Micromorphology and cytochemistry of the branchial glands of the freshwater mullets, *Rhinomugil corsula* (Ham.) and *Sicamugil cascasia* (Ham.)

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Abstract. Scanning electron microscopic studies of the gills of *Rhinomugil corsula* and *Sicamugil cascasia* reveal interspecific variations in distribution, density and architectural plan of the mucous glands and the cytochemical nature of the mucus secreted by them. The gill arch and gill filament epithelium of *Rhinomugil corsula* contains numerous mucous cells, but *Sicamugil cascasia* has few mucous gland openings on its gill arch and gill filament epithelium. In both the mullets the secondary lamellar epithelium lacks mucous gland openings, which is discussed.

The presence of blue, blue-red, red and red-blue mucous cells [AB-pH 2.5 periodic acid-Schiff] in the gills of *Rhinomugil corsula* signifies the acid and neutral nature of the glycoprotein of the mucus secreted by them. However, the presence of only blue mucous cells indicates the presence of acid glycoprotein in the mucus of the gills of *Sicamugil cascasia*.

Keywords. Mullets; micromorphology; cytochemistry; mucous glands.

1. Introduction

The gill units are invested with different kinds of epithelia and cells. These cells are designated as branchial glands. The branchial glands perform various functions under normal and experimental conditions.

Several studies have been made on the general organization of gills with special reference to branchial glands (Munshi 1960; Singh and Munshi 1968; Ojha and Munshi 1974). However, little is known on the surface specializations of branchial glands of Indian fish (Hughes and Munshi 1978).

Previous histochemical studies showed that mucous cells from the epidermis and gills of many teleosts contain glycoproteins (Asakawa 1970; Bremer 1972; Zaccone 1972, 1973; Harris *et al* 1973; Carmignani and Zaccone 1974; Ojha and Munshi 1974) but little is known about the different types of glycoproteins present in the mucous cells of fish gills (Fletcher *et al* 1976). Such histochemical analysis is lacking on the gills of Indian species.

The present work is an attempt to demonstrate the surface ultrastructure and the cytochemical nature of the mucus secreted by the branchial glands of the two mullets inhabiting freshwaters of river Ganges.

2. Materials and methods

Live specimens of *Rhinomugil corsula* (30–50 g) and *Sicamugil cascasia* (2–5 g) were collected from the river Ganges and were transported to and maintained in the animal house of the University.
2.1 Fixation for cytochemical analysis

The fish were anaesthetized by MS 222 (0·01 gl⁻¹) and the gills were carefully removed, washed in Ringer's and fixed in Bouin's and Zenker's fixatives. After processing, the gill pieces were dehydrated in ethanol, embedded in paraffin and sectioned at 5 μm.

2.2 Cytochemical tests

The sections of gills were subjected to various cytochemical tests to demonstrate the chemical nature of the mucus secreted by the branchial glands.

The Schiff's without oxidation technique (Pearse 1968) was used to demonstrate free aldehyde. The periodic acid Schiff's (PAS) method (McManus 1946) was employed to demonstrate glycoproteins in branchial glands. A greenish-blue colour reaction with alcian blue (8GS) adjusted to pH 2·5 with 3% acetic acid (Steedman 1950), was employed to identify the nature of acid glycoproteins. Alcian blue at pH 1·0 (Lev and Spicer 1964) was used to differentiate sulphated acid glycoprotein. After staining with alcian blue (pH 1·0), the sections were blotted dry as recommended by Lev and Spicer (1964).

AB-pH 2·5-PAS technique (Jones and Reid 1973a, b) was used to demonstrate the glands secreting different types of glycoproteins.

2.3 Chemical blockage

The acetylation technique (Lillie 1954) which blocks the hydroxyl groups forming acetyl esters, and the deacetylation technique (Lillie 1954) which hydrolyses the acetyl esters and unblocks the reactive hydroxyl group were also used.

Methylation (Spicer 1960) which blocks the basophilia of acid glycoproteins giving a negative reaction with alcian blue and subsequent saponification (demethylation) which restores the blue staining of acid glycoproteins with alcian blue, were also employed.

2.4 Scanning electron microscopic investigations

For scanning electron microscopy (SEM) 3% phosphate buffered glutaraldehyde was irrigated through the gills of the fish without its prior anaesthetization. This ensured that the pumping action of the gill would quickly rinse the gills. After 5 min the fish seized ventilation and small pieces of gills were fixed in 12% phosphate buffered glutaraldehyde and stored at 4°C for 48 h. The fixed materials were dehydrated and stored in dry acetone. The gill pieces were critically point dried, immediately gold sputtered and examined under scanning electron microscope (PSEM/500) at RSIC, Bose Institute, Calcutta.

3. Observations

3.1 Cytochemistry of branchial glands

Light microscopy and various cytochemical tests indicate the presence of mucous glands in the gill units of the two mullets.
Table 1. A summary of the histochemical tests performed to show the chemical nature of the specialised branchial glands (mucous glands) of *R. corsula* and *S. cascia*.

<table>
<thead>
<tr>
<th>Material</th>
<th>Fixative</th>
<th>Techniques</th>
<th>References</th>
<th>Results</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>Zenker's fluid</td>
<td>PAS</td>
<td>McManus (1946)</td>
<td>+++ + + R</td>
<td>Mucous glands are PAS positive hence contain carbohydrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAS acetylation</td>
<td>McManus and Cason (1950)</td>
<td>–</td>
<td>1:2 glycol groups may be present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAS deacetylation</td>
<td>McManus and Cason (1950)</td>
<td>+++ + R</td>
<td>The restoration of the colour indicates the presence of 1:2 glycol groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Schiff without oxidation with periodic acid</td>
<td>Pearse (1968)</td>
<td>–</td>
<td>Free aldehyde is not present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alcian blue pH 2·5</td>
<td>Mowry (1963), Steedman (1950),</td>
<td>++ + B</td>
<td>The mucous glands are AB positive and contain acid mucopolysaccharide</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Warren and Spicer (1961)</td>
<td></td>
<td>Sulphated mucopolysaccharide present in the mucous glands</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alcian blue pH 1·0</td>
<td>Spicer (1960)</td>
<td>+ B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alcian blue/PAS</td>
<td>Mowry (1956)</td>
<td>++ + + BR</td>
<td>Acid mucopolysaccharide is present in the mucous glands</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild methylation/Alcian blue</td>
<td>Spicer (1960)</td>
<td>+ B</td>
<td>It indicates the sulphated nature of AB positive mucous glands</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild methylation/PAS</td>
<td>Spicer (1960)</td>
<td>++ + R</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild methylation/Alcian blue/PAS</td>
<td>Spicer (1960)</td>
<td>++ + BR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zenker's of Bouin's fluid</td>
<td>Alcian blue pH 2·5</td>
<td>Jones and Reid (1973a,b)</td>
<td>++ R; ++ RB; + + B; + + BR</td>
<td>Only in <em>R. corsula</em> red and red-blue signify the presence of neutral glycoprotein whereas blue or blue-red indicate the presence of acid glycoprotein</td>
</tr>
</tbody>
</table>

B, Blue; BR, blue red; R, red; RB, red blue; +, positive; –, negative.
The PAS positive mucous glands indicate the presence of carbohydrate in mucus, secreted by these specialized branchial glands (table 1). Their negative reaction with PAS, without oxidation with 1% periodic acid, signifies the absence of free aldehyde in the carbohydrate unit of the mucus. They also showed negative results with PAS after acetylation. Deacetylation draws back the PAS positive results indicating the presence of 1:2 glycol-groups. The mucous glands contain glycoproteins and not glycogen, as they resist 1 h treatment with saliva at 37°C and gave PAS positive result after saliva treatment.

The alcian blue tests on the mucous glands at pH 2·5 imparted beautiful colouration, but only a faint reaction was observed at pH 1·0.

The AB-positive mucous glands resist mild methylation for 12 h at 37°C which was clarified by AB, AB-PAS and PAS techniques. These reactions indicate the sulphated nature of the AB-positive mucous glands. Methylation gave a negative reaction with AB-pH 2·5 and subsequent saponification restored the blue staining of acid glycoproteins with AB-pH 2·5.

3.2 Assessment of staining and types of glycoproteins

In *R. corsula* 4 types of mucous glands (blue, blue-red, red and red-blue) were identified by AB-pH 2·5-PAS technique (table 1). However, in *S. cascasia* colour differentiation could not be observed. Blue or blue-red mucous glands signify the presence of acid glycoproteins and red and red-blue ones indicate the presence of neutral glycoproteins in mucus secreted by these glands.

3.3 SEM studies

The epithelia covering the gill head and the gill filaments of the two mullets are differentiated into glandular and non-glandular parts. In *R. corsula* large number of mucous glands surrounded by microridged epithelial cells are discernible on the gill-head and gill filaments (figures 1, 2). The laminated microridges penetrate into the mucous glands (figures 3, 4). The orientation of the mucous glands and the pattern of microridges in the primary epithelial cells are similar to those of the gill arch. The tip of the gill filaments also shows the same distribution pattern and architectural plan of the mucous glands and microridged epithelial cells (figure 5).

*Figures 1–8.* 1. A part of the gills of *R. corsula* showing many mucous gland openings on the gill arch and gill filament epithelia. Secondary lamellae are also seen. 2. Base of the gill filament of *R. corsula* showing mucous gland openings on its epithelium. Secondary lamellae are also seen. 3 and 4. A part of the gill filament epithelium of *R. corsula* showing mucous gland openings and microridged epithelial cells. Micriviilli are also seen in the centre of some microridged epithelial cells (figure 4). 5. The tip of a gill filament of *R. corsula* showing mucous gland openings. Secondary lamellae are also seen. 6. Base of the gill filament of *S. cascasia* showing mucous gland openings and microridged epithelial cells. 7 and 8. A part of the gill filament epithelium of *S. cascasia*. 7. Mucous gland openings and microridged epithelial cells. 8. Enlarged view of the mucous gland opening.

(mgo, Mucous gland opening; ga, gill arch; gf, gill filament; sl, secondary lamellae; mrc, microridged epithelial cells; mv, microvilli; tgf, tip of a gill filament).
SEM studies of *R. corsula* and *S. cascasia*

Figures 1-8.
In *S. cascasia*, the gill arch and the gill filament are provided with mucous glands and microridged epithelial cells (figure 6) but the number of mucous glands are quite low compared to that of *R. corsula*. Each mucous gland has a well-defined rim-like opening (figures 7, 8).

### 4. Discussion

SEM and cytochemical studies reveal interspecific variations in various components of the gills of the two mullets. *R. corsula* has more number of mucous glands in the gill arch than found in *S. cascasia*. The architectural plan of the microridged epithelial cells of *R. corsula* is also different from those of *S. cascasia*. The gill arch and its histology thus appear to be characteristic for these two fish species.

The SEM picture of the gill structure indicates differences in architecture. The differences are mainly in the density and distribution of the mucous glands and the architectural plan of microridges in the primary epithelial cells. The architectural plan of the primary epithelium was similar to that investing the gill arch. From these observations it appears that fishes have inter- and intra-specific variations in density, distribution and architectural plan of the mucous glands and microridged primary epithelial cells.

Each secondary lamella is lined externally by secondary epithelium, which is quite different from the primary epithelium. The secondary epithelium lacks mucous glands and microridged cells which is an adaptation for efficient gaseous exchange. Microridged cells filled with mucus would normally increase the water-blood pathway in the gills which is not favourable for efficient gaseous exchange. Smooth epithelial cells in these secondary epithelium decreases the water-blood pathway and therefore increases gas-exchange efficiency of the fish gills.

#### 4.1 Histochemical analysis of branchial glands

In the gill epithelium of *R. corsula*, blue, blue-red, red-blue and red mucous cells were demonstrated by AB-pH 2.5/PAS technique. The range of glycoproteins by this method was similar to that described at epithelial tissue sites in mammals (McCarthy and Reid 1964; Spicer et al 1974). Blue and blue-red mucous cells in the gills of *R. corsula* suggest the presence of predominantly acid glycoprotein and those staining red-blue and red predominantly neutral glycoproteins. The number of mucous glands secreting acid glycoprotein was more than those secreting neutral glycoproteins. Similar results have been obtained by Fletcher et al (1976) in the gills of plaice, flounder and rainbow trout.

The predominant type of acid glycoprotein in the mucous cells of branchial epithelium has been found to vary between species (Porcelli and Novelli 1970; Zaccone 1972, 1973) consistently. In the gills of *S. cascasia* only blue mucous glands were detected by AB-pH 2.5/PAS technique. This indicates that the mucous cells of this species secrete only acid glycoprotein. The glycoproteins produced by branchial mucous cells thus show interspecific variations.

Mucus secretion in fish is usually assumed to be protective (Jakowska 1963). The secretion further helps to reduce friction (Rosen and Cornford 1971) and to check bacterial and fungal growth. According to Hughes and Wright (1970) the mucus film
covering the secondary gill lamellae in teleost as an important function in relation to
gas, ionic and water exchange at the gill surface. The sulphated acidic mucus
produced by mucous glands of *R. corsula* and *S. cascasia* helps to check bacterial and
fungai growth on gills. The mucus also protects the gills from mud and fine
sediments and thus, gills work efficiently in maintaining gaseous and ionic reg-
ulation. In *R. corsula* and *S. cascasia* no mucus was found to be present in the secondary
epithelium of the lamellae. Similar finding has been reported by Fletcher et al (1976)
in the gills of *Salmo gairdneri*.

SEM and histochemical studies point out the inter- and intra-specific variations in
the density, distribution and architectural configuration of the branchial glands of
the two species of mullets studied presently.

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