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Microarchitectural Adaptations in the Stomach of African Tree Pangolin (*Manis tricuspis*)

Adaptaciones Microarquitecturales en el Estómago del Pangolin Africano (*Manis tricuspis*)

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SUMMARY: The microarchitecture of the pangolin's stomach favouring the high chitinous diet has been less waived into, despite extensive morphological investigations. Histological analysis of the microanatomy will provide powerful tools for interpretation to yield reliable insights. We investigated this by fixing the tissues in 10% formol saline for histological analysis. Serial sections at 5 µm thickness were subjected to general staining methods for light microscopic study (Haematoxylin and eosin, Van Gieson's and Verhoeff's). The results revealed basic structural arrangements in their coats, with a modification of the epithelial lining of cardia and fundus into stratified squamous keratinized epithelium. These modifications were also reflected in the distribution of collagen and elastic fibers in the various layers (coats) of the stomach. The present study has shown that there was an adaptation of the stomach of African tree pangolin to its diet as reflected in the microarchitectural configuration.

KEY WORDS: Stomach; Microarchitecture; Histological analysis; Diet; Coats.

INTRODUCTION

The African tree pangolin (M. trisuspis), a mammal, is commonly found in the western part of Nigeria and consumes a very specific insectivorous diet (Griffiths, 1990; Ofusori *et al*., 2007). Fossil pangolins are first found in the Eocene in Europe. In the Messel shales of Germany they are particularly well preserved, showing that their characteristic scales were already developed. Recent DNA analysis has shown them to be bizarre offshoots of the Carnivora - the carnivores such as cats, dogs, bears, hyenas, civets and mongooses (Schlitter, 2005). Pangolin is an animal with short legs covered in brownish red overlapping scales making it look like a pinecone (Fig. 1.). It has a long, slightly-flattened prehensile tail (equally scaled), and 5 long curved claws on each foot. Its head is small and pointed with very small eyes protected by thickened eyelids, and it has a long sticky tongue for catching ants and termites. This rod-shaped tongue is covered with sticky saliva which is used as a tool to collect prey by inserting it into the termite tunnels (Pangolin Specialist Group, 1996). They have no teeth and their gizzard-like stomach may be specially adapted for grinding food. Ofusori et al. revealed from their work on the morphometric study of the stomach of M. tricuspis that the corpus is more metabolically active than the other parts of the stomach. It is therefore expedient to study the microarchitectural adaptations adopted by pangolin's stomach to cope with their diets.

The general sections of the mammalian gut (including the stomach under investigation) are usually specialized to suit the dietary requirement of particular species (Hildebrand & Goslow, 2001; Schlitter). Pangolin will often choose the larger member of the ant and termite population (for example they will choose against the smaller workers); this decreased the percentages of chitin. Calculating the nutritional value of arthropods is difficult, due to the presence of their hard exoskeleton that serves to protect their bodies. The exoskeleton and the chitin components provide a relatively indigestible substance (Griffiths *et al*.). In addition to the difficulties of digesting the chitin, the latter surrounds and protects the softer nutrients which are rich in fat and protein (Redford, 1983). The sand and ditrus is known to add bulk to the digestive load of insectivores and reduce the caloric proportions of their digestive content (Cohan, 1984). This finding is consistent with other ant and termite eaters (Redford, 1983).

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The mucosa and muscular wall of the stomach are therefore expected to be modified in such a way as to protect it from probable ulceration by the chitin and also provide an effective churning process that will expose the soft internal part which contains the needed nutrients.

Beneath the mucosa which lines the stomach are the laminar propria, muscularis mucosa, sub mucosa, circular and outer longitudinal muscle layers and serosa layer, all of which are arranged in different patterns depending on the part of the gut under consideration (Hildebrand & Goslow) and the gut of the animal in view (Sherwood, 2004).

This study looks at the microarchitectural adaptations adopted by Pangolin's stomach to cope with its high chitinous diet.

MATERIAL AND METHOD

Care of the animals. Eight presumably healthy African pangolins were procured 2 hours before sacrifice at Asejire, a local village in Osun state, south-west zone of Nigeria. They were kept and monitored in the Animal Holdings section of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Nigeria. They were fed with termites and water liberally. All animals were treated in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (1985).

Fig. 1. Photograph of African tree pangolin *M. tricuspis* (Ofusori *et al.*)

Excision of the stomach. Midline laparatomy were performed under slight anesthesia using pentobarbital (6.4mg/100g body weight i.m.). The stomachs were dissected out as shown in Fig. 2 and the stomach excised and cut on a regional basis (i.e. Cardia, Fundus, Corpus, and Pylorus).

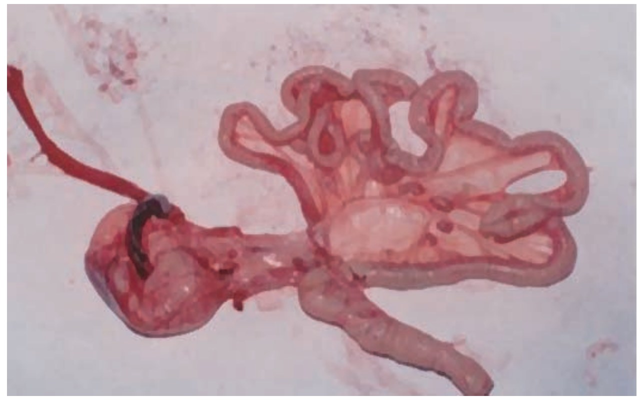

Fig. 2. Photograph of the GIT of African tree pangolin M. tricuspis (Ofusori et al.) Note the absence of colon.

Histological procedures. The excised stomach were cleared of adhered mesentry, washed in physiological saline and immediately fixed in 10% formol saline for 48 hours. After fixation, the tissues were dehydrated through graded alcohol. The tissues were then cleared in three changes of xylene for 1.5 hours duration each. After clearing, the tissues were infiltrated with molten paraffin wax at 56ºC. The tissues were embedded in paraffin wax for a period of 24-48 hours. Sectioning of the tissues was carried out on a rotary microtome (Bright automatic rotary microtome) at 5 µm. The sections were stained with (a) Verhoeff's Haematoxylin elastic tissue stain for the demonstration of elastic fibers, (b) Van Gieson's staining method for the demonstration of collagen fibers and (c) Haematoxylin and eosin for histo-architecture.

RESULTS

It was noted from our present study that the epithelial lining of the stomach are mainly simple columnar with the exception of the cardia and fundus which had stratified squamous keratinized epithelium (Table I. and Fig. 3). The mucosa and muscular coats were also observed to be well organized with a well formidable muscularis externa (Fig. 3) believed to be adopted for the churning of the high chitinous diet. Also, collagen fibers were observed to stain more intensely in the submucosa compared to the mucosa and muscularis externa. The staining intensity was observed to be more in the cardia and fundic regions of the stomach as shown in Fig. 4. Smooth muscle fibers were also observed to stain intensely. Elastic fibers were stained so well in the muscularis externa of all the regions of the stomach. Traces of these fibers were also noticed along the length of the gastric glands in the corpus and pyloric regions Fig. 5.

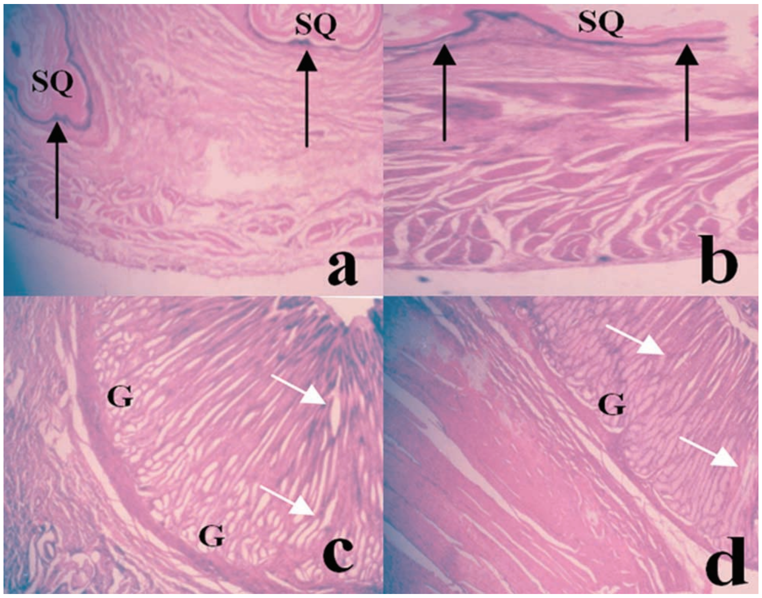

Fig. 3. H & E staining of the transverse section of the stomach (x 100). a) Cardia, b) Fundus, c) Corpus and d) Pylorus. Note the stratified squamous keratinised epithelium (SQ) in a and b well supported by a basement membrane (black arrow), also, note the secretory feature in c and d characterized by gastric glands (G) and gastric pits (white arrow).

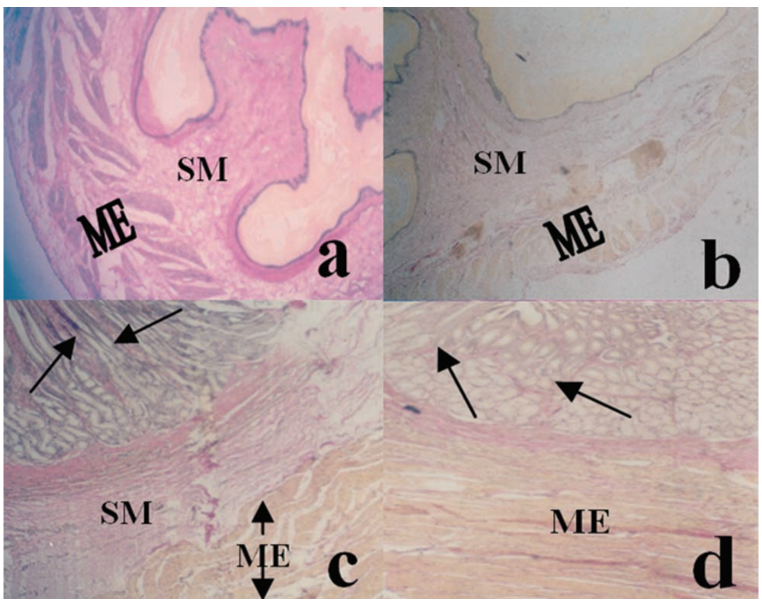

Fig. 4. Van Gieson stain (x 100). a) Cardia, b) Fundus, c) Corpus and d) Pylorus. Note the intense staining of collagen fibers in the sub mucosa (SM) of a, b and c. This staining is also evident in the muscularis externa (ME) and along the length of the gastric gland (black arrow).

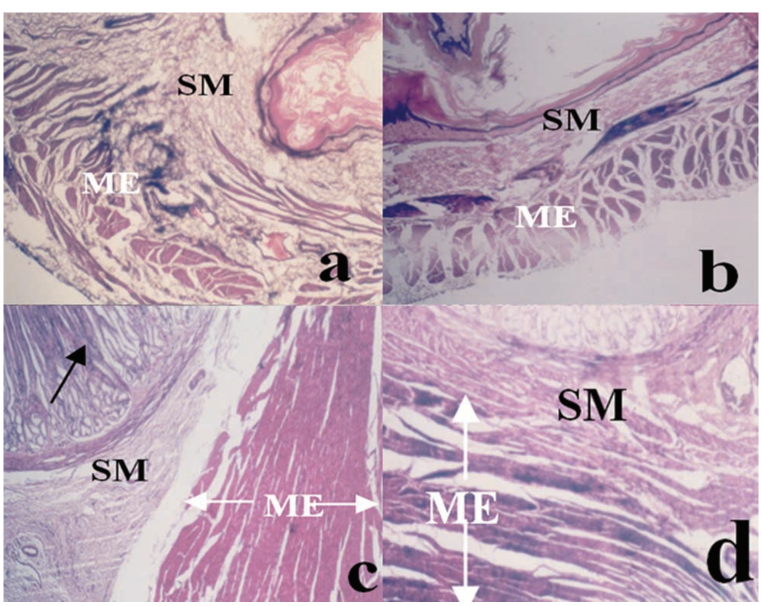

Fig. 5. Verhoeff's stain (x 100). a) Cardia, b) Fundus, c) Corpus and d) Pylorus. Note the high concentration of elastic fibers in the muscularis externa (ME) and to a lesser extent in the submucosa (SM). Also note some few fibers amidst the gastric gland (G) (arrowheads) and blood vessel (re arrow) in c.

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RESUMEN: La microarquitectura del estómago de los pangolines que favorece la alta dieta de chitinous sido poco tomada en cuenta, a pesar de las amplias investigaciones morfológicas. El análisis histológico de la microanatomía proporcionará herramientas de gran importancia para la interpretación, junto con dar una información confiable. Se investigó mediante la fijación de los tejidos en solución salina de formol al 10% para análisis histológico. Las serie de secciones fueron sometidos a métodos de tinción estándar para el estudio con microscopía de luz (hematoxilina y eosina, Van Gieson y Verhoeff's). Los resultados revelaron adaptaciones estructurales básicas en sus capas, con una modificación del revestimiento epitelial del cardias y fundus en epitelio escamoso estratificado (queratinizado). Estas modificaciones también se reflejan en la distribución de colágeno y fibras elásticas en las diversas capas del estómago. El presente estudio ha demostrado que es una adaptación del estómago a la dieta como se refleja en la configuración de la microarquitectura.

PALABRAS CLAVE: Estómago; Microarquitectura; Análisis histológico; Dieta; Capas.

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