Histochemical Studies of Mucus-secreting Cells in the Gut of a Coral Fish, *Chelmon rostratus* Cuvier

By

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Most of the histochemical studies of epithelial mucins secreted by the gut have been carried out in the guinea-pig, mouse, rabbit and rat (Spicer, '60; Spicer and Jarrel, '61; Spicer '65). Similar studies have also been reported in the gut of chicken (Aitken, '58) and snail (Demian and Michelson, '71). In fish, mucus secretion of the integument has been reviewed by Jakowska ('63). The fish gut has an abundance of mucus-secreting cells and a multiplicity of forms has been described (Barrington, '57; Tan and Teh '74) but few detailed histochemical studies appear to have been reported of the mucins secreted by these cells. Only brief studies have been made in the mosquito-fish, *Gambusia affinis* by Bullock ('67), in some salmonids by Bullock ('63) and in *Chelmon rostratus* by Tan and Teh ('74). This paper reports a more detailed histochemical study of the mucins secreted by the gut of *Chelmon rostratus* Cuvier.

Materials and Methods

Live *Chelmon rostratus* measuring 100–150 mm in length were obtained from fishermen and killed with a blow on the head. The abdominal cavity was quickly opened and the gut was dissected out. Pieces of tissues from the oesophagus, stomach, pylorus, pyloric caeca, small intestine and rectum were fixed in calcium acetate formalin (Lillie, '65) for 24 hours, after which they were dehydrated and embedded in paraffin in the usual way. 5μ thick transverse and longitudinal sections were cut and stained with the following:

1. Haematoxylin and eosin
2. Periodic Acid-Schiff Reaction (P. A. S.)—McManus and Mowry ('60)
3. Periodic Acid-Schiff Reaction after Diastase digestion—Barka and Anderson ('63)
4. Southgate's Mucicarmine (MC)—Drury and Wallington ('67)
5. Alcian Blue 8GX (AB)—Mowry ('63)
6. Alcian Blue 8GX after mild acid hydrolysis—Quintarelli et al. ('61)
7. Gomori's Aldehyde Fuchsin (AF): Halmi's modification—Halmi and Davies ('53)
8. Azure A (AzA) at pH's 1.5 and 3.5—Pearse ('68)

Observations

Table 1 summarises the reactions of the mucins secreted by different regions of the Chelmon gut to various histochemical stains.

Table 1. Reactions of Chelmon gut mucins to different histochemical stains.

<table>
<thead>
<tr>
<th></th>
<th>Oesophagus</th>
<th>Pyloric Caeca</th>
<th>Small Intestine</th>
<th>Rectum</th>
<th>Stomach surface mucus cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. A. S.</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Diastase-P. A. S.</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>Mucicarmine</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aldehyde Fuchsin</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>+</td>
</tr>
<tr>
<td>Alcian Blue 8GX pH 2.5</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Acid-hydrolysis-Alcian</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>—</td>
</tr>
<tr>
<td>Blue 8GX</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Azure A: pH 1.5</td>
<td>beta</td>
<td>beta</td>
<td>beta</td>
<td>beta</td>
<td>beta</td>
</tr>
<tr>
<td>pH 3.5</td>
<td>beta</td>
<td>beta</td>
<td>beta</td>
<td>beta</td>
<td>beta</td>
</tr>
</tbody>
</table>

Keys: —: absent
+: weak
#: moderate
##: strong
###: very strong
beta: beta metachromasia

In the oesophagus, mucus-secreting cells are found only in the proximal third. These cells are modified columnar cells and they are periodate reactive (Fig. 1) and diastase resistant. They stain strongly with aldehyde fuchsin (Fig. 2) and are alcianophilic (Fig. 3). The alcianophilia is not removed by mild acid hydrolysis. The mucins show beta metachromasia at pH 1.5 and pH 3.5. Thus, the oesophageal mucus cells secrete acid mucopolysaccharides of the sulphated variety.

In the stomach, no goblet cells are seen. But mucus granules can be seen in the supranuclear region of the surface mucus cells. These granules are periodate-reactive (Fig. 4) and diastase resistant. They
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stain weakly with mucicarmine (Fig. 5) and aldehyde fuchsin and are moderately alcianophilic (Fig. 6). The alcianophilia is abolished by mild acid hydrolysis. The mucins also stain metachromatically with Azure A at pH 3.5. These histochemical reactions suggest that the secretory granules of the surface mucous cells are non-sulphated acid mucopolysaccharides.

The pyloric caeca contain mucus cells which are either of the typical goblet type or of those which appear as elongated profiles (Figs. 7–9). The mucins secreted by these cells are periodate-reactive (Fig. 7) which are diastase-resistant. They are stained dark purplish-brown with aldehyde fuchsin (Fig. 8) and dark bluish-green with Alcian blue 8GX both before (Fig. 9) and after mild acid hydrolysis. They stain metachromatically with Azure A at pH 1.5. These mucins are sulphated acid mucopolysaccharides.

Mucus-secreting cells of the small intestine are of the goblet type. No differences in histochemical reactions are noted between the mucins secreted by the proximal and distal halves of the small intestine. They are all periodate-reactive (Fig. 10), diastase-resistant (Fig. 11) and stain with mucicarmine (Fig. 12). They stain strongly with aldehyde fuchsin (Fig. 13, 15) and alcian blue (Fig. 14, 16). The alcianophilia is not abolished by mild acid hydrolysis. The mucins stain metachromatically with Azure A at pH 1.5 and stain strongly with aldehyde fuchsin, which shows that they are sulphated acid mucopolysaccharides.

The rectum also stains deep purple-red with P. A. S. (Fig. 17) and show no reduction of staining intensity after diastase digestion. The mucins are also alcianophilic (Fig. 18) and the alcianophilia is not abolished by mild acid hydrolysis. They are stained strongly with aldehyde fuchsin and metachromatically with Azure A at pH 1.5. These findings indicate the presence of sulphated acid mucopolysaccharides.

Discussion

The present histochemical study of mucins in the gut of *Chelmon rostratus* has revealed the presence of several types of mucus-secreting cells. A preliminary description of these cells have been presented by Tan and Teh ('74). The secretory granules of all these mucus cells are periodate-reactive and are stained red with mucicarmine. These two histochemical stains have been employed to demonstrate the presence of mucopolysaccharides in general. However, glycogen can also give a positive reaction with P. A. S. (periodic-Schiff reaction) but the reaction will be reduced or abolished if the sections had been previously treated with diastase. Since the granules in the mucus cells
of the Chelmon gut retain their periodate-reactivity and do not show any diminution in staining quality after diastase digestion, it indicates that the P. A. S. positivity had most probably been due to the presence of mucopolysaccharides and not glycogen.

Mucins are classified as neutral or acid mucopolysaccharides (Spicer et al, '65; Pearse, '68). Neutral mucopolysaccharides are P. A. S. reactive but do not stain with Alcian Blue 8GX whereas acid mucosubstances stain bluish-green with the latter. All the mucus cells of Chelmon gut are periodate-reactive and alcianophilic. This indicates that the mucus produced by these cells are acid mucopolysaccharides.

Acid mucopolysaccharides may be sulphated or non-sulphated (Pearse, '68). Sulphated mucopolysaccharides stain deep purplish-brown with aldehyde fuchsin whereas non-sulphated mucins stain weakly (Halmi and Davies, '53). Gastric chief cells also stain deep purple with aldehyde fuchsin (Pearse, '68). Strong affinity for aldehyde fuchsin has been observed in the mucus cells of the oesophagus, pyloric caeca, small intestine and rectum but only a weak reaction is observed in the surface mucus cells of the stomach. This suggests that the mucus cells of the oesophagus, pyloric caeca, small intestine and rectum secrete sulphated acid mucopolysaccharides whereas the surface mucus cells of the stomach secrete non-sulphated acid mucopolysaccharides. Further support for the presence of sulphated acid mucosubstances in the granules of the mucus cells of the oesophagus, pyloric caeca, small intestine and rectum is provided by the demonstration of metachromasia with Azure A at pH 1.5 because non-sulphated mucins stain metachromatically with Azure A at pH 3.5 (Spicer and Jarrels, '61). However, sulphated mucins with partially-masked sulphate esters may also not show metachromasia with Azure A at very low pH's (Spicer, '60; Spicer and Warren, '60).

In the gastric surface mucus cells, the mucus granules are found to be non-sulphated acid mucopolysaccharides which stain metachromatically with Azure A at pH 3.5 and their alcianophilia is abolished after mild acid hydrolysis; this suggests that sialomucins may be present in the secretory granules (Quintarelli et al. '61).

This investigation has shown that the mucins secreted by the Chelmon gut are acid mucopolysaccharides. They are secreted by mucus cells which are modified columnar cells in the oesophagus and goblet cells in the pyloric caeca, small intestine and rectum. No goblet cells are found in the stomach but the surface epithelium shows supranuclear secretory granules which contain non-sulphated acid mucopolysaccharides. The probable functions of these mucosubstances have been discussed previously (Tan and Teh, '74).
Summary

The mucins secreted by the mucus cells in the gut of a coral fish, *Chelmon rostratus*, have been studied histochemically. All the mucins secreted by the gut of this fish have been shown to be acid mucopolysaccharides. In the oesophagus, pyloric caeca, small intestine and rectum, the mucins are sulphated acid mucopolysaccharides whereas in the stomach they are non-sulphated acid mucopolysaccharides.

Acknowledgements

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References

14) Quintarelli, G., Tsuiki, S., Hashimoto, Y. and Pigman, W.: Studies of sialic


Explanation of Figures

Plate I

L.S. (Longitudinal section), T.S. (Transverse section)

Fig. 1. L.S. Oesophagus showing the mucus-secreting cells in the epithelium. P.A.S. positive. ×520.

Fig. 2. L.S. Oesophagus showing the mucus cells strongly stained with aldehyde fuchsin. ×520.

Fig. 3. L.S. Oesophagus showing mucus cells strongly stained with alcian blue 8GX. ×520.

Fig. 4. T.S. Stomach showing surface mucus cells. Supranuclear secretory granules are stained by means of the P.A.S. method. ×660.

Fig. 5. T.S. Stomach showing surface mucus cells. Stained with mucicarmine. ×660.

Fig. 6. T.S. Stomach showing surface mucus cells with secretory granules stained with alcian blue 8GX. ×660.

Fig. 7. T.S. Pyloric caeca showing goblet cells stained by the P.A.S. method. ×130.

Fig. 8. T.S. Pyloric caeca showing the goblet cells strongly stained with aldehyde fuchsin. ×130.

Fig. 9. T.S. Pyloric caeca showing alcianophilia of the goblet cells. Stained with alcian blue 8GX. ×130.
Plate II

Fig. 10. T.S. Proximal part of small intestine showing goblet cells containing P.A.S. positive secretory granules. ×500.

Fig. 11. T.S. Proximal part of small intestine showing mucus cells in the crypt which is strongly stained by the P.A.S. method after diastase digestion. ×500.

Fig. 12. T.S. Proximal part of small intestine stained with mucicarmine. The goblet cells are stained red. ×500.

Fig. 13. T.S. Proximal part of small intestine showing goblet cells stained by aldehyde fuchsin. ×500.

Fig. 14. T.S. Proximal part of small intestine showing strong affinity of goblet cells for alcian blue 8GX. ×500.

Fig. 15. T.S. Distal part of small intestine showing goblet cells stained with aldehyde fuchsin. ×130.

Fig. 16. T.S. Distal part of intestine showing goblet cells stained with alcian blue 8GX. ×520.

Fig. 17. T.S. Rectum stained with P.A.S. ×120.

Fig. 18. T.S. Rectum showing goblet cells in a villus stained with alcian blue 8GX. ×520.