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A histological and histochemical study of the oesophagus and oesogaster of the Senegal sole, *Solea senegalensis*

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SUMMARY

A histological and histochemical study was performed in the buccal cavity and papillae, which were around the teeth, as well as in the oesophagus and oesogaster of the Senegal sole, *Solea senegalensis* adult specimens.

The oesophagus and oesogaster were made up of four distinct layers: mucosa, submucosa, muscular and serous. Two morphological types of epithelial cells were distinguishable in the oesophageal mucosa: the more numerous type cells possessed an electron-dense cytoplasm, whereas the cytoplasm was electron-clear in the other cells. Mucus-secreting cells were the dominant feature of the epithelium throughout the oesophagus. These goblet cells were filled with numerous mucous droplets of low electron-density. The oesophagus was devoid of taste buds.

In the oesogaster mucosa, three types of cells were distinguished: dark, rodlet and light epithelial cells. Dark epithelial cells showed different characteristics from that in the oesophagus: the nucleus was irregular with an electron-dense hyaloplasm, the cytoplasm had a scarce smooth and granular endoplasmic reticulum; a Golgi apparatus consisted of four parallel cisternae, dense granules without membrane, lysosomes and numerous mitochondria. The rodlet cells were elongated, contained rod-like structures and were surrounded by an electron-dense capsule-like structure. The bulk of the rodlet cell was composed of up to 20 extended rodlet units. Light epithelial cells of the oesogaster had the same characteristics as those observed in the oesophagus and contained numerous mitochondria with a dense matrix, abundant smooth endoplasmic reticulum and numerous vesicles.

In the goblet cells of the papillae, sulfomucin was recognised, since they showed alcianophilia (alcian blue pH 1.0 and 0.5). These cells were negative to protein reaction (bromophenol blue) and contained -S-S- and SH groups. Enzymatic activities (alkaline phosphatase, acid phosphatase, ATPase (pH 7.2 and 9.4) and lipid reactions were negative in the goblet cells of the buccal cavity.

Epithelial cells of oesophagus contained a weak presence of acid and neutral mucopolysaccharides. Oesophageal goblet cells contained carboxylated, sulphated (weakly and strongly ionised) mucosubstances and sialic acid. Most goblet cells did not contain proteins and presented disulphide (-S-S-) and sulphydril (-SH) groups. Proteins in general, and in particular those rich in lysine, tyrosine and arginine were present in the

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epithelium, lamina propria, submucosa and muscular layer of the oesophagus.

Lipids in general and phospholipids were observed in the oesophageal epithelium while unsaturated, acid and neutral lipids were not observed. The lamina propria and submucosa contained a weak presence of phospholipids and unsaturated lipids. Acid phosphatase and ATPase (pH 7.2) activities were observed in the lamina propria, submucosa and muscular regions, while ATPase (pH 9.2) activity was weak in these areas. ATPase activity (pH 7.2 and 9.5) was very weak in the epithelium. Oesophageal goblet cells were negative to lipid and enzymatic reactions.

INTRODUCTION

The anatomy and histology of the alimentary tract of teleostean fish have been described for many species (see Barrington, 1957; Khana and Mehrotra, 1971; Vegas-Velez, 1972; Kapoor *et al.*, 1975 for reviews) although there are few studies dealing with the ultrastructure of the digestive tract. Compared to mammals, the teleost digestive tract is histologically simpler, probably because it is so easy to provide an aqueous vehicle for the digestive products and also because, at least in some species, the rate of digestion can be slow, and less complex digestive glands and a less well-developed muscular apparatus are needed (Ferguson, 1995).

The oral cavity and pharynx in most teleostean fish are lined by a thin stratified squamous epithelium containing abundant mucus-secreting cells. Fungiform and filiform papillae may be found, and many species have teeth, which vary greatly in shape. The oesophagus is short and thick walled; the muscularis is comprised of interweaving skeletal muscle fibers that may extend as far as the stomach. The stratified cuboidal or columnar epithelium may be ciliated and contains numerous goblet cells and occasionally taste buds. In addition, multicellular serous or cardiac glands may be found subsequently. The mucosa is thrown into longitudinal folds that end at the stomach, giving way to rugae. The serosa contains prominent nerve fibers of the vagus (Morrison, 1987; Amin *et al.*, 1994).

Some portions of the digestive tract of marine teleosts, specifically the oesophagus and the intestine, are involved in osmoregulation (Kirsch and Laurent, 1975; Hirano and Mayer-Gostan, 1976; Ruiter *et al.*, 1985; Cataldi *et al.*, 1988a; Cataldi *et al.*, 1988b; Ciccotti *et al.*, 1993). The main osmoregulatory tissue, the gills (Karnaky *et al.*, 1977; Zaccone, *et al.*, 1984), as well as the digestive tract, participates directly in this process (Mancera *et al.*, 1993).

The aim of this work was to study the morphology, ultrastructure, as well as enzymatic activity-, lipid-, carbohydrate- and protein distribution in the oesophagus and oesogaster of the Senegal sole, *Solea senegalensis*, as a complement to existing studies on their nutritional physiology, since this flatfish is an excellent economical and commercial species for culture and exploitation in different countries.

MATERIALS AND METHODS

Adult specimens of the Senegal sole, *Solea senegalensis* (mean body weight 370-430 g, total length ranging from 20-30 cm) were obtained from "Cupimar, S.A" fisheries (San Fernando, Cádiz, Spain). Fish were maintained in tanks of 2000 L in the Andalucía Marine Science Institute (CSIC) until their utilisation. Specimens were anaesthetised with benzocaine, 50 mg/L, and the abdominal cavity was dissected.

For light-microscopic studies (carbohydrates and proteins), small samples of buccal cavity, papillae, oesophagus and oesogaster were fixed by immersion in Bouin's fluid for 24 h. After dehydration in graded concentrations of ethanol, samples were embedded in paraffin wax. Sections of 6-8 µm thickness were stained with haematoxylin-eosin and haematoxylin-VOF (Gutiérrez, 1967). Argentic impregnation (kit Bio-Optica milano s.p.a.) was also performed for determining the collagen fibers. Lipid and enzymes were used in unfixed samples from cryostat (Cryocut-E). Histochemical techniques of carbohydrates, proteins, lipids and enzymatic activities are shown in Tables I and II.

Scanning electron microscopy (SEM)

Oesophagus samples for scanning electron microscopy were fixed in 4% glutaraldehyde in (0.1M) Na-cacodylate buffer (pH 7.2) dehydrated through ethanol series, critical point dried with liquid CO_2, coated with gold, and viewed in a Hitachi S 570 scanning electron microscope.

Table I
Histochemical reactions used to detect carbohydrates and proteins. Staining techniques for carbohydrates and proteins were taken from monographs by Martoja and Martoja-Pierson (1970) and Pearse (1985)

Staining techniques	Functions and/or components demonstrated
Carbohydrates	
Periodic acid-Schiff (PAS)	Glycogen, neutral mucosubstances and/or glycoconjugates
Diastase-PAS	Glycogen
Alcian Blue (AB) pH 2.5	Carboxyl-rich glycoconjugates (sulphated or not)
Alcian Blue (AB) pH 2.5/PAS	Neutral and/or acid rich glycoproteins
AB pH 1.0	Sulphated glycoconjugates (weakly ionised)
AB pH 0.5	Sulphated glycoconjugates (strongly ionised)
Neuraminidase or hydrochloric acid hydrolysis -AB pH 2.5	Cleavage of C_4 not acetylated sialic acid
Esterification-AB pH 2.5	Blockage of carboxylated and sulphated groups
Esterification –Saponification-AB pH 2.5	Reactivation of carboxylated groups
Esterification-AB pH 1.0	Blockage of sulphated groups (weakly ionised)
Esterification –Saponification-AB pH 1.0	Unreactivation of sulphated groups (weakly ionised)
Esterification-AB pH 0.5	Blockage of sulphated groups (strongly ionised)
Esterification –Saponification-AB pH 0.5	Unreactivation of sulphated groups (strongly ionised)
Proteins	
Bromophenol blue	Proteins in general
Ninhydrin-Schiff	Proteins rich in lysine (-NH2 groups)
Thioglycolate-potassium ferricyanide (Fe III)	Proteins rich in cystine (-S-S- groups)
1,2 Naphtoquinone-4-sulphonic acid, sodium salt	Proteins rich in arginine
Hg sulphate-sulphuric acid sodic nitrate	Proteins rich in tyrosine
Ferric ferricyanide (Fe III)	Proteins rich in cysteine (-SH- groups)
p-Dimethylaminobenzaldehyde	Proteins rich in tryptophan

Transmission electron microscopy (TEM)

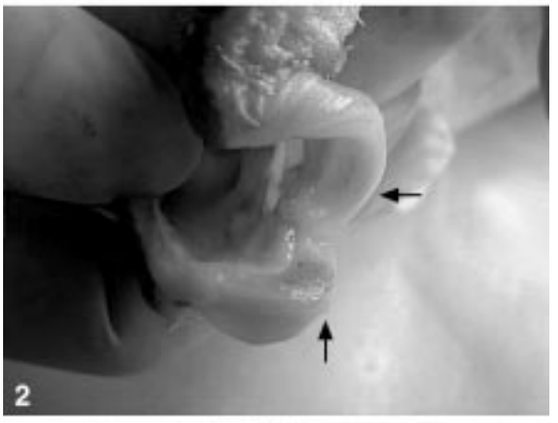
Small pieces of oesophagus and oesogaster were fixed for 2h in cold cacodylate-buffered, 2.5% glutaraldehyde at pH 7.2 (with sucrose 6%), rinsed several times in buffer (without sucrose) and postfixed with 1% OsO_4 in 0.1M cacodylate buffer (rinsed several times in buffer). The samples were dehydrated in a graded series of acetone and embedded in Spurr's medium. Ultrathin sections of 60 to 80 nm thickness (Reichert Jung ultramicrotome) were stained with uranyl acetate and lead citrate prior to examination under the electron microscope (Zeiss EM 9S2).

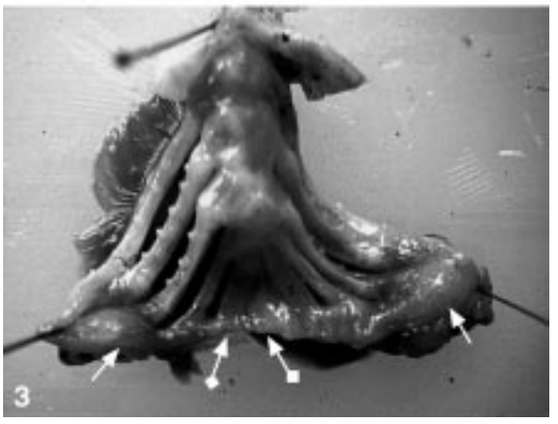
Table II
Histochemical reactions used to detect lipids and enzymes. Staining techniques for lipids and enzymes were taken from monographs by Pearse (1985) and Bancroft and Stevens (1990)

Staining techniques	Functions and/or components demonstrated
Oil Red 0	Neutral lipids
Br- Oil Red 0	Unsaturated lipids
Fe (III) Haematoxylin	Phospholipids
Sudan Black B	Neutral general
Nile Blue	Neutral and acidic lipids
Alkaline phosphatase	Alkaline phosphatase activity
Acid phosphatase	Acid phosphatase activity
ATPase (pH 7.2)	ATPase (pH 7.2) activity
ATPase (pH 9.4)	ATPase (pH 9.4) activity

RESULTS

The alimentary canal of the Senegal sole, *Solea senegalensis* is formed by the buccal-pharynx cavity, oesophagus, stomach and intestine (Fig. 1). In





Figs. 1/3 - **(1)**Intestine (in), liver (l) and gills (g) of *Solea senegalensis.* **(2)**Buccal papillae (→). **(3)**Gill papillae (→) in the arch gills and oesophagus papillae (▪→).

the buccal cavity, on the lips (Fig. 2) and between the pharynx and oesophagus, pairs of symmetrical papillae, around teeth are observed (Fig. 3). The short oesophagus is joined to pharynx papillae.

Histological description

The first part of the digestive tract is constituted by the mouth, teeth and lips. The gill arches are taken as the dividing line between the buccal-pharynx cavity and the rest of the digestive tract.

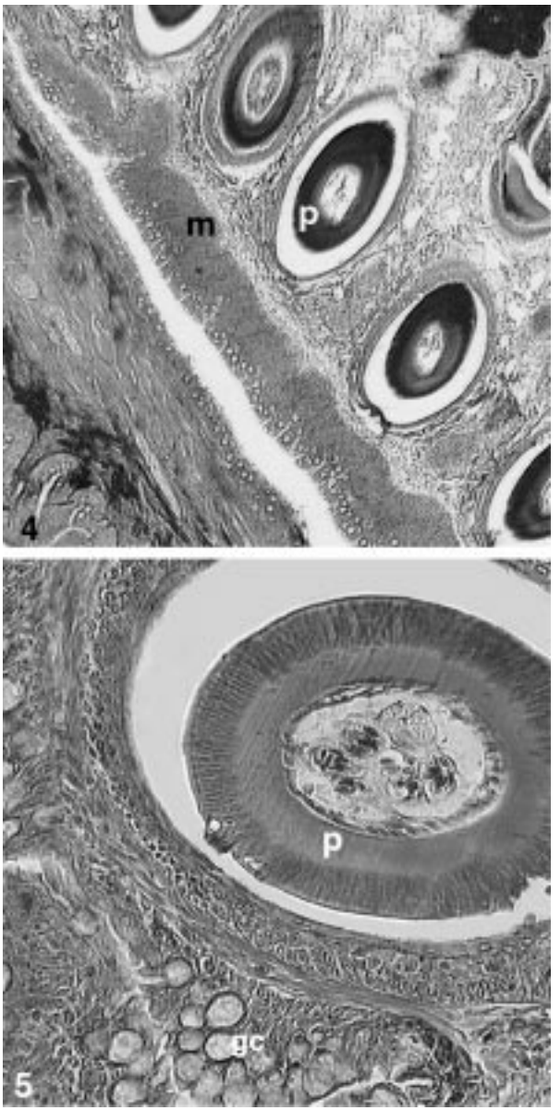
The mouth is small, round and bent, located to the right of the lateral line. The teeth are found in a group in the papillae, which are immersed in the mucosa (Fig. 4 and 5). The mucosa presents a stratified squamous epithelium and under this epithelium densely packed connective tissue fibers oriented parallel to the surface are observed. Striated muscles occur peripheral to the connective tissue where jaw muscles are present.

Scanning electron microscopy of the *Solea senegalensis* oesophagus showed primary longitudinal folds, on which secondary folds (Fig. 6) were also observed. The surface of the epithelial cells exhibited short apical microvilli. In the secondary folds, mucous cells were observed (Fig. 7). Histologically, the oesophagus was composed of the following layers: mucosa, submucosa, muscular and serosa (Fig. 8 and 9). The mucosa was formed by longitudinal folds, which were continuations of those of the pharynx but deeper. Transverse sections showed that minor longitudinal folds occured between the major ones, allowing expansion of the oesophagus. Taste buds were not present. The anterior oesophagus is formed predominantly of a stratified epithelium having a well defined basement membrane, with goblet cells and cubical epithelial cells within the mucosa layer (Fig. 9). The stratified epithelium layer acts as a supporting tissue surrounding and packing the goblet cells. Near the junction of the oesophagus and the stomach (oesogaster), the squamous epithelium is substituted, on the top of the oesophagus folds, by a simple columnar epithelium devoid of goblet cells (Fig. 10). Peripheral to the epithelium there is a thick layer of densely packed connective tissue fibers, the stratum compactum (Fig. 9), which separates the mucosa from submucosa (loose connective tissue). Two layers of striated muscle occur at the periphery of the connective tissue: an inner longitudinal layer, and a thicker outer circular layer. Both muscular layers are still present in the anterior part of the stomach where they are replaced by smooth muscle, and the muscular fibers invert their position. The serosa consists of mesothelial cells and loose connective tissue containing capillaries, and small blood vessels.

Ultrastructural characteristics

Two morphological types of epithelial cells were distinguishable in the oesophageal mucosa: the more numerous type-cells had an electron-dense cytoplasm, whereas the cytoplasm was electron-clear in the other cells (Fig. 11 and 12).

The electron- dense cells contained an irregular nucleus located in the basal half part of the cells; the cytoplasm contained 10-12 mitochondria (Fig. 11), free ribosomes, granular endoplasmic reticulum, small spherical granules and a supranuclear Golgi apparatus consisting of several parallel cisternae, numerous vesicles and abundant tonofilaments.



Figs. 4/5 - (4) Histological section of the papillae, showing dental formation (p) and the mucosa (m). (H/VOF x5). **(5)**Magnification of the dental formation (p) and the goblet cells (gc). (H/VOF x20).

Epithelial cells (electron-dense) of the outer layer exhibited short microvilli and were scarce in the glycocalyx. Neighbouring epithelial cells were intimately joined by interdigitations and many desmosomes (Fig. 13) and the intercellular spaces were dilated.

Light epithelium cells (Fig. 12) were scarce (0-1/each section). In the cytoplasm of these cells, 25-35 mitochondria were detected (Fig. 12). The mitochondrial matrix appeared more electron-dense than the cellular hyaloplasm. The granular endoplasmic reticulum and Golgi apparatus were seldom observed. However, abundant smooth endoplasmic reticulum in tubular shape and fragmented was detected (Fig. 12).

Mucus-secreting cells are the dominant feature of the epithelium throughout the oesophagus. These goblet cells were filled with numerous mucous droplets of low electron-density (Fig. 14). The oesophagus is devoid of taste buds.

The basement membrane was quite thick. Peripheral to the basement membrane were located the fibroblasts and densely packed layers of collagen fibers of the stratum compactum. The submucosal plexus was prominent and contained myelinated (Fig. 15) and unmyelinated nerves, surrounded by Schwann cells. The musculature of the oesophagus was composed of interweaving striated muscle fibers (Fig. 16).

Near the junction of the oesophagus and the stomach (oesogaster), the squamous epithelium was substituted, on top of the oesophagus folds, by a simple columnar epithelium devoid of goblet cells (Fig. 17). In the mucosa of oesogaster, three types of cells were distinguished: dark epithelial, rodlet and light epithelial cells.

Dark epithelial cells (Fig. 17) showed different characteristics from oesophageal cells. The nucleus was irregular with an electron-dense hyaloplasm. The cytoplasm had a scarce smooth and granular endoplasmic reticulum; a Golgi apparatus consisting of four parallel cisternae, dense granules without membrane, lysosomes and numerous mitochondria (45-50/section) with an electron-dense matrix.

Rodlet cells were elongated. They contained rod-like structures and were surrounded by an electron-dense capsule-like structure. The bulk of the cells was composed of up to 20 extended rodlet units

(Fig. 18 and 19). An electron-dense core was observed in the centre of each rodlet unit (Fig. 19).

Light epithelial cells of the oesogaster had the same characteristics as those observed in the oesophagus. Numerous mitochondria with a dense matrix, abundant smooth endoplasmic reticulum and numerous vesicles were observed.

In the oesogaster, the lamina propria was more compact and distinct than in the oesophagus. Numerous fibroblasts were observed in the lamina propria of the oesogaster and the muscular portion was formed of striated muscle fibers.

Histochemistry

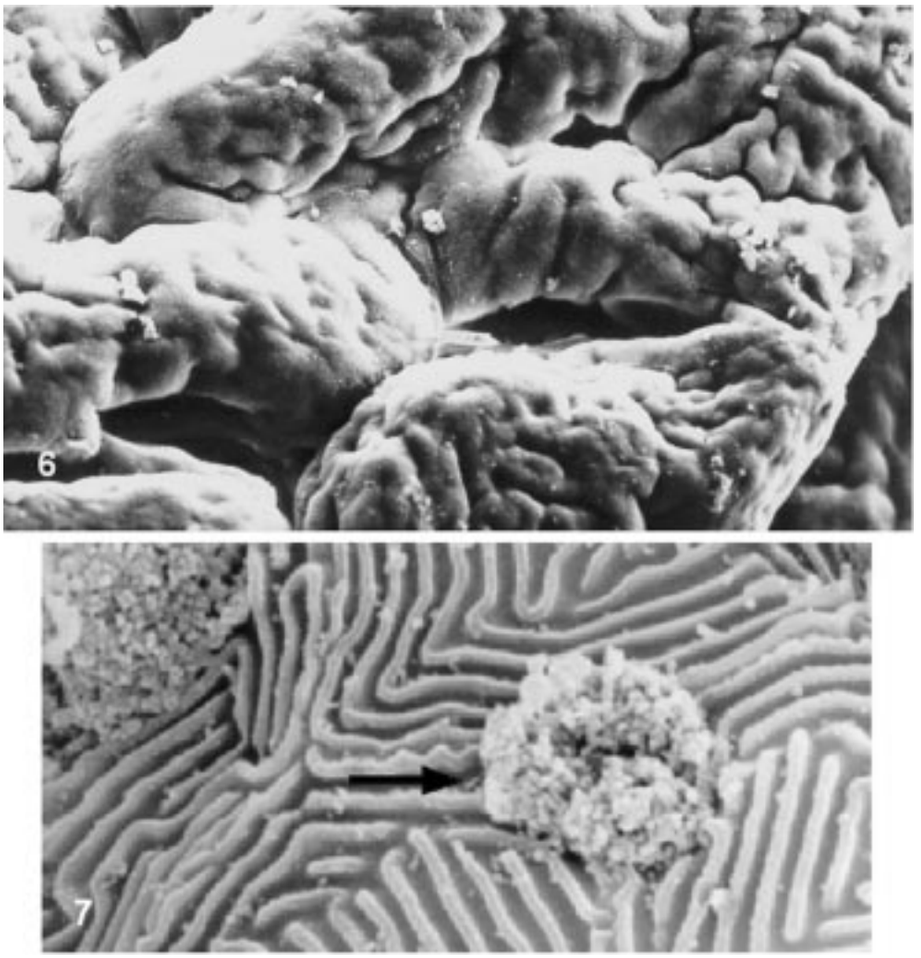
Buccal cavity and papillae

One type of acid mucosubstance was identified in the goblet cells of the papillae by means of histochemical techniques. They were recognised as sulfomucins, since they showed alcianophilia with alcian blue pH 1.0 and 0.5.

The reactions that made evident neutral mucopolysaccharides/glycoproteins (Fig. 20) were positive in the lamina propria and in the dental formation, although they were weakly positive in the epithelial and goblet cells (Table III). On the other hand, the dental formation showed a weak presence of carboxylated mucosubstances (Fig. 21) and glycogen.

Proteins (Fig. 22) were located in the papillae, and in particular, those rich in amino acids (Table III) such as arginine, cystine, cysteine and tryptophan were observed in the dental formation. Proteins rich in arginine were detected in the lamina propria and epithelium of the buccal cavity.

Goblet cells of the papillae (Fig. 22) were nega-



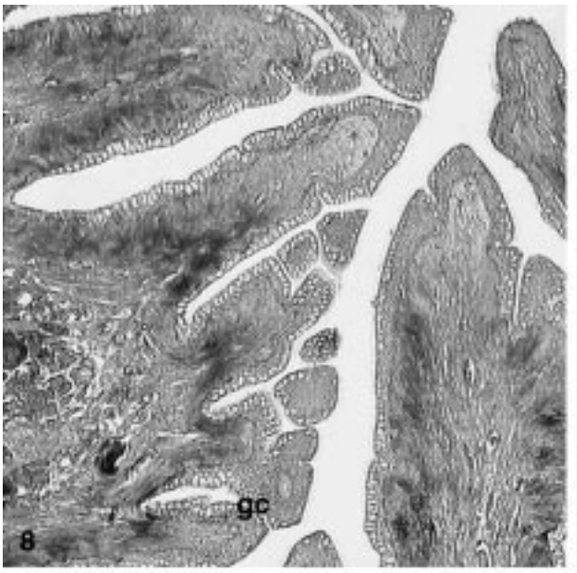
Figs. 6/7 - **(6)**Oesophagus secondary folds (x700). **(7)**Oesophagus goblet cell (→) (x1920).

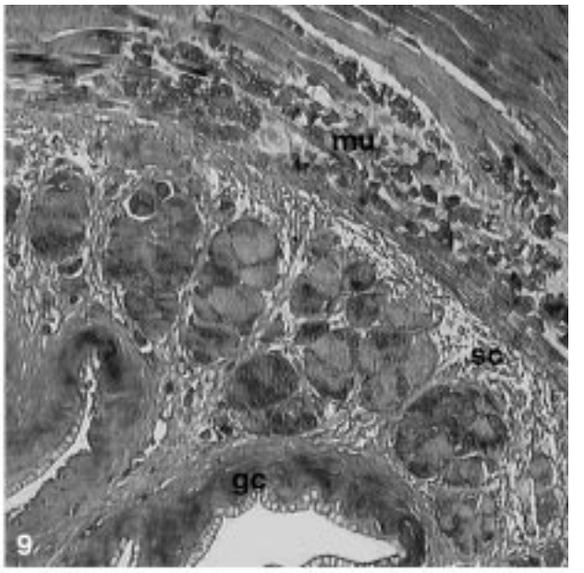
tive to the general protein technique (bromophenol blue) and contained -S-S- and -SH groups (Fig. 23, 24 and 25).

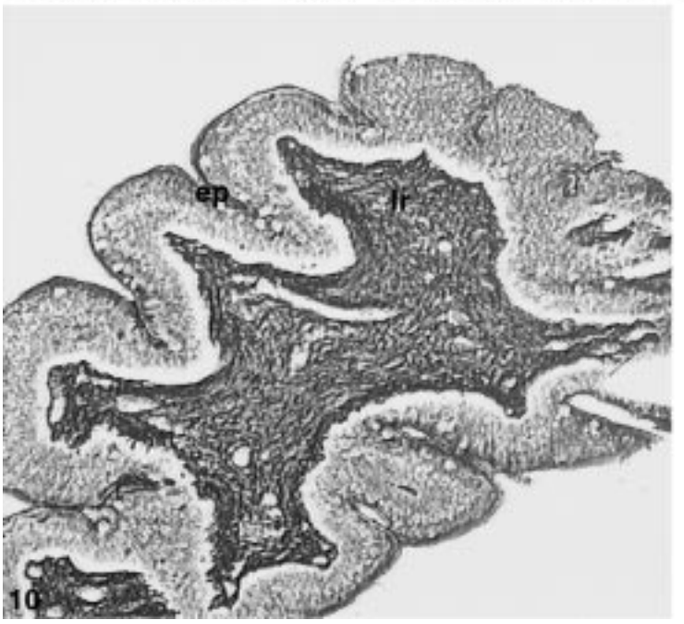
The epithelium and lamina propria of the buccal cavity contained a weak presence of acid, unsaturated and neutral lipids (Table IV). In the buccal cavity, ATPase (pH 7.2) activity was detected in the epithelium and lamina propria, while ATPase (pH 9.2) and alkaline phosphatase activities presented a weak positivity (Table IV). Acid phosphatase activity was observed in the epithelium of the buccal cavity. Enzymatic activities and lipid reactions were negative in the goblet cells.

Oesophagus

A weak presence of acid and neutral mucopolysaccharides were observed in oesophageal epithelial cells. Goblet cells of the oesophagus (Fig. 26) were strongly stained with alcian blue (AB pH 2.5), indicating the presence of carboxylated, sulphated







Figs. 8/10 - (8) Goblet cells (gc) in the epithelium of the anterior oesophagus (H/VOF x5). **(9)**Transverse section of the anterior oesophagus, showing the goblet cells (gc) in the mucosa (m), the "stratum compactum" (sc) in the submucosa and the muscular (mu) (H/VOF x5). **(10)**Epithelium (ep) and the lamina propria (lr) in the posterior oesophagus. (Argentic Impregnation x10).

(weakly and strongly ionised) mucosubstances (AB pH 1.0, 0.5) and sialic acid (neuraminidase or HCl hydrolysis-AB, pH 2.5) (Table V).

Most goblet cells lacked proteins and contained -SH and –S-S- groups (Table V). Proteins (Fig. 27), and in particular those rich in lysine, tyrosine (Fig. 28) and arginine were present in the epithelium, lamina propria, submucosa and muscular layers (Table V).

Lipids in general and phospholipids were observed in the mucosa epithelium, while unsaturated, acid and neutral lipids were not observed. Lamina propria and submucosa contained a weak presence phospholipids and unsaturated lipids (Table VI).

Goblet cells of the oesophagus and the buccal cavity were negative to lipid and enzymatic reactions. Acid phosphatase (Fig. 29) and ATPase (pH 7.2) activities were observed in the lamina propria, submucosa and muscular portion of oesophagus, while ATP-ase (pH 9.2) activity was weak in these areas. ATPase (pH 7.2 and 9.5) were feeble in the epithelium (Table VI).

DISCUSSION

The structure of the oesophageal wall of the Senegal sole, *Solea senegalensis* is similar to other teleostean fish (Grau *et al.*, 1992; Gargiulo *et al.*, 1996) and consists of four layers: mucosa, submucosa, muscular and serosa. The lining epithelium is stratified and the muscularis consist of striated muscled fibers, as in *Sparus aurata*, *Seriola dumerili* and *Tilapia* sp (Elbal and Agulleiro, 1986a; Grau *et al.*, 1992; Gargiulo *et al.*, 1996).

Fingerprint-like microridges on the squamous epithelial cells of the gut mucosa of the Senegal sole have been described in other species (Meister *et al.*, 1983; Morrison, 1987; Grau *et al.*, 1992) and these microridges may protect the surface from trauma from ingested materials and anchor the mucus secreted by goblet cells (Ezeasor and Stokoe, 1980; Humbert *et al.*, 1984; Uehara and Miyoshi, 1988). In the Senegal sole, the lamina propria was practically indistinguishable, and the stratum compactum, which separated the mucosa from the loose connective tissue –the submucosa- was well developed, such as Grau *et al.* (1992) observed in *Seriola dumerili*. On the other hand, *S. Senegalensis* presented powerful layers of striated muscle in the oesophageal surface that according to Grau *et al.* (1992) might act as the main grinding device.

A notable characteristic of the oesophagus of the Senegal sole is the absence of taste buds, as occurs in seabream and tilapine (Elbal and Agulleiro, 1986a; Gargiulo *et al.*, 1996). However, taste buds have been observed in different fish species (Reifel and Travill, 1977; Martin and Blaber, 1984).

In the oesophagus of the Senegal sole, two different morphological types of epithelial cells were distinguishable: the more numerous cells had electron-dense cytoplasm, whereas the cytoplasm was electron-clear in the other cells. Light epithelial cells contained numerous mitochondria. Similar epithelial cells present in the oesophagus of *S. Senegalensis* have been described in *Salmo gairdneri* (Weinreb and Bisbal, 1955) and in *Sparus aurata* (Elbal and Agulleiro, 1986a), although the authors did not notice any ultrastructural differentiation between clear and dense epithelial cells. The electron-dense cells in *S. senegalensis* were not excessively mitochondria-rich but the intercellular spaces were dilated. Meister *et al.*, (1983) observed similar cell types in mid and posterior oesophagus of sea-water fish species. According to different authors (Kirsh, 1978; Berridge and Oschman, 1972; Yamamoto and Hirano, 1978; Meister *et al.*, 1983), the association of mitochondria and dilated intercellular spaces with the absence of significant transepithelial net water fluxes (Kirsch, 1978) is generally related to an active ion transport on lateral membranes and rapid "entrainment" of solutes in intercellular spaces with baso-lateral water recycling (Yamamoto and Hirano, 1978). Similar intercellular spaces as observed in the Senegal sole have been described in the columnar epithelium of the *Anguilla japonica* oesophagus during sea water adaptation, being characterised by a selective ion permeability independent of that of water (Yamamoto, 1978). The electron-clear epithelial cells were observed in the oesophagus and oesogaster of *S. Senegalensis*. These cells, rich in mitochondria, showed elements of a tubular system similar to those described by Pisam (1981) for chloride cells of teleost gills; and according to Arellano (1999) these cells were also stained with osmium tetraoxide by using the technique of Watrin and Mayer-Gostan (1996), a widely used technique which does not employ antibodies. This substantiates the hypothesis that these clear dense epithelial cells are ion-transporting cells. According to Meister *et al.*, (1983), dilated intercellular spaces were not necessarily related to ion absorption.

In the oesogaster of *S. Senegalensis*, rodlet cells were observed throughout the digestive tract (Arel-

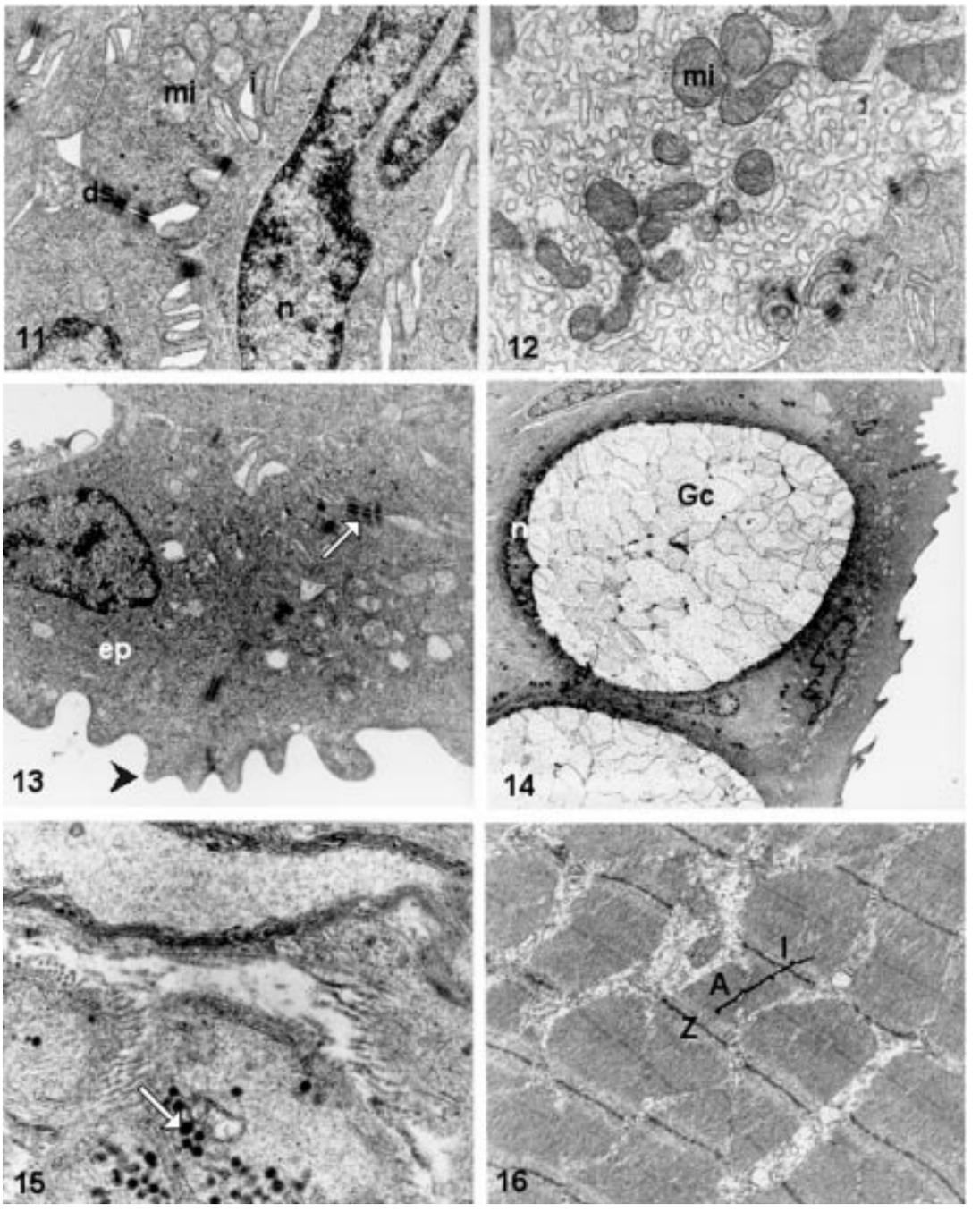


Fig. 11/16 - (11) Oesophageal epithelium showing the dark cells with cytoplasm electron-dense and a plasmalema with numerous interdigitations (i) and desmosomes (ds). The nucleus (n) is long and irregular, and in the cytoplasm are observed numerous mitochondrias (mi) (x20000). **(12)**Light epithelial cells showing numerous mitochondria (mi) and abundant smooth endoplasmic reticulum in tubular shape and fragmented (x25000). **(13)**Epithelial cells (ep) in the oesophagus apical showing microvilli and desmosomes (x16000). **(14)**Goblet cell (gc) detected in the nucleus (n) (x4000). **(15)**The submucosal plexus, showing myelinated nerves (→) (x25000). **(16)**The musculature of the oesophagus is composed of interweaving striated muscle fibers (x10000).

Table III
Histochemical distribution of carbohydrates and proteins in the papillae of *S. senegalensis*

	Epithelium	Goblet cells	Lamina propria	Teeth
Glycogen	0	0	0	0-1
Neutral glycoproteins	0-1	0-1	2-3	3
Carboxylated groups	0	2-3	0	0-1
Sulphated glycoconjugates (weakly ionised)	0	3	0	0
Sulphated glycoconjugates (strongly ionised)	0	2-3	0	0
Hydrochloric acid hydrolysis AB pH 2.5	0	0	0	0
Esterification-AB pH 2.5	0	0	0	0
Esterification –Saponification-AB pH 2.5	0	2-3	0	0-1
Esterification-AB pH 1.0	0	0	0	0
Esterification –Saponification-AB pH 1.0	0	0	0	0
Esterification-AB pH 0.5	0	0	0	0
Esterification –Saponification-AB pH 0.5	0	0	0	0
Proteins in general	2	0	2-3	3
Proteins rich in lysine	0-1	0	0-1	0-1
Proteins rich in tyrosine	0-1	0	0-1	0-1
Proteins rich in arginine	1	0	2	2
Proteins rich in tryptophan	2-1	0	2-1	2-3
Proteins with cysteine residues	2	2-3	2	2
Proteins with cystine residues	2	3	2	2

Results are expressed as semiquantitative assessment of colour intensities by independent scores of two investigators. Estimated scale ranging from 0 (unreactive) to 3 (strongly reactive).

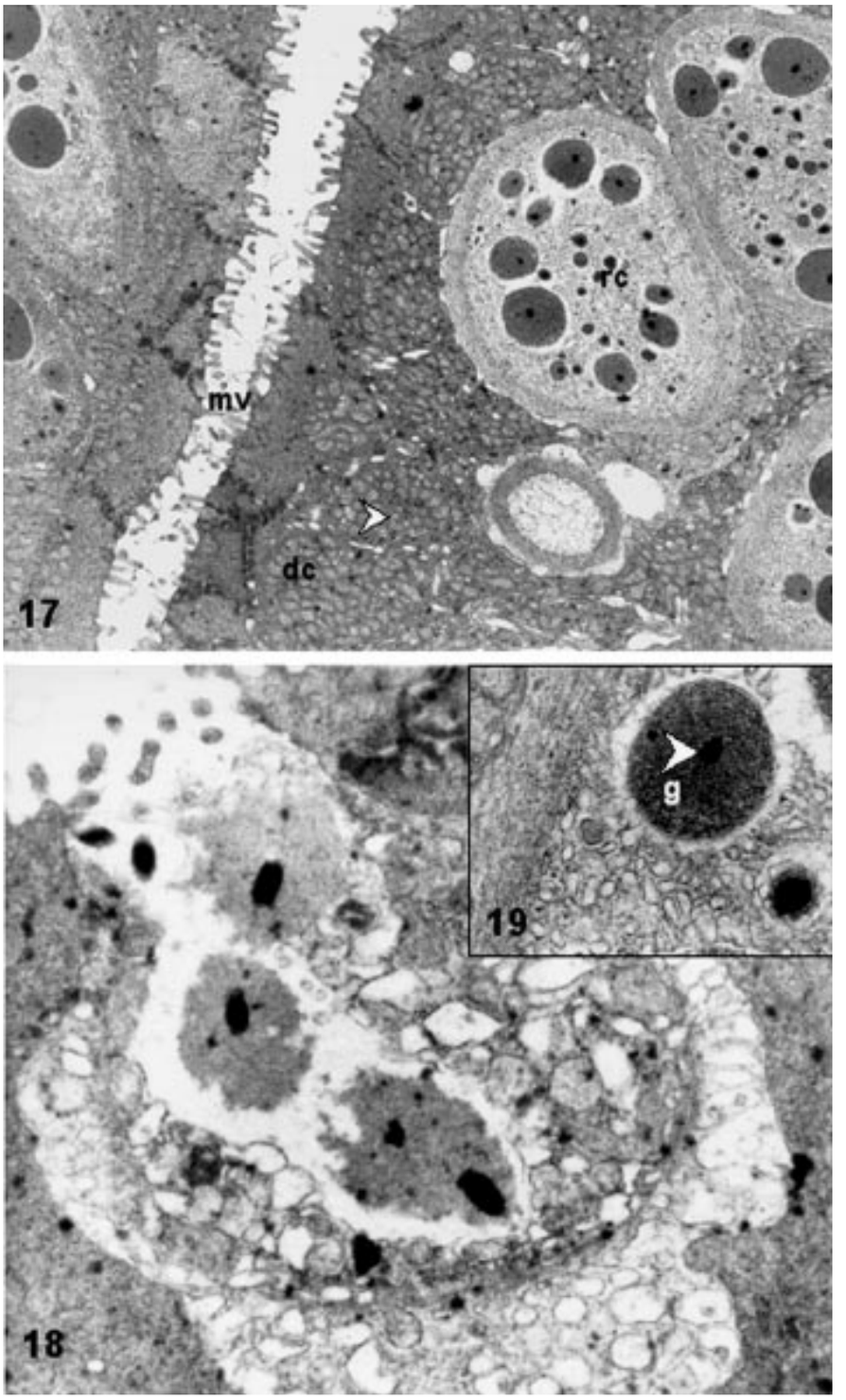
lano, 1999). These cells are present in the epithelium of numerous organs/tissues (gills, digestive tract, heart, thymus, skin, etc.) of both freshwater and marine teleostean fish (Leino, 1981; Iger and Abraham, 1997; Dezfuli *et al.*, 1998). Although the nature of these cells remains controversial, studies on their fine structure have supported the view that rodlet cells are secretory/enzymatic cells (Leino, 1981). These cells could be found under conditions of experimental wounding and after exposure to lead (or cadmium) polluted water, diluted seawater, manured water, acidified water, after thermal elevation or after exposure to polluted water (Iger and Abraham, 1997).

An integral component of the digestive mucous layer are the mucins, which in conjunction with locally secreted components are crucial for the functioning of epithelial cells. Mucins are high molecular weight glycoproteins forming intricate networks. They protect the mucous membranes of the gastrointestinal system, and other bodily portions (skin, gills) by forming a viscoelastic gel which acts as a physical barrier between the mucosa and environmental agents (Pajak and Danguy, 1993). Sulfomucins, sialomucins and neutral mucosubstances/glycoproteins were observed in mucous

Table IV
Histochemical distribution of lipids and enzymatic activities in buccal cavity of *S. senegalensis*

	Epithelium	Goblet cells	Lamina Propria
Unsaturated lipids	1	0	0-1
Neutral lipids	1	0	0-1
Acid lipids	1	0	0-1
Phospholipids	0	0	0
Alkaline phosphatase	0-1	0	0-1
Acid phosphatase	1-2	0	0
ATPase (pH 7.2)	2-3	0	3
ATPase (pH 9.4)	0-1	0	0-1

Results are expressed as semiquantitative assessment of colour intensities by independent scores of two investigators. Estimated scale ranging from 0 (unreactive) to 3 (strongly reactive).



Figs. 17/19 - **(17)**Oesogaster epithelium, showing numerous rodlet cells (rc), dark cells (dc) (numerous mitochondrias (➢) and the microvilli (mv) (x5800). **(18)**Rodlet cell spilling its contents (x26600). **(19)**Magnification of the rodlet cell showing the dense granules (g) and droplet in the middle (➢) (x2400).

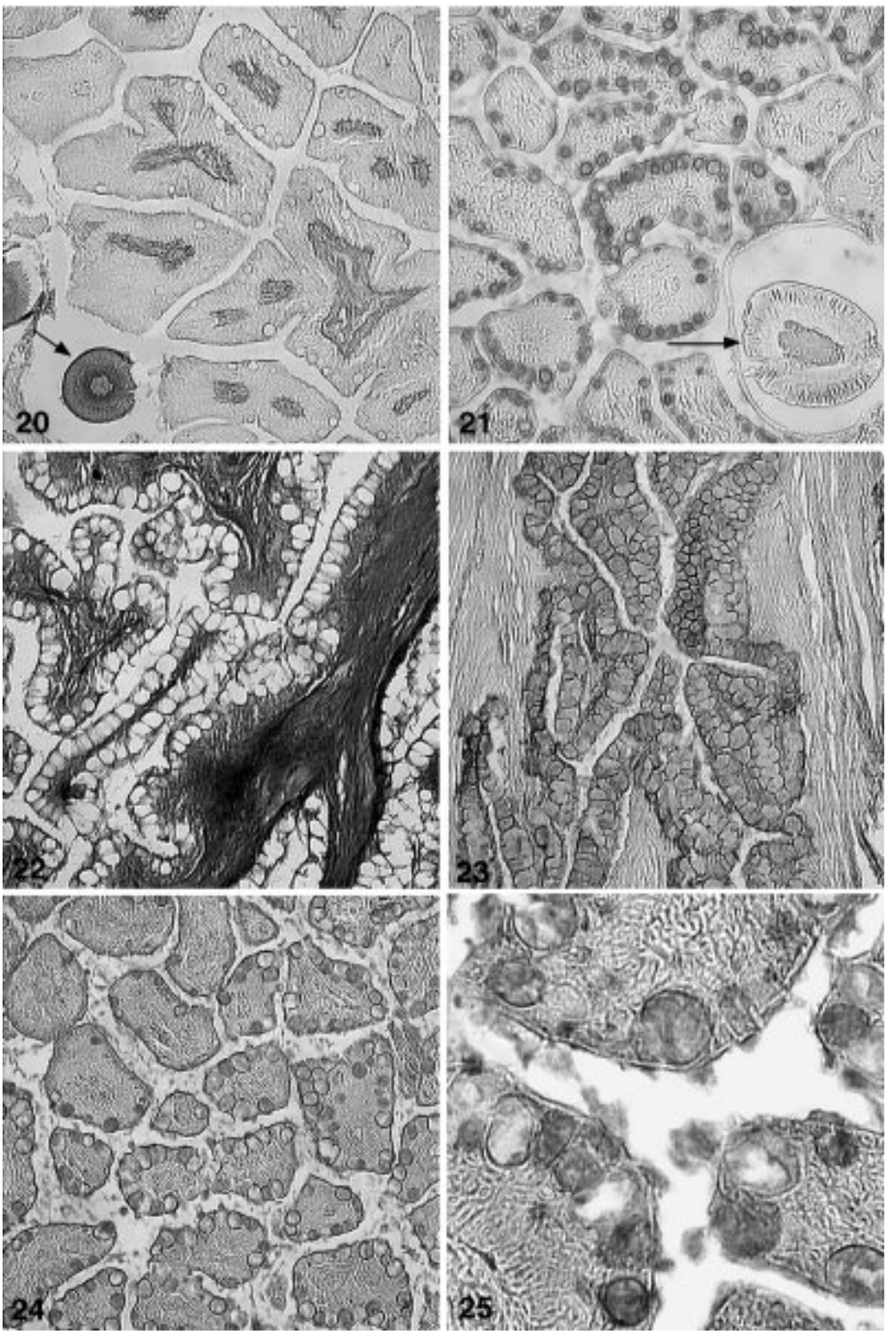


Table V
Histochemical distribution of carbohydrates and proteins in oesophagus of *S. senegalensis*

	Epithelium	Goblet cells	Lamina Propria	Submucosa	Muscular
Glycogen	0	0	0	0	0
Neutral glycoproteins	0-1	0-1	2	1-2	1
Carboxylated groups	0-1	3	0	0	0
Sulphated glycoconjugates (weakly ionised)	0-1	2-3	0	0	0
Sulphated glycoconjugates (strongly ionised)	0	2	0	0	0
Hydrochloric acid hydrolysis AB pH 2.5	0	0-1	0	0	0
Esterification-AB pH 2.5	0	0	0	0	0
Esterification –Saponification-AB pH 2.5	0-1	3	0	0	0
Esterification-AB pH 1.0	0	0	0	0	0
Esterification –Saponification-AB pH 1.0	0	0	0	0	0
Esterification-AB pH 0.5	0	0	0	0	0
Esterification –Saponification-AB pH 0.5	0	0	0	0	0
Proteins in general	2	0-1	2-3	2-3	2-3
Proteins rich in lysine	2	1	2	1-2	2
Proteins rich in tyrosine	2	0	1	1	2
Proteins rich in arginine	1	0	2	2-3	3
Proteins rich in tryptophan	0	0	0	0	0
Proteins with cysteine residues	3	2-3	2-3	2-3	2-3
Proteins with cystine residues	2	2	2	2	2-3

Results are expressed as semiquantitative assessment of colour intensities by independent scores of two investigators. Estimated scale ranging from 0 (unreactive) to 3 (strongly reactive).

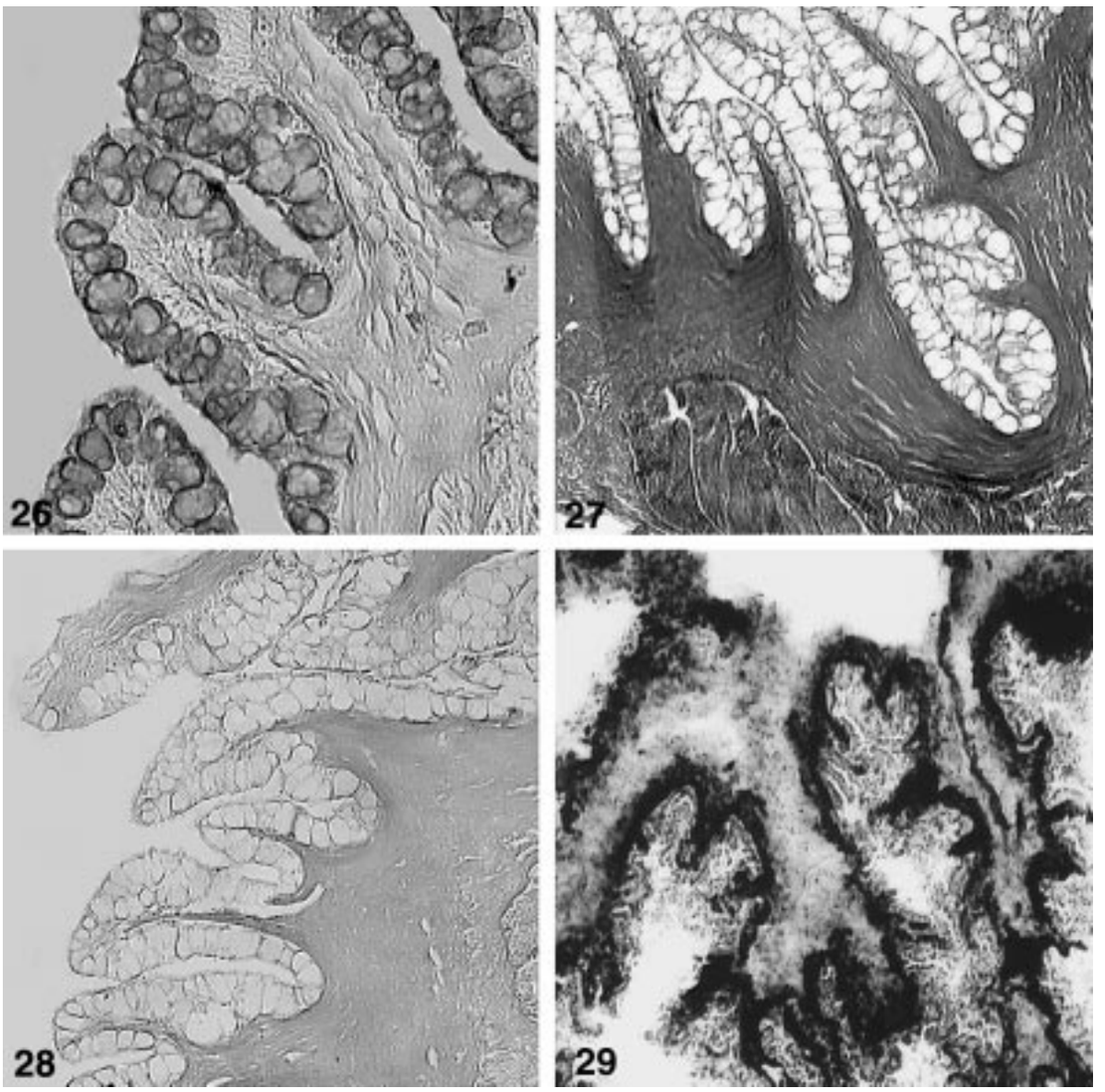
cells of the oesophagus of *S. senegalensis*, as in *Mugil saliens* (Elbal and Agulleiro, 1986b); mucous secretion could protect from the abrasion that nutritive particles produce (Elbal and Agulleiro, 1986b). Glycoproteins containing sialic acid were also observed in the oesophageal mucous cells of *S. senegalensis*. Reifel and Travill (1977) postulated that the large amount of mucus secreted does not seem to be explicable simply as a lubricant. It is important to note that salivary glands are lacking in most fish species so the oesophageal mucous cells could have the same function as mammalian saliva in protecting the mucosa of the entire alimentary canal. This notion was also expressed by Scocco *et al.* (1998) regarding the tilapine oesophagus althought here the sialoglycoconjugates displayed an organisation which was unusual in mammals (Klein *et al.*, 1992; Wu *et al.*, 1994). As Zimmer *et al.* (1992) reported, the presence of sialic acid residues in mucous cells prevents viruses from recognising their receptor determinants and also protects the mucosa from attack by sialidase produced by bacteria.

Neutral and acid mucins were also detected in intestinal goblet cells of *Solea solea* larvae (Boulhic and Gabaudan, 1992), and sialosulphoglycoproteins and neutral glycoconjugates were observed during larval development of *Sparus aurata* (Sarasquete *et al.*, 1995; Domeneghini *et al.*, 1998). Weakly ionised sulphomucins were detected in intestinal goblet cells of adult (Arellano *et al.*, 1999), larvae and juvenile *Solea senegalensis* specimens (Ribeiro *et al.*, 1999; Vieira, 2000).

The weak presence of neutral mucosubstances and acid mucins in the oesophagus of *S. senegalensis* was also observed in *Seriola dumerili* (Grau *et al.*, 1992). According to Kapoor *et al.* (1975), the oesophagus of fish species has a secretory rather than an absorptive function.

The protein content of the different portions of the digestive tract of teleost has rarely been investigated using histochemical tests. Goblet cells of the Sene-

Figs. 20/25 - **(20)**Dental formation (→). PAS (x10). **(21)**Dental formation (→). Alcian blue pH 2.5 (x10). **(22)**Papillae mucosa. Bromophenol blue (x10). **(23)**Papillae mucosa. Cysteine (x10). **(24)**Papillae mucosa. Cystine (x10). **(25)**Magnification of goblet cells. Cystine (x40).



Figs. 26/29 - **(26)**Oesophageal mucosa. Alcian blue pH 2.5/PAS (x20). **(27)**Oesophageal mucosa and mucular. Bromophenol Blue (x10). **(28)**Oesophageal mucosa. Tyrosine (x10). **(29)**Oesophageal mucosa. Acid phosphatase activity (x5).

gal sole oesophagus contained –S-S and –SH groups; proteins rich in lysine, tyrosine, arginine, cystine and cysteine were observed in the epithelial cells, lamina propria, submucosa and muscularis. Similar results were observed in *Sparus aurata* oesophagus (Arellano, 1995).

As in other fish species (Arellano, 1995; Grau *et al.*, 1992; Sarasquete *et al.*, 1995; Ribeiro *et al.*, 1999), the mucous cells of the oesophageal and buccal cavity of *S. senegalensis* were negative for the lipid reactions and enzymatic activities.

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Table VI
Histochemical distribution of lipids and enzymatic activities in oesophagus of *S. senegalensis*

	Epithelium	Goblet cells	Propria Lamina	Submucosa	Muscular
Unsaturated lipids	0	0	1	1	1
Neutral lipids	0	0	0	0	0
Acid lipids	0-1	0	0	0	0-1
Phospholipids	1	0	1	1	0
Alkaline phosphatase	0	0	0	0	0
Acid phosphatase	1	0	2	2	2-3
ATPase (pH 7.2)	1	0	2	2	2
ATPase (pH 9.4)	1	0	1	1	1

Results are expressed as semiquantitative assessment of colour intensities by independent scores of two investigators. Estimated scale ranging from 0 (unreactive) to 3 (strongly reactive).

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