THE OESOPHAGUS OF THE AFRICAN PALM SQUIRREL (*EPIXERUS EBII*): A MICRO ANATOMICAL OBSERVATION

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ABSTRACT

The oesophageal micromorphology of the rodent, African Palm Squirrel was investigated to fill the dearth of information on the histology of this organ from available literature and help in understanding its digestive tract biology. The organ after harvesting was subjected to routine histological procedure for light microscopy. The organ microanatomy was typical of the histology of mammalian tubular organ. The well-developed epithelium was of stratified squamous cells. The laminar propria containing elastic tissue fibres was apparently smaller than the large epithelium and muscularis mucosae. The muscularis mucosae striated muscle was arranged longitudinally. The small submucosa contained thin regular connective tissue. The tunica muscularis was made up of skeletal muscle cells which were arranged in an inner circular and outer oblique orientation, with the myenteric plexus located between these two muscle layers. The adventitia contained blood vessels. The well-developed epithelium is an adaptation to rough and coarse feed it consumes in the wild especially the fibrous coat of the oil palm fruit and other hard nuts.

Keywords: Histology, Myenteric plexus, African palm squirrel, Elastic fibres, Nigeria

INTRODUCTION

The oesophagus has been described as the transit tube connecting the oro-pharyngeal cavity to the stomach (Nzalak *et al*., 2010; Ikpegbu *et al*., 2012). The morphology of the oesophagus has been described in some domestic animals (McGeady *et al*., 2006), African giant rat (Nzalak *et al*., 2012), gopher snake (Khamas and Reeves, 2011), camel (Dellmann *et al*., 1968; Jamdar and Ema, 1982), laboratory mouse (Berghes *et al*., 2010); African catfish (Ikpegbu *et al*., 2012), but none has been documented in the African palm squirrel (APS).

Variations have been reported on the different coats of this organ. These include type of epithelium (Schummer *et al*., 1979; Berghes *et al*., 2010), nature and composition of the lamina propria (Sukon *et al*., 2009), presence or absence of the muscularis mucosae, even the type of muscle cell (Nzalak *et al*., 2010), presence and nature of oesophageal glands (Rossi *et al*., 2006) and type and orientation of the muscles in the tunica muscularis (Sukon *et al*., 2009). As part of continued study of wild rodent biology, the finding on the histology of this species is reported. Results of the present study will serve as a base-line for future reference in rodent oesophageal histology. The results will not only fill the knowledge gap, but also help in understanding the digestive tract biology and its role in adaptation of the African palm squirrel in the wild. Moreover, result of the microstructure of the African palm squirrel oesophagus will serve as a lead for future clinical and therapeutic intervention of diseased conditions of the digestive tract of the rodent.

MATERIALS AND METHODS

Five adult African palm squirrels of both sexes captured in the wild from Olokoro, Umuahia in Abia State, Nigeria from March 2013 to September 2014 using metal cage traps, were used for the study. Olokoro, Umuahia is in the rainforest vegetation of southern Nigeria characterized by heavy rains and thick mangrove forest.

The rodents were immediately transferred to the Veterinary Anatomy Laboratory of Michael Okpara University of Agriculture, Umudike, for acclimatization. During this period, the animals were fed with grasses, oil palm fruit and water *ad libitum.* The squirrels were sedated with chloroform and sacrificed by cervical decapitation. Animal weight was taken with Mettler balance (Model Ohaus Scout PRO-200) with a sensitivity of 0.1 g. The animal was placed on dorsal recumbency and cut open through mid-ventral incision from the inguinal region to the mandibular symphysis. The oesophagus was dissected out and slices fixed in 10 % neutral buffered formalin.

The tissues were processed by firstly, dehydrating them in graded ethanol. Thereafter, they were cleared in xylene, impregnated and embedded in paraffin wax. Sections 5 µm thick were obtained with Leitz Microtome Model 1512. They were stained with Haematoxylin and Eosin for light microscopy examination (Bancroft and Stevens, 1990). The slides were examined and photomicrographs taken with Motican 2001 camera (Motican, UK) attached to Olympus microscope.

RESULTS

The APS oesophagus at low magnification presented prominent epithelium, muscularis mucosae and tunica muscularis (Figure 1). At higher magnification, the stratified squamous epithelium was composed from surface to underlying laminar propriaviz: stratum corneum containing 5 – 8 layers of cell, stratum granulosum, stratum spinosum and deep stratum basale (Figure 2).

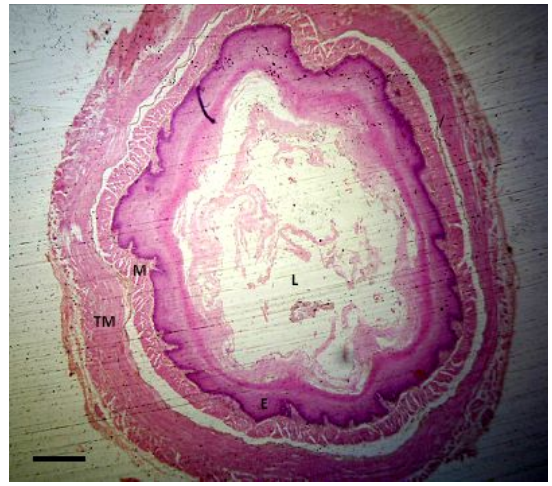

Figure 1: Transverse section of the oesophagus the lumen L, epithelium E, muscularis mucosae M, and tunica muscularis TM. Note the lumen L, containing desquamating cells. H & E (Scale bar = 4 µm)

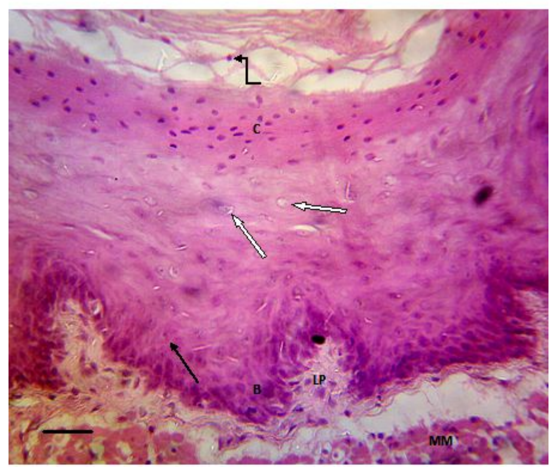

Figure 2: Section of oesophagus showing the epithelium containing stratum corneum C, stratum granulosum (white arrow), stratum spinosum (black arrow), and stratum basale B. Note the laminar propria LP, muscularis mucosae MM, and the desquamating cells (Z-shaped arrow head). H & E (Scale bar = 40 µm)

Polygonal to regularly shaped fibres containing desquamating squamous cells were seen attaching to the apical surface of the epithelial stratum corneum, hence, becoming the most proximal structure surrounding the oesophageal lumen (Figures 2 and 3). The laminar propria contained lymphocytes and elastic fibres which formed papillary layer that interdigitated with the epithelial ridges or pegs. The well-developed muscularis mucosae were of skeletal muscle arranged in a longitudinal fashion (Figures 2 and 4). The submucosa was relatively small and contained thin layer of regular connective tissue fibres (Figure 4).

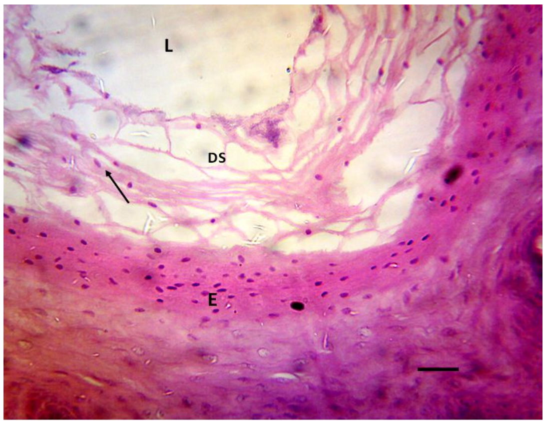

Figure 3: Section of oesophagus showing desquamating cells (black arrow), detaching from the epithelium E. Note that the desquamating cells layer DS, now surrounds the lumen L, directly. H & E (Scale bar = 40 µm)

The tunica muscularis contained skeletal muscle cells arranged in an inner circular and outer longitudinal to oblique orientation (Figures 4 and 5). No smooth muscle was observed. Myenteric plexus was observed between the two muscle layers of the tunica muscularis (Figure 5). The tunica adventitia of loose irregular fibrous coat contained blood vessels (Figures 5 and 6).

DISCUSSION

This study has attempted to expound the microstructure of the oesophagus of the African palm squirrel. Result of the study showed that the rodent APS oesophageal histology is typical of mammalian tubular organs. This finding has been reported in the *Chinchilla laniger* (Calamar *et al*., 2014).

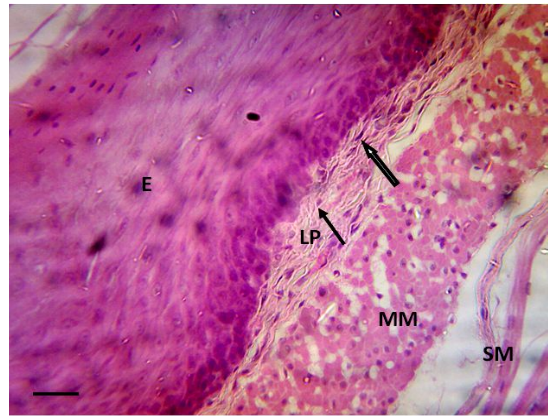

Figure 4: Section of the oesophagus showing the epithelium E, laminar propria LP, containing elastic fibres (white arrow), and lymphocytes (black arrow), muscularis mucosae MM, containing longitudinally arranged skeletal muscle cells. Note the submucosa SM, containing collagen fibres. H & E (Scale bar = 40 µm)

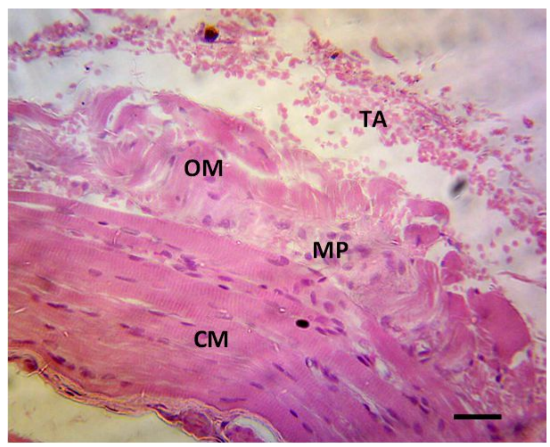

Figure 5: Section of the oesophagus showing the tunica adventitia TA, skeletal muscles of tunica muscularis arranged in an inner circular layer CM and outer oblique layer OM. Note the myenteric plexus MP, in-between the two tunica muscularis layers. H & E (Scale bar = 40 µm)

The stratified squamous cell is a protective epithelium against rough food materials (Sukon *et al*., 2009). This epithelium type may be related to its feeding habit and the presence of desquamating cell layer (Dellmann and Brown, 1987).

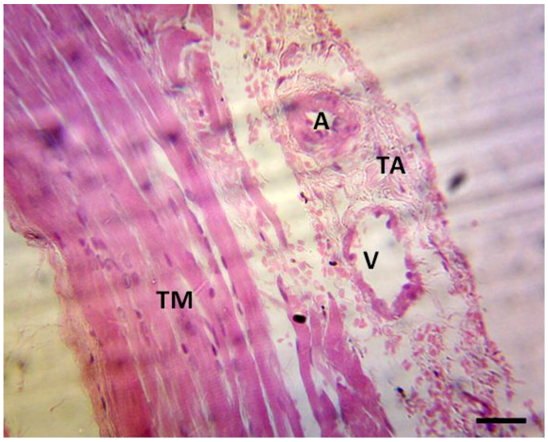

Figure 6: Section of oesophagus showing the Tunica adventitia TA containing artery A, and vein V. Note the tunica muscularis containing circular skeletal muscle. H & E (Scale bar = 40 µm)

This type of keratinized epithelium has been described in ruminants, horse and rodents, but non-kreatinized stratified epithelium as seen in this study, has been reported in the humans, dogs, cats and African giant rat (Schummer *et al.*, 1979; DeNardi and Riddell, 1991; Fawcett and Raviola, 1994, Nzalak *et al.*, 2010). A simple columnar to psuedostratifed columnar epithelium has been described in the gopher snake *Pituophis catenifer* (Khamas and Reeves, 2011), while a ciliated columnar epithelium with mucous secreting goblet cells has been reported in *Varanus niloticus* (Ahmed *et al.*, 2009).

Oesophageal gland was not seen in this study. This could be the reason for the observed luminal desquamating cells, which represents high cellular turnover. Also, the absence of the oesophageal glands resulted to the unavailability of intra-regional lubricant to ingesta on transit through the tube. The absence of oesophageal submucosal gland as seen in this study has been reported in the gopher snake (Khamas and Reeves, 2011), laboratory mouse (Berghes *et al.*, 2010), African giant rat (Nzalak *et al.*, 2010), but its presence has been reported in the oesophageal anatomy of the Llama – *Lama glama* (Sukon *et al.*, 2009), where it is associated with increased buffering capacity and absorption of volatile fatty acids.

The presence of elastic fibres in the lamina propria will allow some elasticity of the oesophagus when large volume of food is consumed. This elasticity will help reduce friction on the epithelium, thus a compensatory adaptation for lacking secretory glands. It is also possible that the documented salivary glands products may be aiding in food softening in the oral cavity before transit through the tube (Parillo *et al.*, 2005; Selvan *et al.*, 2008; Nzalak *et al.*, 2010; Ikpegbu *et al.*, 2013), but more work should be done on the salivary glands organ-volume ratio to ascertain this suggestion. Oesophageal lamina propria mucous glands have been described in the bird *Rhynchotus rufescens* (Rossi *et al.*, 2006).

The well-developed longitudinally oriented skeletal muscularis mucosae relative to smaller submucosa may be an adaptation to re-enforce the contraction of the predominant circular skeletal muscle of the tunica muscularis and the outer oblique muscle. This complimentary contraction under voluntary control will ensure uniform but regular peristaltic movement from all directions during deglutition. This will reduce incidence of food stasis in the tube, a phenomenon if it occurs will be dangerous in the wild where the animal must consume enough food in a short time because of prey-predator relationship and also competition from man that considers their foraging habit as pest activity. This observation agreed with the Sarosiek and McCallum (2000), who stated that this organ is highly specialized for the thrust of food from the mouth to the stomach. Absence of muscularis mucosae has been reported in the African giant rat (Nzalak *et al.*, 2010).Oesophageal muscularis mucosae containing smooth muscle cells have been reported (Dellmann and Brown, 1987).

Variations exist in the type and orientation of muscle cell of the tunica muscularis. In humans, horses, cats and pigs, the upper and lower portions of the esophagus are composed of striated and smooth muscles, respectively, with a mixed portion between them (Schummer *et al.*, 1979). In dogs, ruminants and rodents including mice, rats and hamsters, the muscle layer of the esophagus consists mostly of striated muscle fibers (McGeady *et al.*, 2006). In the laboratory mouse, the inner circular and outer longitudinal muscle coats are entirely striated muscle that changes to smooth muscle at oesophageal sphincter (Berghes *et al*., 2010), in the African catfish it is entirely striated muscle (Ikpegbu *et al*., 2012). In this study, the tunica muscularis was composed of mostly inner circular and smaller outer obliquely oriented striated muscle, the arrangement reported in the cranial two-thirds of horse oesophagus is the inner circular and outer longitudinal (Schummer *et al*., 1979); in ruminant, and camel, the arrangement of the tunica muscularis striated muscle fibers in the inner and outer layers is mixed, oblique and spiral (Getty, 1975; Jamdar and Ema, 1982).

The myenteric plexus observed in the study was very prominent like that reported in the cattle (Vittoria *et al*., 2000; Teixeira *et al*., 2001), but in the camel and Ilama, it was reported to be scant and difficult to locate (Jamdar and Ema, 1982; Sukon *et al*., 2009). Though the nature of function of the striated esophageal muscle myenteric plexus is still largely unknown (Diamant, 1989; Conklin and Christensen, 1994; Wörl and Neuhuber, 2005; Clouse and Diamant, 2006; Sukon *et al*., 2009; Shiina and Shimizu, 2012), but recent reports in literature have indicated dual innervation from both nerve fibers from the vagus and the myenteric plexus on the endplates of oesophageal skeletal muscle in the guinea-pig and rat (Zhou *et al*., 1996; Neuhuber *et al*., 2001).

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