# Histomorphology of the stomach mucosa of Emin's mole rat (*Heliophobius emini*)

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#### SUMMARY

Histomorphological structure of the stomach mucosa of Emin's mole rats (n=10) was studied at five sampling points along the greater curvature. The first sampling point was at the esophagus-stomach junction and the fifth close to the pyloric sphincter. The stomach mucosa was glandular starting with a very narrow branched tubular mucous gland. Branched and coiled tubular mucous glands were again seen at sampling point three and five. The gastric gland bodies were deepest at sampling point number four. Parietal cells were the principal cells on the gland bodies except at sampling points three and five where mucous cells dominated. Presence of two areas with mucous glands suggests an evolved mechanism of protecting the stomach wall against physical and chemical damages, especially against the indicative highly acidic gastric environment. The large number of parietal cells on the stomach mucosa signifies a decreased gastric pH and very likely indicates a high bactericidal environment in the Emin's mole rat.

Key words: histomorphology, gastric mucosa, Emin's mole rat

#### **INTRODUCTION**

The mammalian order Rodentia has the largest number of species which display a wide range of dietary needs ranging from insectivory, granivory. omnivory and herbivory. Of the latter, include those feeding on shoots and leaves to fossorial or sedentary rodents that feed on roots, bulbs and tubers. Such a wide range in dietary reflected needs shall be on the morphophysiological adaptations of the digestive tract. The histomorphology of the stomach of various species of rodents has been widely studied. In the mouse, rats and hamsters, the stomach mucosa has two distinct parts, a non-glandular and a glandular part (Ghoshal and Bal, 1989). The same structural pattern is reported in the Laotian rock (Laonastes rat aenigmamus) (Scopin et al., 2015), muroid rodents of Saudi Arabia (Meriones rex, Meriones libycus, Acomys dimidiatus, Acomvs cahirinus and Dipodillus dasyurus), the South African spiny mouse (Acomvs spinosissimus) (Boonzaier et al., 2013, Walter et al., 2014), the white-tailed rat (Mystromys albicaudatus), and the Iraq local guinea pig (*Cavia porcellu*) Maddock and Perrin, 1981, Al-Rhman, 2016). However, some rodents such as the *Oligoryzomys* nigripes (Sub-family Sigmodoninae) has a glandular stomach (Borghesi et al., 2015).

Within mole rats, some variation on the morphological structure of the stomach has been reported in two species that consume a similar diet of roots and tubers. The Northeast African mole-rat (*Tachyoryctes splendens*, Family: *Spalacidae*) has a compound stomach while the Emin's mole

rat (*Heliophobius emini*, Family: *Bathyergidae*) has a simple glandular stomach (Kotze *et al.*, 2010; Sahd *et al.*, 2017).

The gastric mucosa of mammals in general, is lined by five major types of cells; columnar epithelial cells; mucous cells that secrete an alkaline mucus that protects the epithelium against shear stress and acid; parietal cells that secrete Hydrochloric acid (HCl); chief cells that secrete pepsinogen, a proteolytic enzyme and enteroendocrine cells that secrete a variety of regulatory peptides. Within the mammals, the occurrence and distribution of the various cells of the gastric glands varies considerably (Perrin and Curtis, 1980; Ghoshal and Bal., 1989; Jo and Brian, 2006).

Mole rats are subterranean mammals that spend their life in dark, poorly ventilated environment. Most of them are known for their exceptionally long lifespan that exceeds 30 years compared to the other rodents such as the house mouse that have a maximum life span of about 4 years (Tian et al., 2013). One of the mole rats, the naked mole rat (*Heterocephalus glaber*) has been shown to have fibroblasts that have anti-carcinogenic property (Tian et al., 2013). Many organs forming the digestive system of the moles are also used as source of stem cells, and there are promising results for the use of these cells in regenerative medicine (Brittan and Wright, 2002).

The Emin's mole rats are widely distributed in East, Central and Southern Africa including Tanzania and they could form a potential animal model in cancer and regenerative medicine research. Much as the general structure of the stomach has been described, there is no study yet that has looked at the microscopic structural changes of the stomach mucosa from the esophagus junction to the pyloric sphincter. The present study aimed at studying and documenting the histomorphology of the gastric mucosa of the Emini's mole rat (*Heliophobius emini*).

### MATERIALS AND METHODS

### Study site, tissue sampling and processing

The Emin's mole rats which are considered as agricultural pests were collected in February, 2017 from Mlali ward which is located about 18 kilometres southeast of Morogoro town. Mlali ward lies approximately at latitudes S 06° 58' and longitude 37°32'. Biophysically, lowland and river valleys are the predominating agro-ecological zone of the ward. The elevation is between 520 and 760 meters above sea level. The ward's sub-humid climate is dominated by a bimodal distribution of rainfall. The heavy and long rains start in mid-March and last until late May, while the short rains occur from November to January. The average annual precipitation is 890 mm and average annual temperatures is 24°C (Paavola, 2008)

A total of ten adult Emin's mole rats (considered adult if with 4 four cheek teeth per quadrant, 7 females and 3 males) were collected by excavation of the burrowing tunnels. The animals were transported to Sokoine University of Agriculture (SUA) where sample collection, tissue processing and analysis were performed. Each animal was euthanized using an overdose of chloroform (RANKEM, RFCL Limited, New Delhi, India), thereafter (within five minutes of death), the abdominal cavity was opened at the linea alba and the abdominal cavity organs exposed. Α thorough gross examination of the abdominal viscera was performed. Lack of

gross pathological lesions was interpreted as an indication of a healthy status, and was thus followed by tissue sampling.

The greater and lesser omenta were removed, and the digestive tract resected at distal end of the esophagus and at the cranial duodenum. The length of the greater curvature of the stomach was measured followed by its opening along the lesser curvature and the contents washed out with phosphate-buffered saline (PBS, pH 7.4). The stomach was immersion-fixed in 10% neutral buffer formaldehyde for at least 5 days. Thereafter, gastric wall samples were collected at five points (Fig. 1) and processed for paraffin embedding using a standard procedure (Kiernan, 1990) in a tissue processor (Shandon Southern Duplex processor, UK). The paraffin blocks trimmed followed were bv sectioning at a thickness of 5 microns (Baird Tatlock Chadwell Health, Essex, UK), stained with Hematoxylin-Eosin (Kiernan, 1990) and examined with the a light microscope.



**Figure 1.** Schematic drawing of the stomach indicating the sampling points 1-5. E, esophagus; D, duodeum.

### **Evaluation of the tissue samples**

For every tissue sample, the general structure of the gastric wall was studied at x10 and x20 objectives. Where there were

histological differences between the two ends of a section, the dominating histological structure was taken as the characteristic feature of that particular section. Similarly where there were histological differences between animals on a particular sampling point, the dominating (observed in  $\geq 6$  animals) one was taken as the characteristic feature of that particular sampling point. For each section, five gastric glands vividly showing the gastric pit, neck, body and the dilated end (fundus) were used for measuring the gastric gland pit and body depths. A calibrated graticule (Olympus, Japan) was used for this purpose. The relative populations of parietal and chief cells along gland body were evaluated by arbitrarily assessing the dominating cell type. Student's t-test was used to compare means of the gastric gland body and pit depths.

### RESULTS

### **Gross anatomy**

The stomach was a dorso-ventrally flattened sac located in the left cranial quadrant of the abdominal cavity and had a prominent fundus. The mean length of the greater curvature of the stomach was  $4.3 \pm 1.2$  cm.

### **Microscope anatomy**

## Microscopic structure of the abdominal esophagus

The esophagus was lined by a highly keratinized stratified squamous epithelium (Fig. 2). Smooth muscle cells formed the *lamina muscularis mucosa* that formed a complete layer beneath the *lamina propria*. The *tunica muscularis* was formed by skeletal muscles (Fig. 2) which formed a thicker inner circular and outer longitudinal layer.



**Figure 2.** Light micrograph of the esophageal-gastric mucosa. The epithelium of the esophagus (A) is highly keratinized and under laid by a prominent *lamina muscularis mucosa* layer (D) composed of smooth muscles. The gastric mucosa starts with very prominent branched tubular mucous glands (C). Many parietal cells were evident in the initial part of the glandular mucosa (B). Note the *tela submucosa* (E) and the striated muscles of the *tunica muscularis* (F). (H.E., x50)

### General microscopic structure of the stomach wall

The stomach wall was divided into four main lavers. tunica mucosa tela submucosa, tunica muscularis and tunica serosa/adventitia. The lamina epithelialis of the mucosal layer was composed of tall or low simple columnar. Mucus-secreting cells characterized by basally located deeply staining (dark) nucleus also covered some parts of the epithelium. Such mucous cells extended into the gastric pits and in sampling point number three, these mucous cells lined the entire gastric glands. The distribution of the mucous cells did however; vary along the sampling points.

Parietal cells were characterized by their large size (average of 2 µm in diameter) and an eosinophilic cytoplasm. These cells were observed at all sampling points except sampling point number three. The least was at sampling point number 5 while the highest numbers were at sampling point number four. Chief cells were characterized by basal cytoplasm that stained basic, a round nucleus and an apical cytoplasm that appeared vacuolated. The population of chief cells was much lower

than that of the parietal cells at all sampling points.

The *lamina muscularis mucosa* consisted of smooth muscle cells while the *tela submucosa* consisted of loose connective tissue with a very prominent vasculature and submucosal nerve plexus. A thick inner circular and an outer thinner longitudinal smooth muscle fibre characterized the *tunica muscularis*.

### **Glandular morphometry**

Table 1 summarizes mean  $\pm$  SD of glandular body depth and gastric pits depth of mucosal glands at the various sampling points. The mean glandular body depth increased craniocaudally reaching the maximum at sampling point number 4 (552.1\pm68.6) and becoming lower again at sampling point number 5 (69.4\pm28.7). The gastric pits were deepest at sampling point number 5 (193.5 ± 28.7 µm) although it did not differ with of sampling point number one (161.0±21.1, p>0.05).

**Table 1.** Mean  $\pm$  SD (in  $\mu$ m) of glandular body and gastric pit depths of mucosal glands at the various sampling points. Means in the same column with different superscripts are significantly different (p<0.05)

Sampling	Depth of	Depth of
point	gastric gland	gastric gland
number	bodies	pits
1	$^{a}138.4 \pm 12.7$	$^{a}161.0 \pm 21.1$
2	$^{\circ}201.6 \pm 49.9$	$^{\mathrm{b}}98.6 \pm 40.2$
3	$^{c}250.1 \pm 70.9$	<sup>b</sup> 96.6 ±39.1
4	$^{d}552.1 \pm 68.6$	$^{\circ}55.2 \pm 15.0$
5	${}^{\mathrm{b}}69.4 \pm 28.7$	$^{a}193.2 \pm 28.7$

# Histological structure of the stomach mucosa at sampling point 1

The highly keratinized stratified epithelium of the esophagus changed abruptly into a glandular epithelium (Fig. 2). The initial part of the glandular epithelium had a prominent simple-branched tubular mucous gland (Fig. 2) which was followed by a mixture of simple tubular and simple branched tubular glands. The gastric pits were lined with columnar cells some of which were mucous in nature. However, mucous cells were not observed in the crypts. Parietal cells dominated the gland bodies. The skeletal muscles of the esophageal *tunica muscularis* extended into the very initial part of the glandular stomach. Thereafter, the *tunica muscularis* consisted of mingled smooth and skeletal muscles before becoming purely smooth muscles.

### Histological structure of the stomach mucosa at sampling point 2

At this sampling point, the gastric glands were both straight-tubular and branched tubular. The lining epithelium was low columnar with mucous cells that extended into the crypts (Fig. 3). Parietal cells dominated the gland bodies while some chief cells were observed mainly at the dilated ends (fundus) of the glands.



**Figure 3.** Stomach mucosa at sampling point 2 with gland bodies dominated by parietal cells (thin arrow). Note the chief cells in the dilated ends (fundus) of the glands (thick arrows), the *lamina muscularis mucosa* (L) and the mucous cells in the gastric pits and neck of the gland (M). (H. E., x250)

### Histological structure of the stomach at sampling point 3

Straight, branched and coiled tubular mucous glands characterized the mucosa at sampling point 3 (Fig. 4).

Parietal cells were very scanty at this sampling point.



**Figure 4.** Stomach mucosa at sampling point 3. Note the straight (S), branched (B) or coiled (C) tubular mucous glands, the columnar luminal epithelium (arrow) lining the gastric gland pit (P) and lumenal surface (arrow) (H.E. x 125)

### Histological structure of the stomach at sampling point 4

The mucosa at this sampling point was occupied by simple straight-tubular and branched gastric glands. This sampling point had the deepest gland bodies, and which were as at sampling point number 2, dominated by parietal cells (Fig. 5). The gland pits were also the shortest (Table 1). Chief cells were very rare being located close to the *lamina muscularis mucosa*. Mucous cells lined both the lumenal epithelium as well as the gland pits



**Figure 5a.** Gastric glands at sampling point 4 showing the deepest gland bodies and shortest pits (P). Parietal cells are the dominant cells of the gastric glands. M is the *lamina muscularis mucosa*. (H. E. x 125) Inset (b): A higher magnification (x250) of the glands with the *lamina muscularis mucosa* (M)

### Histological structure of the stomach at sampling point 5

At this point, coiled and branched tubular glands characterized the mucosa. The

gastric pits were the deepest amongst the sampling points (Table 1). Parietal cells were also evident (Fig. 6).



**Figure 6.** Stomach mucosa at sampling point 5. Note the deep gastric pits (A) and the tubular mucous glands (G). A few parietal cells (arrows) are also evident (H. E. x250).

#### DISCUSSION

The present work has documented a markedly keratinized epithelium of the esophagus, which is generally observed in adult rodents and ruminants and is believed to be correlated with the diet. However, unlike in the domestic mammals, the lamina muscularis mucosa formed a complete layer, a phenomenon that is seen in the primates.

In dogs, ruminants and rodents including mice, rats and hamsters, the muscle layer of the esophagus consists mostly of striated muscle fibers. Studies on the contractile properties of esophageal striated as compared to the cardiac and skeletal

muscles in rats demonstrate that esophageal muscle has properties similar but not identical to those of skeletal muscle and that some specific properties may be esophageal for peristalsis beneficial (Takahito et al., 2010). Esophageal motility is controlled centrally by an extrinsic neuronal mechanism and peripherally by an intrinsic neuronal mechanism (Cunningham and Sawchenko. 1990: Conklin and Christensen, 1994; Park and Conklin, 1999; Jean, 2001; Wörl and Neuhuber, 2005; Clouse and Diamant, 2006; Neuhuber et al., 2006; Goyal and Chaudhury, 2008). Whether presence of striated muscles enables the mole rat to regurgitate or vomit as in ruminants and carnivores remain to be determined.

In agreement to other reports (Kotze et al., 2009, 2010; Sahd et al., 2017), the present study demonstrated the mucosal lining of the stomach of Emin's mole rat is entirely glandular. The gastric mucosa in the domestic mammals and man is generally divided into three distinct glandular regions namely cardiac, fundic and pyloric. The distribution of these glandular regions differs between species. Microscopically, each of these glandular regions differs from the other too (Eurel and Frappier, 2006). For example, the cardiac glands do not have parietal cells, except in the carnivores. In the guinea pig (Cavia porcellus) and Laotian rock rat (Laonastes aenigmamus) the cardiac gastric glands as it is in most of the domestic mammals, also lacks parietal and mucous cells predominate cells (Scopin et al., 2015; Al Rhman, 2016). In the Oryzomys rodent, cardiac glands are of mucous in nature (Borghesi et al., 2015). In the present study, typical fundic glandular region (with shortest pits and longest gastric gland bodies) was seen at sampling point number four and therefore, very narrow. A similar finding is reported in the Laotian rock rat (Scopin et al., 2015).

mucous gland followed by glands that had a large number of parietal cells. Large mucous glands were also seen at sampling points 3 and they also dominated at sampling point number 5, which is similar to the guinea pig (Al Rhman, 2016). Studies in mice have also shown that mucous producing cells (mucous cells) had the highest population and percentage densities in the gastric mucosa (Dare et al., 2012). The mucus that is secreted onto the surface of the stomach acts as a lubricant, reducing physical damage to the epithelium by ingested material, acts as a physical barrier against luminal pepsin and HCl, and as a trap of bacteria (Forstner, 1978; Belley et al., 1999). Most likely therefore, the presence of a large mucous gland at the esophagus-cardiac junction and at sampling points three and five ensures availability of mucus to protect the stomach lining against physical and chemical injury by the HCl which is secreted in more than 60% of the stomach mucosa. In this study, parietal cells were the

The gastric glands started with a prominent

dominating cell of the gastric glands (except where the glands were mucous). Chief cells have also been reported to be very scarce in the guinea pig (Ghoshal and Bal, 1989). Reduced chief cells are thought to be an evolutionary adaptation to reduce the secretion of enzymes that degrade the symbiotic microorganisms in the stomach of rodents (Scopin et al., 2011). Dominance of parietal cells in the gastric glands were also reported in the reddishgrey musk shrew, Crocidura cyanea and guinea pigs (Ghoshal and Bal, 1989). Charleton (1973) had suggested that a reduction of parietal cells in the glandular epithelium might result in decreased HCl secretion, and that an increased gastric  $p^{H}$ , prolonged salivary amylase activity and increased carbohydrate (CHO) digestive efficiency. Hypochlorhydria and

achlorhydria increase the risk of and exacerbate the severity of bacterial and certain parasitic infections (Giannella et al., 1973). The presence of a large population of parietal cells in about 60% of the stomach mucosa in the Emin's rat therefore signifies a decreased gastric  $p^{H}$ and very likely therefore, a decreased CHO digestion efficiency. However, this will also very likely indicate a high bactericidal environment in the Emin's mole rat. The produced HCl in combination with other substances including immunoglobulins and lactoferrin are capable of reducing bacterial colonization of the stomach. Nevertheless, resistance of the stomach lining to irritants and injury is also mediated by a number of autacoids including prostaglandins, gaseous mediators (nitric oxide and hydrogen sulphide) and neuropeptides such calcitonin gene-related as peptide (Wallace, 2008).

Although some studies have indicated a histological difference between the greater curvature and lesser curvature walls in rabbits (Oliveira *et al.*, 2001), this remain to be determined in the Emini's mole rat. However, the observed presence of a mucous glandular zone at the proximal end of the gastric mucosa and at the mid part, and presence of parietal cells at all sampling points except number three is a very unique histomorphological feature of the Emin's mole rat.

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