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OBSERVATIONS ON THE HISTOCHEMISTRY OF THE ALIMENTARY CANAL OF *MYSTUS GULIO* (HAM.) (SILURIFORMES : BAGRIDAE)

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The mucopolysaccharides of mucous secreting cells and gastric glands present in the alimentary canal of *M gulio* have been investigated. Different types of mucous secreting cells were observed in the entire digestive tract ranging from a mixture of both neutral and carboxylated mucins in oesophagus and striated border of stomach to the highly acidic carboxylated mucins in the mucous cells of the intestine. Versatility of the oesophagus is seen in having club cells consisting of proteins and phospholipids. The nature of mucous secretions in the different parts of the alimentary tract are seen to be associated with the omnivorous nature of feeding of the fish.

INTRODUCTION

Histology of the alimentary canal of *Mystus gulio* was studied by Kamal Pasha (1964), who described the structure and distribution of secretory cells in the alimentary canal plays diverse role in the digestive process of the fish. There are different types of mucous secretory cells as described by Weinreb & Bilstad (1955), Bullock (1967), Bishop & Odense (1976), Bucke (1971), Western (1971), Suvorova & Treschuk (1973) and Danguy *et al.* (1985). Keeping in view the diversity and functional importance of the mucous secreting cells in the alimentary canal of *M. gulio*, emphasis in the present study is laid on the histochemistry of the different types of mucous secreting cells.

MATERIAL AND METHODS

Specimens of *M gulio* were maintained in the laboratory and after a

starvation of 6–10 days the fish were cut open and parts of the alimentary canal in the regions of oesophagus, stomach and intestine were removed and fixed in susa, Bouin's fluid and formol calcium. After fixation the material was dehydrated, cleared in xylene and embedded in paraffin wax of 58°C melting point. Transverse serial sections of 6–8 μm thickness were cut. For general histological observations, sections were stained with Azan; and Haematoxylin counter stained with eosin.

A number of histochemical tests have been conducted for elucidating the chemical nature of different layers of alimentary canal. Details of the techniques followed were mostly from Pearse (1968) and Bancroft (1975).

OBSERVATIONS

General histology of the alimentary canal

Histologically, the wall of the alimentary canal has four layers, like that of any typical vertebrate digestive tract. These are from inside, mucosa, submucosa, muscularis and serosa. The mucosal epithelium of oesophagus consists of mucous secreting cells and club cells. A few taste buds are found in the anterior oesophagus But they are more in number in buccopharyngeal mucosa.

The mucosa of stomach consists of columnar-epithelial cells and gastric glands. The columnar cells are tall, cylindrical and provided with a distinctly striated mucous secreting free border called "striated border" or "top plate". The gastric glands of stomach are simple, tubular, consisting of only single type of secretory cells.

The mucosal epithelium of the intestine is composed of two types of cells, the columnar epithelial cells and mucous secreting cells. The mucous secreting cells are few and scattered.

The submucosa which forms the second tunic is made up of highly vascularised connective tissue. A core of connective tissue of submucosa penetrates into the folds of the mucosa, forming tunica propria.

Muscularis forms the third layer of the wall of the alimentary canal consisting of two types of muscles, namely, outer circular muscles and inner longitudinal muscles in oesophagus. But in stomach, intestine and rectum the order is reversed.

The serosa forms the other most layer. It is very thin consisting of a layer of calls having subserous connective tissue.

Histochemistry

The mucous cells in the mucosal layer of oesophagus (Fig. 1A), intestine and striated border of stomach were intensely positive to periodic acid Schiff's (PAS) test showing rich quantities of carbohydrates and carbohydrate containing groups in them, while gastric glands of stomach were moderately positive to PAS. A negative reaction was obtained with Schiff's without oxidation The PAS reaction was found to be resistant to saliva digestion suggesting the absence of glycogen. The PAS reaction was blocked in the acetylation solution and was restored after deacetylation showing the presence of 1:2 glycol groups.

When subjected to alcian blue (AB) staining technique at pH 1.0 and 2.5 striated border of stomach (Fig 1B) and the mucous cells of oesophagus and intestine (Fig 1C) were intensely positive whereas club cells of oesophagus, gastric glands of stomach and all the other layers gave a negative reaction.

In the combined technique of AB (pH 1.0 and pH 2.5) followed by PAS, mucous cells of oesophagus (Fig. 1D) and striated border of stomach took a bluish purple colour indicating the presence of a mixture of both acid and neutral mucins Gastric glands of stomach were positive to PAS showing the presence of neutral mucins only In the case of intestine the mucous cells were intensely positive to AB suggesting the presence of acid mucins only

In a combined staining with aldehyde fuchsin followed by alcian blue (AF/AB) mucous cells of oesophagus and intestine and striated border of stomach were alcianophilic. The mucous cells of oesophagus and intestine and the striated border of stomach exhibited metachromasia with toluidine blue They were also metachromatic with Azure A at pH 3.0 and pH 4.0.

When methylation tests (mild and active) were carried out followed by AB, the alcianophilia of mucous cells was abolished and restored after subsequent saponication. When methylation tests were carried out followed by AF/AB staining, the mucous cells of intestine showed affinity to AB in preference to AF, indicating the presence of carboxylated mucins.

Protein predominance in club cells of oesophagus (Fig. 1E) was revealed by bromophenol blue (BPB) a basic protein stain to which they were intensely

positive. Gastric glands of stomach and tunica propria, submucosa and muscularis of all the regions were moderately positive to BPB. With ferric ferricyanide, club cells of oesophagus and gastric glands of stomach were moderately positive, whereas, with $KMnO_4$/AB test, striated border of stomach, in addition to mucous cells of both oesophagus and intestine, gave an intense positive reaction. All the other layers such as tunica propria, submucosa and muscularis were positive to millon's reaction and ferric ferricyanide test and negative to p-DMAB

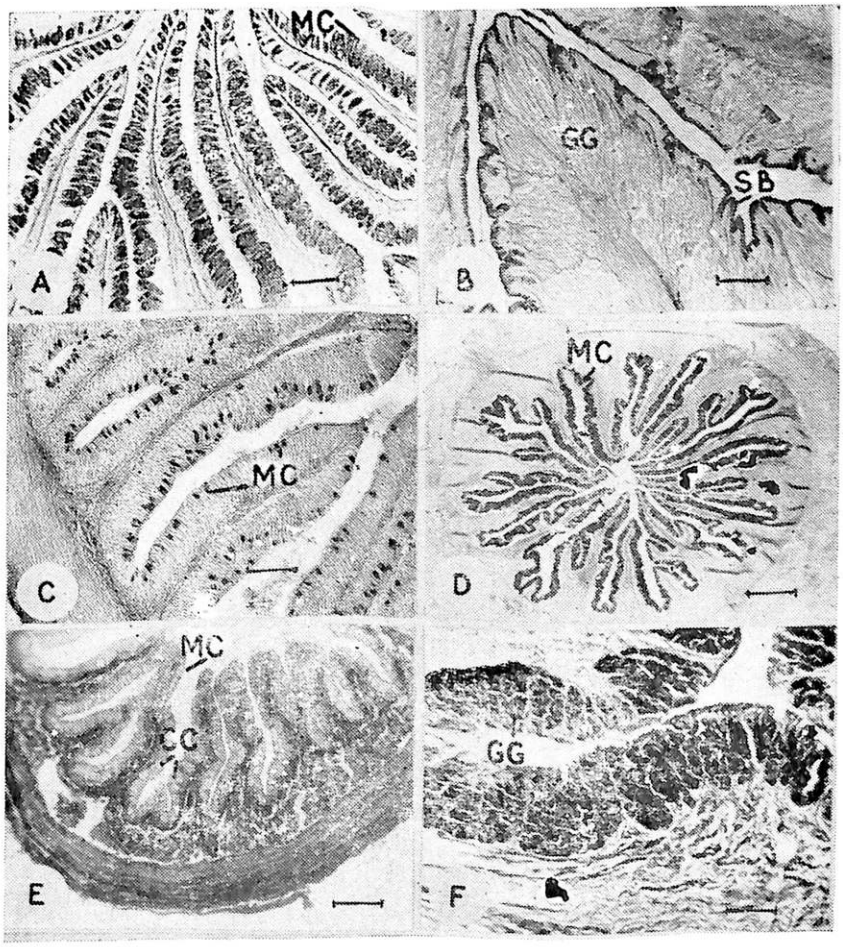

Fig. 1 A-F. A. Photomicrograph of oesophagus showing intensely stained mucous cells (MC) (PAS: Scale bar = 0.062 mm), B. Intensely stained striated border (SB) of stomach (AB pH 2.5 : Scale bar = 0.122 mm), C. Intense positivity of mucous cells (MC) of intestine (AB pH 2.5 : Scale bar = 0.076 mm), D. Showing stained mucous cells (MC) of oesophagus (AB pH 2.5/PAS : Scale bar = 0.231 mm), E. Showing positivity of club cells (OC) of oesophagus (Bromophenol blue : Scale bar = 0.147 mm), F. Intensely stained gastric glands (GG) of of stomach (Copper phthalocyanin : Scale bar = 0.097 mm).

nitrite test and $KMnO_4$/AB test, showing the presence of tyrosine and sulphydril groups of proteins in the former and absence of tryptophan and disulphide groups of proteins in the latter. Tunica propria and submucosa (to the exclusion of all the other layers) were however positive to Congo red, indicating the presence of glycoproteins. With Ninhydrin/Schiff's test, club cells of oesophagus gave a moderate reaction, showing the presence of protein bound amino groups.

The detection of lipids and phospholipids was made by using Sudan black B and copperphthalocyanin respectively. Club cells of oesophagus and gastric glands of stomach (Fig. 1F) stained intensely to copperphthalocyanin whereas tunica propria, submucosa and muscle layers stained moderately. But, mucous cells of both oesophagus and intestine; and striated border of stomach gave a negative response.

DISCUSSION

The mucous cells of oesophagus in *Mystus gulio* are provided with both neutral mucopolysaccharides and carboxylated mucins as in the case of *Thymallus arcticus baicalensis* (Suvorova & Treschuk, 1973). All grades of acid mucous secretions are found in the oesophagus of the omnivorous *M. gulio* showing the functional versatility of the particular region of the alimentary tract, unlike in other fishes. Both plant and animal matter have to be prepared for further easy digestion in the rest of the alimentary tract of the omnivorous fishes.

The main digestive part of the alimentary canal is the stomach, which is well developed in *M. gulio*. In fishes, according to Fang & Grove (1979), maximal acidity is observed a few hours after food intake, whereas in the absence of food, the gastric fluid may be weakly acidic or neutral. The present histochemical tests revealed that the gastric glands of stomach consists of neutral mucosubstances while the striated border of stomach consists of carboxylated mucins in addition to neutral mucins. The weakly acidic nature of gastric glands is perhaps due to starvation and emptiness of stomach. The granules of gastric glands are however eosinophilic as in *Gadus morhua* (Bishop & Odense, 1966), whereas in a freshwater catfish *Amieurus catus* lipid granules were reported in gastric glands (Romanin, 1959).

The intestinal tract of *M. gulio* is found to have strongly acidic mucous cells as in the case of several other fishes (*Cottus gobio*, *Enophrys bubalis* (Western, 1971), *Thymallus arcticus baicalensis* (Suvorova & Treschuk, 1973) and *Esox lucius* (Bucke, 1971). Bullock (1967) reported the presence of aldehyde

fuchsin positive goblet cells in the intestine of an insectivorous fish *Gambusia affinis* As *M. gulio* is an omnivore with fragments of cellulose in the intestine also, the presence of carboxylated mucins in the intestine, shows that digestion continues into the intestine, which is neither very long as in herbivores like *Ctenopharyngodon idella* nor very short as in carnivores like *Esox lucius*. Earlier studies on estuarine catfishes, also showed that cellulose producing microorganisms were found to play important role in the digestion of cellulose (Stickney & Shumway, 1974). The same may be expected in *M. gulio* which is also an estuarine catfish containing fragments of algae in the intestine. At the same time, the presence of acidophilic mucous cells, as in the carnivorous *Esox lucius* (Bucke, 1971) shows the adaptability of *M. gulio* to an omnivorous mode of feeding.

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