Short Communication

Morphometric study of the stomach of African pangolin (*Manis tricuspis*)


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General sections of the mammalian gut are usually adapted to suit the dietary requirements of the particular species. It is to be anticipated therefore that pangolin, which has no teeth unlike some other mammals, may have adopted a means of coping with her high chitinous diet, hence the need for this study which is expected to reveal functional implications of the different regions of the stomach. The animals were sacrificed by cervical dislocation and the harvested stomach cut on a regional basis that is (cardia, fundus, corpus and pylorus) and fixed in 10% formol saline for histological analysis. The stained sections were subjected to morphometric analysis at a magnification of x40 using the eye piece micrometer procedure. The results revealed that the corpus is more metabolically active than the other parts as evidenced by its total thickness (4422 ± 227.26 µ). This may be related to its active role in the churning process. The cardia and fundus on the other hand are the main recipients of the pressure initiated by the contraction of the corpus leading to the exposure of the soft internal part of the ingested arthropods. The pylorus on the other hand, performs its role of propelling the chyme into the small intestine. It is therefore evident that the parts of the stomach investigated are relevant to the high chitinous diet.

Key words: Stomach, diet, chitinous, functional, implication, pangolin.

INTRODUCTION

The mammalian stomach usually presents a cardiac, fundus, corpus, and pyloric part. These parts are known to vary histologically due to their peculiar functions and the animal in view (Sherwood, 2004). These variations are specialized to suit the dietary requirements of the particular species (Hildebrand and Goslow, 2001). The wall of the stomach has all the layers of a typical tubular organ all of which are arranged in different patterns depending on the parts of the stomach under consideration (Hildebrand and Goslow, 2001). The tunica mucosa is composed of epithelium, a laminar propria (of collagen, elastic, and reticular fibers) and muscular mucosae. The submucosa contains collagen fibers, fat and the submucosa nerve plexuses. No submucosa glands are present (Williams et al., 1989). The tunica muscularis externa has two coats: an inner circular and outer longitudinal layer. The tunica serosa is composed of mesothelium overlying a later of loose connective tissue (Heath et al., 1999). The work of David and Hladic (2001) on morphology of the...
Table 1. Morphometry of different layers (coats) of the of pangolin’s stomach.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Parts of the stomach (µ)</th>
<th>Cardia</th>
<th>Fundus</th>
<th>Corpus</th>
<th>Pylorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscularis externa</td>
<td></td>
<td>579 ± 48.26</td>
<td>620 ± 0.48°</td>
<td>2684 ± 81.66*</td>
<td>1756 ± 47.28*</td>
</tr>
<tr>
<td>Submucosa</td>
<td></td>
<td>154 ± 6.67</td>
<td>162 ± 0.01°</td>
<td>179 ± 7.67*</td>
<td>155 ± 21.93*</td>
</tr>
<tr>
<td>Mucosa</td>
<td></td>
<td>350 ± 5.01</td>
<td>340 ± 8.02°</td>
<td>1376 ± 21.93*</td>
<td>911 ± 38.71*</td>
</tr>
<tr>
<td>Total Thickness</td>
<td></td>
<td>2879 ± 30.24</td>
<td>2989 ± 82.65°</td>
<td>4422 ± 227.26*</td>
<td>2164 ± 103.89*</td>
</tr>
<tr>
<td>Relative thickness of the mucosa</td>
<td></td>
<td>0.13 ± 0.16</td>
<td>0.14 ± 0.05°</td>
<td>0.30 ± 0.10*</td>
<td>0.41 ± 0.39*</td>
</tr>
<tr>
<td>Relative thickness of the sub mucosa</td>
<td></td>
<td>0.05 ± 0.29</td>
<td>0.06 ± 0.01°</td>
<td>0.04 ± 0.02°</td>
<td>0.06 ± 0.23*</td>
</tr>
<tr>
<td>Relative thickness of the muscularis externa</td>
<td></td>
<td>0.28 ± 0.83</td>
<td>0.27 ± 0.11°</td>
<td>0.60 ± 0.36*</td>
<td>0.81 ± 0.47*</td>
</tr>
</tbody>
</table>

Measurements are in microns (µ; mean ± S.E.M). n = 6.
*Not significantly different; P > 0.05 vs. cardia.
*Significantly different; P < 0.05 vs. cardia.

The comparisons of the mean values of the thicknesses of the regions of the stomach are revealed in Table 1. The first part of the stomach (cardia) was compared with other parts. The results revealed a non-significant difference (P > 0.05) between the mean values of cardia and fundus. This was however different in the case of corpus which had the thickness of its coats significantly different in virtually all the layers (P < 0.05). The pylorus also showed a significant difference in its muscularis externa, mucosa, total thickness, relative thickness of the mucosa and the relative thickness of the muscularis externa as shown in Table 1.

The results revealed the possible functions of the regions of the stomach. This is in light of how metabolically different gastrointestinal tract in primates and comparison with other mammals in relation to diet revealed that the ratio of stomach and large intestine to small intestine (by area, weight, and volume) are low in frugivores and high in folivores; the continuous spread of coefficients reflect the different degrees of adaptation of these two dietary extremes. Udo et al. (2005) histologically examined by light microscopy the morphology of the principal sections of the gastrointestinal system of two Antarctic seals with different dietary habits, namely, the Weddell seal (Leptonychotes weddellii) and the crabeater seal (Lobodon carcinophagus). The tissue layers of the gastrointestinal tract of both seals are almost identical to those observed in most other mammals and no major differences in principle organization could be found between the two seal species.

The insectivorous Manis tricuspis is expected to have adopted various mechanisms, which will allow the stomach to cope with the hard chitinous diet. Some of these mechanisms, which will be made evident by the morphometric assessment, will give an insight to the peculiar functions of the regions of the stomach. This present study aims at measuring the thicknesses in the layers of the different parts of the stomach including the muscular coats which will be a pointer to how metabolically active a part is, in reference to the work of Adams (1967).

MATERIALS AND METHODS

Care and sacrifice of animals

We studied the stomach sections from eight pangolins of both sexes, after being procured and brought to the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Nigeria. The stomach were harvested following the sacrifice of the animals by cervical dislocation, fixed in 10% formal saline and processed for light microscopic study using Haematoxylin and eosin stain. The care and handling of the animals conform to the rules and guidelines of the animal right committee of the Obafemi Awolowo University.

Morphometrical assessment

The stained sections were subjected to morphometric assessment recommended by W.H.O (1991) which included: dividing the eye piece occlurometer into two 100 small divisions, the stage micrometer scale was made up to 1 mm divided into 0.1 mm divisions and each 0.1 mm was divided into 0.01 mm. The eye piece scale (occulometer) was inserted into the eye piece of the microscope by removing the superior lens thus placing the scale on the field stop, the stage micrometer was also placed on the stage of the microscope, the stage scale was focused by the low power objective lens (x4), the stage and the eye piece scales were adjusted until there was a parallel point between the two scales, the number of the eye piece divisions and its corresponding stage measurements was noted (if 70 occlurometer divisions equal to 14 µm, all the objective lens were thus calibrated). Calibration was needed for each microscope use. The occlurometer fixed into the Olympus Microscope was then focused through stained sections of the tissue to allow for the measurement of the thicknesses of the layers of the stomach.

Statistical analysis

Data were expressed as mean ± standard error of mean (S.E.M). Student’s t-test was then used to determine the level of significance. Except where otherwise stated, p<0.05 was taking as the significant level.

RESULTS AND DISCUSSION

The comparisons of the mean values of the thicknesses of the regions of the stomach are revealed in Table 1. The insectivorous Manis tricuspis is expected to have adopted various mechanisms, which will allow the stomach to cope with the hard chitinous diet. Some of these mechanisms, which will be made evident by the morphometric assessment, will give an insight to the peculiar functions of the regions of the stomach. This present study aims at measuring the thicknesses in the layers of the different parts of the stomach including the muscular coats which will be a pointer to how metabolically active a part is, in reference to the work of Adams (1967).
active a region is, as made evident by the thicknesses of the smooth muscle fibers which agrees with the investigation of Adams (1967) who concluded that increased thickness of the smooth muscle fibers connotes increased metabolic activity. The significant increase in the total thickness of the corpus (4422 ± 227.26 µ) as shown in Table 1, points to its active role in both the chemical and the mechanical (churning) process which requires a high metabolic activity. There is a significant increase in the mucosa of the corpus which is believed to have an important role in the chemical process. In a related work on the chemical activities of the stomach, Nisa et al. (2005) revealed that the stomach of Malayan pangolin has three regions of mucous gland, one oxyntic gland and one pyloric gland. Cells immunoreactive for chromogranin, serotonin, somatostatin, BPP and glucagon were also detected in all of the gastric glands, while gastrin-immunoreactive cells were found in the entire gastric gland except for the oxyntic gland. The significant increase in the thickness of the muscularis externa in corpus (2684 ± 81.66 µ) (Table 1), could be due to increased mechanical role required to successfully cope with the high chitinous diets. This observation conform to the works of Heath et al. (1999) who enumerated the functions of the stomach in man to include the mechanical breakdown of solid food in to chyme by the strong muscular activities of the corpus. The cardia and the fundus on the other hand showed a non-significant different when compared (P>0.05). This may however be attributed to the similar role played in the churning process. Though the cardia may serve as the entrance to the stomach and the fundus as the space above the cardiac opening, they play other role in pangolin as made evident by the work of Ofusori and Caxton-Martins (2005). Their functions were reported to be very significant in the churning process as they present a stratified squamous epithelium (keratinized). They are the main recipient of the pressure and forceful contraction initiated by the corpus leading to the proper grinding and exposure of the soft internal part of the ingesta rich in fats and proteins (Redford and Dorea, 1984). The pylorus plays its role of squirting the chyme into the duodenum as evidenced by a significant increase in the thickness of its muscularis externa (P < 0.05).

Our conclusion revealed that the various regions of the stomach have their specific role most especially in the churning process to compensate for the lack of teeth in African pangolin (M. tricuspis).

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REFERENCES