Complex Carbohydrate Histochemistry of the Surface and Gastric Pit Epitheliums of the Japanese Macaque

By

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Summary : Complex carbohydrate histochemistry of the surface and gastric pit epitheliums of the Japanese macaque was performed and the following conclusions were made:
1. Reactions to neutral and acid mucopolysaccharides are generally moderate, however, reactions to neutral mucosubstances in the body and pylorus are fairly clear.
2. Neutral mucopolysaccharides do not surpass weak acid ones.
3. Reactions to strong acid mucopolysaccharides are extremely faint.
4. Complex carbohydrates in the surface and gastric pit epitheliums bear some resemblance to those of type II mucous cells (Imai et al.).
5. There is no difference in the degree of reaction between the surface and gastric pit epithelium; however, Tsujimura recognized such a difference in the dog.

The surface and gastric pit epitheliums are composed of mucous cells. However, the staining properties of mucopolysaccharides are weaker than those of ordinary mucous cells. Some scholars consider that the same epitheliums chiefly secret neutral mucopolysaccharide.

The authors investigated mucopolysaccharides in the above-mentioned epithelium in the cardia, fundus, lesser and greater curvatures, and pylorus of the Japanese macaque and compared them with those in the mucous cells in the salivary glands of the same animal.

Materials and Methods

Three adult Japanese macaques who were fasted were used in this study. The animals were killed by a rapid intravenous injection of 1.5 cc of Nembutal. The samples were taken in as fresh a state as possible from the above-mentioned portions (Fig. A). The tissues were fixed in buffered formalin, embedded in paraffin and cut into 6μ-thick sections. The sections were stained with the techniques shown in Table 1.
Table 1. Staining methods

Acetylation-PAS reaction
Acetylation-saponification-PAS reaction
Sulfation (H$_2$SO$_4$: ether 1:1)-toluidine blue (pH 2.5) method
Sulfation (H$_2$SO$_4$. ether 1:1)-alcian blue (pH 1.0) method
Alcian blue (pH 2.5)-PAS reaction
Periodic acid-paradiamin (PAD) method (Spicer)
Low iron diamin (LID) treated with H$_5$IO$_6$-alcian blue (pH 2.5) method (Spicer)
High iron diamin (HID) treated with H$_5$IO$_6$-alcian blue (pH 2.5) method (Spicer)
PA-Con A-HRP-alcian blue (pH 2.5) method
Mucicarmine stain (Meyer)
Alcian blue (A.B.) (pH 2.5) stain
LID-alcian blue (pH 2.5) method (Spicer)
HID-alcian blue (pH 2.5) method (Spicer)
PA-Red-Con A-HRP-alcian blue (pH 2.5) method
Alcian blue (A.B.) (pH 1.0) stain
Alcian blue (A.B.) (pH 0.5) stain
Toluidine blue (T.B.) (pH 7.0) stain (Ohno)
Toluidine blue (T.B.) (pH 4.1) stain (Ohno)
Toluidine blue (T.B.) (pH 2.5) stain (Ohno)

Observations

1. Neutral mucopolysaccharide

Acetylation-PAS, acetylation-saponification-PAS and sulfation-T.B.(pH 2.5) techniques showed the presence of neutral mucopolysaccharide (Fig. 1), and those reactions in the epithelium of the pylorus were especially obvious. The sulfation-A.B. (pH 1.0) technique reacted indistinctly to the same substance in both epithelia, whereas A.B. (pH 2.5)-PAS (Fig. 2) and Spicer’s PAD methods clearly reacted to neutral mucopolysaccharide in the same epithelia. Moreover, LID treated with H$_5$IO$_6$-A.B.(pH 2.5) (Fig. 3) and HID treated with H$_5$IO$_6$-A.B. (pH 2.5) (Fig. 4) showed the presence of the same substance. The PA-Con A-HRP-A.B. (pH 2.5) technique reacted to neutral mucous of types II and III in the epithelia of the pits at the fundus, greater curvature and pylorus.

2. Acid mucopolysaccharides.

Mucicarmine clearly demonstrated acid mucopolysaccharide in the surface and gastric pit epithelia. However, it was impossible for this stain to distinguish different types of the same substance. The authors tried several techniques to solve this problem.

a. Weak acid mucopolysaccharide

The surface and gastric pit epithelia in the cardia, fundus and pylorus contained considerable concentrations of A.B. (pH 2.5) positive weak acid mucopolysaccharide (Fig. 5). The same substance in the lesser and greater curvatures was fairly well stained by the same method (Table 2.). The LID-A.B. (pH 2.5) (Fig. 6) and HID-A.B. (pH 2.5) (Fig. 7) techniques also demonstrated weak acid mucopolysaccharide in the surface and gastric pit epithelia. PA-Con A-HRP-A.B. (pH 2.5) demonstrated type I acid mucous. The presence of acid mucous of types I and II in the above-mentioned epithelia was shown by a positive reaction to the PA-Red-Con A-HRP-A.B. (pH 2.5) technique (Fig. 8). The same reaction was remarkable especially in the epithelia of the cardia and fundus, while the reaction was faint in the pylorus (Table 3).

b. Strong acid mucopolysaccharide

A.B. (pH 1.0), A.B. (pH 0.5) and LID-A.B. (pH 2.5) techniques showed the presence of extremely small quantities of acid mucopolysaccharide containing the SO$_4$ radical (Tables 2 and 3).

3. Hyaluronic and chondroitin sulfuric acids

T.B. (pH 4.1) and T.B. (pH 2.5) techniques indicated the absence of these acids in the surface and gastric pit epithelia (Table 3).
Table 2. Histochemical reactions in the surface and gastric pit epitheliums

<table>
<thead>
<tr>
<th>Epithelium in the cæcum</th>
<th>Acetylation-PAS</th>
<th>Acetylation-saponification-PAS</th>
<th>Sulphation-T.B. (pH 2.5)</th>
<th>Sulfation-A.B. (pH 10)</th>
<th>A.B. (pH 2.5)</th>
<th>Spicier's PAS</th>
<th>LID treat with H$_2$IO$_4$-A.B. (pH 2.5)</th>
<th>HID treat with H$_2$IO$_4$-A.B. (pH 2.5)</th>
<th>PA-ConA-HRP-A.B. (pH 2.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>surface</td>
<td>- (±)</td>
<td>+++++</td>
<td>m.v.+</td>
<td>- (+)</td>
<td>R.+ +</td>
<td>Br.++++</td>
<td>R.B.</td>
<td>1.Br. (g.B.)</td>
<td>Bl.</td>
</tr>
<tr>
<td>surface</td>
<td>- (±±±)</td>
<td>+++</td>
<td>m.v.+</td>
<td>- (+)</td>
<td>R.+ +</td>
<td>Br.</td>
<td>R.B.</td>
<td>1.Br. (g.B.)</td>
<td>Bl.</td>
</tr>
<tr>
<td>gastric pits</td>
<td>- (±±±)</td>
<td>+++</td>
<td>m.v.+</td>
<td>- (+)</td>
<td>R.+ +</td>
<td>Br.</td>
<td>R.B.</td>
<td>1.Br. (g.B.)</td>
<td>Bl.</td>
</tr>
</tbody>
</table>

Notes: - m.: metachromasia, 1.: light, g.: granule, R.: red, V.: violet, Br.: brown, Bl.: blue, B.: black

Discussion

The authors proved the presence of neutral and weak acid mucopolysaccharides in the surface and gastric pit epithelia. However, reactions to strong acid mucopolysaccharide were faint. The authors have also identified two types of mucous cells in the salivary glands of the Japanese macaque. They tentatively named these types I and II. Both types have neutral and weak acid mucopolysaccharides. Distinct reactions to strong acid mucopolysaccharide were recognizable in type I. However, the same reactions to type II were wanting. The surface and gastric pit epithelia contained acid mucous of types I and II, and both of the mucous cell types had the same substances. Several mucosubstances in the surface and gastric pit epithelia resembled those in type II mucous cells. Neutral mucous of types II and III was recognizable in the pit epithelium of the fundus, greater curvature and pylorus. However, neutral mucous was unrecognizable in two kinds of mucous cells. Type III acid mucous was demonstrated in type I mucous cells, while type II mucous cells and epithelium of the stomach did not contain this acid mucous.

In terms of the secretion of mucosubstances in the surface and gastric pit epithelia, the authors failed to determine that the quantity of neutral mucopolysaccharide exceeded that of weak acid mucopolysaccharide.
Table 3. Histochemical reactions in the surface and gastric pit epitheliums

<p>| Table 3: Histochemical reactions in the surface and gastric pit epitheliums |
|---------------------------------|---------|-----------------|-----------------|-----------------|---------|---------|---------|---------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Mucicarmine</th>
<th>A.B. (pH 2.5)</th>
<th>LID-A.B. (pH 2.5)</th>
<th>HII-D-A.B. (pH 2.5)</th>
<th>PA-Red-ConA-HRP-A.B. (pH 2.5)</th>
<th>A.B. (pH 1.0)</th>
<th>A.B. (pH 0.5)</th>
<th>T.B. (pH 7.0)</th>
<th>T.B. (pH 4.1)</th>
<th>T.B. (pH 2.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelium in the forestomach</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Surface</td>
<td>++</td>
<td>✦✦✦✦</td>
<td>Bl. (B.R.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
</tr>
<tr>
<td>Gastric pits</td>
<td>++(✦)</td>
<td>✦✦✦✦</td>
<td>Bl. (B.R.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
</tr>
<tr>
<td><strong>Epithelium in the fundus</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>++</td>
<td>✦✦✦✦</td>
<td>Bl. (B.R.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
</tr>
<tr>
<td>Gastric pits</td>
<td>✦✦✦✦</td>
<td>Bl. (B.R.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
<td></td>
</tr>
<tr>
<td><strong>Epithelium in the greater curvature of the body</strong></td>
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<td></td>
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</tr>
<tr>
<td>Surface</td>
<td>✦✦✦✦</td>
<td>✦✦✦✦</td>
<td>Bl. (R.B.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
</tr>
<tr>
<td>Gastric pits</td>
<td>✦✦✦✦</td>
<td>✦✦✦✦</td>
<td>Bl. (R.B.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
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<tr>
<td><strong>Epithelium in the lesser curvature of the body</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Surface</td>
<td>✦✦✦✦</td>
<td>✦✦✦✦</td>
<td>Bl. (R.B.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
</tr>
<tr>
<td>Gastric pits</td>
<td>✦✦✦✦</td>
<td>✦✦✦✦</td>
<td>Bl. (R.B.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
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<td><strong>Epithelium in the pylorus</strong></td>
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</tr>
<tr>
<td>Surface</td>
<td>✦✦✦✦</td>
<td>✦✦✦✦</td>
<td>Bl. (R.B.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
</tr>
<tr>
<td>Gastric pits</td>
<td>✦✦✦✦</td>
<td>✦✦✦✦</td>
<td>Bl. (R.B.)</td>
<td>Bl.</td>
<td>Bl.++</td>
<td>✦~✦(✦)</td>
<td>g.✦(✦+)</td>
<td>✦</td>
<td>✦</td>
<td>✦</td>
</tr>
</tbody>
</table>

Notes: — m.: metachromasia, g.: granule, R.: red, V.: violet, Bl.: blue, B.: black

Tsujimura⁴ observed mucosubstances in the surface and gastric pit epitheliums of the dog, and concluded as follows:

a. Reactions to the neutral mucopolysaccharide and weak acid mucopolysaccharide in the surface epithelium at the lesser and greater curvatures and pylorus, and the epithelium at the entrance of the gastric pits are moderate, and such epithelium surpass the epithelium in the deep portion of the pits in terms of the quantity of the above-mentioned mucosubstances.

b. Reactions to weak acid mucopolysaccharide in the epithelium at the deeper portion of the gastric pits in the lesser and greater curvatures are weaker than those in the surface epitheliums at the lesser and greater curvatures.

c. The epithelium at the deeper portion of the gastric pits in the lesser and greater curvatures strongly reacts to strong acid mucopolysaccharide, while it reacts weakly to the same substance in the pylorus.

d. Reactions to strong acid mucopolysaccharide in the surface epithelium at the lesser and greater curvatures and the epithelium at the entrance of the gastric pits are weaker than those at the deeper region of the same pits. Reactions to the same substance in the pylorus are scarcely recognizable.

Thus there are differences between the Japanese macaque and the dog.
References


Explanation of Figures

Plate I

(All are of the Japanese macaque)

Fig. 1. Pylorus. Sulfation-T.B. (pH 2.5). x 100
Metachromasia ++ (violet).

Fig. 2. Pylorus. A.B. (pH 2.5)-PAS. x200
Epithelium of the mucous membrane: red +++, blue red +++.
Epithelium of the gastric pit: ~-~.

Fig. 3. Fundus. LID treated with H5IO6-A.B. (pH 2.5). x200
Reaction color: red black.

Fig. 4. Pylorus. HJD treated with H5IO6-A.B. (pH 2.5). x200
Reaction color: light brown.

Fig. 5. Cardia. A.B. (pH 2.5). x200
Degree of reaction: +~+++.

Fig. 6. Cardia. LID-A.B. (pH 2.5). x200
Reaction color: blue black.

Fig. 7. Lesser curvature. HID-A.B. (pH 2.5). x200
Reaction color: blue.

Fig. 8. Fundus. PA-Red-Con A-HRP-A.B. (pH 2.5). x200
Epithelium of the mucous membrane: Reaction Color: blue ++.
Epithelium of the gastric pit: Reaction color: blue +.

Fig. 9. Lesser curvature. A.B. (pH 1.0). x200
Degree of reaction: +.