Observations on the anatomy, histology and histochemistry of the stomach of the vespertilionid bat *Miniopterus schreibersii* (Kuhl)

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Abstract. Both the openings of the stomach of *Miniopterus schreibersii* are directed cranially and the fundus is large and rounded. The longitudinal layer of muscles is thin but the circular layer is thick particularly at the apex of the fundic caecum. In addition to the usual types of cells, namely pepsinogen cells, parietal cells, surface mucous cells and neck mucous cells, a new type of cell, the interstitial cell of the gastric mucosa, which stains intensely pink with PAS, has been identified for the first time in the gastric glands. In general there is a predominance of neutral mucins particularly in the pyloric region where blebs of mucus are seen to ooze out into the lumen. Acidic mucins are present in moderate amounts in the surface mucous cells at the apex of the fundic caecum.

Keywords. Stomach of bat; *Miniopterus schreibersii* (Kuhl).

1. Introduction

Although bats represent a group of mammals with highly specialised and unique characters and have been included in one taxonomic order—Chiroptera—they have extremely varied but specific feeding habits such as frugivorous, nectarivorous, pollenivorous, insectivorous, piscivorous, carnivorous and sanguivorous kinds (Grasse 1955). The fact that the morphology of the alimentary tract of mammals is highly adaptive and varies with the diet was mentioned by several early workers (Huxley 1865; Langley 1880; Rollet 1971; Robin 1881; Beddard 1902). In recent years considerable anatomical, histological and histochemical differences in the gastro-intestinal tract of bats have also been reported by Schultz (1965) and Forman (1971a, b, 1972, 1973). It was therefore felt that a detailed study of the alimentary canal of bats with different food habits may reveal interesting facts which may enable us to postulate a working hypothesis for understanding the relationship between the food habits and digestive physiology of bats. The present paper embodying descriptions of the anatomy, histology and the histochemistry of the stomach of *Miniopterus schreibersii* is a part of the extensive programme of study.

2. Material and methods

Adult specimens of *Miniopterus schreibersii* with body weight ranging from 10.0 to 10.8 g were collected from the Robber's cave near Mahabaleshwar in Western
Maharashtra between 1 pm-4 pm when the stomach is nearly empty. The specimens were killed by chloroform and their stomachs and a part of the duodenum were fixed in 10% neutral formalin or calcium acetate formol or Rossman's fixative. After fixation, the tissues were processed for histological and histochemical study.

The sections were stained by following procedures, Ehrlich's haematoxylin and eosin, Cason's (1950) one step modification of Mallory-Heidenhain azan procedure. Masson's (1923) technique for demonstrating argentaffin cells was slightly modified by employing 0·1% of potassium bromide solution instead of a solution of gold chloride.

The classification given by Spicer et al. (1965) was adopted for localisation and identification of different types of mucopolysaccharides and the techniques for different types of mucopolysaccharides as given by Lillie (1976) were employed.

3. Observations

The stomach of *M. schreibersii* is small, widely rounded along the greater curvature. Consequently the two openings of the stomach—one into the oesophagus and the other into the duodenum—lie close to each other and both are directed cranially. Figure 1 illustrates the internal anatomy of the stomach of *M. schreibersii*. The cardiac vestibule is narrow at the junction of the oesophagus but becomes progressively wide caudally and towards the fundic caecum. There are no valves at the

![Figure 1. Semischematic drawing to show the general anatomy of the stomach of Miniopterus schreibersii. B.gl: Brunner's glands; c.v: cardiac vestibule; duo: duodenum; f.c: fundic caecum; ces: oesophagus; py: pylorus; ph.sph. pyloric sphincter; t.st: tubular stomach; tr.z: transition zone.](image-url)
juncture of the oesophagus and stomach, and the mucosa of the stomach charac-
terised by the presence of tubular glands commences immediately after the
terminal part of the oesophagus (figure 2). The tubular region of the stomach
lies immediately below the cardiac vestibule, and the cavity of the stomach reaches
its widest expanse in this region where the rugal folds lie parallel to the greater
curvature.

The fundic caecum is large and rounded and the rugae in this region appear
like irregularly arranged protuberances of varying sizes and heights, some bulbous
and others conical, projecting into the lumen of the stomach (figure 5). The
pylorus is short and is guarded by a pyloric sphincter. The circular muscle layer,
which forms the sphincter, is thick along the greater curvature and becomes
progressively reduced in thickness towards the lesser curvature. The sphincter
is conical with a terminal central aperture and projects towards the duodenal
side of the gastro-duodenal junction.

Whereas the two muscle layers, namely the outer longitudinal and the inner
circular, are nearly equally thick (50 to 70 μ) on the sides of the fundic caecum,
the longitudinal muscle layer is thinner and the circular layer is considerably thicker
in the rest of the regions of the fundic caecum. Since the circular layer of muscles
occurs in the form of numerous successive bundles it presents a serrated and notched
appearance in sectional views (figure 3). In the region of the fundic caecum the
muscles project into the bases of the rugal folds (figure 4). The circular layer of
muscles is well developed at the caudal portion of the lesser curvature and along
the greater curvature (150 to 200 μ in thickness), and it becomes progressively
thin towards the two orifices of the stomach.

3.1. Organisation of the gastric mucosa

The gastric mucosa (figures 5–7) shows the following types of cells:

(i) Pepsinogen or zymogen cells: They are conical to ellipsoidal in shape
and are located at the bases of the glands. These cells have vesicular nuclei lying
at the bases of the cells. Their cytoplasm has an affinity for haematoxylin.

(ii) Parietal cells: These are large and spherical cells containing conspicuous
centrally placed nuclei and eosinophilic cytoplasm and are located between the
region of the pepsinogen cells at the base of the glands and the neck of the glands.

(iii) Surface mucous cells: These cells are tall columnar with vesicular basally
located nuclei surrounded by a large amount of cytoplasm that does not have an
affinity for eosin but takes a slightly bluish stain with haematoxylin. Variations
in the shape of these cells may occur in different regions of the stomach. Whereas
in some places they are short and contain very little cytoplasm, in the pyloric
region the distal end of these cells are ruptured and a large amount of mucus
appears to ooze out of the broken distal end of these cells.

(iv) Mucous neck cells: These cells are not very different from the surface
mucous cells except that these cells are intact and do not exhibit ruptured distal
ends. They have basally located nuclei and they imperceptibly merge into the
surface mucous cells so that it is difficult to distinguish them from the latter. These
cells lie at the bottom of the gastric pits.

(v) Interstitial cells of the gastric mucosa: Numerous small irregularly shaped
cells with darkly staining small nuclei lie closely packed between the parietal
cells (figure 6) and are stained deeply by the PAS staining procedure (figure 7). They contain very little cytoplasm which extends out in the form of two or three processes. These cells have not been so far described by earlier workers, and their exact function is not known.

The glands are simple and tubular in the region of the cardiac vestibule which is characterised by the absence of rugae. The mucosa has a height of 300 to 400 μ and the zymogen cells occupy the basal region. The major part of the gland, which extends from the region of the zymogen cells to the region of the neck mucous cells, is composed of parietal cells and numerous interstitial cells of the gastric mucosa. The surface and neck mucous cells occupy a small distal segment of the gland.

The number of pepsinogen cells within the gastric glands becomes progressively reduced from the fundic caecum towards the transition zone. Further, the gastric glands appear to be branched at their distal segments in the fundic caecum. The glands in the pylo-fundic transition zone have very few pepsinogen cells. This region leads into the pylorus where there is a profuse secretion by the surface mucous cells (figure 10). The mucosa in this region has a height of 250 μ and the mucous cells occupy more than half the length of the gland.

Taking the stomach as a whole the pepsinogen cells form about 5 to 7%, the parietal cells about 50 to 60% and the surface mucous cells about 20%. The interstitial cells and the neck mucous cells compose the rest of the glands.

Patches of lymphoid tissue occur scattered throughout the mucosa of the stomach (figure 8). A conspicuous muscularis mucosae exists at the base of the mucosa and in many places it appears to project deep into the rugal folds (figure 4). Below the muscularis mucosae the rugal folds enclose a submucosa containing blood vessels, connective tissue and lymphatics.

The Brunner's glands occupy a small region on the duodenal side of the gastro-duodenal junction. These glands are separated from the intestinal mucosa by a thin muscularis mucosae. A noteworthy feature of the Brunner's glands of Miniopterus schreibersii is their arrangement in bundles. Each bundle contains a few to several acini and is surrounded by thin fibrous connective tissue (figure 11).

3.2. Mucin histochemistry

Tables 1 and 2 show the results of the histochemical tests employed.

The apical cytoplasm of the surface mucous cells in the gastro-oesophageal junction and the entire surface mucous cells of the pylorus give an intense positive reaction to PAS staining. The secretion occurs as blebs within the cell and is very profuse in the latter region (figure 8). The secretory blebs lose their identity after coming out of the cells. Intense PAS-positive reaction is also seen in the surface and neck mucous cells at the apex of the fundic caecum (figure 9). The interstitial cells of the gastric mucosa are also intensely PAS-positive. The intensity or the extensiveness of the PAS-positive reaction is not altered in any way after digestion with salivary amylase which eliminates glycogen.

Sequential staining with alcian blue (pH 2.5), followed by PAS, gives a slightly bluish-purple reaction at the gastro-oesophageal junction. However, an intense blue-purple colour is observed in the surface mucous cells at the apex of the fundic caecum (figure 9).
Figures 2-5. 2. Gastro-oesophageal junction to show the abrupt ending of the oesophageal epithelium (arrow) and the commencement of the gastric mucosa. × 100. 3. Part of the fundic region of the stomach to show bundles of circular muscles (arrow). × 100. 4. Part of the wall of the fundic caecum. Note the folded mucosa and the occurrence of muscularis mucosae (arrow) projecting into the rugae. × 100. 5. Part of the gastric mucosa in the fundic region. Note the darkly staining pepsinogen cells (arrow) at the bases of the glands. Arrowhead points towards the parietal cell. × 640.
Figures 6-9. 6. Part of the basal portion of the fundic glands. Note the deeply staining interstitial cells (arrow). × 1330. 7. Part of the gastric mucosa to show the darkly staining interstitial cells (PAS staining). × 560. 8. Part of the gastric mucosa in the fundic region to show the large lymphoid patch (arrow) and blood capillaries (arrowhead). × 154. 9. Part of the apex of the fundic caecum (CIPAS staining) to show the intensely stained surface and neck mucous cells. × 340.
Figures 10 and 11. 10. Part of the gastro-duodenal junction to show the Brunner’s glands. The glands are composed of numerous acini arranged in small bundles separated by connective tissue septa (PAS staining). × 154. 11. Part of the pyloric region to show the intensely stained surface and neck mucous cells (PAS staining). × 104.
Table 1. Distribution of mucins in different types of cells.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Pepsinogen cells</th>
<th>Parietal cells</th>
<th>Interstitial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>± R</td>
<td>4 R</td>
<td></td>
</tr>
<tr>
<td>Saliva-PAS</td>
<td>± R</td>
<td>4 R</td>
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<tr>
<td>Cl-PAS</td>
<td>± R</td>
<td>4 R</td>
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<tr>
<td>AB (pH 2·5)-PAS</td>
<td>± R</td>
<td>4 R</td>
<td></td>
</tr>
<tr>
<td>AB (pH 1·0)-PAS</td>
<td>± R</td>
<td>4 R</td>
<td></td>
</tr>
<tr>
<td>AF</td>
<td>2 V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF-AB (pH 2·5)</td>
<td>2 V</td>
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</table>

Table 2. Distribution of mucins in the mucous cells of different regions.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Cardiac vestibule</th>
<th>Fundus</th>
<th>Apex of fundic caecum</th>
<th>Transition zone</th>
<th>Pylorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>2 R</td>
<td>1 R</td>
<td>4 R</td>
<td>1 R</td>
<td>4 R</td>
</tr>
<tr>
<td>Saliva-PAS</td>
<td>2 R</td>
<td>1 R</td>
<td>4 R</td>
<td>1 R</td>
<td>4 R</td>
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<tr>
<td>Cl-PAS</td>
<td>2 RP</td>
<td>1 R</td>
<td>4 RP</td>
<td>1 R</td>
<td>4 R</td>
</tr>
<tr>
<td>AB (pH 2·5)-PAS</td>
<td>2 RP</td>
<td>1 R</td>
<td>4 R</td>
<td>1 R</td>
<td>4 R</td>
</tr>
<tr>
<td>AB (pH 1·0)-PAS</td>
<td>2 R</td>
<td>1 R</td>
<td>4 R</td>
<td>1 R</td>
<td>4 R</td>
</tr>
<tr>
<td>AF</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Methyl-ation</td>
<td></td>
<td></td>
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<tr>
<td>(37° C)-AB (pH 2·5)</td>
<td></td>
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<tr>
<td>Demethylation (37° C)-AB (pH 2·5)</td>
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<tr>
<td>Hyaluronidase-AB (pH 2·5)</td>
<td>1 B</td>
<td>1 B</td>
<td>± B</td>
<td>± B</td>
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<tr>
<td>Sialidase-AB (pH 2·5)</td>
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Key to symbols used: 1—weak; 2—moderate; 3—intense; 4—very intense; ± very weak, R—red; B—blue; BP—blue predominates pink; RP—pink predominates blue; V—violet.

With alcian blue (pH 1·0)-PAS sequential staining it was observed that similar results were obtained as with only PAS staining since alcian blue (pH 1·0) did not stain the cells. With aldehyde fuchsin (AF)-alcian blue (pH 2·5) sequential staining whereas the pepsinogen cells stained with aldehyde fuchsin, the surface mucous cells at the gastro-oesophageal junction took a moderate stain with AB. The AB in this sequential staining also stains the surface mucous cells at the apex of the fundic caecum.

On mild methylation at 37° C the alcianophilia is abolished and is restored on saponification. This indicates that the staining is due to the presence of carboxymucins.

Results similar to those as with AB (pH 2·5)-PAS sequential staining were obtained with colloidal iron-PAS sequential staining too.

On treatment with hyaluronidase and subsequent staining with AB (pH 2·5) no alteration in the intensity of staining due to AB was observed in the treated
sections. This indicates the absence of hyalomucins. On treating the sections with sialidase and subsequent staining with AB (pH 2.5), however, there was a reduction in staining due to AB. This indicates the presence of sialomucins.

From the above it is evident that there is a predominance of neutral mucins in the mucosa of the stomach of *Miniopterus schreibersii*. Of the acidic mucins, sialomucins are present only in a small area of the stomach, namely the gastro-oesophageal junction and the surface mucous cells at the apex of the fundic caecum.

4. Discussion

Although the general shape of the stomach of *Miniopterus schreibersii* is tubular, it differs to some degree from the typical insectivorous type (Eisenraut 1950; Grasse 1955; Schultz 1965; Forman 1972) in the greatly rounded fundus, the very closely approximated cardiac and pyloric ends and a small but conspicuous cardiac vestibule. The last is very well developed in the fruit eating Megachiroptera and in the Phyllostomatidae (which are also partly fruit eaters) and to a lesser extent in *Natalus* and *Plecotus* (insectivorous), and *Pizonyx* (piscivorous) (Forman 1972).

The cardiac glands of *Miniopterus schreibersii* have as many pepsinogen cells as the fundic glands. The cardiac glands in other species of bats examined (Rouk and Glass 1970; Forman 1971a, b, 1972) have been shown to contain a few to no pepsinogen cells. Forman (1972) also observed that there is a little to no staining reactivity in the pepsinogen cells towards PAS in the bats he examined with the exception of a consistently strong staining of zymogenic granules in two species of *Noctilio*. The present investigation has revealed that the pepsinogen cells in *Miniopterus schreibersii* are PAS-negative but are selectively stained with AF. Kolb (1954) indicated that the cardiac glands of *Nyctalus* and *Rhinolophus* probably function as a source of lubricant in the stomach.

Allison (1948) observed that the parietal cells of species of Insectivora are progressively smaller from the lower to the upper boundary within an individual fundic tubule. Forman (1972) observed this condition only in *Rhynchonycteris* among the 13 species of bats he examined. Of the remaining 12 species he noticed a reverse situation (that is, the largest cells were towards the surface of the mucosa) in 10 species. No difference in the size of the parietal cells at different parts of the gland exists in *Miniopterus schreibersii*.

The presence of interstitial cells of the gastric mucosa has not been reported so far. They react negatively to tests for acid mucins. Their exact function is not known. Although the possibility of the interstitial cells being, in fact, enteroendocrine cells cannot be ruled out completely since the techniques for detecting the latter were not employed in the present study, such a possibility seems rare considering the relative abundance of these cells in the entire stomach.

Sections of the stomach that were subjected for the detection of argentaffin cells did not show the presence of these cells.

With regard to the population of the different types of cells in the gastric mucosa, the term 'chief cell' is very confusing since they constitute only 5 to 7% of the total cell population. It is here suggested that these cells be called only as 'pepsinogen cells' or 'zymogen cells' on the basis of the secretory material.
The stomach of *Myotis lucifugus lucifugus* (Ito and Winchester 1963) and that of other species bats (Forman 1971a, b, 1972, 1973; Rouk and Glass 1970; Hart 1971) have been shown to contain the usual types of cells found in other mammals.

The presence of abundant amount of neutral mucins in the pyloric region of *Miniopterus schreibersii* suggests that it acts as a lubricant and prevents ulceration and possibly enzymatic action on the gastro of the intestinal mucosa. Probably the Brunner's glands also serve a similar purpose. This was also suggested by Mathis (1928). Hollander (1951) has pointed out that mucus ‘adheres to the underlying tissue with great tenacity, is capable of maintaining a considerable thickness instead of flowing off rapidly as the acid secretion does, is generally impermeable to pepsin because of its absorptive properties’. A predominance of neutral mucins over acidic mucins has also been reported in mouse, rat, hamster, gerbil, guineapig, rabbit, cat, dog and man by Sheahan and Jervis (1976).

In *Miniopterus schreibersii* sialomucins were observed only in surface mucous cells at the gastro-oesophageal junction and in the neck mucous cells and surface mucous cells in the apex of the fundic caecum. Forman (1972) in his thesis (which was published later than his paper) mentioned that he obtained negative results with colloidal iron in every species of bats he examined but he mentioned that such results could be due to improper fixation. But in a paper which was published earlier than his thesis Forman (1971b) obtained positive results with colloidal iron in the cardiac glands of most of the species he studied. He also observed (Forman 1972) that duodenal glands of Brunner showed a strongly positive reaction to AB in only one species, namely *Carollia*. In the present study the glands of Brunner show a negative reaction to AB (pH 1·0 and pH 2·5) as well as colloidal iron. Further, the occurrence of these glands as distinct bundles lined by connective tissue is not reported in any other bat.

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