endoplasmic reticulum, and broad bands of microfilaments, were usually located in both the supranuclear and the basal cytoplasm. (Figs.6.19, 6.23). Occasional dense bodies and several small mitochondria were also present.

Type III These pear-shaped cells had irregularly-indented basal nuclei (Fig.6.24). The luminal plasma membrane was smooth, and had a very scanty glycocalyx cover, in marked contrast to the neighbouring mucous cells (Fig.6.25). The apical cytoplasm contained centrioles, microtubules, microfilaments and clear vesicles.

The concave face of the moderately-developed, paranuclear Golgi apparatus was directed towards the basal cytoplasm, which contained numerous granules (Fig.6.24). These were spherical (1.4 - 1.8nm in diameter), and displayed graded electron-densities (Fig.6.26). They comprised very dense granules surrounded by regular halos, moderately dense, looser granules with ill-defined halos, and vesicular granules with scanty, flocculent cores. Some granules were closely associated with the basal plasma membrane: a few were located in the supranuclear and apical cytoplasm. The few mitochondria present were supranuclear. Broad perinuclear bands of microfilaments were common, whilst cisternae of granular endoplasmic reticulum were often noted in the basal cytoplasm.

### 3.4.3 ABSORPTIVE CELLS

#### 3.4.3.1 INTESTINE

The luminal border of a typical absorptive cell carried many small microvilli (Fig.7.1). The lateral plasma membrane established typical junctional complexes at its apex, whilst towards the base, there arose a few finger-like processes (Fig.7.2). Transverse sections of the middle portions revealed that certain edges of each cell extended as flanges which were wedged between adjacent cells (Fig.7.3). Desmosomes were observed along the lateral plasma membranes, while the basal membrane which was smooth, rested upon a characteristic basal complex (Fig.7.2). The oval nuclei of the cells contained one or two nucleoli and thin heterochromatin margins (Fig.7.4).

Each microvillus measured approximately 0.9 um long and 0.06 um wide, and was covered by a thin coat of flocculent material (Fig.7.5). This coating was particularly dense towards the tip, where it constituted a capitulum (Fig.7.1). The central core contained some 25 - 35 fine parallel filaments, which extended from the tip into the terminal web (Fig.7.5).

Filaments running parallel to the luminal surface were scanty, and were mainly confined to the lower portion of this zone. Membrane-bound tubular and round profiles appeared to originate from invaginations of the plasma membrane between the microvilli.

The zone immediately beneath the terminal web, contained large amounts of smooth endoplasmic reticulum which presented round, tubular, or irregular profiles (Fig.7.5). Microtubules and multivesicular

bodies were also present in this region, which merged with the supranuclear cytoplasm. The latter contained numerous cisternae of granular endoplasmic reticulum oriented parallel to the long axis of the cell and interspersed with mitochondria and free ribosomes (Fig.7.1). Dense spherical bodies of varying sizes containing unidentified heterogeneous material were also prominent.

The Golgi complex was located in the central supranuclear cytoplasm, and consisted of flattened sacs, small vesicles on the concave face, with large, irregular, dilated sacs and vacuoles on the convex face. Short profiles of granular endoplasmic reticulum abutted these vacuoles (Fig.7.3).

The infranuclear cytoplasm contained several extended contours of granular endoplasmic reticulum, free ribosomes, occasional microtubules and bundles of microfilaments (Fig.7.3, 7.6).

Lamellar structures, similar to those described by Yamamoto (1966) and by Iwai (1968) in the intestinal absorptive cells of young and adult rainbow trout, and also noted in other teleosts by Noaillac-Depeyre & Gas (1973;1974) and Ozaki (1965), were observed (Figs.7.2, 7.4, 7.6). They extended through the paranuclear cytoplasm, bounding narrow passages which either ended blindly or communicated with the exterior of the cell. These passages occasionally branched or anastomosed, or sometimes formed loops which partially or completely surrounded mitochondria (Fig.7.7). Desmosomes were noted occasionally across the lumen of the tubule, portions of the opposing lamellae being modified into attachment plaques, the cytoplasmic aspects of which were associated with microfilaments (Fig.7.6).

Occasional cells possessed markedly larger numbers of mitochondria than their neighbours (Figs.7.8, 7.9). In these cells, the small, oval or spherical mitochondria, with moderately dense matrices, tubular cristae and a few intercristal dense bodies, closely packed the supranuclear and basal cytoplasm. A few cisternae of granular endoplasmic reticulum, and small dense bodies were disposed between the mitochondria.

### 3.4.3.2 CAECA

The absorptive cells of the caeca resembled their intestinal counterparts: major differences proved to be the presence of more apical lysosomes, and the increase in volume and complexity of the infranuclear lamellar structures (Fig.7.10, 7.11).

### 3.4.3.3 CORRELATIONS BETWEEN FUNCTION AND MORPHOLOGY OF INTESTINAL & CAECAL ABSORPTIVE CELLS

The intestine and caeca have already been shown to be the main sites for fat absorption in young and adult rainbow trout (Iwai, 1968; Bergot and Flechon, 1970a; b), and in this study, materials which closely resembled the very low density lipoprotein described in mammalian liver by Claude (1970), were observed within the epithelia of these regions. These materials were particulate and measured 33 - 46nm in diameter. In tissues primarily fixed in osmium tetroxide, the granules revealed inner dense, and peripheral pale portions (see Fig.7.13). Such particles occurred singly in the cisternae of the smooth endoplasmic reticulum in the basal cytoplasm (Fig.7.12). They accumulated in the vacuoles on the peripheral face of the Golgi apparatus (Fig.7.13), and within the lamellar structures, especially at their points of anastomosis (Fig.7.14). Large localised accumulations occurred along

the deeper parts of the intercellular spaces, extending from the level of the Golgi complex to the cell base (Figs.7.13, 7.15). Some of these were located opposite the points where the lamellar structures were continuous with the lateral plasma membrane, whilst similar large quantities occurred between the intraepithelial leucocytes, and the bases of absorptive cells (Fig.7.12). Such particles were also observed on both surfaces of the lamina densa, and amongst the collagen fibrils of the propria (Fig.7.15). They were noticeably scant in the lumina of capillaries, which revealed markedly fenestrated walls (Fig.7.16).

In the caecal epithelium, the intercellular spaces and the lamellar structures appeared to play increased roles in absorption. Both exhibited a series of oval dilatations which were filled with the particulate materials (Figs.7.17, 7.18). Communications between the lumina of the lamellar structures and the dilatations of the intercellular spaces were common.

3.4.3.4 RECTUM

The absorptive cells of the rectum revealed a gradation in some features of the apical cytoplasm - such as intermicrovillous invaginations, tubules, vacuoles and lysosomal bodies - which provided a basis for their histological classification into non-vacuolated and vacuolated types.

The microvilli of the non-vacuolated cells for instance, varied in height, but apical invaginations were few (Fig.7.19). The terminal web resembled that of the intestinal absorptive cells, but the zone below contained longitudinally-oriented tubules, and a few multivesicular bodies. The succeeding zone contained several elongate mitochondria and clusters of free ribosomes. Granular endoplasmic reticulum was

conspicuously absent except in the immediately supranuclear cytoplasm, which contained the Golgi apparatus (Fig.7.20). The nuclei resembled those of intestinal absorptive cells. The infranuclear cytoplasm contained a few lamellar structures, free ribosomes, and occasional fat droplets (Fig.7.21).

The vacuolated cells had uniform microvilli somewhat smaller (0.6nm x 0.06nm) than those of the intestinal absorptive cells (Fig.7.22). The intervillous plasma membranes formed deep, tubular invaginations, most of which traversed the terminal web to terminate in the sub-apical zone amongst many longitudinally-oriented tubular and vesicular profiles.

The internal surfaces of these, as well as those of the invaginations bore a thin coat of delicate, filamentous material (Fig.7.23). The hyaloplasm between the tubules and the vesicles contained longitudinally-oriented bundles of microfilaments and microtubules. The tubules opened into small vacuoles, which obviously coalesced to form the numerous larger vacuoles which were characteristic of the mid-apical cytoplasm (Fig.7.22, 7.24). These vacuoles were round or oval, and varied in size and content, and in cells showing moderate cytoplasmic activity, the zone was filled with a series of vacuoles of an average diameter of 2 um. Some of the vacuoles displayed short blunt processes, which lodged in congruent depressions of neighbouring vacuoles (Fig.7.24). Thus the vacuoles gave the appearance of being arranged in chains, some of the 'links' of which occasionally coalesced with one another (Fig.7.25).

The matrix of the more apical vacuoles comprised a scanty, loose flocculent material (Fig.7.23). Deeper in the cytoplasm, some of the vacuoles were uniformly filled with finely-granular, and fibrillar

material (Figs.7.24, 7.25) while in others, a more compact, dense material with scattered lucent patches was present (Fig.7.22). These matrices also contained myelin figures, and tiny round, oval or rod-shaped, coated vesicles, with small mitochondria, and multivesicular bodies disposed between the vacuoles.

The immediate supranuclear cytoplasm often contained so many vacuoles that the Golgi complex and other organelles were displaced towards the periphery (Fig.7.25). Occasional large lysosomes with a heterogeneous internal composition, were observed in the supranuclear cytoplasm (Fig.7.26, 7.27). The matrices of some of these consisted of a series of myelin bodies, embedded in a clear, finely-granular background, while in others, denser lamellated bodies, together with membrane-bound bodies containing both dense particles and filamentous materials were present.

The nucleus resembled that of the intestinal absorptive cell: the infranuclear cytoplasm contained granular endoplasmic reticulum, free ribosomes, filaments, microtubules, mitochondria and well-developed lamellar structures.

Accumulations of particulate material, resembling low density lipoprotein similar to those described in the intestine and caeca were observed in the basal cytoplasm (Fig.7.28). These were also present in the dilations of the lamellar structures, and in the intercellular spaces, especially those around intraepithelial leucocytes. Other particles of this nature were seen within, and on both surfaces of, the lamina densa.

Occasionally, membrane-bound accumulations of finely-granular material, intermingled with tiny vesicles resembling the supranuclear vacuolar contents, were wedged between the cell bases (Fig.7.29). Pale flocculent materials were also observed within the capillaries of the propria.

### 3.4.4 CYTOCHEMISTRY OF THE ABSORPTIVE CELLS

#### 3.4.4.1 ALKALINE PHOSPHATASE ACTIVITY

Lead phosphate precipitate was abundant in the absorptive cells. The microvilli were strongly positive, more so in the intestine and caeca than in the rectum (Figs.7.30, 7.31). The precipitate was evidently located only on the external surfaces of the intestinal microvilli, but on both aspects of the rectal microvilli. The intervillous invaginations and some of the smooth membrane outlines in the region of the terminal web also showed some deposit, though the terminal web itself was for the most part negative. In the intestinal cells, the cisternae of the smooth endoplasmic reticulum were strongly positive in contrast to the lumina of the tubular profiles of the corresponding zone in the rectum, which showed no reaction, although there was a non-specific sprinkling of deposits in this zone.

The multivesicular bodies revealed only a slight reaction, but the supranuclear dense bodies were very strongly positive. Some showed a uniform deposition of precipitate while the myelin inclusions within other bodies showed heavier deposits (Fig.7.32). The cisternae of the granular endoplasmic reticulum contained much less deposits than those of the smooth endoplasmic reticulum. The Golgi cisternae were positive, with occasional particularly dense areas visible (Fig.7.33). The vacuoles containing low density lipo-proteins, and also the dense

bodies in the Golgi zone were positive. A few deposits were also disposed on the inner surface of the nuclear envelope while the nucleolus was very strongly positive (Fig.7.34).

Sections incubated in substrate-free media, or in the presence of L-phenylalanine, showed no reaction at all.

3.4.4.2 ACID PHOSPHATASE ACTIVITY

As a whole, lead phosphate deposits were abundant, being concentrated on the supranuclear dense bodies. Some myelin bodies seemed to comprise thin cortical and medullary portions, with the precipitate limited to the medulla (Fig.7.35). Deposits were often confined to the dense components of the large heterogeneous bodies of the rectal epithelial cells (Fig.7.36). The myelin bodies within large vacuoles also showed deposits, but Golgi cisternae, and profiles of smooth and granular endoplasmic reticulum, were negative.

3.4.5 GOBLET CELLS

The luminal border of the undischarged goblet cell carried microvilli which were as high, though more slender and more sparse than those of adjacent absorptive cells (Fig.7.37). The oval basal nucleus showed a few undulations, thin marginal heterochromatin and a nucleolus (Fig.7.38), whilst the apical and supranuclear cytoplasm of the cells showed structural variations which reflected its secretory state.

The supranuclear cytoplasm of a young cell for instance, was characterized by the presence of many concentrically-arranged cisternae of granular endoplasmic reticulum, dilated with flocculent material (Fig.7.39).

The cisternae surrounded a Golgi complex, the convex aspect of which was related to several electronlucent vacuoles; the concave face was related to both immature and small, coated vesicles. The latter face also demonstrated both dense and lamellated bodies, while several small oval mitochondria were located between the cisternae of the granular endoplasmic reticulum.

Older cells were conspicuous by virtue of the appearance of mucous vesicles at the concave face of the Golgi apparatus, which progressively accummulated in the central apical cytoplasm (Fig.7.40). These vesicles were enclosed in thin smooth membranes, and had loose fine fibrillar cores which exhibited some difference in electron density (Fig.7.37, 7.41). The intervesicular cytoplasm, where it was obvious, contained short profiles of dilated granular endoplasmic reticulum but it was generally so reduced that only a narrow shell was left on the lateral aspect. The microvilli and terminal web were usually intact in loaded, but non-discharging cells, and in one such cell, a large multivesicular body was observed above the vesicles (Fig.7.37).

In discharging cells, the terminal web and most of the central apical membrane, including the microvilli, were disrupted (Fig.7.41). Disruption of some vesicular membranes and coalescence of their mucous contents often occurred in intact cells, but massive rupture of the majority of apical vesicles was also often observed, with the resultant amorphous mucus, which was paler than that of intact vesicles, being released in bulk (Fig.7.42).

In those epithelia in which alkaline phosphatase activity was localised, the goblet cell outlines were clearly delineated by the

deposition of the reaction product over the entire plasma membranes (Fig.7.43). The Golgi cisternae were also positive, but no deposits were ever observed within the cisternae of granular endoplasmic reticulum.

### 3.4.6 INTESTINAL ENDOCRINE CELLS

Two cell types were distinguished according to their granular characteristics. One closely resembled gastric Type I cells; the other resembled the gastric Type II cell but did not exhibit so much granular pleomorphism. Cells corresponding to gastric Type III were not observed.

Type I These cells were triangular in outline, with oval, generally cordate nuclei. In contrast to those of gastric Type I cells, their apices did not reach the luminal surface, while their intracytoplasmic granules (100 - 105nm in diameter), were spherical, very dense and bounded by close-fitting membranes (Figs.7.44, 7.45). The granules were more or less evenly distributed throughout the cytoplasm although they had a tendency to be arranged marginally towards the middle and basal regions.

Type II Cells of this type were columnar, and extended much more through the epithelium than Type I, although again, actual contact with the lumen was not observed. Their oval nuclei occurred at the same level as those of the absorptive cells (Fig.7.46). A moderately-developed Golgi complex was supranuclear (Fig.7.47). Granules were highly electrondense and though scattered throughout the cytoplasm, they appeared to be concentrated in the basal portions (Fig.7.48). They were mostly ovoid (116 x 100nm), though a few comma-shaped and

spherical granules were present. Otherwise, the granules were located eccentrically within loose-fitting, investing membranes.

These cells, like the absorptive cells, contained several slender, longitudinally-oriented mitochondria, and also lamellar structures in both the supranuclear and basal cytoplasm (Figs.7.48, 7.49). The elongated profiles of granular endoplasmic reticulum in the supranuclear cytoplasm were dilated while occasional microfilaments, microtubules and myelin figures were also noted (Fig.7.46).

### 3.4.7 PEAR-SHAPED CELLS

These cells were limited by very thick walls, measuring 0.3um thick over the basal and middle portions which became thinner towards the apex, where they established desmosomes with adjacent absorptive cells (Figs.7.50, 7.51). Nuclei were lobed and eccentric (Fig.7.52), while the cytoplasm contained several paddle-like structures containing dense rods which were axial and continuous with the long, apical 'handles' of the paddles (Fig.7.50). A series of vertically-oriented membranes, and some granular endoplasmic reticulum and mitochondria were observed between these structures. The apical aspect of the cells contained numerous microtubules and a few vesicles (Fig.7.51).

### 3.4.8 INTRAEPITHELIAL LEUCOCYTES

These were consistently observed wedged between the bases of the absorptive cells of the intestine, caeca and rectum. The majority appeared to be either lymphocytes or neutrophils.

The nuclei of the lymphocytes were mostly oval but with indented outlines (Fig.7.53). The nucleoplasm contained an eccentric nucleolus,

and an irregular band of heterochromatin which was attached to the inner surface of the nuclear envelope. The cytoplasm showed a well-developed Golgi complex, and a centriole surrounded by several small, oval mitochondria, and short cisternae of granular endoplasmic reticulum. Most parts of the cytoplasm contained aggregates of free ribosomes and occasional vesicles.

Neutrophils were characterized by having smaller, eccentric and often lobed nuclei, which showed heterochromatin configurations similar to those of the lymphocytes (Fig.7.54). The nucleoli were also eccentric. A moderately-developed Golgi apparatus was located close to the nucleus (Fig.7.55). Small mitochondria were distributed throughout the cytoplasm. The most prominent feature, however, was the presence of numerous membrane-bound granules of varying shapes, sizes and internal structure. Most were large and fusiform, or oval. These had cores of fibrillar material aligned with the long axes of the granules. The fewer, large spherical granules contained finely granular material; the small spherical granules had uniformly dense cores. Profiles of granular endoplasmic reticulum were arranged around the circumference. Free ribosomes were present, and a few dense and lamellated bodies were also observed (Fig.7.54).

A third cell type, characterized by an irregularly-indented nucleus, and numerous spherical intracytoplasmic vesicles, was occasionally observed (Fig.7.54). Some of the vesicles of this cell had dense cores while others had loose osmiophilic material within them.

## 3.5 MAJOR COLLAGEN ELEMENTS AND ASSOCIATED CELLS

### 3.5.1 LIGHT MICROSCOPY

(A) Organisation of collagenous elements

A homogeneous, collagenous sheath occurred beneath the basal lamina of the epithelium in the anterior oesophagus (see Fig.3.1). This sheath became very loose towards the posterior oesophagus (see Fig.3.3), but in the stomach, it was reorganized at a deeper level within the lamina propria into the stratum compactum. It maintained this position in the remaining parts of the gut.

In transverse sections, the compactum appeared markedly corrugated, and appropriate longitudinal sections revealed that within the intestino-rectal valve, it folded back on itself, much in the manner of a hair-pin. Distally, it formed triangular creases at the bases of the rectal mucosal folds. Occasionally, it was pierced by vascular and nervous elements (Fig.8.1).

In the stomach, the collagenous elements of both internal and external strata granulosa were most distinct, forming networks which maintained continuity with the compactum by means of narrow strands (Fig.8.2). In the intestine, caeca and rectum, only the granulosum externum was well-defined, and the collagen within it consisted of two or three thin concentric coats, linked by short radial strands (Fig.8.3). It proved impossible to distinguish the collagen of the superficial propria from those of the granulosum internum, though radial strands did extend from the deepest band to the inner surface of the compactum.

(B) Fibroblasts

A continuous layer of fibroblasts was closely applied to the superficial surface of the stratum compactum (Fig.8.4). Often, these were intermingled with granule cells (Fig.8.5). The fibroblasts appeared flattened or irregular, with several cytoplasmic processes. Their nuclei were fusiform or oval, and were surrounded by lighter-staining cytoplasm. Cells resembling fibroblasts, but having long attenuated processes, were observed amongst the granule cells in the granulosum externum (Fig.8.4).

(C) Granule Cells

In the anterior oesophagus, granule cells were not associated with the sub-epithelial collagen sheath. However, large numbers were scattered within the looser collagenous tissue of the propria and the submucosa (Fig.8.6). In the stomach, some were noted in the connective tissue surrounding the glands, but a much greater concentration occurred in the internal and external granulosa, and in the submucosa (Fig.8.2).

In the caeca, intestine and rectum, most of the granule cells were contained within the stratum granulosum externum, where they were arranged in three or four layers (Fig.8.3). The internal stratum, and the sub-epithelial layers of the propria, contained much fewer numbers (Figs.8.3, 8.5). Occasional granule cells were observed within the circular muscle coat and in the vascular connective tissue between the muscle coats.

On occasions, it was noted that the granule cells and vascular elements were associated, the cells being closely applied to the walls

of lymphatic and blood vessels (Fig.8.7). Some, in fact, were observed to be lying freely within the lumina of occasional lymphatics (Fig.8.8).

The granule cells were oval, round or spindle-shaped, varying greatly in size. Each possessed a large eccentric nucleus, and the cytoplasm contained numerous round granules of varying sizes. In plastic sections, the granules stained deeply with 1% toluidine blue in 1% borax, contrasting sharply with the pale background. The granules stained pink with haematoxylin and eosin and bright red with Masson's trichrome. They were PAS negative, alcianophilic at pH1 and pH2.5, and stained purple with aldehyde fuchsin (see Fig.4.9 ). Whilst they did not stain with Bierbrich scarlet in glycine buffer at pH9, 9.5 or 10, they proved positive to Baker's haematein test for phospholipids.

### 3.5.2 ELECTRON MICROSCOPY

(A) Collagenous elements

Both the sub-epithelial sheath of the anterior oesophagus and the stratum compactum were composed of several plies of tightly-packed collagen fibrils, which showed dark and light cross-banding, ranging from 26nm to 60nm in diameter. All the fibrils were arranged in one direction, and weaved their course through each ply. The direction of the fibrils in adjacent plies alternated, resulting in an orthogonal pattern, which proved to be particularly regular in the sub-epithelial sheath, where up to 20 plies were observed (Fig.8.9). In contrast, the plies were interrupted in the stratum compactum, and occasional stray fibrils extended obliquely through much of its thickness (Figs.8.10, 8.11). A few microfilaments also intermingled with the fibrils.

(B) Fibroblasts

These cells possessed many dilated cisternae of granular endoplasmic reticulum, which contained flocculent material of medium electrondensity (Fig.8.12). Occasional cisternae of smooth endoplasmic reticulum, with a beaded appearance, were also observed. Bands of microfilaments were located around the nucleus between the cisternae of granular endoplasmic reticulum, and beneath the plasma membrane, where they were interspersed with numerous free ribosomes and spherical vesicles, containing some loose material (Fig.8.13). Mitochondria were small and sparse and a few dense bodies were present (Fig.8.12).

(C) Granule Cells

These cells existed either singly, or in groups of up to four. They were usually partially or completely ensheathed by the cytoplasmic processes of special connective tissue cells (Figs.8.14, 8.15). A gap, 20nm to 30nm wide, separated the two cells. The plasma membrane of the ensheathed portion was smooth, while the naked portion was in close contact with collagen fibrils. In these areas, occasional bifurcated pseudopodia projected from the cell surface (Fig.8.14).

The nuclei were mostly oval and peripheral in position. Some showed a few shallow depressions on the nuclear outline but in most cells several intracytoplasmic granules were seated into impressions of the nuclear envelope (Figs.8.14, 8.15). The nucleoplasm contained an eccentric nucleolus, and in most cases, dense chromatin masses were distributed against the inner surface of the nuclear envelope.

The Golgi complex was located near the nucleus and comprised

four to six curved flattened sacs which, in some cells, showed large oval electronlucent expansions (Figs.8.16, 8.17). Both the convex and concave faces were associated with small, and thus, presumably young granules measuring about 0.1 um in diameter. Larger, maturing granules up to 0.6 um in diameter were also noted closeby, while further away, and filling most of the cytoplasm, were numerous, mature granules ranging from 0.5 um to 1.3 um in diameter (Fig.8.15).

Each granule comprised a highly dense homogeneous core, separated by a regular narrow space from a thin membrane. Structural variations in the cores were frequently observed however. In some granules, for instance, the core was misshapen, or spotted with irregular lucent patches (Figs.8.18, 8.19, 8.20). In other cases, small myelin figures were associated with a localised peripheral depletion of the core matrix, while larger myelin figures sometimes surrounded or even replaced the entire matrix. In such situations the spaces between the cores and their surrounding membranes were markedly increased and irregular, and often contained loose flocculent material. Where the cores appeared to be intact, radial finger-like tags extended from the membranes to their surfaces (Fig.8.21).

A centriole with associated microtubules, was located close to the concave surface of the Golgi complex (Fig.8.22). Free ribosomes and short narrow cisternae of granular endoplasmic reticulum were also uniformly distributed throughout the cytoplasm, while a few mitochondria containing tubular cristae and moderately dense matrices were present (Fig.8.17).

A portion of the cytoplasm, usually located at one pole of the cell, was largely devoid of organelles other than ribosomes (Fig.8.18, 8.20) and it was separated from the rest of the cytoplasm by a delicate fibrillar arc. Some smooth membrane-bound tubular profiles, often continuous with the plasma membrane, occurred near the level of demarcation.

Considerable difficulties were encountered with the preservation of these cells. The technique which proved to give the most consistent results, was initial fixation with 6.25% glutaraldehyde, followed by post-fixation with 1% osmium tetroxide. These fixatives were buffered with 0.1M cacodylate at pH7.3. With lower concentrations of glutaraldehyde, the results were inconsistent for, though the granules were usually preserved, there was marked distortion of the remaining cytoplasm. Primary fixation with osmium tetroxide on the other hand, resulted in loss of the granular matrix as well as distortion of the cytoplasm.

(D) Ensheathing Cells

These were highly-irregular, fibroblast-like cells with a propensity to hug the granule cells with attenuated cytoplasmic processes (Fig.8.23). In places, these processes were held together by desmosomes (Fig.8.21). Many coated and smooth vesicles were associated with the plasma membrane (Fig.8.24). Those parts of the cell surface away from the granule cells were undulating and were in contact with collagen fibrils.

The cell nuclei were large, and showed extensively-folded outlines. Nucleoli were invariably present and chromatin was rather dispersed.

A small Golgi apparatus and occasional centriole were found close to the nuclei, and free ribosomes and dilated cisternae of endoplasmic reticulum were spread throughout the cytoplasm. Several large, spherical or oval mitochondria with prominent cristae in a relatively dense matrix were also present, while membrane-bound accumulations of fine or coarse granular material and a few dense bodies were observed. Bands of microfilaments were found in the perinuclear cytoplasm and within the cytoplasmic processes, where some extended to the desmosomal plaques. Microtubules were observed close to the centrioles and in the cytoplasmic processes (Figs.8.25, 8.20).

### 3.5.3 ULTRAHISTOCHEMISTRY OF THE GRANULE CELLS

(A) Carbohydrates

P.A.S. positive carbohydrates proved to be absent in the granules, using the techniques of Rambourg et al (1969) and Seligman et al (1965). However, with the application of Ruthenium red, after Jollie & Triche (1971), fine deposits were observed in some of the granules, the smaller granules showing the more positive reaction (Fig.8.26).

(B) Basic proteins

Acrolein fixation which is recommended for the detection of proteins, failed to preserve the granule cells; even so, no deposits were noted in the free granules. Deposits however, were observed in the nuclei, and in the nuclei of several neighbouring cells, which provided an adequate control for the technique.

(C) Localization of Enzymes

(1) Alkaline phosphatase

Lead phosphate appeared as coarse deposits on the reactive

cytoplasmic organelles. The cisternae of granular endoplasmic reticulum were consistently reactive, as were the smooth membrane-bound tubules within the organelle-free zone of the cytoplasm (Figs.8.27, 8.28). The Golgi apparatus was also reactive (Fig.8.29). Here, the deposits occurred within the flattened sacs and in the vacuoles on the convex face.

The granules varied in their reactivity. In some cells, none showed deposits although surrounded by positively-reacting organelles. Occasional cells were encountered in which most of the granules reacted, and here, the reactions took the form of irregular deposits on the dense matrices. The myelin figures between some of the matrices and their membranes also contained deposits (Fig.8.27).

No reaction was observed in control sections incubated in substrate-free medium.

(2) Acid Phosphatase

Attempts to localise this enzyme gave erratic results. The positive reaction of some granules could not be repeated in later attempts, which were also aimed at preserving the cells better. However, when the reaction was positive, fine deposits were found only on several granules without any other cell constituent reacting (Fig.8.30). In the majority of reactive granules, the reaction product formed a thin peripheral rim, though occasionally, a considerable peripheral area of the matrices also contained deposits. In such cases, it appeared as if the reactive front was encroaching towards the granule centre. The reaction product was sometimes seen as prominent blobs protruding from the surface of the dense matrix (Fig.8.31).

Granules showing marked peripheral reaction as well as a fine overall deposit were also observed (Fig.8.32).

The control sections did not show any reaction.

(3) 5-Nucleotidase

The lead phosphate reaction product appeared as fine particles concentrated around the granules (Fig.8.33). In occasional cells, particulate deposits were scattered throughout the matrices of a few granules, with other cytoplasmic organelles and other granules failing to react (Fig.8.34). The reaction was not at all evident in control sections.

(4) Arylsulphatase

Though cell preservation with this technique was very poor, reaction product was noted as dense particles scattered between, and concentrated around the granules (Fig.8.35). No deposits were observed in control sections.

(5) Peroxidase

Slight deposits of reaction product were confined to the myelin figures (Fig.8.36). No other deposits were found, neither were deposits observed in control sections.

## 3.6 INTRINSIC INNERVATION

### 3.6.1 GENERAL DISTRIBUTION

Acetylcholinesterase positive elements representing nerve fibres and cell bodies, were consistently observed in all parts of the gut as brown deposits. These findings were at slight variance with those of Burnstock (1959b), who failed to find cell bodies in the submucosa of the gut of the brown trout, using silver impregnation and methylene blue techniques. Examination of plastic sections did in fact confirm the presence of nerve cell bodies in the submucosa of the stomach, and in the corresponding zone of the intestine and rectum (Figs.9.1, 9.2, 9.3). Furthermore, nerve cell bodies were also found within the muscularis mucosae of the rectal folds (Fig.9.4).

The vagal nerve trunks of the oesophageal serosa, and their continuations in the myenteric plexus of the stomach, consisted of myelinated and unmyelinated fibres, and nerve cell bodies enclosed in a collagenous capsule (Fig.9.5). Nuclei of supportive cells were also observed. In the stomach, additional nerve cell bodies and fibres were scattered in the connective tissue outside the trunks (Fig.9.6). Similar elements constituted the myenteric plexus in the remainder of the gut, though the vagal trunks were not found beyond the stomach.

A dense sub-epithelial network of acetylcholinesterase positive elements was observed in the oesophagus and stomach. The muscle coats also showed the presence of positive fibres, especially in the oesophagus where they formed a complex network.

## 3.6.2 ELECTRON MICROSCOPY

### 3.6.2.1 MYENTERIC PLEXUS

The myenteric plexus consisted of nerve cell bodies and myelinated and unmyelinated nerves associated with satellite and Schwann cells respectively. These were invested successively by a thin basal lamina, collagen fibrils and interstitial cells. They existed as discrete units in the vagal trunks, to which the myelinated fibres were confined. The trunks were also encapsulated by several layers of collagen fibrils, separated by thin processes of fibroblasts (Fig.9.7).

Elements of the myenteric plexus were generally embedded in a collagenous connective tissue which was relatively scant in the caeca. The tissue was particularly well vascularized in the rectum. Granule cells, isolated smooth muscle cells, and migratory leucocytes were other components noted in the connective tissue.

NERVE CELL BODIES

These occurred singly or in groups of two or three, varying both in size and cytoplasmic features. Small nerve cell bodies, ranging from 5 um to 20 um in length and 4 um to 6 um in thickness, and larger neurons, up to 50 um in length and 11 um in thickness, were both observed.

The nerve cell bodies were distinguished by their large round, oval or fusiform nuclei, which were central or eccentric in position, and contained one or two prominent nucleoli. The nucleoplasm was finely granular, and only rarely were slight heterochromatin condensations observed against the nuclear envelope. The cell bodies showed various types of processes and local modifications of the plasma membrane.

The Golgi complex was generally moderately developed, and was often found to be associated with granular vesicles, composed of electron dense cores separated from their investing membranes by halos. Lysosomal bodies were prominent and comprised multivesicular, lamellated and dense forms. The mitochondria were usually small, and were distributed throughout the cytoplasm. Free ribosomes were abundant. Centrioles and smooth endoplasmic reticulum were occasionally observed. The granular endoplasmic reticulum, microfilaments, microtubules and granular vesicles however, showed variations in amount and organisation, thus providing a basis for distinguishing the cell bodies further.

(1) Small Nerve Cell Bodies

These were numerous and proved to comprise at least three different cell types. Most of these contained granular endoplasmic reticulum appearing as uniformly-distributed isolated cisternae (Fig.9.8), or as stacks, located towards either one, or both poles (Figs.9.9, 9.10). Such cells generally had a wide distribution being noted within the cores of the vagal trunks, and constituting the only cell type observed in the caeca.

A number of small cells encountered only in the stomach contained abundant microfilaments and a few microtubules in the perinuclear cytoplasm (Fig.9.11). There was little granular endoplasmic reticulum, but clusters of free ribosomes were preponderant in the peripheral cytoplasm. Long slender mitochondria were also present.

The third cell type was characterised by moderate to large numbers of large intracytoplasmic granular vesicles, 90nm - 140nm in diameter (Figs.9.12, 9.13). These vesicles appeared to be of Golgi origin and

in one cell, a granular vesicle was observed in the process of budding from the lateral margin of a flattened cisterna (Fig.9.13). Stacks of granular endoplasmic reticulum were located only at one pole of the cell body (Fig.9.12).

(2) Large Nerve Cell Bodies

These were found in all parts of the gut except in the caeca. At least two types were identified.

The first type, noted in the stomach, contained large amounts of interlacing microfilaments and microtubules within its central cytoplasm. Clusters of free ribosomes were abundant and evenly distributed. (Fig.9.14). A few isolated cisternae of granular endoplasmic reticulum were located mainly at the periphery, while several short cisternae of Golgi sacs were located in the central cytoplasm. Cell processes and modifications of the plasma membrane were especially noticeable.

The second type of cell was more widely distributed, showing sparse microfilaments and microtubules. More cisternae of granular endoplasmic reticulum were present however, either singly, or in extensively-distributed stacks (Figs.9.15, 9.16). Intracytoplasmic granular vesicles, 55nm - 70nm in diameter, occurred mostly in the region of the Golgi cisternae (Fig.9.15).

(3) Plasma Membrane Modifications

In glutaraldehyde-fixed, osmium tetroxide post-fixed material, the plasma membrane of the nerve cell bodies showed dense areas at certain points of contact with the cytoplasmic processes of satellite

cells, or with vesiculated axons (Fig.9.14). Portions of the plasma membrane overlying the subsurface cisternae, first described by Rosenbluth (1962), appeared relatively denser. These subsurface cisternae, which were noted in both small and large neurons, comprised single smooth membrane-bound flattened sacs, with bulbous lateral expansions (Figs.9.14, 9.17, 9.18). They ranged from 115nm - 540nm in length, and conformed closely to the local outline of the overlying plasma membrane. The membranes became increasingly dense along their flattened middle portions. The parts of the neuronal surface showing subsurface cisternae were invariably covered by satellite cell cytoplasmic processes. Micropinocytic vesicles were occasionally associated with the plasma membranes (Fig.9.18).

(4) Neuronal Processes

Apart from the axons, processes of varying number, shape and size stemmed from the cell bodies. The majority made contact with vesiculated axons of other neurons, and they comprised:

(a) Large processes with elongated triangular profiles, which either arose abruptly, or directly continued the taper of a fusiform nerve cell body (Fig.9.19). The bases of these processes were characterized by extensive cisternae of granular endoplasmic reticulum arranged in remarkably parallel arrays. Mitochondria, clear vesicles, free ribosomes and multivesicular bodies were located distally;

(b) Rectangular and club-shaped processes with cores similar to the main cytoplasm, which arose from any part of the cell body (Figs.9.18, 9.20);

(c) Small conical 'spike-like' processes which took origin from the main body of the neuron or from a type (b) process (Figs.9.21, 9.22, 9.23). These varied in length, and had amorphous cores of moderate density. Smooth membrane-bound tubules converged at the base of one example (Fig.9.21) and the core of another process contained similar tubules and microfilaments (Fig.9.23). Most of these processes showed thickenings of the cell membrane at the points of contact with vesiculated axons;

(d) Large blunt processes which contained numerous microtubules in parallel arrays, and several large granular vesicles, about 120nm - 140nm in diameter (Fig.9.24). Smaller processes of this category lacked the microtubules, but contained similar granular vesicles together with mitochondria (Fig.9.25);

(e) Occasional foot-like processes which contained a few smaller granular vesicles, about 67nm in diameter, together with microfilaments and mitochondria (Fig.9.26).

AXON PROFILES

Myelinated axons occurred as single oval or round profiles in transverse section of the vagal trunk and as previously noted, were enclosed by the cytoplasm of Schwann cells (Fig.9.27). The myelin lamellae were highly osmiophilic and surrounded a lucent axoplasm which contained microtubules, microfilaments, and mitochondria oriented parallel to the long axis. Occasional dense bodies, and large smooth membrane-bound irregular vesicles were present. Granular vesicles, about 67nm in diameter, were also observed in some of these axons (Fig.9.28).

The unmyelinated axons within the vagal trunks showed wider variation in diameter, and existed singly or in small groups, bound together by Schwann cell cytoplasm (Fig.9.27). These axons consistently contained large numbers of vesicles of varying size and density, as well as microtubules, microfilaments and smooth endoplasmic reticulum (Fig.9.29).

Much larger aggregates of axons were another consistent feature of the rest of the myenteric plexus (Fig.9.30). The vesicles were scattered along the axons but were more numerous in the club-shaped enlargements or varicosities that occurred along their course (Figs.9.30, 9.31). The vesicles were often associated with mitochondria and on rare occasions, they were intermingled with dense particulate material (Figs.9.24, 9.25).

Three classes of vesicle were observed: electronlucent, small granular, and large granular. Vesicles of all classes were mostly round and their diameters were 30nm - 120nm, 45nm - 70nm and 90nm - 150nm respectively. Flattened and oval forms of all three types were occasionally observed. The granular vesicles contained electron dense material separated from the investing membranes by lucent annular zones. Such halos were well-defined in tissues fixed in glutaraldehyde and post-fixed in osmium tetroxide (Fig.9.31). In contrast, in primarily osmium tetroxide-fixed tissues, the halos were indistinct, especially in the large granular vesicles, where they were filled with material of medium electron density (Fig.9.32).

Three types of varicosity were distinguished by the proportion of types of vesicle they contained. In the main, Group I varicosities

contained round lucent vesicles of varying sizes, which were normally associated with a few large granular vesicles (Figs.33, 9.34).

Group II varicosities contained small granular vesicles intermixed with large numbers of small flattened, lucent vesicles (Fig.9.34). Some of the varicosities in this group also contained dense particulate material (Figs.9.24, 9.25).

Group III varicosities were characterised by large numbers of large granular vesicles, which either constituted the whole vesicle population, or were mixed with numbers of small flattened lucent vesicles (Figs.9.34, 9.35).

Varicosities of all types were involved in the formation of axo-somatic and axo-dendritic synapses. Occasionally, two morphologically-different axon varicosities synapsed on the same nerve cell body (Figs.9.25, 9.26). The pre- and post-synaptic membrane thickenings were asymmetric, the latter appearing denser and accordingly more prominent. The synaptic cleft showed a width of 20nm - 35nm. When a synapse was on a small somatic or dendritic spine-like process, the varicosity completely covered that process. Any variation in post-synaptic membranes occurred along the entire border of the small process (Fig.9.21) or in some instances, as localised patches at the bases of longer processes (Fig.9.22).

In synapses involving varicosities of all types, the electron-lucent vesicles were usually closest to the pre-synaptic membranes (Figs.9.36, 9.22, 9.21). However, in a few Group III varicosities involved in axo-somatic synapses, most of the vesicles were concentrated

in the middle, with the large granular vesicles displaced to both ends of the profile (Fig.9.37). In one such example, the post-synaptic membrane showed two thickened areas. Electronlucent vesicles were associated with the presynaptic membrane opposite one area, whilst a group of large granular vesicles was located opposite the other.

NON-NEURONAL ELEMENTS

(a) Satellite Cells

These cells were characterised by their dense fusiform or irregular nuclei, which were surrounded by a narrow band of cytoplasm (Figs.9.38, 9.39). Their thin cytoplasmic processes closely surrounded the nerve cell bodies, except where the latter were approached by vesiculated axons. Occasional slight densities existed at the points of contact of the plasma membranes (Fig.9.15). The outer surfaces of the satellite cells were invariably invested by a thin basal lamina. The cytoplasm was generally dense but dilated cisternae of granular endoplasmic reticulum and mitochondria were prominent. A few microfilaments were found close to the plasma membrane densities.

(b) Schwann Cells

These constituted the most common non-neuronal components of the myenteric plexus. Their nuclei were fusiform, oval or irregularly-indented, rich in heterochromatin and with eccentrically-placed nucleoli (Figs.9.40, 9.41). The nuclei of those cells associated with myelinated axons in the vagal trunks had coarser heterochromatin, and were denser than those of the cells associated with unmyelinated axons (Fig.9.41). The thin rim of cytoplasm surrounding the nucleus usually contained a Golgi apparatus, a centriole associated with microtubles,

small mitochondria, free ribosomes and a few short cisternae of granular endoplasmic reticulum (Fig.9.40). The attenuated processes, which completely or partially surrounded the unmyelinated axons, contained free ribosomes, granular endoplasmic reticulum and occasional mitochondria (Figs.9.40, 9.42). Like the satellite cells, the Schwann cells were covered by a thin basal lamina.

(c) Interstitial Cells

Each of these comprised a cell body and long cytoplasmic processes which were often held together by desmosomes (Fig.9.43, 9.38). The processes enclosed fibrils of collagen, either Schwann cells or satellite cells, and axons or nerve cell bodies. The cell body contained the nucleus, within which was a central nucleolus and some peripheral heterochromatin. The Golgi complex and a prominent centriole were located close to the nucleus (Fig.9.43). Granular endoplasmic reticulum was rare but there were abundant free ribosomes, prominent mitochondria and dense bodies. The cytoplasmic processes contained short dilated cisternae of granular endoplasmic reticulum and occasional mitochondria. Micropinocytic vesicles which were associated with the plasma membrane, seemed likely to coalesce within the cores of the processes, to form electronlucent sacs (Fig.9.44).

(d) Vascular elements

These included numerous capillaries, arterioles, venules and lymphatics, and of these, the lymphatics presented the most distinctive features, in that the endothelial cells contained numerous intracytoplasmic granules which appeared to originate from a well-developed Golgi apparatus, and to pass through several developmental and autolytic stages within the cytoplasm (Fig.9.45). Immature granules were

recognised by their pale flocculent cores and irregular outlines, while the mature granules appeared denser, more spherical, with densely-packed, fibrillar material within their cores (Fig.9.46). The autolytic stages were characterized by several myelin figures forming within the substances of the granules and overall autolysed areas were recognised as electronlucent patches.

### 3.6.2.2 SUBMUCOUS PLEXUS

Elements of the submucous plexus existed at two levels within the gastric submucosa. In the intestine and rectum, the submucous plexus was confined to a narrow connective tissue layer interposed between the stratum granulosum externum and the circular muscle coat. Occasionally, parts of the plexus extended into the circular muscle coat. As in the myenteric plexus, nerve cell bodies and axons were associated with satellite and Schwann cells respectively, and were usually surrounded by interstitial cells.

#### NERVE CELL BODIES

In the stomach, the nerve cell bodies of the first level were located close to the inner aspect of the circular muscle coat. The second level comprised nerve cell bodies scattered amongst the smooth muscle fibres and granule cells in the deeper part of the submucosa. The relative thinness of the circular muscle of the intestine and rectum often permitted observation of the elements of both plexuses in the same field (Fig.9.47)and again it was possible to distinguish small and large cell types.

A number of the small cells resembled those which formed the major elements of the myenteric plexus (Fig.9.48), but a few, in different

parts of the gut, showed significant structural differences.

One such type (19 um x 5 um), observed in the deeper part of the gastric submucosa, was distinguished by its more highly-developed Golgi complex, which comprised some five groups of highly-vacuolated cisternae, and electronlucent vesicles arranged in the form of a horse-shoe (Fig.9.49). Large granular vesicles, 130nm - 150nm in diameter, were located near the cisternae, while closeby, large round lysosomal bodies with finely-lamellated cores were found. Cisternae of granular endoplasmic reticulum were few but clusters of free ribosomes were abundant.

A second distinctive cell type (8.2 um x 7.6 um) was located against and partially embedded in the circular muscle coat of the intestine (Fig.9.50).

These cells contained granular vesicles, about 65 nm in diameter, distributed at random within the cytoplasm.

The large nerve cell bodies resembled those of the second category described in the myenteric plexus. The cytoplasm contained moderate amounts of granular endoplasmic reticulum (Fig.9.51). The Golgi complex was associated with small granular vesicles whilst on one occasion, a vesicle in the process of budding from the extremity of a cisterna was observed (Fig.9.52).

Plasma membrane modifications and neuronal processes were less commonly observed than in the myenteric plexus. Some cell bodies were associated with satellite cells while others, especially in the rectum

were embedded together with contiguous axons, in the infoldings of Schwann cells (Fig.9.48).

AXON PROFILES

For short distances, the axons ran in groups in close contact with each other, occasionally making contact with neurons and smooth muscle cells without the intervention of Schwann cells (Fig.9.48). They also exhibited varicosities. The intervaricose profiles contained microtubules, microfilaments, mitochondria and large numbers of various types of vesicle (Figs.9.53, 9.54).

The three classes of vesicles and three groups of varicosities described in the myenteric plexus were present, and varicosities of all groups formed axo-somatic synapses (Figs.9.48, 9.53). A group II varicosity was observed in close proximity to the sarcolemma of an innermost circular muscle cell (Fig.9.48).

3.6.2.3 MUSCLE AND MYONEURAL RELATIONSHIPS

(A) Striated Muscle

Only the muscles of the oesophageal wall were striated. The fibres were bounded externally by a thin basal lamina. Micropinocytic vesicles were frequently associated with the sarcolemma while numerous fusiform nuclei were located at the periphery of the fibres (Fig.9.55). Their outlines were often very irregular, and in some cases, appeared to be drawn out into villiform processes. Nucleoli were central.

The perinuclear sarcoplasm was scanty and contained a few mitochondria, large quantities of glycogen granules, and dense bodies.

Most of the fibre was made up of myofibrils, which comprised myofilaments closely resembling their counterparts in the mammal. The fibrils showed the normal striations: A, I, Z, H and M bands (Figs.9.55, 9.56) but the Z bands were most prominent, limiting sarcomeres which measured about 2.5 um. The sarcoplasm around the myofibrils contained anastomosing sarcoplasmic reticulum, occasional mitochondria, and moderate amounts of glycogen. Transverse tubules formed triads at the level of the Z bands. A few glycogen granules were noted between the myofilaments, sometimes in linear arrangement. The extremities of the muscle fibres were rounded and contained organising myofilaments, numerous mitochondria, smooth endoplasmic reticulum, lysosomes and fat deposits (Fig.9.57).

The broad connective tissue strips between the bundles of muscle fibres contained capillaries, single myelinated and groups of unmyelinated axons, all with Schwann cells and collagen fibrils, still enveloped by satellite cells (Fig.9.58). The nerve terminals involved in the neuromuscular junctions, contained many lucent synaptic vesicles, measuring 40nm - 120nm in diameter (Fig.9.59). Some terminals contained small granular vesicles and in addition, had paler background material, Large mitochondria with prominent tubular cristae and lucent matrices were associated with the vesicles.

The pre-synaptic membranes showed areas of increased density and maintained occasional continuity with membranes of adjacent synaptic vesicles (Fig.9.59). The primary synaptic cleft measured about 24nm in width, and contained an intermediate basal lamina. The post-synaptic membrane was thicker than the pre-synaptic membrane and secondary synaptic clefts were absent, or simply represented by shallow

depressions which were often continuous with the necks of caveolae (Fig.9.60).

The post-synaptic membrane was smooth ("en-plaque" type ending: Fig.9.61), or it exhibited trough-like depressions within which the nerve endings lay ("en-grappe" type endings: Fig.9.60). Small granular vesicles were noted in the "en-grappe" type, and lucent vesicles were associated with certain thickened portions of the pre-synaptic membrane.

The sarcoplasm below the axonal endings contained numerous glycogen granules, large mitochondria and smooth endoplasmic reticulum (Fig.9.59).

(B) Smooth Muscle

The longitudinal and circular coats of the gastro-intestinal tract were made up of smooth muscle cells. As expected, they were fusiform and bore central oval nuclei. The sarcolemma was associated with numerous caveolae and attachment plaques (Fig.9.62). The extra-fibrillar sarcoplasm adjacent to the poles of the nuclei, contained granular endoplasmic reticulum, free ribosomes and mitochondria with medium dense matrices, tubular cristae and intercristal dense bodies. The remaining sarcoplasm contained myofilaments amongst which were electrondense fusiform bodies, free ribosomes, and mitochondria. The intercellular spaces between the muscle fibres was filled with collagen.

As in the gut of many vertebrates (Gabella, 1972a) the longitudinal muscle coat did not show the presence of nerve

processes. Nerve processes however, were readily observed in the circular muscle coat. Large bundles with their associated Schwann cells and adjacent collagen fibrils were ensheathed by satellite cells. The axons showed vesicle-rich varicosities, which established close contact with muscle cells.

In the stomach, vesiculated axons were situated mostly in the broad radial connective tissue strips in the circular muscle coat (Fig.9.63). Group II and III varicosities were observed amongst these axons, but the distance between these varicosities, and the muscle cells, was several hundred nanometers; closer contacts were rare.

In the caeca, intestine and rectum, bundles of unmyelinated axons were usually buried about three layers deep within the circular muscle coat (Figs.9.64, 9.65). Vesiculated axons were disposed towards the surfaces of the bundles, and were rarely covered by processes of supporting cells. In some places, however, the gap between the axons and the muscle fibres, which contained a basal lamina and collagen fibrils, measured up to 600nm. Elsewhere typical close contacts involving varicosities of all types were observed (Figs.9.64, 9.65, 9.66). Single isolated naked axons also occurred between the muscle layers, where their varicosities made contacts with muscle cells.

A gap of about 14nm to 20nm separated the varicosities from the sarcolemma with which many caveolae were associated in this zone (Fig.9.67). An interesting observation at this point was the apparent segregation of vesicles within the Group III varicosities. Here, the electronlucent vesicles appeared to be related to that portion of the axolemma facing the caveolae along the sarcolemma.

The muscularis mucosae of the rectum consisted of smaller and more loosely-arranged smooth muscle cells of very irregular outline (Fig.9.68). Each cell had several blunt processes which made contacts with those of their neighbours. Small nerve cell bodies (8.8 um x 5.6 um) and numerous highly-vesiculated axons were associated with the muscle cells. These nerve cell bodies had no notable features other than their presence at this level of the gut wall. Though all three types of axon varicosity were observed, Group III varicosities were clearly predominant. Occasional longitudinal axonal profiles comprising series of varicosities were were observed and segregation of the two types of vesicles was commonly noted. In one varicosity, the small lucent vesicles were associated with a thickened portion of the axolemma (Fig.9.68).

Individual relationships, involving muscle cells and varicosities, were relatively more frequent in this area of rectum than in other regions of the gut. Relationships involving one muscle cell and two similar varicosities (Group III), and one muscle cell and two different varicosities (Group I, III), were observed (Figs.9.69, 9.70).

# 4.0 DISCUSSION

## 4.1 CORRELATION OF DIET, GUT LENGTH AND FUNCTIONAL SURFACE AREA

According to Wood, et al. (1957), when compounding artificial diets for use in commercial fish farms, considerable emphasis is placed on simulating as far as is possible the diet of the fish in the wild state. With regard to the rainbow trout, such a diet should be basically high in protein and moderately low in fat and carbohydrates. It is therefore expected that the features of the gut of the farmed rainbow trout described in the present study should be fairly representative of the species. Thus, when translated into anatomical terms, the implication is that the gut of the domestic trout has all the morphological features of a typical carnivorous fish.

That feature which has been stressed above all as characteristic of such a fish, is the presence of a short gut (Kapoor, et al. 1975). Initial dissections as indicated in Section 1 of this work would seem to bear out the validity of this statement, certainly in regard to physical length, where the overall body length/gut length ratio varied around a mean of 5:6. It is well-established however, that shortness of the gut does not necessarily reflect the functional surface area. As has been demonstrated in the present study, the rainbow trout is one species in which this is especially true, since the compensation for loss in length of gut is achieved in a variety of ways.

The first and the most notable of these is the presence of the intestinal caeca. In the specimens examined, their average number per fish varied from 42 to 64, measuring 3 - 4 mm in basal diameter, and from roughly 1 - 4 cm in length. It is of interest to note, at this stage, that Bergot, et al. (1975) estimated that the total caecal

length in the rainbow trout was more than 6 times the length of the intestine, and that the caecal surface area was more than 3.2 times that of the anterior intestine and more than twice that of the whole intestine altogether. It is also of interest to note that this significant increase in gut surface area mentioned by these authors did not include that afforded by the longitudinal mucosal ridges seen under the SEM in Section 2 of this work.

The arrangement of the caeca in descending order of their length and their disposition in two major groups around the stomach seems, from this study, to be highly significant from the point of view of affording the fish a definitive centre of gravity, and so proper equilibrium in the water. The trout thus achieves this enormous increase in gut surface area without compromising its stability.

Despite the fact that Kapoor, et al. (1975) did not acknowledge their presence in teleosts, scanning EM in the present study shows that effective gut surface area in the intestine is increased by means of villi similar in structure to their counterparts in the mammal as shown by Marsh & Swift (1969).

The rectum, on the other hand, gains the same effect by means of unique mucosal folds. The complex surface patterns as seen in scanning micrographs also indicate that the folds provide for longer retention, and effective absorption of food, without interfering with defaecatory function which appears to be the main function of the central tubular passage. This point will be discussed later in more detail.

Finally, it is notable that digestive epithelium commences in the distal third of the oesophagus, in the so-called oesogaster. By

effectively providing an anterior extension of the fundic zone of the stomach, the oesogaster constitutes another way whereby the digestive surface area is increased.

## 4.2 FUNCTIONAL ADAPTATIONS OF THE EPITHELIUM

### 4.2.1 OESOPHAGUS

The general epithelial structure of the oesophagus indicates that a measure of protection is achieved without any cornification. The copious amounts of mucus provided by the epithelium serves lubrication purposes primarily and the presence of taste buds indicates some sensory functions.

#### 4.2.1.1 FILAMENT-CONTAINING CELLS

As regards the protective nature of the epithelium, it is apparent from this study that this depends on the filament-containing cells which abound in the anterior third of the organ. The mechanical property of these cells, in turn, depends on the large amounts of tonofilaments combined with the extensive plication and interdigitation of the plasma membranes and their association with desmosomes. The relationship of the basal complex with both the stratum compactum and the basal cells, as shown in the present study, indicates a firm attachment of the epithelium, an essential adaptation for any structural function. Roberts, et al. (1970) emphasize this point when they suggest that the density of the basal plasmalemma of basal cells of the skin of salmon, similar to that described in this study, functions as a half-desmosome attaching the cells to the basal complex. In this study, this idea is reinforced by the obervation of annular filaments which secure the epithelium to the convolutions of the basal complex.

At the same time, the filament-containing cells undergo changes in secretory function as they migrate towards the lumen. The cells in the lower layers appear to be mainly involved with the synthesis of the tonofilaments, which are retained in the peripheral cytoplasm. In the

higher layers though, the Golgi apparatus clearly becomes active, secreting pale, fibrillar material contained in small vesicles. Such vesicles, Schliwa (1975) has suggested, are involved in the renewal of the external coat and the cell membranes of the surface cells. This study certainly indicates the validity of this theory by virtue of the close relationship between the vesicles and the luminal plasma membranes, the marked reduction of vesicles in the surface cells compared with the number in the underlying cells, and the very obvious glycocalyx coat.

A further feature of the surface cells which has received much attention in the literature, is the formation of microridges from the luminal plasma membranes. Weinreb and Bilstad (1955) for instance, observed these as striations under the light microscope, and Linss (1969), noting their ultrastructural profiles in the oesophagus of pike, believed them to represent microvilli. That they are indeed true microridges is confirmed by this study but unfortunately, the complex nature of their patterns makes precise description difficult.

The origin of the ridges is most likely related to the plication of the plasma membranes in the mid-epithelial filament-containing cells, with microfilaments being added to the cores of the ridges when the cells reach the surface. Thus the final form could well be determined by the dynamic processes set up by the relocation of microfilaments, and the formation of extra plasma membrane by the glycocalyx-renewing vesicles already discussed. The microridges, therefore, may have arisen in the process of fortification of the frontline of an epithelium which has a primarily structural function. In this respect, since microridges have previously been shown to exist on epithelial surfaces such as skin and cornea (Harding, 1973), which are subject to mechanical insult, there

appears to be an obvious advantage in having a sculpted surface rather than a smooth one for absorbing traumatic impacts.

Microridges have also been implicated in the retention of mucus by authors such as Andrews (1975), Hawkes (1974), and Schliwa (1975). The present study certainly suggests that a relationship between microridges and mucus exists in the trout oesophagus, particularly amongst opposing cells lining the grooves surrounding the elevations in the anterior oesophagus. In many cases, a sheet of mucus appeared suspended by the apices of microridges and the streamed, fibrillar appearance of the mucus suggests some movement or spread. Such an observation would make the suggestinn of Bereiter-Hahn (1971) that the spread is brought about by active contraction of the microdiges through the presence of tonofilaments a possibility. It is far more likely though, that by virtue of the concentration of mucous cells in the bases of the grooves, a back pressure is created by sheer quantity of the mucus which, combined with passive sliding movement between the opposing cells, transport the mucus to the oesophageal lumen. The eventual spread of the mucus is likely to be brought about by differential movement between the mucosa and the ingested material. The role of spreading of mucus ascribed to the inter-ridge troughs by Sperry and Wassersug (1976), is doubtful because of the viscosity of the mucus and the narrowness of the troughs.

### 4.2.12 MUCOUS CELLS

The epithelial transition characteristic of the trout oesophagus involves a progressive posterior-ward loss of filament-containing cells and the replacement of Type I mucous cells by Type II which are organized into multicellular glands. This represents a shift of

emphasis from a mainly structural to an entirely secretory role. The change in the pattern of the histochemical profiles of the mucous cells is also of interest. Type I mucous cells, functioning as unicellular glands, share the production of neutral, carboxylated and sulphated muco-substances which are released in an apocrine manner. In contrast, each Type II mucous cell produces the three types of mucosubstance which it releases in a merocrine manner. The significance of these histochemical and morphological variations is obscure. It would appear that the amount of mucus produced in the mid-oesophagus is in excess of that required for mere lubrication purposes. It may well be, as suggested by Reifel and Travill (1978), that the mucus has a digestive function.

4.2.1.3 SENSORY FUNCTIONS

There is a measure of controversy concerning the presence of taste buds in the fish oesophagus, and it seems from study of the literature that whilst they are present in some species, they are notably absent in others.

The taste buds presently observed, appear similar to those described by Hirata (1966) save for the prominence of the intermediate cells. These may well represent an independent cell type, or a stage in the differentiation of the light cells. Obviously the characteristics of the intermediate cells cannot be totally resolved by study of the apical processes with the SEM. The shortest and most numerous of the processes however, are generally accepted as representing the apices of the dark cells (Reutter et al. 1974) Ovalle and Shinn, 1976).

The club-shaped processes on the other hand, show a distribution similar to the more conical types thought to represent the apices of

light cells, and their occurrence could legitimately be regarded as a species variation peculiar to the rainbow trout. Thus, the longest filiform processes, with smaller, clubbed apices, would, by process of elimination, belong to the intermediate cells, though admittedly, further studies are needed to justify this claim.

Irrespective of cell-types however, earlier scanning studies report that piscine taste buds fall into three categories, an assumption again by Reutter et al.(1974) based on external surface morphology. In this study, only one type was observed, and since they were found at the general level of the epithelium, they were taken to represent Reutter's and his co-workers' "Type III" taste buds.

They postulated that "Type III" buds are essentially chemoreceptors, and accordingly, the anterior oesophagus would appear to play a critical role in deglutition. In this respect, Barrington (1957) concluded that the co-existence of striated muscle and such taste buds could only indicate the possibility of food rejection at oesophageal level.

The presence of many backwards-directed teeth in the oro-pharynx however, make this a rather unlikely supposition in the trout. It would seem much more likely that the taste buds do in fact stimulate deglutition, and possibly initiate gastric responses such as secretion or even emptying.

### 4.2.2 STOMACH

In the present study, the term 'corpus' or 'body' has been preferred to denote the long anterior dorsal arm of the stomach instead of 'cardia' as used by Weinreb & Bilstad (1955). This is because the

cardiac stomach, as defined in histological terms, is absent in the trout. Moreover, the present terminology agrees with the recommendations of Barrington (1957).

4.2.2.1 SURFACE MUCOUS CELLS

This current investigation is the first to present the normal surface ultrastructure of the stomach of trout, by means of the high resolving power and depth of field of the scanning electron microscope. The most striking feature of the luminal surfaces of the epithelial cells is the marginal arrangement of their microvilli, surrounding a relatively smooth, central area, which contrasts with the surface of mammalian gastric epithelial cells, which have been shown to have an overall covering of microvilli (Pfeiffer, 1970). This increase in peripheral surface area of trout gastric mucous cells could well be related to absorption, with the central zone being mainly involved with the release of mucous vesicles.

The well-developed secretory system of the cells, demonstrated with the transmission electron microscope, provides the copious mucus which protects the gastric mucosa from both physical and chemical injury. In this respect, the association of the Golgi complex with several multivesicular and dense bodies, suggests that the cells have an in-built mechanism for autolysing excess secretion. The sequestered membraneous elements within some of the dense bodies, possibly represent lipoid materials which are known to take a longer time to digest (Novikoff et al., 1964). The manner of release of the mucous vesicles has been demonstrated to be by exocytosis.

Microfilaments are widely distributed in the cytoplasm of the

surface mucous cells, features which have been shown to exist in the co coral fish and perch (Ling & Tan, 1975) Noaillac-Depeyre & Gas, 1978). In the latter species though, microfilaments were reported to be absent in the cores of the microvilli. However, the organization of microfilaments in the basal cytoplasm into annular bands, which surround the impressing convolutions of the basal complex appears to be unique to the cells of the trout stomach. This feature emphasizes the structural role of the microfilaments, which together with the extensive interdigitations along the lateral plasma membranes, will enable the epithelium to withstand the possible shearing forces caused by ingested material. The smoothness of the basal complex and the reduction in amounts of microfilaments in the pits cells, suggests that the structural role is confined, and logically so, to the surface cells.

The clear vesicles intermixed with the mucous vesicles may be involved with transport of materials across the epithelium. Their increased presence in the cells at the bottom of the pits, along with the presence of structures resembling tubulo-vesicles, may be a developmental feature, since it is generally accepted that epithelial cells originate here, and mature as they migrate towards the surface. On the other hand, it could be that these cells contribute to the production of acid, for these are also the main features of the oxyntic cells. In fact such a possibility has been suggested by Rehm (1950) and Rehm, et al., (1953).

The abrupt nature of the transition from pit to gland cells was clearly borne out by both present light and electron microscopic studies. This situation is in contrast to the observation of Weinreb

& Bilstad (1955), who actually described mucous neck cells in the stomach of the trout. Nevertheless, mucous neck cells have been reported to occur in the plaice stomach by Dawes (1928), and their ultrastructure has been demonstrated in the perch by Noaillac-Depeyre & Gas (1978). Thus, this study does not critically disagree with the suggestion by Smit (1968), that amongst teleosts, gastric glands can be of two types; those with and those without mucous neck cells.

4.2.1.2 OXYNTIC CELLS

The ultrastructural characteristics of the oxyntic cells of the trout appear consistent with that ability to produce both acid and pepsinogen generally attributed to such cells in teleosts (Smit, 1968; Holstein, 1975). The relationship between the tubulo-vesicles and the apical plasma membrane was close enough in places to suggest a continuity between the two elements, and in this context, several authors have presented evidence which also indicates that tubulo-vesicles are a differentiation of the apical plasma membrane in teleosts and other vertebrates (Noaillac-Depeyre & Gas, 1978; Forte & Forte, 1970; Leeson, 1973).

The effect of distension on the oxyntic cells, presented in this study, shows that the cells are extremely active. This capability of the cells to undergo such dynamic changes, can be accounted for by the presence of many large mitochondria in their basal cytoplasm. The depletion of the tubular elements in the apical cytoplasm was complete, and the increase in surface area of the apical plasma membrane enormous, which changes coincide in the main with those described in correspondingly stimulated cells in the frog by Sedar(1965), and in chicken by Toner (1963).

The basal cytoplasm of the trout cells, in addition, exhibited a rather unique feature, in that the massive membrane turnover also affected the basal plasma membrane plications. They decreased in number, and considerable amounts of membrane became internalized in the form of annular lamellae. The existence of short linear densities towards the periphery of each lamella, possibly indicates that the membranes are sealed off from the cytoplasm. The significance of the lamellae however remains obscure, especially since they represent a reverse of the events in the apical cytoplasm.

4.2.2.3 GASTRIC ENDOCRINE CELLS

The present study adequately shows an abundance of endocrine cells in the trout stomach, and further, demonstrates that various types can be distinguished on the basis of the ultrastructural features of their intracytoplasmic granules. The establishment of such features in these cells is necessary, by virtue of the scant knowledge of their presence in teleosts in the literature. Until now, the ultrastructural features have largely been undetermined although Noaillac-Depeyre & Gas (1978) noted their presence in the gastric epithelium of the perch.

The apices of most of the endocrine cells in this study reached the gastric lumen and, in addition, exhibited such modifications as the presence of microvilli, cilia, reduced glycocalyx cover, centrioles, vesicles and abundant, longitudinally-oriented microtubules. These structures may be responsible for the recognition of stimuli which trigger or inhibit the release of the granules. Stressing this possible function, Fujita & Kobayashi (1977) referred to such endocrine cells as "taste cells in the gut".

The cytoplasm of most of the cells shows a well-developed secretory apparatus, which obviously elaborates the granules and, as mentioned previuosly, the present study revealed certain differences in the distribution of the granules. These were largely supranuclear in Type I, distributed overall in Type II, and basal in Type III. This pattern of distribution would indicate some polarity in the release of the granules, but the significance of such a process, is not at all clear despite the fact that morphological evidence of exocytosis of the granules was demonstrated in Types II and III.

The variations in electrodensity of the granules which are quite evident in Types II and III cells, could be due to a fixation fault, or an escape of granular contents through intact limiting membranes. This has been so widely noted in mammals however, that Forssmann & Orci (1969) proposed that the granules are released by a diacrine mechanism, which being a combination of the aforementioned mechanism, and exocytosis.

The different cell types described in this section may actually secrete different hormones, since biochemically, several hormones have been shown to exist in the gut of teleosts (Nilsson, 1970). The granules of Type I cells did not exhibit any clear features which would justify a direct comparison with known mammalian cell types. The abundance, distribution and granular characteristics of Type II cells on the other hand, closely resemble those described in enterochromaffin cells (EC) in the mammalian stomach by Solcia, et al. (1975). The fluoreseence observed within the gastric epithelium of the rainbow trout by Read & Burnstock (1968a) supports this identification. The Type III cells bear close resemblance to the G-cells, which are the source of gastrin in the mammalian stomach.

4.2.3 ABSORPTIVE CELLS

The findings of the present study demonstrate clearly that regional differences in structure exist amongst the absorptive cells lining the post-gastric gut of the adult rainbow trout. The demonstration of an extensive accumulation of very low density lipo-protein particles within the epithelium of the intestine and caeca suggests that these regions are primarily involved with absorption of fats. The vacuolated nature of the rectal absorptive cells, which was very obvious in both light and electron microscopy, is particularly significant in that these cells resemble the absorptive cells described in the rectum of the rainbow trout alevin by Iwai (1968), and at the same time, cells lining the middle portion of the gut in other stomachless teleosts by authors such as Yamamoto (1966), Gaunthier & Landis (1972), Noaillac-Depeyre & Gas (1973; 1976), and Stroband & Debets (1978).

The vacuolated nature of these cells obviously indicates their ability to take up proteins by pinocytosis, even though this differs from the views of Yamamoto (1966), who did not recognise any differences between the absorptive cells of the intestine and the rectum in the adult rainbow trout. Nontheless, his conclusion is particularly interesting since he expected such a difference between the trout, which has a well-developed stomach, and the stomachless teleosts because in the latter, there is no peptic digestion, and the pancreatic proteolytic enzymes do not completely hydrolyse protein elements.

This interpretation was considered so logical that even when Iwai (1968) did find variations similar to those seen in this study in the alevin, he hypothesized that immaturity of the stomach, and so low

proteolytic activity, was responsible. The implication was that the rectal cells would lose their vacuoles when the stomach became competent. This study has convincingly shown that in addition to a fully-developed and functional stomach, the absorptive cells of the rectum in the adult trout do in fact retain their capability to pinocytose proteins as a primary function.

4.2.3.1 ABSORPTIVE CELLS OF THE CAECA AND INTESTINE

The present study shows that in the inactive, and so presumably in the fasting, state, the structure of the absorptive cells of the intestine and caeca is very similar to those of the typical vertebrate intestinal absorptive cells. One major difference is the presence of lamellar structures in the supranuclear and basal cytoplasm, a situation which is currently regarded as a general feature of the absorptive cells of the gut of teleosts. The present study has shown this feature to be true throughout the whole gut length, with the lamellae being particularly prominent in the cells of the caeca.

The distribution of low density lipo-proteins within active cells is in agreement with that demonstrated by Bergot & Flechon (1970a) after feeding fasted adult trout with long chain fatty acids. Thus, it can be deduced that once fat has been absorbed, it is transported from the cells by three main pathways. In the first place, the appearence of single, membrane-bound fat particles both in the apical cytoplasm and at the corresponding level in the intercellular spaces, suggests exocytosis of those particles at that level. Secondly, most of the particles appear to be concentrated in the Golgi vacuoles and exocytosed at that level, thereby accounting for the large numbers found between the level of the intercellular spaces and the basal lamina. It is

important to note at this point that there was evidence to show that some of these accumulations are converted into dense bodies, suggesting that the cells exert a measure of control over the amount of fat which actually reaches the circulation. Lastly, single, membrane-bound particles may either reach the intercellular spaces directly by exocytosis, or they are actually channelled there by the lamellar structures. Evidence from present studies would seem to suggest that the first and third methods constitute the major pathways by means of which fat particles are transported in the caeca. At the same time, the moderate accumulation of fat particles in the perivascular connective tissue, in contrast to their relative scantiness in the lumina of the capillaries, may simply be a chance observation, since Bergot and Flechon (1970a) convincingly demonstrated large quantities of fat particles in both capillaries and lymphatic vessels. On the other hand, since the function of both types of vessel in the evacuation of fat was demonstrated experimentally in their work, the present observation may mean that under natural conditions, the capillaries play only a supplementary role.

The significance of the accumulation of large numbers of mitochondria in both the supranuclear and basal cytoplasm could not be determined. However, such mitochondria were much smaller in size than those contained in apparently functional cells, and the presence of dense intercristal granules could well be related to the accumulation of calcium ions, resulting from a decrease in oxidative phosphorylation, as has been shown by Hackenbrock & Caplan (1969). These cells may, therefore, be in a dormant state, since cells with similar features have been described in the intestinal epithelium of fasted grasscarp, by Stroband and Debets (1978).

In the present study, lamellar structures were observed in all the absorptive cells located in the post-gastric gut. These exhibited anastomoses and occasional continuity with both lateral and basal plasma membranes, so much so that with the occasional formation of desmosomes across their lumina it would suggest that they are actually infoldings of the plasma membrane. The role of the lamellae in lipid transport has already been discussed, but the close relationship between those in the basal cytoplasm and large elongate mitochondria indicates that they are also involved in such energy-dependent activities as water and ion transport, functions which, incidentally, are consistent with those ascribed to epithelia with similar morphological characteristics by Berridge and Oschman (1972).

4.2.3.2 ABSORPTIVE CELLS OF THE RECTUM

The present study indicates that rectal absorptive cells have a characteristic apical tubular system, which represents a differentiation of the intermicrovillous plasma membrane. This system obviously constitutes the portal of entry of the protein into the cells, and the property of pinocytosis ascribed to such a system, is likely to be achieved by a combination of factors contributed to by the loose filamentous coat of the tubules and the abundance of microtubules and microfilaments in the intertubular cytoplasm. Whilst the latter may well bring about some movement in the form of a 'milking' effect, Lambson (1966) suggests that the filamentous coating provides points of attachment for protein molecules.

The increase in density of the vacuoles towards the nucleus, certainly indicates a parallel gradient of digestive activity within them. The coated vesicles usually found in the matrices of the more apical vacuoles, may be the source of hydrolytic enzymes as hypothesised

by Friend & Farquhar (1967). The progressive increase in the quantity of myelin figures, and the eventual conversion of the vacuoles into heterogeneous dense bodies, most probably represent a progressive accumulation of undigested lipoid material.

The presence of membrane-bound accumulations in the basal intercellular spaces, similar to those seen in the apical vacuoles would seem to indicate that some material reaches the circulation without o being digested within the cells, a point supported by the work of Noaillac-Depeyre & Gas (1974) on the posterior intestine of the carp. They showed that a fraction of experimentally-fed horseradish oxidase reached the intercellular spaces without penetrating the large vascular system.

Morphological evidence from this study, supported by the extensive biochemical evidence reviewed by Smit (1968), indicates that the conditions in the gut of trout are adequate to ensure the complete intraluminal digestion of proteins. The persistence of the vacuolated cells in the rectum of the trout therefore, creates somewhat of a problem, but one for which two main solutions can be advanced. Firstly, since the trout is noted for its high protein requirement, the co-existence of intracellular and extracellular protein digestion may well be a method of ensuring maximum absorption of ingested protein, and indeed, the gross modification of the rectal mucosa is a point in favour of this hypothesis. Alternatively, that the stomach has physical limitations as a storage organ is obvious, when one considers that the wall contains the continuous inelastic band of the stratum compactum. When food is plentiful, gastric emptying may be more frequent, thus resulting in incomplete hydrolysis of proteins. In such circumstances,

the pinocytosis of proteins by the rectal cells would accordingly serve as a complementary mechanism to that of the stomach.

It is reasonable to assume that the rectal epithelium of the trout presents a cell renewal system, comparable to the intestinal epithelium of the goldfish as illustrated by Vickers (1962). Thus, the non-vacuolated cells lining the basal portions of the troughs between the mucosal folds, would necessarily be the generative cells. The non-vacuolated cells lining the apical parts of the primary folds facing the central lumen on the other hand, closely resemble the cells described in the posterior intestine of fasted fish by Noaillac-Depeyre & Gas (1973). It seems logical therefore to assume that these cells go through alternate phases of dormancy and activity, since they line that part of the fold whose main function is defaecatory.

Lamellar structures were again very evident in the basal cytoplasm of the rectal absorptive cells, and the presence of particulate material, much like lipid particles within them, both above and within the lamina densa would suggest that the rectal cells also play a minor role in fat absorption.

## 4.3 MAJOR COLLAGENOUS ELEMENTS AND ASSOCIATED CELLS

### 4.3.1 STRATUM COMPACTUM

The attachment of the stratum compactum to the basal lamina complex of the epithelium in the anterior oesophagus and its significance has already been discussed and the deeper situation which the compactum assumes from stomach to vent must obviously impart similar mechanical stability to this region of the gut. This is achieved by means of collagenous bands, which run from the more superficial portions of the propria and from the circular muscle coat to merge with the compactum. Stability is also provided without inhibiting peristalis however, by its having a corrugated form, thus making some allowance for the movement of softer tissues around it.

Otherwise, the outstanding ultrastructural features of the stratum compactum are the density of the collagen fibrils, their layered arrangement, the unidirectional arrangement of the fibrils within a layer, and not least, the regular alternation of these layers which results in a remarkable plywood, or orthogonal pattern. These features naturally constitute the basis of the layer's mechanical strength, but it also inevitably follows that once the crenations have been eliminated, there is no chance of further expansion. These observations subscribe to the conclusion reached elsewhere (Burnstock, 1959a) that the stratum compactum acts as a girdle, restricting over-distension of the gut. This brake on over-distension is thus present throughout the length of the gut, especially that from the stomach to the vent. Thus, arguably, the presence of the stratum compactum in the post-gastric gut might not be necessary if the pyloric sphincter is as efficient as that in mammals, in permitting small amounts of chyme to pass through at intervals. Such controlled release would have made the precautionary structural adaptation

against over-distension unnecessary in the succeeding parts of the gut.

In the light of the above reasoning therefore, it is legitimate to hypothesize that a physiological pyloric incontinence exists, which is mediated through a highly-integrated nervous reflex, doubtless also involving endocrine cells, which permits the trout to pack in as much available food into as much space as the whole gut can afford.

The resultant quick passage of food through the stomach, and the organ's relationship with the pinocytosis of proteins by rectal cells has already been discussed. This hypothesis may be particularly relevant to the wild trout which by virtue of seasonal variations in the availability of food, may be forced into feeding by surfeit to compensate for periods of dearth. From this point of view, it would be interesting to monitor the long-term effects of domestication on the structure of the stratum compactum.

### 4.3.2 GRANULE CELLS

Although granule cells have been demonstrated to be abundantly present in the submucosa of the oesophagus and stomach in the present study, by far a greater concentration showed a preferred association with the loose collagenous bands under the stratum compactum, to constitute the stratum granulosum. A preliminary investigation of their development, not reported in Section 3, showed that both strata are absent in the alevin and fry. When the layers do develop, the stratum compactum appears first, closely followed by a stratum of granule cells. Such an ontogenetic relationship can be inferred from the observations of Bolton (1933), Burnstock (1959a) and Smith (1975),

and it is thus likely that the stimulus which triggers their development, is related to the natural change in diet, or a necessary change at that stage in status of bodily defence mechanisms.

A cursory examination of the positional relationship of the fibroblasts, the stratum compactum and the granule cells, especially under the light microscope, creates an impression reminiscent of the situation in the epiphyseal plate of a long bone. In this way, whilst the fibroblasts on the internal surface are involved, with the process of fibrinogenesis, possible fibrinolysis is occurring on the external surface. The implication therefore is that the granule cells could well be involved in regulating the thickness of the stratum compactum. Although occasional intimate contact between the granule cells and the stratum compactum has been demonstrated in this study, there is no evidence to show that this occurs as a physical process. The existence of a humoral effect is however possible, whereby a substance may well be elaborated by the granule cells which would stimulate or inhibit fibrinogenesis.

Another possible explanation of this kind of relationship is to regard the arrangement as some kind of depot, or even as a semi-diffuse organ, either of which could be cast very much in the mode of a lymphoid organ. That this is a reasonable hypothesis can be gauged from the fact that the gut is as much exposed to the external environment as the skin, and therefore constitutes a vulnerable area through which the fish is exposed to pathogens or toxic materials. In this respect, the stratum compactum could play a back-up role by providing a mechanical barrier to the penetration of parasites and other organisms.

The ultrastructural features of the granule cells certainly suggest that they constitute an independent cell population, possessed of a well-developed secretory apparatus, which elaborates the granules. The decreasing prominence of the secretory apparatus which becomes apparent in large granule-laden cells indicates that the cells produce the granules at an early stage in their life cycle, after which the secretory apparatus involutes. Any over-crowding of the cytoplasm with granules seen on occasions, may have resulted in some of them being embedded in the nuclear envelope.

Taken overall however, these, as well as the obvious presence of active fibroblasts occupying a fairly definitive position in relation to the stratum compactum, make the suggestion of Burnstock (1959a) that the granule cells arise by the action of fibroblasts developing granules in their cytoplasm during fibrinogenesis, seem very unlikely.

The presence of polarized, organelle-free zones within the granule cells clearly indicates that they are capable of some movement. This can be deduced from the demonstration during phagocytosis, by Zucker-Franklin & Hirsch (1964), of similar processes in motile rabbit leucocytes. The relationship between the granule cells and the walls of vascular elements, and the admittedly rare demonstration of a granule cell within the lumen of a vessel, which could possibly be explained on the grounds that only healthily-farmed fish were used in this study , may in fact represent evidence of such potential. In this context, it would appear that any motility is exhibited only in pathological situations, since Roberts (1972) reported a massive invasion of the nasal epithelium by these cells in salmon suffering from ulcerative dermal necrosis.

The present work reveals that the granules contain phospholipid, acid mucopolysaccharide and a lack of basic proteins. This contrasts with the investigations of Weinreb & Bilstad (1955) and Bullock (1963) who concluded that each granule possessed a protein core of arginine, and an outer lipid-phospholipid shell with a trace of non-acid mucopolysaccharide. Smith (1975) on the other hand, certainly confirmed the presence of phospholipid, but did not detect the non-acid mucoploysaccharide and arginine, which as mentioned, is in agreement with present findings.

Alkaline phosphatase activity was demonstrated within the granules, and the secretory apparatus of the granule cells, in this study. It thus appears that this enzyme is parcelled into the matrix of the granules via the granular endoplasmic reticulum and the Golgi elements in a manner parallel to that of enzyme incorporation into granules of mammalian leucocytes. There is no evidence though, to show that the hydrolases, also shown to be present, pass through the same process. Acid phosphatase, for instance, seemed to be associated with the depletion of the granules in which they were noted. At the same time, 5-nucleotidase and arylsulphatase activities appeared to be concentrated at the periphery of the granules. Consequently, it is not clear whether these hydrolases form an integral part of the granules, or are merely concerned with their auto-digestion, evidence of which is shown morphologically by similarly-located myelin figures. The localization of peroxidase is interpreted as rather non-specific.

The present study reveals a special and intriguing relationship between the granule cells and 'ensheathing' cells. The processes of the 'ensheathing' cells are held together by desmosomes, and so they

constitute a fixed cell population. The granule cells are contained in a three-dimensional meshwork formed by these cells. By virtue of their possessing secondary lysosomes, microfilaments and coated vesicles within their cytoplasm, these ensheathing cells show a marked similarity to the reticular cells found in mammalian lymphoid organs, especially those of the thymus. The ectodermal origin of the thymic reticular cells is established and in this respect, they may differ from the ensheathing cells. Their physical relationship to lymphocytes and granule cells respectively appear very similar however. Consequently it is logical to liken the ensheathing cells to fixed macrophages, capable of taking up and degrading particulate matters within their cytoplasm, and at the same time, exchanging materials with the granule cells along their extensive common borders. Such a hypothesis is supported by the presence of considerable lysosomal activity within the ensheathing cells, in contrast to the situation in neighbouring granule cells. Furthermore, coated vesicles are associated with the plasma membranes closest to the granule cells, which would suggest that the functions of the cells could also include support, nourishment, and possibly, transmitting inductive instructions to the granule cells.

At this point, it is reasonable to hypothesize as has been touched on earlier, that the stratum compactum, the ensheathing cells and granule cells constitute a composite defence mechanism, both mechanical and humoral, which develops in response to environmental demands. It would appear that the ensheathing cells can pick up and degrade particulate matters and at the same time, induce the granule cells to release their granules, which could very well have detoxicant and anti-microbial effects. Under pathological conditions, the granule cells disengage and actually migrate towards the lesion. The actual

significance of the chemical combination within the granules is obscure, but it is clear that any protective function would, without doubt, be dependent upon the chemical constitution of the granules. The mechanism of this function remains to be worked out.

## 4.4 INNERVATION OF THE GUT

The most significant finding in the present study of the morphology of the innervation of the gut is the demonstration of nerve cell bodies in the submucous plexus throughout the gastro-intestinal tract. This contrasts directly with the findings of Burnstock (1959b), and Read & Burnstock (1968b) who failed to demonstrate nerve cell bodies at that level in the trout. The capricious nature of the silver impregnation and methylene blue staining techniques used by Burnstock (1959b) could well account for the presence of the cells not being recorded. The fact remains however that their presence has far-reaching implications. In the first place, it necessarily invalidates the general schema for teleost gut innervation proposed by Burnstock (1969), since that is entirely based upon his findings in the trout gut. Secondly, the undoubted presence of nerve cell bodies calls for a review of the hypothesis of Burnstock (1969) to the effect that their presence in the submucous plexus is purely an evolutionary feature, confined to higher vertebrates. This idea incidentally is based in part on his schema, and in part on the reported absence of nerve cell bodies in the submucous plexus of the gut of amphibians by Gunn (1951).

Nerve cell bodies were also noted in the muscularis mucosae of the rectum. Thus it would appear that there is a diffuse intrinsic network of nerve cell bodies in the gut wall of the trout, suggesting an existence of a comparatively high level of intrinsic control of peristaltic activity. However, evidence of extrinsic control is also provided by the presence of the vagus, and the anterior and posterior splanchnic nerve trunks, which supply parts of the gut.

### 4.4.1 MYENTERIC PLEXUS

#### 4.4.1.1 NERVE CELL BODIES

The nerve cell bodies of the myenteric plexus are scattered in the meshes of the plexus, and are not organised into large ganglia as in birds and mammals. The present study is the first attempt to classify them according to their ultrastructural features. The difficulty in formulating precise criteria for classifying such highly structurally-variable cells from thin sections is obvious. Nevertheless, the cells present reasonably clear structural characteristics which definitively indicate that they constitute a heterogeneous population. The differences in size, and the presence, absence or varying pattern of the distribution of cytoplasmic organelles, may well be the morphological expression of varying functions of the cell bodies. In this regard, it should be pointed out that several workers (Nishi & North, 1973; Hirst, et al., 1974; Wood, 1974) have demonstrated the existence of different types of intrinsic neurons in the mammalian gut, distinguishable on the basis of spike discharge.

In an early light histological classification of myenteric neurons in the brown trout, Burnstock (1959b) recognised three types, and most of the cell bodies classified as small in the present study, are comparable to Burnstock's Type III or smallest neuronal type. As mentioned, this study confirms their presence in large numbers, and at the same time, demonstrates further differences based on their ultrastructure.

At least, three sub-types of these Type III neurons have been shown to exist. The two sub-types of the large nerve cell bodies observed in this study fall into the same size range as Types I and II of Burnstock's classification. The most distinctive of all the nerve cells presently

observed however, were the small granulated neurons. Although occasional granular vesicles were found close to the Golgi complex in many nerve cell bodies, the sheer number of granules in these cells distinguish them as a definitive cell type. It is reasonable to suggest therefore that these cells are the source of large granular vesicles of the Group III varicosities.

The processes stemming from the nerve cell bodies, and the membrane specializations demonstrated in the present study, resemble those described in the guinea-pig myenteric neurons by Gabella (1972b). Many axo-somatic and axo-dendritic synapses were also demonstrated, a fact supported by the light microscopical observation of Burnstock (1959b), who described many pericellular nerve endings on the nerve cell bodies of the myenteric plexus of the brown trout. Three morphologically-different types of axon profile formed these synaptic contacts with a single nerve cell body, often forming synaptic contact with two types of axon profile. Such relationships have been observed in the mammalian myenteric plexus (Gabella, 1972b; Cook & Burnstock, 1976) and they suggest that spike discharge from a neuron is controlled by several inputs.

4.4.1.2 AXON PROFILES

Group I axon profiles containing a homogeneous population of round electronlucent vesicles (30-120nm in diamter), and other axon profiles containing these vesicles intermixed with a few large granular vesicles, are here interpreted as representing cholinergic innervation. This interpretation is aptly supported by the similarities between these vesicles, and those contained in the nerve terminals at the motor end plates demonstrated in this study. The latter are generally believed to contain the cholinergic transmitter substance, acetylcholine.

Group II axon profiles containing small granular vesicles (45-70nm in diameter) and large numbers of small, lucent vesicles most probably represent adrenergic innervation. Yamamuchi & Burnstock (1968) observed similar profiles in the trout heart, and considered them to be adrenergic. Moreover, Campbell & Gannon (1976) reinforced this point when they demonstrated catecholaminergic axons in the myenteric plexus of the rainbow trout by fluorescent histochemistry.

Axon profiles containing a homogeneous population of large granular vesicles, and a mixture of these with small, and often flattened, lucent vesicles, were of all three types, the most commonly encountered. It is possible that they represent sampling variations of the same structural type.

The nature of the transmitter substance contained in these large granular vesicles is a subject of a very interesting controversy in the current literature, and it still remains to be resolved. Unfortunately the scope of the present study did not permit any examination of this controversy. However, by demonstrating the abundance of these axon profiles, the present study does highlight the important role possibly played by the unidentified transmitter in the mediation of peristalsis in the trout gut.

Several candidates have been suggested as being the transmitter contained in these vesicles. The phenomenon of 'rebound' contraction, for instance, which occurs after the cessation of nerve stimulation in the trout gut, has been thought to involve neurons which inhibit smooth muscle by releasing a transmitter, unrelated to either acetylcholine or noradrenaline (Campbell & Burnstock, 1968). Burnstock (1972) presented

extensive evidence to show that the transmitter is adenosine triphosphate or a related substance, thus introducing the term 'purinergic' innervation. At the same time, Baumgarten et al. (1973), presented morphological evidence to show that such axons, and even the nerve cell bodies containing large granular vesicles, are serotoninergic. The subject for their experiment was Lamptera fluviatilis which is not particularly far removed on the evolutionary scale from the trout.

On the other hand, the axon profiles may represent Substance P, a highly potent polypeptide, which has been isolated from the gut of teleosts, and has been demonstrated to stimulate the motility of isolated intestine of several fish species (Euler & Ostland, 1956; 1957). Relevant to this is the recent immunohistochemical localization of Substance P in the neuronal perikarya and axons of the mammalian myenteric plexus by Pearse & Polak (1975), and its ultrastructural localization in the large granular vesicles within terminal axons (Pickel et al. 1977).

#### 4.4.1.3 VAGALANERVE TRUNKS

The vagus nerve trunks formed a significant part of the myenteric plexus in the stomach, occurring in the form of encapsulated units, consisting of different types of nerve cell bodies, distributed at different levels along their length, together with myelinated and unmyelinated axons. Groups II and III varicosities were also present within the trunks. Obviously, the myelinated axons lost their myelin on emergence from the capsule, and it would appear, as suggested by Burnstock (1959b), that most of the axons terminated on the neurons lying outside the nerve trunks, since the innervation of the circular muscle coat appeared sparse. The mixed nature of the vagus nerve has

therefore been confirmed ultrastructurally, in agreement with Campbell and Burnstock (1968) who referred to the vagus in the trout as a vagosympathetic trunk. Correlating these observations with the state of knowledge of the affect of vagal stimulation on the trout stomach however, is extremely difficult. Nonetheless, three reasons for the 'rebound' contraction can be advanced. In the first place, it could be due to the stimulation of axons containing large granular vesicles. Secondly, adrenergic fibres which have been demonstrated in the vagus, may contribute to such an effect. Alternatively, the wide distribution of neurons along the length of the trunks may itself make the response a compound effect, which need not be entirely attributable to any one type of axon profile.

### 4.4.2 SUBMUCOUS PLEXUS

Like those of the myenteric plexus, the nerve cell bodies of the submucous plexus showed variations in ultrastructure, and at least four different types were distinguished. The small nerve cell body with the well-developed Golgi complex, containing large granular vesicles, is probably similar to the granular neurons of the myenteric plexus. The features of the second distinctive type, which notable include the presence of small granular vesicles, are particularly significant, for it is not unreasonable to suppose that such a neuron is the source of the small granular vesicles found in Group II varicosity profiles. This nerve cell body should thus necessarily be interpreted as being adrenergic. Were this interpretation to be confirmed histochemically, it would constitute yet again one of the many features of the innervation of the trout gut, distinguishing it from the widely-accepted vertebrate pattern. In this regard, it is important to note that Campbell & Gannon (1976) demonstrated adrenergic nerve cell bodies in the myenteric

plexus of the stomach of the rainbow trout by fluorescent histochemistry, in contrast to the situation in mammals, where intrinsic adrenergic neurons have only been demonstrated in the proximal colon of the guinea-pig (Furness & Costa, 1971).

The two-tier arrangement of the elements of the submucous plexus in the stomach of the trout, is in accord with its arrangement in the gut of mammals as shown by Gunn (1968) and Stach (1973). In the post-gastric gut of the trout though, the decline of the submucosa brought about by the proximity of the stratum compactum and the inner muscle layer, confines the elements to a single tier.

The relationship between an en passage Group II varicosity and the innermost circular smooth muscle coat of the rectum shown in this study, is consistent with the suggestion of Stach (1973) that the submucous plexus provides motor innervation to the inner third of the circular muscle. The tier on the mucosal aspect of the plexus may also provide motor innervation to the muscularis mucosae in the stomach, as well as sensory innervation to the entire gut mucosa.

### 4.4.3 INNERVATION OF THE RECTAL MUSCULARIS MUCOSAE

The innervation of the muscularis mucosae in the rectum is obviously more complex by virtue of the presence of small nerve cell bodies, and the preponderance of Group III varicosities, which establish very close contact relationships with the muscle fibres, which themselves extended processes towards their neighbours.

In all these respects, the neuromuscular arrangement here differs markedly from that in the remaining parts of the gut. Functionally, such an elaborate arrangement suggests that the response to nerve

stimulation is prompt, since the transmitter must rapidly reach high concentrations in the small neuromuscular spaces, with little room for diffusion. This neuromuscular arrangement thus indicates a highly-efficient control of the smooth muscle, by the unknown transmitter previously mentioned in connection with the Group III varicosities. Such control is illustrated vividly by the scanning electron microscope in this study, which showed three neighbouring rectal mucosal folds, each exhibiting a unique architectural pattern. These have obviously been caught as a wave of contraction was passing through them. In summary therefore, it can be said that such a highly-efficient pattern is in fact necessary in that part of the gut, which is sculptured to slow down the passage of food in order to maximise absorption, and at the same time permit defaecatory functions.

## REFERENCES

AL-HUSSAINI, A. H. (1949). On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: Cytology and physiology.
Quart. J. Microsc. Sci. 90, 323-354

ANDREWS, P. M. (1975). Microplicae: morphology, origin and possible functional significance.
J. Cell Biol., 67, 11a.

BAINTON, D. F., FARQUHAR, M. G. (1970). Segregation and packaging of granule enzymes in eosinophilic leucocytes.
J. Cell Biol. 45, 54-73

BARDACH, J. E., RYTHER, J. H., McLARNEY, W. O. (1972). Aquaculture. The farming and husbandry of freshwater and marine organisms.
New York, London, Sydney, Toronto: Wiley-Interscience.

BARKA, T., ANDERSON, P. J. (1962). Histochemical methods using hexazonium para-rosanilin as coupler.
J. Histochem. Cytochem. 10, 741-753.

BARRINGTON, E. J. W. (1957). The alimentary canal and digestion.
In: "The Physiology of Fishes" (M. E. Brown, ed.), vol.I, Ch.3, pp.109-161. New York: Academic Press.

BAUMGARTEN, H. G., BJORKLUND, A., LACHENMAYER, L., NOBIN, A., ROSENGREN, E. (1973). Evidence for the existence of Serotonin-, Dopamine-, and Noradrenaline-containing neurons in the gut of Lamptera fluviatilis.
Z. Zellforsch. mikrosk. Anat. 141, 33-54.

BEREITER-HAHN, J. (1971). Light and electron microscopic studies on function of tonofilaments in teleost epidermis.
Cytobiologie 4, 73-102.

BERGOT, P., FLECHON, J.-E. (1970a). Forme et voie d'absorption intestinale des acides gras a chaine longue chez la truite arc-en-ciel (Salmo gairdneri, Rich.) I. Lipides en particules.
Anns Biol. anim. Biochem. Biophys. 10, 459-472.

BERGOT, P., FLECHON, J.-E. (1970b). Forme et voie d'absorption intestinale des acides gras a chaine longue chez la truite arc-en-ciel (Salmo gairdneri, Rich.) II. Lipides "etales".
Anns Biol. anim. Biochem. Biophys. 10, 473-480.

BERGOT, P. , SOLARI, A., LUQUET, P. (1975). A comparison of absorptive surfaces of pyloric caeca and intestine in the rainbow trout (Salmo gairdneri, Rich.)
Ann. Hydrobiol. 6(1), 27-43.

BERRIDGE, M. J., OSCHMAN, J. L. (1972) Structural characteristics of transporting cells. In: "Transporting Epithelia" New York & London: Academic Press.

BOLTON, L. L. (1933). Basophile (mast) cells in the alimentary canal of Salmonid fishes.
J. Morphol. 54, 549-582.

BULLOCK, W. L. (1963). Intestinal histology of some salmonid fishes with particular reference to the histopathology of acanthocephalan infections.
J. Morphol. 112, 23-34.

BURNSTOCK, G. (1959a). The morphology of the gut of the brown trout (Salmo trutta).
Quart. J. Microsc. Sci. 100, 183-198).

BURNSTOCK, G. (1959b). The innervation of the gut of the brown trout (Salmo trutta).
Quart. J. Microsc. Sci. 100, 199-219.

BURNSTOCK, G. (1969). Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates.
Pharmac. Rev. 21, 247-324.

BURNSTOCK, G. (1972). Purinergic Nerves.
Pharmac. Rev. 24(3), 509-581.

CAMPBELL, G., BURNSTOCK, G. (1968). Comparative physiology of gastro-intestinal motility. In "Handbook of Physiology". Section 6. Alimentary Canal (C.F.Code, ed.), pp 2213-2266. New York & London: Academic Press.

CAMPBELL, G., CANNON, B. J. (1976). The splanchnic nerve supply to the stomach of the trout, Salmo trutta and S. gairdneri.
Comp. Biochem. Physiol. 55C, 51-53.

CLAUDE, A. (1970). Growth and differentiation of cytoplasmic membranes in the course of lipoprotein granule synthesis in the hepatic cell.
J. Cell Biol. 47, 745-766.

COOK, R. D., BURNSTOCK, G. (1976). The ultrastructure of Auerbach's plexus in the guinea pig. I. Neuronal elements.
J. Neurocytol. 5, 171-194.

CULLING, C. F. A. (1974) Handbook of histopathological techniques. 3rd ed. London: Butterworths.

DAWES, B. (1929). The histology of the alimentary tract of the plaice (Pleuronectes platessa).
Quart. J. Microsc. Sci. 73, 243-274.

EL-BADAWI, A., SCHENK, E. A. (1967). Histochemical methods for separate, consecutive and simultaneous demonstration of acetyl-cholinesterase and norepinephrine in cryostat sections.
J. Histochem. Cytochem. 15, 580-588.

EULER, U. S. von, OSTLUND, E. (1956). Occurrence of a Substance P-like polypeptide in fish intestine and brain.
Brit. J. Pharmac. Chemother. 11, 323-325.

EULER, U. S. von, OSTLUND, E. (1957). Effects of certain biologically occurring substances on the isolated intestine of fish.
Acta Physiol. scan. 38, 364-372.

FORSSMANN, W. G., ORCI, L. (1969). Ultrastructure and secretory cycle of the gastrin-producing cell.
Z. Zellforsch. mikrosk. Anat. 101, 419-432.

FORTE, T. M., FORTE, J. G. (1970). Histochemical staining and characterization of glycoproteins in acid-secreting cells of frog stomach.
J. Cell Biol. 47, 437-452.

FRIEND, D. S., FARQUHAR, M. G. (1967) Functions of coated vesicles during protein absorption in the rat vas deferens.
J. Cell Biol. 35, 357-376.

FUJITA, T., KOBAYASHI, S. (1977). Structure and function of gut endocrine cells.
Int. Rev. Cytol. Suppl. 6, 187-233.

FURNESS, J. B., COSTA, M. (1971). Morphology and distribution of intrinsic adrenergic neurones in the proximal colon of the guinea-pig.
Z. Zellforsch. mikrosk. Anat. 120, 346-363.

GABELLA, G. (1972a). Innervation of the intestinal muscular coat.
J. Neurocytol. 1, 341-362.

GABELLA, G. (1972b). Fine structure of the myenteric plexus in the guinea-pig ileum.
J. Anat. (Lond.) 111(1), 69-97.

GAUNTHIER, G. F., LANDIS, S. C. (1972). The relationship of ultra-structural and cytochemical features to absorptive activity in the goldfish intestine.
Anat. Rec. 172, 675-702.

GOLDFISCHER, S. (1965). The cytochemical demonstration of lysosomal aryl sulfatase activity by light and electron microscopy.
J. Histochem. Cytochem. 13, 520-523.

GREENE, C. W. (1912). Anatomy and histology of the alimentary tract of the king salmon.
Bull. U. S. Bur. Fisheries 32, 73-100.

GULLAND, G. L. (1898). The minute structure of the digestic tract of the salmon and the changes which occur in fresh water.
Anat. Anz. 14, 441-445.

GUNN, M. (1951). A study of the enteric plexuses in some amphibians.
Quart. J. Microsc. Sci. 92, 55-77.

GUNN, M. (1968). Histological and histochemical observations on the myenteric and submucous plexuses of mammals.
J. Anat. (Lond.) 102, 223-239.

GURR, E. (1962). Staining, practical and theoretical.
London: Leonard Hill, Baltimore: Williams and Wilkins.

HACKENBROCK, C. R., CAPLAN, A. I. (1969). Ion induced ultrastructural transformations in isolated mitochondria. The energized uptake of calcium.
J. Cell Biol. 42, 221-234.

HARDING, C. V. (1973). Corneal epithelial surfaces in elasmobranchs and teleosts as seen with the scanning electron microscope.
Biol. Bull. 145, 438.

HARRIS, J. E., WATSON, A., HUNT, S. (1973). Histochemical analysis of mucous cells in the epidermis of brown trout (Salmo trutta).
J. Fish Biol. 5, 345-351.

HAWKES, J. W. (1974). The structure of fish skin. I. General organization.
Cell Tiss. Res. 149, 147-158.

HENRIKSON, R. C., MATOLTSY, A. G. (1968). The fine structure of teleost epidermis. I. Introduction and filament-containing cells.
J. Ultrastruct. Res. 21, 194-212.

HIRATA, Y. (1966). Fine structure of the terminal buds on the barbels of some fishes.
Arch. histol. jap. 26, 507-523.

HIRST, G. D. S., HOLMAN, M. E., SPENCE, I. (1974). Two types of neurons in the myenteric plexus of the duodenum in the guinea-pig.
J. Physiol. (Lond.) 236, 303-326.

HOLSTEIN, B. (1975). Gastric acid secretion in a teleostean fish: a method for continuous collection of gastric effluence from a swimming fish and its response to histamine and pentagastrin.
Acta physiol. scand. 95, 417-423.

HUGON, J. S., BORGERS, M. (1966). Ultrastructural localization of alkaline phosphatase activity in the absorbing cells of the duodenum of mouse.
J. Histochem. Cytochem. 14, 629-641.

ITO, S., WINCHESTER, R. J. (1963). The fine structure of the gastric mucosa in the bat.
J. Cell Biol. 16, 541-577.

IWAI, T. (1968). Fine structure and absorption patterns of intestinal epithelial cells in rainbow trout alevins.
Z. Zellforsh. mikrosk. Anat. 91, 366-379.

JOLLIE, W. P., TRICHE, T. J. (1971). Ruthenium labelling of micropinocytic activity in the rat visceral yolk-sac placenta.
J. Ultrastruct. Res. 35, 541-553.

KAPOOR, B. G., SMIT, H., VERIGHINA, I. A. (1975). The alimentary canal and digestion in teleosts. In: Advances in Marine Biology, vol. 13, pp. 109-239. (F. S. Russell, M. Yonge, eds.). New York: Academic Press.

KARNOVSKY, M. J., ROOTS, L. (1964). A 'direct-coloring' thiocholine technique for cholinesterases.
J. Histochem. Cytochem. 12, 219-221.

KEMPSON, S. A. (1975). Personal communication.

KIMURA, N. (1973). Fine structure of the epithelial cells in the pyloric caecum of the rainbow trout, Salmo gairdneri. Jap. J. Ichthyol. 20(1), 13-24.

KIMURA, N., KUDO, S. (1975). Fine structure of the stratum granulosum of the pyloric caeca of the rainbow trout. Jap. J. Ichthyol. 22(1), 16-22.

LAMBSON, R. O. (1966). An electron microscopic analization of transport across rat visceral yolk-sac. Am. J. Anat. 118, 21-51

LEESON, T. S. (1973). Canaliculi and tubulovesicles of rat parietal cells. Am. J. Anat. 136, 541-547.

LEV, R., SPICER, S. S. (1964). Specific staining of sulphate groups with alcian blue at low pH. J. Histochem. Cytochem. 12, 309.

LILLIE, R. D. (1965). Histopathologic technique and practical histochemistry. 3rd ed. New York: McGraw-Hill.

LING, E. A., TAN, C. K. (1975). Fine structure of the gastric epithelium of the coral fish, Chelmon rostratus, Cuvier. Okajimas Fol. anat. jap. 51, 285-310.

LINNS, W. (1969). Electronenmikroskopische Untersuchungen am Oesophagus des Hechtes (Esox lucius). III. Die Feinstruktur der indifferenten Zellen des Epithels. Anat. Anz. 125, 113-127.

MacCRIMMON, H. R. (1971). World distribution of rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Canada 28, 663-704.

MacRAE, E. K., MEETZ, G. (1970). Electron microscopy of the ammoniacal silver reaction for histones in erythropoietic cells of the chick, J. Cell Biol. 45, 235-245.

MARSH, M. N., SWIFT, J. A. (1969). A study of the small intestinal mucosa using the scanning electron microscope. Gut 10, 940-949.

McMANUS, J. F. A. (1948). Histological and histochemical uses of periodic acid. Stain Technol. 23, 99-108.

McMANUS, J. F. A., MOWRY, R. W. (1958). Effects of fixation on carbohydrate histochemistry. J. Histochem. Cytochem. 6, 309-316.

MOWRY, R. W. (1956). Alcian blue technique for the histochemical study of acidic carbohydrates. J. Histochem. Cytochem. 4, 407.

NILSSON, A. (1970). Gastrointestinal hormones in the holocephalian fish, Chimaera monstrosa (L.).
Comp. Biochem. Physiol. 32, 387-390.

NISHI, S., NORTH, R. A. (1973). Intracellular recording from the myenteric plexus of guinea-pig ileum.
J. Physiol. (Lond.) 231, 471-491.

NOAILLAC-DEPEYRE, J., GAS, N. (1973). Absorption of protein macromolecules by the enterocytes of the carp (Cyprinus carpio L.).
Z. Zellforsch. mikrosk. Anat. 146, 525-541.

NOAILLAC-DEPEYRE, J., GAS, N. (1974). Fat absorption by the enterocytes of the carp (Cyprinus carpio, L.).
Cell Tiss. Res. 155, 353-365.

NOAILLAC-DEPEYRE, J., GAS, N. (1976). Electron microscopic study on gut epithelium of the tench (Tinca tinca L.) with respect to absorptive functions.
Tiss. Cell 8(3), 511-530.

NOAILLAC-DEPEYRE, J., GAS, N. (1978). Ultrastructural and cytochemical study of the gastric epithelium in a freshwater teleostean fish (Perca fluviatilis).
Tiss. Cell 10(1), 23-37.

NOVIKOFF, A.B., ESSNER, E., QUINTANA, N. (1964). Golgi apparatus and lysosomes.
Fedn. Proc. 23, 1010-1022.

OPPEL, A. (1896). Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbelthiere.
Bd. 1, 70; Bd. 2. 7.

OVALLE, W. K., SHINN, S. L. (1977). Surface morphology of taste buds in catfish barbels.
Cell Tiss. Res.178, 375-384.

OZAKI, N. (1965). Some observations on the fine structure of the intestinal epithelium of some marine teleosts.
Arch. histol. jap. 26, 22-38.

PEARSE, A. G. E. (1968). Histochemistry, theoretical and applied.
vol. I, London: J. & A. Churchill.

PEARSE, A. G. E., POLAK, J. M. (1975). Immunocytochemical localization of Substance P in the mammalian intestine,
Histochemistry 41, 373-375.

PFEIFFER, C. J. (1970). Surface topology of the stomach in man and the laboratory ferret.
J. Ultrastruct. Res. 33, 252-262.

PICKEL, V. M., REIS, D. J., LEEMAN, S. E. (1977). Ultrastructural localization of Substance P in neurons of rat spinal cord.
Brain Res. 122, 534-540.

RAMBOURG, A., HERNANDEZ, W., LEBLOND, C. P. (1969). Detection of complex carbohydrates in the Golgi apparatus of rat cells.
J. Cell Biol. 40, 395-414.

READ, J., BURNSTOCK, G. (1968a). Fluorescent histochemical studies on the mucosa of the vertebrate gastrointestinal tract. Histochemie 16, 324-332.

READ, J., BURNSTOCK, G. (1968b). Comparative histochemical studies of adrenergic nerves in the enteric plexuses of vertebrate large intestine. Comp. Biochem. Physiol. 27, 505-517.

REHM, W. S. (1950). A theory of the formation of HCl by the stomach. Gastroenterology 14, 401-417.

REHM, W. S., SCHLESINGER, H., DENNIS, W. H. (1953). Effect of osmotic gradients on water transport, hydrogen ion and chloride ion production in the resting and secreting stomach. Am. J. Physiol. 175, 473-486.

REIFEL, C. W., TRAVILL, A. A. (1978). Structure and carbohydrate histochemistry of the oesophagus in ten teleostean species. J. Morph. 152, 303-314.

REUTTER, K., BREIPOHL, W., BIJVANK, G. J. (1974). Taste buds in fishes. II. Scanning electron microscopical investigations on Xiphophorus helleri, Heckel (Poecilidae, Cyprinodontiformes, Teleostei). Cell Tiss. Res. 153, 151-165.

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

ROBERTS, R. J. (1972). Ulcerative dermal necrosis (UDN) of salmon (Salmo salar L.). In: "Diseases of fish". Proceedings of Symposium, No. 30, Zoological Society, London, May 1971, (Mawdeslay-Thomas, L. E., ed.) pp. 53-81. New York & London: Academic Press and the Zoological Society.

ROBERTS, R. J., SHEARER, W. M., ELSON, K. G. R., MUNRO, A. L. S. (1970). Studies on ulcerative dermal necrosis of salmonids. I. The skin of the normal salmon head. J. Fish Biol. 2, 223-229.

ROSENBLUTH, J. (1962). Subsurface cisterns and their relationship to the neuronal plasma membrane. J. Cell Biol. 13, 405-422.

SABATINI, D. D., BENSCH, K., BARRNETT, R. J. (1963). Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. 17, 19-58.

SCHLIWA, M. (1975). Cytoarchitecture of surface layer cells of the teleost epidermis. J. Ultrastruct. Res. 52, 377-386.

SEDAR, A. W. (1965). Fine structure of the stimulated oxyntic cell. Fedn. Proc. 24, 1360-1367.

SELIGMAN, A.M., HANKER, J.S., WASSERKRUG, H., DMOCHOWSKI, H., KATZOFF, L. (1965). Histochemical demonstration of some oxidised macromolecules with thiocarbohydrazide (TCH) or thiosemicarbazide (TSC) and osmium tetroxide.
J. Histochem. Cytochem. 13, 629-639.

SMIT, H. (1968). Gastric secretion in the lower vertebrates and birds. In: "Handbook of Physiology". Section 6: Alimentary Canal, (C. F. Code, ed.), vol V, ch.135, pp. 2791-2805. Washington: American Physiological Society.

SMITH, H. (1975). Eosinophilic granular cells of salmonids.
M.Sc. Thesis, University of Stirling.

SOLCIA, E., CAPELLA, C., VASSALLO, G., BUFFA, R. (1975). Endocrine cells of the gastric mucosa.
Int. Rev. Cytol. 42, 223-286.

SPERRY, D. G., WASSERSUG, R. J. (1976). A proposed function for microridges on epithelial cells.
Anat. Rec. 185, 253-258.

SPICER, S. S., (1965). Diamine methods for differentiating muco-substances histochemically.
J. Histochem. Cytochem. 13, 211-234.

SPICER, S. S., HORN, R. G., LEPPI, T. J. (1967). Histochemistry of connective tissue mucopolysaccharides. In: Connective tissue (Wagner, B. M., Smith, D. E., eds.), pp. 251-303. Baltimore: Williams and Wilkins.

SPICER, S. S., MEYER, D. B. (1960). Histochemical differentiation of acid mucopolysaccharides by means of combined aldehyde fuchsin - alcian blue staining.
Am. J. Clin. Pathol. 33, 453-460.

STACH, W. (1973). Light and electron microscopic investigations on the innervation of the musculature of the colon.
Folia Morph. (Warsz.) 32(4), 457-467.

STROBAND, H. W. J., DEBETS, F. M. W. (1978). The ultrastructure and renewal of the intestinal epithelium of the juvenile Grasscarp, Ctenopharyngodon idella (Val.).
Cell Tiss. Res. 187, 181-200.

THIERY, J. P. (1967). Mise en evidence des polysaccharides sur coupes fines en microscopie electronique.
J. Microscopie 6, 987-1018.

TONER, P. G. (1963). The fine structure of resting and active cells in submucosal glands of the fowl proventriculus.
J. Anat. (Lond.) 97, 575-583.

VICKERS, T. (1962). A study of the intestinal epithelium of the goldfish, Carassius auratus: its normal structure, the dynamics of cell replacement, and the changes induced by salts of cobalt and manganese.
Quart. J. Microsc. Sci. 103, 93-110.

WACHSTEIN, M., MEISEL, E. (1957). Histochemistry of hepatic phosphatases at physiologic pH.
Am. J. Clin. Pathol. 27, 13-23.

WEINREB, E. L., BILSTAD, N. M. (1955). Histology of the digestive tract and adjacent structures of the rainbow trout, Salmo gairdneri irideus.
Copeia 3, 194-204.

WOOD, E. M., YATUSAKE, W. T., WOODALL, A. N., HALVER, J. E. (1957). The nutrition of salmonid fishes. I. Chemical and histological studies of wild and domestic fish.
J. Nutrition 61, 465-478.

WOOD, J. D. (1974). Neurophysiology of ganglia of Auerbach's plexus.
Am. Zoologist 14, 973-989.

YAMAMOTO, T. (1966). An electron microscopic study on the columnar epithelial cell of fresh water teleosts: goldfish (Carassius auratus) and rainbow trout (Salmo irideus).
Z. Zellforsch. mikrosk. Anat. 72, 66-87.

YAMA UCHI, A., BURNSTOCK, G. (1968). An electron microscopic study on the innervation of the trout heart.
J. comp. Neurol. 132, 567-588.

ZUCKER-FRANKLIN, D., HIRSCH, J. G. (1964). Electron microscopic studies on the degranulation of rabbit peritoneal leucocytes during phagocytosis.
J. Exp. Med. 120, 569-576.

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