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Histochemical study of skin and gills of Senegal sole, *Solea senegalensis* larvae and adults

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Summary. A battery of horseradish peroxidase-conjugated lectins (Con A, WGA and DBA), as well as conventional histochemical techniques (PAS, saponification, Alcian Blue pH 0.1, 1, 2.5, chlorhydric hydrolisis, neuraminidase, Bromophenol blue, Tioglycollate reduction and Ferric-ferricyanide-FeIII) were used to study the content and distribution of carbohydrates, proteins and glycoconjugate sugar residues on the skin and gills of Senegal sole, *Solea senegalensis* larvae and adults.

During larval development of *Solea senegalensis* (from hatching until day 45 posthatching), epidermal sacciform, as well as branchial and epidermal chloride cells were unreactive with all cytochemical tests performed in this paper. Mucous or goblet cells of the corporal skin and gills containing strongly sulphated acid glycoproteins were evident on days 15-20 of larval development, as well as in epidermal and branchial mucous cells of adult specimens, which also contained GlcNAc and/or sialic acid. In adult specimen, the proteic content was higher in branchial mucous cells than in epidermal cells. In larvae, variable amounts of glycoproteins containing sialic acid, GlcNAc, GalNAc, Man and/or Glc residues were observed in epithelial cells and/or cuticle. GlcNAc and/or sialic acid sugar residues were only weakly detected in glycoproteins of some epidermal and branchial mucous cells of larvae by day 45, because from hatching until metamorphosis, lectin reactions (WGA, Con A and DBA) were negative in mucous cells.

Key words: Skin, Gills, Glycoproteins, Carbohydrates, Proteins, Histochemistry, Larvae, Adults, *Solea senegalensis*

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Introduction

In fish, the skin is the primary barrier against the environment, allowing normal internal phisiological function, so its condition is important in many disease processes. It serves in the exchange of gases, water, ions and large molecules (respiration and osmoregulation) and it has a variety of secretory (mucoid and cuticular) and sensory (chemo- and neurosensory) functions. The mucous or goblet cells produce the mucus which covers the body and they are found distributed over the skin, on the gills and lining the digestive tract (Roberts, 1978; Gona, 1979; Groman, 1982; Sarasquete et al., 1995, 1996, 1998). The glycoproteins found in mucus are either neutral or acid. Neutral glycoproteins stain magenta/red when subjected to the PAS staining procedure, while acid glycoproteins take on a blue coloration when the tissue is stained with Alcian Blue (AB) pH 2.5. Acid glycoproteins, however, may contain either sialic acid (sialomucins) or sulphated esters (sulphomucins). Only the sulphated glycoproteins continue to stain blue when the pH of AB ranges between 0.5 and 1 (Mittal and Banerjee, 1975; Gona, 1979; Sarasquete et al., 1989, 1996). Mucous or goblet cells of *Anguilla anguilla*, *Halobatrachus didactylus* and *Sparus aurata* skin contain glycoproteins rich in sialosulphomucins. The absence of ß- and α-galactose (Gal), N-acetyl-galactosamine (GalNAc) and α-L-fucose (Fuc) glycoconjugate sugar residues were notable in cuticle, germinative and/or goblet cells of the skin of these species (Illana, 1993; Sarasquete et al., 1998).

On the other hand, the skin mucus may be important in natural defense against parasites and pathogenic microorganisms (Fletcher, 1978). Changes in the number and dimensions of mucous and chloride cells can be an indicator of pathological or inflamatory processes induced by the environment (Lemoine and Olivereau, 1971; Wendelaar-Bonga and Lock, 1992; Lee et al., 1996a). The sialic acid content has been used to estimate the degree of skin mucification in fishes (Arillo et al., 1979). Moreover, sialitation and sulphation of the glycoproteins, components of the mucous cells, may be

important for increasing the resistance of mucus to bacterial degradation (Rhodes et al., 1985).

In fish, chloride cells -ionocytes or mitochondria-rich cells- are the primary osmoregulatory cells located in gills and skin (Mittal and Banerjee, 1975; Marshall and Nishioka, 1980; Lee et al., 1996a,b). These cells contain large basal nuclei, numerous cytoplasmic vesicles, and mitochondria with enlarged separated cryptae and also contain microvilli on their free surface (Groman, 1982). In adult fish, the function of chloride cells is reflected in their ionic composition and their specific enzyme equipment and they have been shown to contain Na^+/K^+, Ca^{++} and Mg^{++} ATPases (Marshall and Nishioka, 1980; Naon and Mayer-Gostan, 1983). Recently, Watrin and Mayer-Gostan (1996) described a method which allows the simultaneous recognition of ionocytes and mucous cells in the gill epithelium of turbot.

In teleosts, active ionoregulation begins in the early stages of the development. Since gills and kidney are still under -or poorly- developed at these stages, the skin has been presumed to be the functional site of ionoregulation (Weisbart, 1968; Guggino, 1980a,b; Morrison, 1993). Instead of branchial respiration, fish larvae rely on cutaneous gas exchange. Therefore, even if the larvae possessed morphologically differentiated gills, gill respiration would be rather ineffective (Segner et al., 1994). The skin of the newly-hatched fish has several important physiological functions. Apart from the protective role common to all intergumentary surfaces it has to act as a selective osmoregulatory membrane and the site for respiratory transfer, since the gill bars lack filaments at hatching and there is no functional blood vascular system (Roberts et al., 1973; Lee et al., 1996a). Thus, it is essential for efficient respiratory and osmotic function that the epidermis of larvae be relatively thin to allow gas exchange at this requirement takes precedence over the structural strength required for the physical protection function of the adult (Roberts et al., 1973; Morrison, 1993).

In fish larvae, chloride cells may be related with osmoregulatory and respiratory functions and during larval development, these cells can be located in yolk-sac, pectoral fins, gill chambers and/or body skin (Lasker and Threadgold, 1968; Hwang, 1989; Ayson et al., 1994; Padros, 1994; Lee et al., 1996a). However, Leatherland and Lin (1975) found no chloride cells in skin of *Clupea harengus* larvae and in yolk sac of embryo and alevin of *Oncorhynchus kisutch*. Homologies and differences between epidermal and branchial chloride cells of larvae have been discussed (Lasker and Threadgold, 1968; Roberts et al., 1973; Padros, 1994).

Senegal sole, *Solea senegalensis* is a species well adapted to warm climates and commonly exploited in extensive aquacultural production of some southern European countries, such as Spain (Drake et al., 1984; Rendón et al., 1997) and Portugal (Dinis, 1992; Ribeiro et al., 1997). Important mortalities and/or diseases have been described during larval development of different species. In flatfish larvae, pathological alterations have been related with pathogen agents, swimbladder alterations, starvation, inert diets, defficient enzymatic activities, respiratory problems, abnormal pigmentation, etc (Padros, 1994). In this paper, we report some histological-histochemical characteristics of the skin and gills of the Senegal sole, *Solea senegalensis* larvae (from hatching until day 45) and adult specimens.

Materials and methods

Senegal sole, *Solea senegalensis* larvae from hatching until day 45 posthatching, as well as skin and gills of adults specimens were fixed in Bouin's solution or in 10% v/v buffered formaldehyde (pH 7.2) and embedded in paraffin. Sections of 5-7 μm thickness were stained with Haematoxylin-eosin and Haematoxylin-V.O.F (Sarasquete et al., 1993, 1995). Cytochemical tests for carbohydrates (PAS, diastase-PAS; Alcian Blue pH 0.5,1 and 2.5; neuraminidase-type V from *Clostridium perfringens*, Sigma St Louis, M.O.- and/or chlrorydric hydrolisis; acetylation and saponification), glycoproteins (lectins) and proteins (Bromophenol blue, Ferric-Ferricyanide-FeIII and reduction with Tioglycollate) performed in this paper are described in Pearse (1985) and Bancroft et al. (1990).

For study of lectins, sections were treated with 0.3% hydrogen peroxide for 10 minutes (to inhibit the endogenous peroxidase) in Tris-buffered saline (TBS) at pH 7.2. The sections were incubated for 30 minutes at room temperature in horseradish peroxidase-conjugated lectins (HPR-lectin conjugated) dissolved in TBS (20 μg/ml): Con A (Mannose -Man- and/or Glucose -Glc-), WGA (N-acetyl-D-glucosamine -GlcNAc- and/or sialic acid -NANA-) and DBA (N-acetyl-D-galactosamine -GalNAc-). After three washes in TBS, peroxidase activity was visualized with TBS containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride and 0.015% hydrogen peroxide. Sections were washed in running taper water for 10 minutes, dehydrated, cleared and mounted in Eukitt. Substitution of lectin-HPR conjugates by TBS and pre-incubation of the sections with sugar inhibitors (Methyl-α Man, D-GlcNAc and D-GalNAc) were used as controls.

Results

The skin of *Solea senegalensis* adults was composed of a cuticle, epidermis, dermis and hypodermis. The stratified squamous epithelium of the epidermis was constituted by germinative or basal cells, epithelial or malpighian cells and mucous or goblet cells. The mucous cells were of variable dimensions, open on the surface by small pores through which they discharged their secretions forming a thick coat on the surface of epidermis (Fig. 1A-D). The mucous cells were more numerous in tail and fins than in corporal skin. While some chloride cells were present in *Solea senegalensis*

skin, clavate cells were not observed in this species.

Some histochemical reactions performed in this paper (Table 1) were positive in epidermal and branchial mucous cells of adult specimens (Fig. 1) but chloride cells of larvae and adults were unreactive for carbohydrate, protein and lectin reactions. Thus, some mucous cells were weakly positive to PAS reaction and strongly stained with Alcian Blue 0.5 and 2.5 (Fig. 1B, C, E), and specially with Alcian Blue pH 1. When Alcian Blue pH 2.5-PAS reaction was performed, most mucous cells stained blue (acid mucins), some mucocytes stained red (neutral mucins) and a few mucous cells stained purple, indicating a combination of neutral and acid mucins (Table 1). PAS reactivity was weakly increased after saponification process, suggesting the presence of acetylated syalic acid. Moreover, sialic acid was also evidenced in epidermal and branchial mucous cells (Fig. 1D) with WGA-lectin (N-acetyl-D-glucosamine -GlcNAc-and/or Sialic acid -NANA-), because a slight decrease in WGA staining and a decreased alcianophilia (Alcian Blue pH 2.5), after neuraminidase treatment, was observed in these goblet cells. Glycoproteins containing acetylated sialic acid, GlcNAc, GalNAc, as well as Man and/or Glc sugar residues were detected in cuticle and epithelial cells of the skin (Table 1). In epidermal and branchial mucous cells, glycoproteins containing acetylated sialic acid and sulphated groups (weak and strongly ionizated) were observed (Fig. 1E). Proteins rich in cysteine-bound sulphydryl (-SH) and cystine disulphide (-S-S-) groups were detected in branchial mucous cells (Fig. 1F), but these reactions were very weak or negative in epidermal mucous cells (Table 1).

At hatching, the skin of *Solea senegalensis* larvae consisted of only two layers of squamous cells overlying a considerable dermal space. The majority of epithelial cells appeared densely stained and developed into malpighian cells of the adult specimens. The other components of the skin larvae were the numerous «empty» sacciform/mucous cells (Fig. 2A,B), as well as the chloride cells, located specially on the rostral region. At this period, PAS and Alcian blue reactions (pH 0.5, 1 and 2.5) were negative in the sacciform/mucous and chloride cells. At the beginning of larvae development, the mouth had not yet opened, and the digestive system was still rudimentary. Only part of the pharynx had formed and was connected with lateral gill chambers, which eventually opened to the outer medium. Numerous chloride cells occurred on the inner surface of the gill chambers and skin near the openings of the branchial chambers, in head and tail.

During larval development, and from day 12-13 onwards, we observed an increase in the mucous cell number (Fig. 2C-F), which first started to secrete neutral mucins (PAS and diastasa-PAS positive) and later (by day 15-20) also synthesized acid (carboxylated and sulphated) mucins. The dermis remained poorly developed although the amount of melanine (Fig. 2C) present in the upper dermis (*stratum spongiosum* in adults) was increased.

In 17-20-day-old larvae, the skin presented a dermis with conective fibers -collagen-; an acidophilic basal membrane was now evident between epidermis of dermis. During *Solea senegalensis* larvae development, the number of chloride cells appeared to increase in the opercular areas and through gill epithelium. Chloride cells observed in gills and skin of larvae and adults (Fig. 2E, F) were unreactive with all histochemical tests performed in this study. From Senegal sole metamorphosis (around 17-20th day), the epidermal mucous cells containing carboxylated and sulphated glycoproteins (Alcian blue pH 0.5, 1 and 2.5) increased progressively, while the «clear or empty» sacciform cell number appeared to decrease parallely. From hatching until metamorphosis of *Solea senegalensis*, the mucous

Table 1. Histochemical results of the skin and gills of *Solea senegalensis* adults.

	CUTICLE	EPITH	GERM	MucS	MucG
Schiff (Free aldehydes)	0	0	0	0	0
PAS (glycoconjugates with 1,2 gycols or amino-ol)	1	1	1	1	2
Diastase/PAS (Glycogen)	1	0-1	0-1	1	2
KOH/PAS (Reactivity of acetylated hydroxyl and methylated carboxyl groups)	1	1	1	1	3
Alcian Blue 2.5-PAS (Acidic and neutral mucins)	P	R	R	B/R/P	R/B/P
Alcian Blue 2.5 (Acidic groups, specially Carboxylated)	1	0	0	2	2
Neuraminidase or Chlroridric hydrolisis/ Alcian Blue 2.5 (Sialic acid)	0-1	0	0	1	1
Alcian Blue 0.5 (Sulphated groups strongly ionizated)	0	0	0	2	3
Alcian Blue 1 (Sulphated groups weakly ionizated)	0	0	0	1	3
WGA (GlcNAc/NANA)	1	0	0	1-3	1-3
Neuraminidase/WGA	0-1	0	0	0-1	0-1
ConA (Man/Glc)	1	1	1	0	0
DBA (GalNAc)	0-1	1	1	0	0
Bromophenol Blue (General Proteins)	1	1	1	0-1	2
Ferric-Ferricyanide-FeIII (Cysteine groups)	0	0	0	0	2
Tioglycollate/ Ferric-Ferricyanide -FeIII (Cystine groups)	1	1	1	0-1	1

Results are based on a subjectively estimated scale ranging from 0 to 3 with 0 being unreactive and 3 strongly reactive. EPITH: epithelial cells; GERM: germinative or basal cells; MucS: mucous cells of skin; MucG: mucous cells of gills. R: red; B: Blue; P: purpure: a mixture of neutral (R) and acidic (B) groups.

cells were unreactive with WGA, DBA and Con A lectins. In 45-day-old larvae, a few epidermal and branchial mucous cells were weakly (Fig. 2D) or strongly (Fig. 2E) stained with WGA and a weak staining was observed with Bromophenol blue reaction (general protein) (Fig. 2C). In adult specimens, these reactions were more intense in some mucous cells (Table 1), and epidermal and branchial mucous cells contained protein-rich cystine disulphide (-S-S-) groups. Cysteine-bound sulphydryl groups were observed in mucous branchial but not in epidermal mucous cells (Fig. 1; Table 1). Cuticle and epithelial cells of *Solea senegalensis* skin larvae and adult specimens were stained with different lectins (Table 1).

From hatching, several neuromasts were observed on the head epithelium of the *Solea senegalensis* larvae skin. Melanocytes (Fig. 1B, C) were also observed from hatching, isolated under epidermis; they increase during larval development and specially during metamorphosis (about the 20th day). Melanophores were located in both the *stratum spongiousum* and the hypodermis. Some eosinophilic cells were observed in 45-day-old larvae skin. They appeared for the first time in the basal regions of the fin epithelium and later throughout the skin epithelium. The cytoplasm of these cells contained acidophilic granules and showed similar characteristics to eosinophil-leucocytes.

Discussion

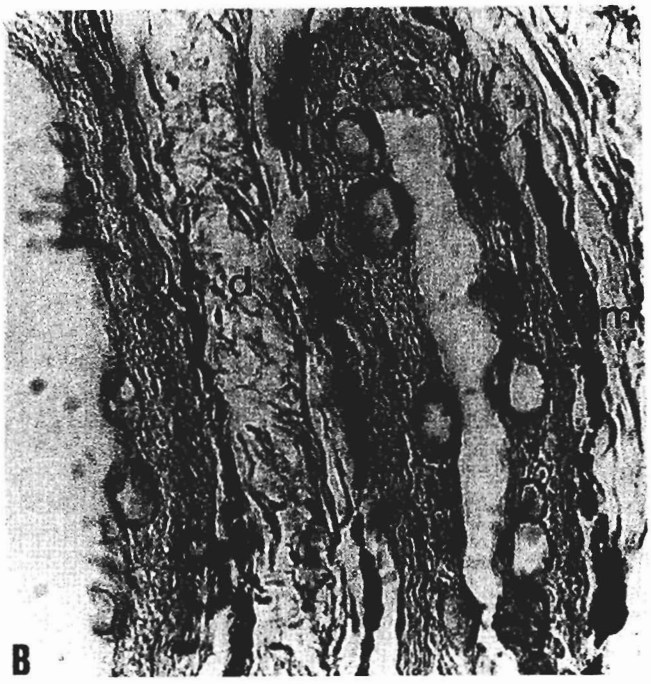
The skin of Senegal sole, *Solea senegalensis* adult shows its general morphology similar to other species without scales. The epidermis is a stratified squamous epithelium covering the surface corporal. The epidermis is usually thicker in species like eel, *Anguilla* sp. with negligible scale cover and with numerous mucous cells (Roberts, 1978; Fouda, 1979; Sarasquete et al., 1989) and consists of a tightly condensed layer of malpighian cells with the basal cells showing columnar orientation and above these a transition to horizontal flattening; mucous cells are interspersed and appear as spheres opening to the surface within the uper zone. Goblet or mucous cells usually originate in the middle layers of the epidermis; they increase in size and elaborate secretions, mainly glycoproteins, as they approach the surface (Roberts, 1978).

Mucous secretion of the teleost skin presents interspecific differences (Fletcher and Grant, 1968; Askawa, 1970; Harris et al., 1973; Sarasquete et al., 1989; Illana, 1993). Mucous cells of the *Solea senegalensis* skin contain sialosulphoglycoproteins, because these mucous cells were weakly stained with PAS and diastase-PAS (neutral mucins), and strongly stained with Alcian Blue pH 0.5, 1 and 2.5 (acidic mucins). These results, as well as the decreased reactivity after neuraminidase treatment previous to WGA stain, could suggest the presence of sialic acid, as well as the absence of glycogen (diastase-PAS positive) such as was evidenced in other species, whose mucous cells were unreactive with the Bromophenol blue reaction (general proteins) (Bullock et al., 1976; Sarasquete et al., 1989). However, the Bromophenol blue technique and the PAS reaction were positive in some mucous cells of the *Sparus aurata* skin (Sarasquete et al., 1997) and specially in branchial mucous cells of *Solea senegalensis* adults. Basic and acid proteins were observed in mucous cells of *Channa striata* epidermis (Mittal and Banerjee, 1975). According to Harrison et al. (1987) variability in staining within a given cell could be attributed to a temporal sequence of biosynthesis of the mucous secretion. Biosynthesis of mucin glyco-conjugates includes at least two post-transcriptional modifications to the secretory protein; firstly glycosilation of the protein followed by modifications to the sugar moiety (Phelps, 1978). Those cells that did not stain with PAS contained only proteins; mucous cells staining with PAS could be related to the stage when the cell was producing mainly glycoproteins: these cells would stain with Alcian Blue when the glycoproteins had been carboxylated and the presence of sulphated glycoproteins would coincide with the stage when the sulphated groups had conjugated to the glycoprotein (Els and Hennerberg, 1990).

Proteins rich in cystine disulphide (-S-S-) groups were observed in cuticle, epithelial and mucous cells of skin and gills of *Solea senegalensis* adults. In skin of different species, the presence of both cysteine and cystine groups and the presence of protein-bound NH_2 groups suggests that the epithelial cells of the outermost layer in the epidermis are undergoing the process of keratinization (Mittal and Banerjee, 1975; Mittal et al., 1976). Proteins rich in basic groups -lysine and arginine- are present in epidermal and branchial goblet cells (data not presented) of *Solea senegalensis*; -SH groups were observed only in branchial mucous cells while cystine-bound disulphide (-S-S-) groups were also detected in cuticle, epithelial and mucous cells of the skin. In *Sparus aurata* skin, the presence of cystine bridges have been related to the glycoproteic nature of the mucous secretion (Sarasquete et al., 1998).

Sialic acid has been used to estimate the degre of skin mucification in different species (Lemoine and Olivereau, 1971; Harris et al., 1973; Pickering, 1974). Histochemically, Illana (1993) showed the presence of

Fig. 1. Histological sections of skin and gills of *Solea senegalensis* adult specimens. **A.** Some epidermal mucous cells (mc) contain proteins. Bromophenol Blue reaction. **B.** Sulphated glycoproteins in mucous cells (mc) of the skin. Melanocytes (m) are detected between epidermis (e) and dermis (d). Alcian Blue pH 0.5. **C.** Acidic glycoproteins in epidermal mucous cells (mc). Alcian Blue pH 2.5. **D.** A few epidermal mucous cells (mc) are strongly stained with WGA lectin. **E.** Neutral and acidic glycoproteins in branchial mucous cells (mc). The chloride cells (cc) are negative. Alcian Blue pH 2.5-PAS. **F.** Branchial mucous cells (mc) containing cystine-bound disulphide (-S-S-) radicals. Chloride cells (cc) are not stained. Tyoglycollate reduction-Ferric-Ferricianyde- Fe III. x 400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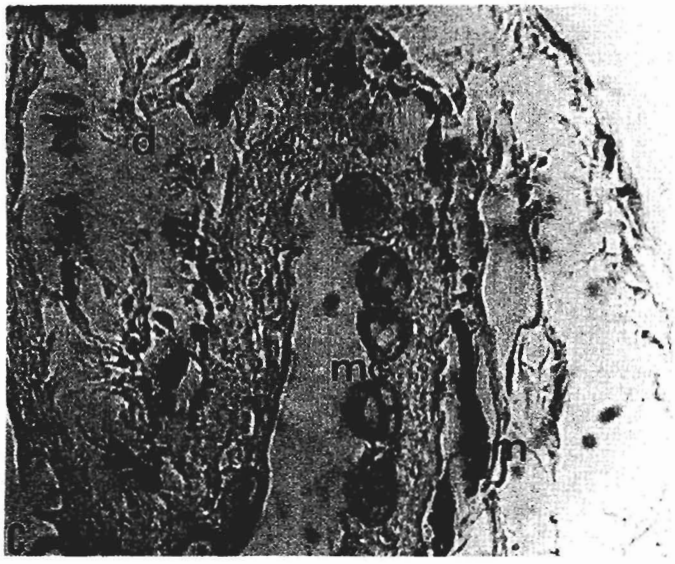
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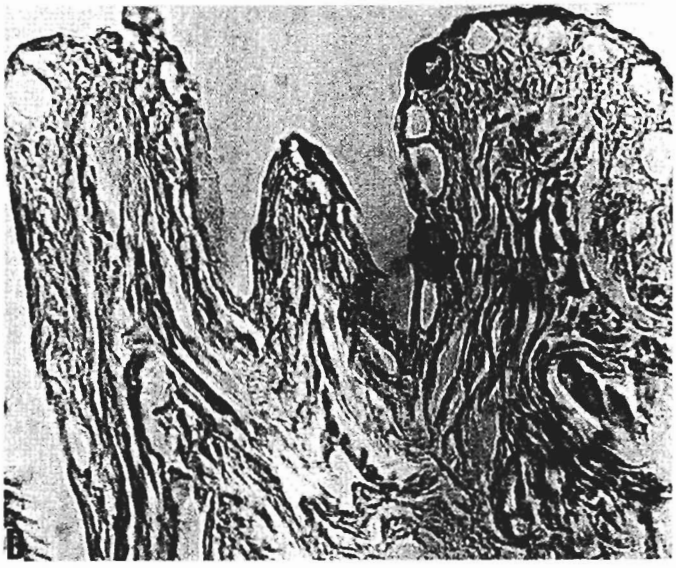

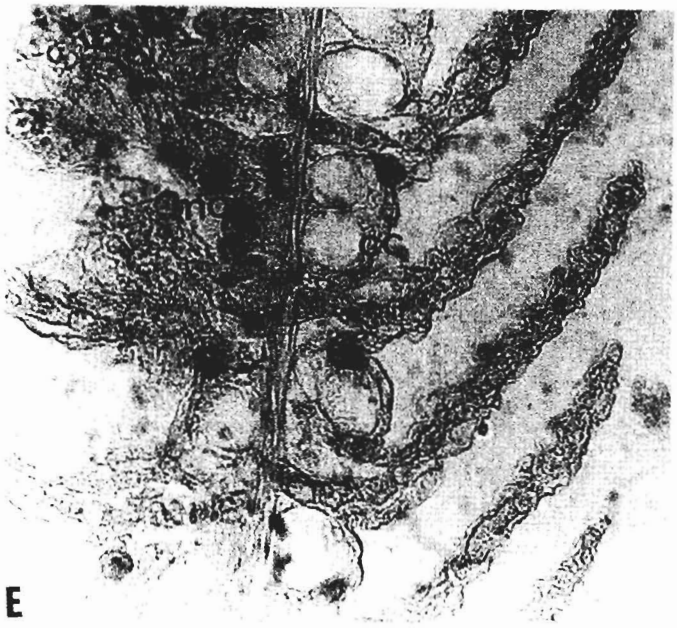
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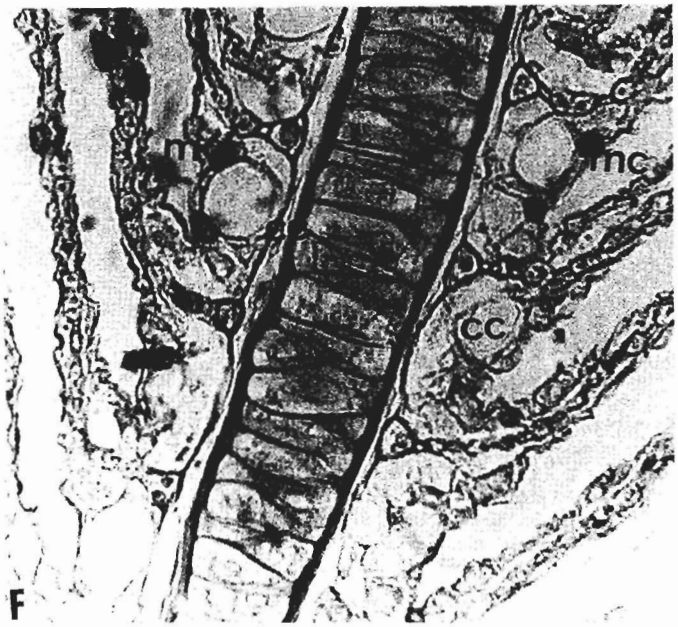
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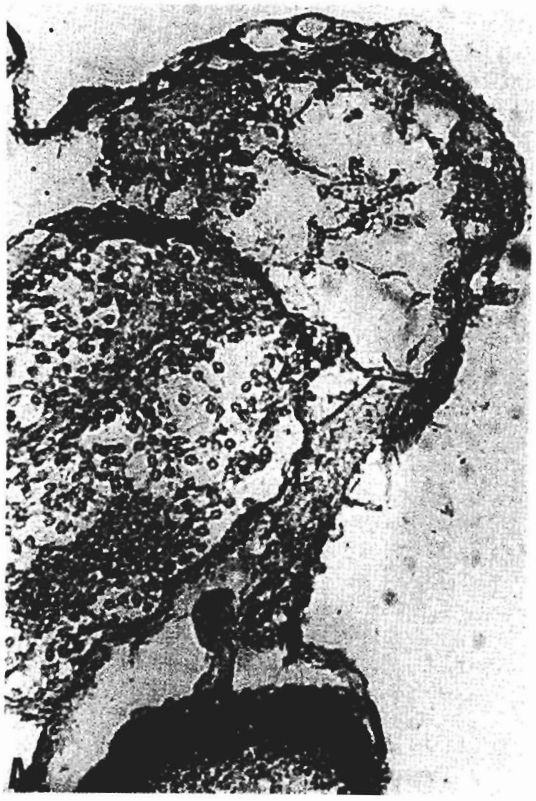
sialic acid, GlcNAc, as well as Glc and/or Man glycoconjugate sugar residues in goblet cells and cuticle of *Halobatrachus didactylus* and *Anguilla anguilla* epidermis. According to this author, goblet cells and cuticle of *Halobatrachus didactylus* failed to bind DBA, indicating the absence of GalNAc residues, which were detected in *Anguilla anguilla*. In *Sparus aurata*, the results obtained by conventional mucin histochemistry and by using lectins suggest that WGA-reactivity in epidermal goblet cells was due to GlcNAc and sialic acid residues (Sarasquete et al., 1998), such as was observed in skin (cuticle and mucous cells) of *Solea senegalensis* adults; glycoconjugates containing these saccharide residues were weakly detected in some mucous cells of skin and gills of larvae about the 45th day, because from hatching until metamorphosis, the WGA lectin was negative in epidermal and branchial mucous cells of larvae.

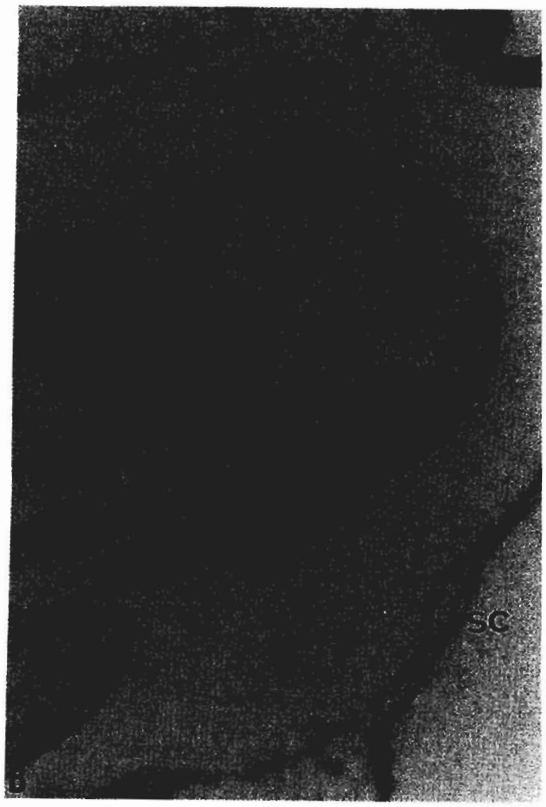
During larval development of Senegal sole, *Solea senegalensis*, as in other species, the skin shows significant morphological and physiological modifications. The thickness of the epidermis, the obliteration of dermal space with connective fibers, as well as the epidermal cells varies greatly during development (Roberts et al., 1973; Shen and Leatherland, 1978; Morrison, 1993; Padros, 1994). Epidermal sacciform and/or mucous cells, malphigian or epithelial cells, as well as chloride cells («clear cells» according to Roberts et al., 1973) have been described during larval development of different species (Lasker and Threadgold, 1968; O'Connell, 1981; Morrison, 1993; Padros, 1994; Lee et al., 1996a). Some differences beween chloride cells located in branchial and epidermal epithelia of larvae have been pointed by Roberts et al. (1973).

The skin of the newly-hatched Senegal sole, as in other species (Roberts et al., 1973; Whitear, 1986; Padros, 1994) consists of only two layers of squamous epithelial cells through which gas exchange probably takes place, since the gill filaments are not yet developed. A large, fluid-filled space separates the epidermis from the mesoderm. As the Senegal sole larvae develops, the mucous cell number and their acidic glycoproteic secretion increase, while the «clear» sacciform cell number appeard to decrease. Padros (1994) in *Scophthalmus maximus* suggested that possibly the sacciform cells are transformed, during development, into active mucus-secreting cells. In Senegal sole larvae, sacciform cells were unreactive for all morphological and histochemical tests performed in this study. The metamorphosis, about the 17-20th day in Senegal sole (Ribeiro et al., 1997), denotes a major change in the ecology and physiology of the fish, and it might be expected that changes would occur in the developing skin. During Senegal sole larval development, and specially in 12-15-dayold larvae, mucous cells are increased and they start to synthesize carboxylated and sulphated mucins (weakly and strongly ionizated). In *Pleuronectes platessa*, these acid mucins appear later in 30-day-old larvae (Roberts et al., 1973). According to Singh and Mittal (1990), the mucous cells in initial stages of carp development, located in deeper layers, appear to synthesize only neutral mucopolysaccharides, the acid mucopolysaccharide moieties, both sulphated and non-sulphated, being gradually incorporated as they are further differentiated and pushed towards the surface. Similar results are observed in *Solea senegalensis* larvae.

Chloride cells are present in skin of *Solea senegalensins* larvae at hatching, such as was observed in other species (Hwang, 1989; Morrison, 1993; Padros, 1994). From metamorphosis, chloride cells are located, specially, in branchial and opercular epithelia, as well as in rostral region and tail of the Senegal sole larvae. According to Roberts et al. (1973) in *Pleuronectes platessa* larvae, between the 30-60th days, all chloride cells («clear cells», according this author) are showing partial or complete breakdown of the membrane systems of the mitocondria and smooth-walled tubules and appear in many cases to be breaking up on the surface. Lasker and Threadgold (1968) assumed that the mitochondria-rich cells which they found in the sardine skin were the larval equivalent of the so-called «chloride cells» of the teleost gill lamellae to which has been ascribed the function of osmoregulation under changing conditions of salinity. However, Roberts et al. (1973) pointed out that although these «clear or chloride cells» may be related to osmoregulatory and respiratory functions, they may well have a different function from the chloride cells of gills. In *Pleuronectes platessa*, and in sardine, *Sardinops caerulea* (Lasker and Threadgold, 1968) larvae, these cells were not eosinophilic. In different fish larvae, chloride cells show different staining affinities (Hwang, 1989). In *Solea senegalensis* skin larvae, both sacciform and chloride cells were clear or empty cells, without apparent tinctorial affinity by acid or basic stains. The typical gill chloride cells or mitochondrion-rich cells (Pisam and Rambourg, 1991; Ayson et al., 1994) are eosinophilic and they are not mucous secreting cells (Roberts et al., 1973; Padros, 1994). Similar observations were noted in branchial

Fig. 2. Histological sections during development of *Solea senegalensis* larvae. **A.** Head epithelium of a larva on day 2 posthatching with numerous empty clear- sacciform cells (sc) between the squamous epithelial cells (ec). Haematoxylin-VOF. **B.** Larvae at day 9. WGA-unreactive sacciform cells. The striated border (ss) of the digestive epithelium is strongly stained (positive control in Sarasquete et al., 1996). WGA lectin. **C.** Larvae at day 45. Absence or a very small presence (*) of proteins in mucous cells (mc) of the epidermis. The epithelial cells (ec), troncal musculature (tm), as well as the supranuclear vacuoles (sv) of the digestive system are stronlgy stained (positive control). Bromophenol Blue reaction (general proteins). **D.** Larvae on day 45 posthatching. Weak staining in cuticle (cu) and in epidermal mucous cells (mc). WGA lectin. **E.** Absence of sulphated glycoproteins in branchial and chloride cells (cc) around 10-15 day-old larvae. Alcian Blue pH 0.5. **F.** Gills of a larvae at day 45. Numerous WGA reactive mucous cells (mc) and unreactive chloride cells (cc) are observed. WGA lectin. a, c-e, x 400; b,f, x 250

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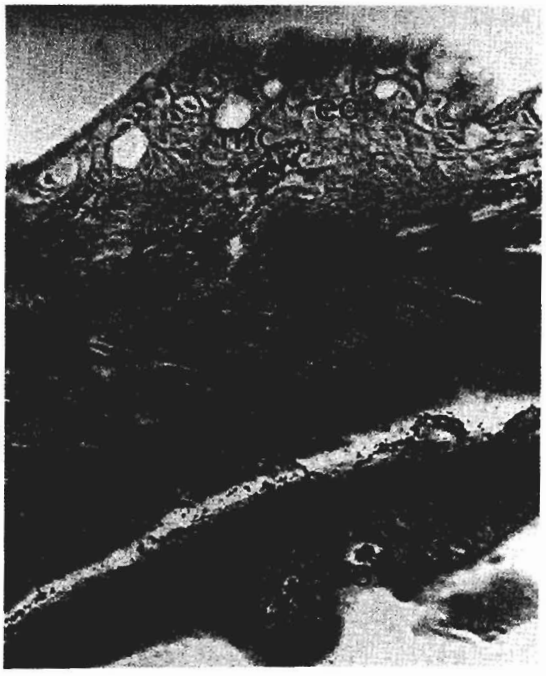

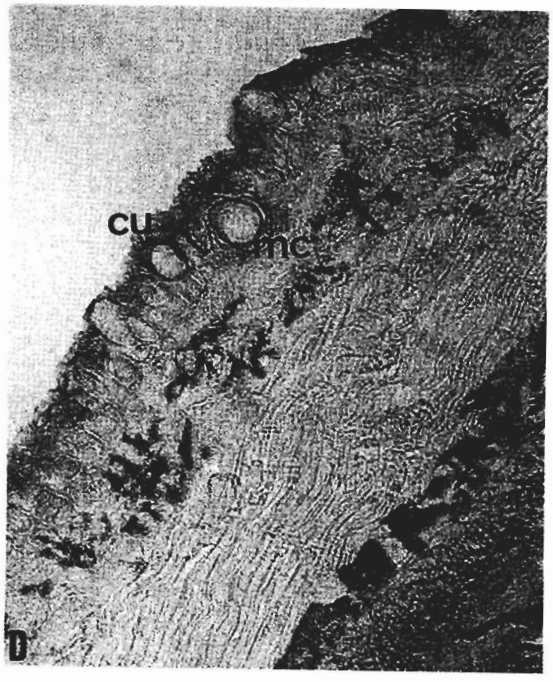
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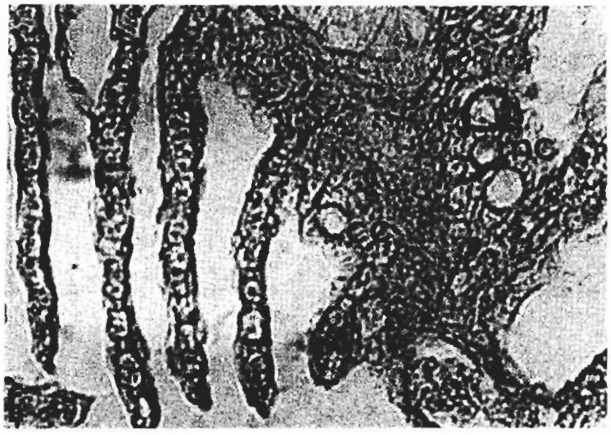

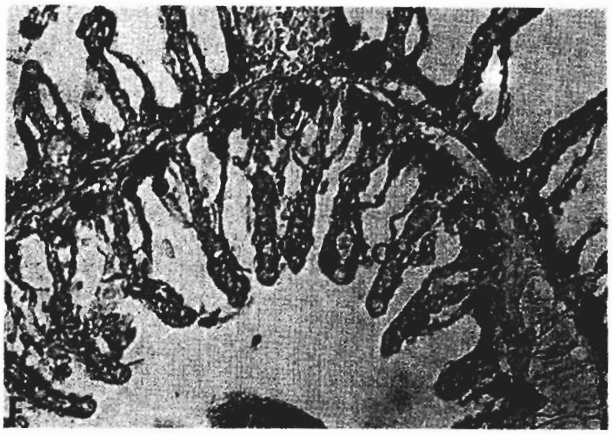

chloride cells of *Solea senegalensis larvae* and adults.

In *Solea senegalensis* larvae development, by the 45th day, such as was shown by Roberts et al. (1973) in *Pleuronectes platessa*, some eosinophilic granular cells (eosinophils) were observed in the epidermis. It is generally assumed that eosinophils are granulocytic leukocytes which are derived from multi-potent stem cells in haematopoietic tissue and, in fish, they have been implicated in specific immunological general defense processes (Powell et al., 1990). Eosinophils occur in abundance in skin of *Solea senegalensis* adults, as well as in blood, haematopoietic tissues, gut and gills of different species (Roberts et al., 1972; Sarasquete and Gutiérrez, 1982; Powell et al., 1990; Barnett et al., 1996). According to Mittal et al. (1976), the tryptophan present in eosinophilic granular cells in the epidermis of *Barbus sophor*, may be converted into 5 hydroxytryptamine -a potent vaso constrictor- to facilitate the antibody transfer across the epidermis, and thus may play an important role in the local defense mechanisms.

In *Senegal sole* larvae from hatching, as in other teleosts (Morrison, 1993; Padros, 1994), numerous neuromasts are observed on the epidermal head epithelium. In *Scophthalmus maximus* larvae (Padros, 1994), the presence of neuromasts has been related to a poor development of olfatory and visual systems.

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