

SOME HISTOLOGICAL STUDIES ON THE DIGESTIVE SYSTEM OF THE SPALAX LEUCODON (LESSER BLIND MOLE RAT) INHABIT THE EGYPTIAN NORTH COAST

O.S. Tammam¹, S.A. Emara² and Dalia Abo Sedera¹

¹*Survey of Natural Resources in Environmental System Dept., Environmental Studies & Research Institute, University of Sadat City, Egypt.*

²*Cytology & Histology Dept., Faculty of Veterinary Medicine. University of Sadat City, Egypt.*

ABSTRACT

The aim of the present study is to reveal some anatomical and histological structure of the digestive system of the blind mole rat (*Spalax leucodon* Egyptianus). Nine blind mole rats of both sexes were used for this study. The digestive system of the lesser blind mole rat resembles that of other rodents except for some variations. The lips are very small as a flap of skin. The incisor teeth are long, curved and powerful. The stomach is unilocular compound type. The proventriculus is demarcated from the glandular gastric regions by a limiting ridge. The glandular region contains simple tubular glands, lined by three types of cells: mucous neck cells, parietal cells and chief cells. The outer longitudinal muscle layer of both cecum and colon showed teniae.

We can conclude that the digestive system of *Spalax leucodon* Egyptianus is similar to that of other rodents, and observed differences are due to adaptation to habitat and their diet.

INTRODUCTION

The genus *Spalax* contains the blind, fossorial or subterranean mole rats, which are one of several types of rodents that are called mole rats (**Macdonald, 2006**). In Egypt, the lesser blind mole rat (*Spalax leucodon* Egyptianus) lives in the el-Daba zone, Marsa Matruh Governorate, in the north coast (**Tammam & Omar, 2009**). Their eyes are completely covered by a layer of skin (**Richard Hoath, 2003**). The animals use their powerful front teeth for digging through soil (**Dewey, 2003; Hutchins, 2004; Schlitter et al., 2008**). The digestive tract is an important system in living organisms and plays a vital role in food processing and absorption (**Hill et al., 2008**). Investigation of the digestive system of the lesser blind mole rat is very important, where it is a rodent belonging to herbivorous animals which prefer special food such as plant roots, tubers and bulbs that grow underground (**Dewey, 2003; Hutchins, 2004; Shehab, 2004; Ozkan, 2007; Schlitter et al., 2008; Tammam & Omar, 2009 and Tammam & Refai, 2013**).

Several morphological studies have been performed to relate the variation in gastrointestinal structure to different feeding habits (**Chivers & Hladik, 1980; Perrin & Curtis, 1980; Langer, 2002**). However, there is still a need for a better understanding of the digestive system.

The goal of our study is to carry out some morphological and histological investigations of the digestive system of *Spalax leucodon* Egyptianus, which lives in Egypt at the north coast in the el-Daba zone.

MATERIAL &METHODS:

Nine mature apparently healthy lesser blind mole rats (*Spalax leucodon egyptiacus*) of both sexes were used in this investigation. They were obtained from El- Dabaa zone Marsa Matrouh Governorate, Egypt. The animals were sacrificed after slight anesthesia under chloroform inhalation. The digestive system was dissected and examined anatomically. Small pieces were taken directly after sacrifice from different parts of the digestive system of the lesser blind mole rats and fixed in 10% neutral buffered formalin. The samples were dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in soft paraffin, then blocked in hard paraffin wax (60 - 62°C, melting point). Sections of 5-7 micrometer thick were cut using rotary microtome, and stained with Harri's hematoxylin and eosin, Crossmon's trichrome stain, Alcian blue method "pH 2,5" and Periodic acid Schiff (PAS) technique (**Bancroft et al., 1996**).

RESULTS:

1-MACROSCOPICAL OBSERVATION:-

The oral cavity was bounded by two lips (Fig .3) The upper lip was larger than the lower one; each one was represented by a thin flap of skin surrounding the incisor teeth. The rest of the upper lip appeared as bilateral thickening covered externally with skin and contained with the upper jaw. The incisor teeth were long, curved and separated outside from the rest of the mouth by a flap of skin. So, they are still on the outside when the animal closes its mouth (Fig .3). The tongue was elongated flat and formed from: root, body and tip. The dorsal aspect of the tongue contained slight thickening at the posterior region toward the root represented as elevation or prominence as torus lingue(Fig.4). The stomach was unilocular and appeared as C-shape dilated sac (Figs.1&2). The wall of the stomach was thin in the proventriculus and thick in the glandular part with folds (rugae).The proventriculus was demarcated from the glandular gastric part by clear ridge (limiting ridge) (Fig. 5). The small intestine was divided into three parts; duodenum, jejunum, and ileum (Figs.1&2). The large intestine was formed from three distinct parts: cecum, colon, and rectum (Figs.1&2). The liver was large and situated in the right cranial abdominal cavity just behind the diaphragm. The liver was formed from five lobes left, right, right central, spigelian, and caudate lobes. The investigated liver contained gall bladder that presented in a depression in the right lobe of the liver .(Fig .6).

MICROSCOPICAL OBSERVATION:

Each of two lips was formed from central muscle mass lined internally by mucous membrane and covered externally with skin. The lamina epithelialis of internal surface was formed from stratified squamous keratinized epithelium. The skin contained hair follicles sweat glands and sebaceous glands. The hair follicles appeared compound , each was formed from groups of hair follicles surrounded by dense fibrous connective tissue capsules (Fig.7).The labial glands were lobulated and mainly mucous in nature .They gave faint reaction to alcian blue stain (Fig .9),while they appeared negative with PAS technique . The lamina epithelialis of the tongue was stratified squamous keratinized epithelium. The ventral surface of tongue appeared smooth and thin with thin keratin layer. The dorsal lingual surface was rough and thick with thicker keratin

layer, it contained lingual papilla. The filiform papillae were predominated on the dorsum of the tongue mainly on tip and body. The filiform papillae were appeared as thread-like projections directed caudally, its connective tissue core was not extended over the dorsal surface of the tongue(Fig.8) .The fungiform papillae were present inbetween the filiform papillae. The central muscle mass of the tongue was formed from striated (skeletal) muscle bundles which arranged in different directions .The tunica mucosa of the esophagus was appeared folded and forming longitudinal folds, the lamina epithelialis was formed from stratified squamous highly keratinized epithelium. The lamina propria was formed from loose connective tissue. The lamina muscularis mucosa was thin layer of smooth muscle bundles .The tunica submucosa formed from loose connective tissue (Fig .10). The tunica muscularis externa appeared thick and formed from two layers ,the inner circular and outer longitudinal smooth muscle fibers .The adventitia was noticed in the cervical part of esophagus and was formed from loose connective tissue continued with the other of the neighboring organs. The tunica serosa was appeared in both the thoracic and abdominal parts.

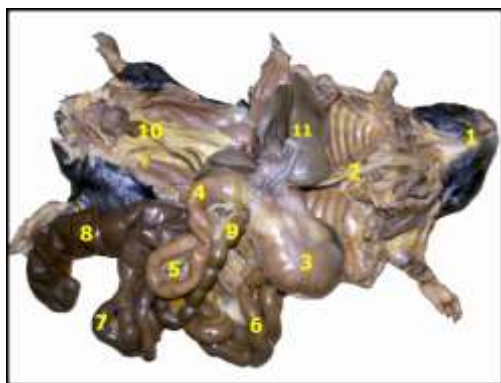
The first region of the stomach was small nonglandular part and was lined by stratified squamous highly keratinized epithelium (Fig.11), while the other region appeared glandular and was lined by simple columnar epithelium (Fig .12). Small redge or limiting ridge of fibrous connective tissue was separated between the nonglandular and glandular gastric regions (Fig .12).

The fundic gland region was the largest zone in the stomach; the lamina propria was appeared thick and occupied by simple branched tubular glands (Fig.13). These tubular glands were lined by mucous neck cells, parietal cells and chief cells. The mucous cell was short columnar cell; its cytoplasm appeared lightly basophilic and contained mucigenous granules. The nuclei were oval in shape and situated toward the base of the cell. The parietal cells were triangular or pyramidal in shape and appeared more obvious, mainly in the body of the tubular gland. Their cytoplasm was more acidophilic, their nuclei were spherical to ovoid in shape and situated toward the base of the cells (Fig. 14).The chief cells were more numerous cells, specially at the body and the base of the tubular glands, the cells were low columnar with lightly basophilic cytoplasm containing granules as zymogenic granules, their nuclei were spherical, vesicular and located toward the base of the cells (Figs.15) .

The intestinal villi of jejunum were more longer than that of duodenum and ileum .The lamina epithelialis was formed from simple columnar cells with brush border on their free surface and goblet cells inbetween(Figs.16).The intestinal glands were appeared in the lamina propria opened at the base of villi in the intervillar spaces .The intestinal glands were appeared as crypts (Fig.16). The Peyer's patches were observed in case of ileum .They were formed from aggregation of lymph nodules and lymphocytes in the lamina propria and sometimes extended into submucosa. The tunica submucosa was formed from loose connective tissue. In case of dudenum ,the submucosa showed mucous submucosal glands .The tunica submucosa appeared free from glands in case of jejunum and ileum.The tunica muscularis externa was formed from two layers of smooth muscle fibers. The tunica serosa of small intestine was thin and formed from loose connective tissue covered with mesothelium.

The tunica mucosa of the cecum appeared folded and contained plicae circularis. The lamina epithelialis was simple columnar epithelium with goblet cells inbetween. Lamina propria was formed from loose connective tissue (Fig.17) and occupied by long simple branched tubular glands (intestinal glands. They were lined by simple columnar cells and increased number of goblet cells. The lamina muscularis mucosa was thin and formed from smooth muscle fibers (Figs.17). The tunica submucosa was formed from loose connective tissue (Figs.17). The tunica muscularis externa appeared relatively thin in cecum and thick in colon and rectum. It was formed from two muscular layer of smooth muscle bundles. The outer longitudinal muscle layer contained teniae. Tunica serosa was thin. The mucosal folds of colon were long and less thick than that of the cecum and the plicae circularis were not distinct. The lamina propria contained short intestinal crypts, inbetween them loose connective tissue. The outer longitudinal muscle layer contained tinae coli. The rectum was contained thick tunica mucosa, with few mucosal folds. The lamina epithelialis was simple columnar epithelium with goblet cells (Figs.18). The lamina propria contained short intestinal crypt surrounded by loose connective tissue, also it contained lymph nodules and lymphocytes mainly at the caudal part of the rectum. The anal canal was continued with rectum by recto-anal junction. Its lamina epithelialis was stratified squamous keratinized epithelium. Propria submucosa formed from fibrous connective tissue contained blood vessels, lymph vessels and nerve fibers. Also it contained sebaceous gland. The tunica muscularis was skeletal muscle bundles.

The liver lobules were illdistinct, where the interlobular connective tissue was very few, but the classical hepatic lobule was appeared as polygonal in shape, its center contained the central vein while the peripheries contained portal zones "portal lobules". The hepatocytes were arranged side by side forming hepatic plates that arranged radially from the central vein toward the periphery of the hepatic lobule (Fig.19).



(Fig.1) gross morphology of digestive system tube

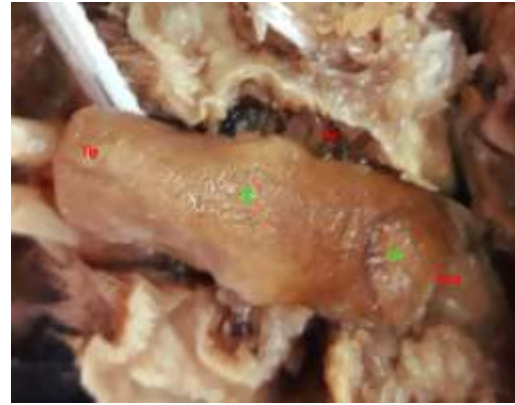
Oral cavity (1), esophagus (2), stomach (3), duodenum (4), pancreas (5), jejunum (6), ileum (7), cecum (8), colon (9), rectum (10) and liver (11) .



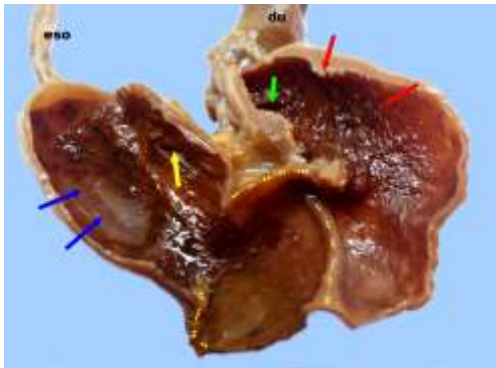
(Fig.2) anatomical structure of gastrointestinal



(Fig.3) Face of lesser blind mole rat showing long incisor teeth extended outside tongue



(Fig.4)The tongue of lesser blind mole rat in the lower jaw , showing :- tip , body (*) and root of the ,elevation or prominence (th) , molar teeth (mo) .

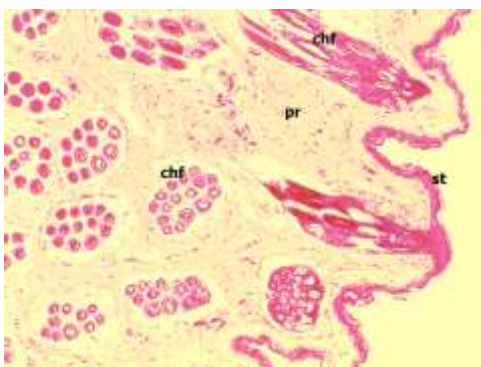


(Fig.5)The inner surface of the stomach ,

showing : gastro-esophageal opening (yellow arrow) , gastro- duodenal opening (green arrow) ,smooth surface of non-glandular region (blue arrow) , folded



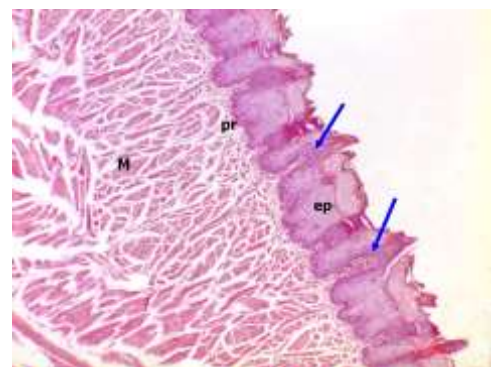
(Fig.6) The liver lobes (1-6) of lesser blind mole rat



mucosa of the glandular region (red arrow) , the limiting ridge (yellow dots) mole rat (spalax leucodon egyptiacus)

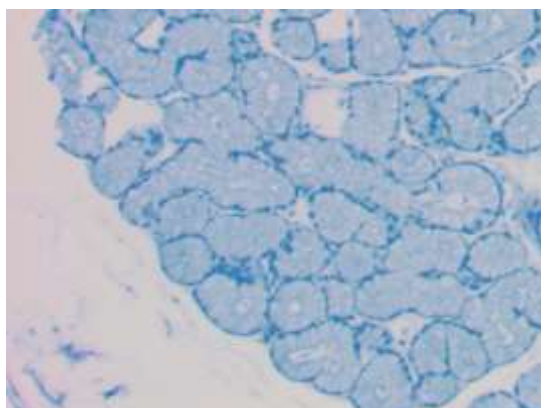
(Fig.7)External part of lip of the spalax leucodon muscle mass of skeletal muscle bundles (M) lamina

sebaceous gland (black arrow) and compound hair follicles (blue arrow) .

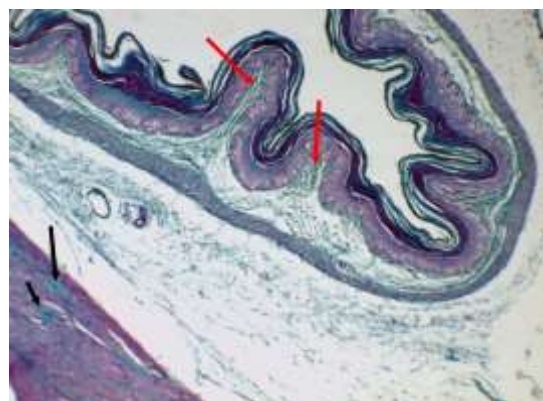


(Fig.8)dorsal surface of the tongue of spalax leucodon filiform papillae (arrow) , dense fibrous connective tissue

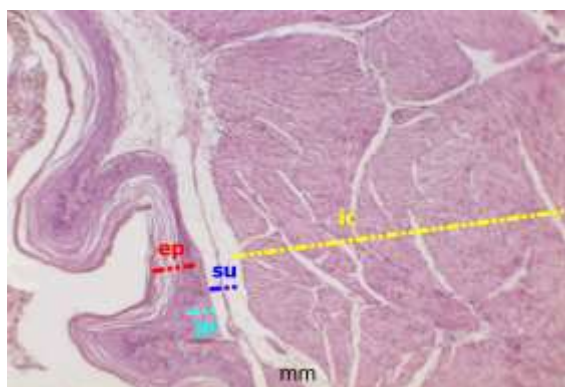
propria (pr) and the central muscle mass of skeletal muscle bundles (M) .



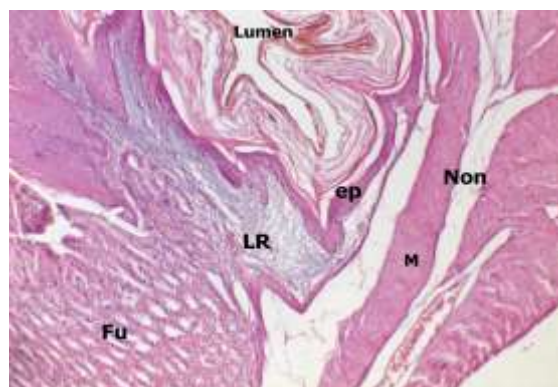
(Fig.9)labial gland of spalax leucodon with faint Reaction of the alcian blue stain



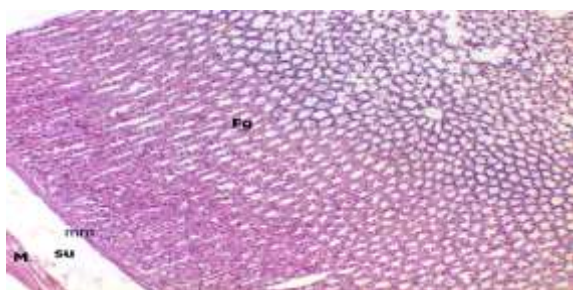
(Fig.10) C.S of the esophagus of spalax leucodon distribution of collagen fiber Crossmon's trichrome



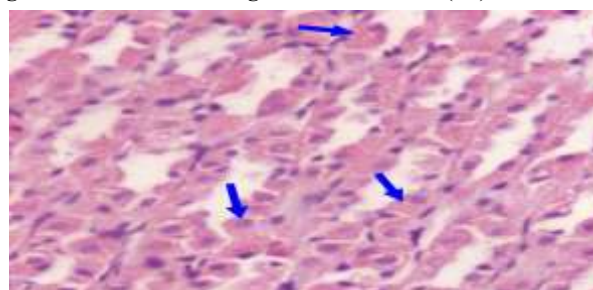
(Fig.11) Non-glandular stomach of lesser blind mole rat Spalax leucodon egyptiacus non-



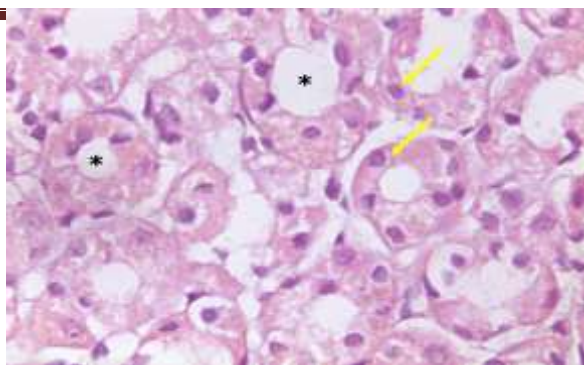
(Fig.12)The junction between the non-glandular and Glandular Spalax leucodon egyptiacus non-glandular stomach (Non),limiting ridge (LR) separates between the glandular stomach and glandular stomach (fu).



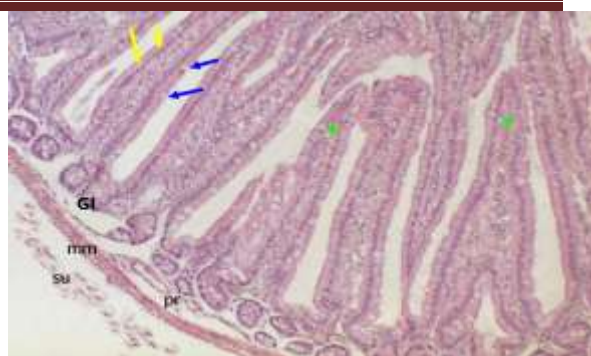
(Fig.13)C.S of simple branched tubular gland of parietal cells Glandular stomach of the spalax leucodon



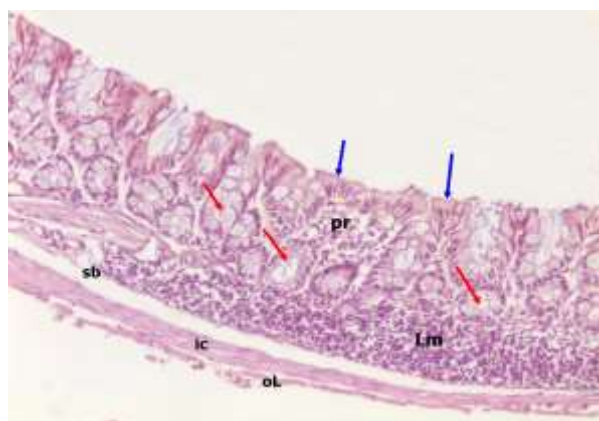
(Fig.14)glandular stomach showing of the spalax leucodon egyptiacus



(Fig.15)glandulr stomach showing chief cells of the Spalax leucodon egyptiacus



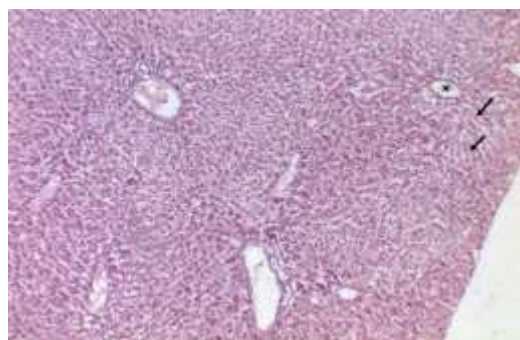
(Fig.16) the jejunum of the lesser blind mole rat showing long villi and crypts



(Fig.17) The rectum of lesser blind mole rat Showing intestinal gland lined by goblet cell



(Fig.18)The cecum of lesser blind mole rat showing collagen fiber in lamina propria with crossmon's Trichrom stain



(Fig.19) liver of the lesser blind mole rat showing : central vien (*) , radiated hepatic plates (arrows) .Note : the interlobular connective tissue is very few so , the lobulation didn't appeared .

DISSCUSION:

The structure of the gastrointestinal tube of the Egyptian lesser blind mole rat showing similar structure of the other rodent, that was studied by several authors' .(Chivers & Hladik 1980 ; Ghoshal and Bal .1989 ; Ali et al 2008 ; Nzalak et al 2010 ; Mohamadpour 2011 ; perez et al2011; Boonzaier 2012; AL Mahmodi 2014; Scopin et al 2015). Shehab, (2004) and Ozkan ,2007 mentioned that the upper incisors of mole rat are dorsoventrally directed and the lower incisors are craniodorsally inclined. The present study revealed that the tongue is rounded with rough dorsal surface and contains prominence on its caudal part, the same result was described Nasr et al., 2012 in rat where the anterior margin of the prominence is semicircular

and rises over the lingual body . we can concluded that the rough dorsal surface and prominence junction for protection. The dorsal surface of the tongue was rough and containing lingual papillae , the same result was mentioned by **Nasr et al., (2012)** and **Ghassemi&Cheshni (2014)** in rat; **Erdogan et al., (2015)** in Meerkat (*Suricata suricatta*). While ,**Stangl and Pfau (1994)** found that the tongue of geomyid and heteromyid ,contains lingual papillae on the dorsum and contains on to the sides. The present study showed that the esophagus is musclomembranous tube connects between pharynx and stomach , **Nzalak et al.,(2010)** in AGR rat & **Scopin et al., (2011)** in the Laotian rock rat , mentioned that the oesophagus is rather short and resembles a narrow pipe a short tube extending from the distal end of pharynx to the stomach . The present study showed that stomach of Egyptian lesser blind mole rat is c-shaped and formed from one compartment. It is divided into non-glandular and glandular parts by a distinct limiting ridge .the bordering fold stretched from the angular incisures of the lesser curvature to the greater curvature opposite the angular incisures .The same results were recorded by **Ghoshal and Bal (1989)** in mouse ,rat , hamster and gerbil and **Jacklynn et al.,(2014)** in different rodents ,while the demarcation between non-glandular and glandular parts are not distinct in the stomach of Guinea pigs . The present study showed that the small intestine is divided into non-demarked three parts duodenum , jejunum and ileum .The same result was mentioned by **Ali et al., (2008)** in AGR ; **Pérez et al.,(2011)** in chinchilla . The present study showed that the large intestine is formed from three demarketed parts :caecum ,colon , and rectum . The same result is mentioned by **Pérez et al.,(2011)** in chinchilla; **Stan et al., (2014)** in rabbit . The caecum in the Egyptian lesser blind mole rat is well developed and large as in rabbit (**Stan et al., 2014**) . The present study revealed that the liver of in the lesser blind mole rat is formed from five lobes left ,right ,right central ,spigelian or central , and caudate lobes . The same result was mentioned by **Abdel Aziz (1997)** in albino rats and **Boshra El salkh et al.,(2008)** in fat sand rat *psammomys obesus* . The lamina epithelialis of oral cavity of Egyptian blind mole rat is formed from stratified squamous keratinized epithelium rest on lamina propria of dense fibrous connective tissue. The same finding was recorded by **Delalande et al (2014)** who added that the rodent species have thinner and keratinized mucosa .

The lamina epithelialis covering the tongue is stratified squamous keratinized epithelium, which is thick with thicker keratin layer on dorsal surface and thin with thinner keratin layer on ventral surface. The dorsal surface is rough and contained lingual papillae. The same structure was mentioned by **Jackowiak,& Godynicki (2005)** in the bank vole , **Nasr et al (2012)** in rat and **Mustapha et al.,(2015)** in AGR. The filiform papillae are distributed on the dorsum of tongue mainly on tip and body, in between them few fungiform papillae. The same result was observed by **Nasr et al (2012)**, **Mustapha et al.,(2015)** and **Erdogan et.al2015** in rat . The esophagus of lesser blind mole rat is lined by stratified squamous keratinized epithelium with thin lamina muscularis mucosae and absence of esophageal glands. This finding confirms the same result which recorded by **Wilczynska (1999)** in *Apodemus flavicollis* , **Shina et al.,(2004)** in rodent ,and **Scopin et al.,(2015)** in the Laotian rock rat . While , **Nzalak et al.,(2010)** mentioned that the lining epithelium of the esophagus of African giant rat is non keratinized stratified squamous epithelium with no lamina muscularis and glands . The wall of the stomach of lesser blind mole rat is formed from four basic tunics , from inward outward are mucosa ,submucosa, muscularis and serosa . this confirms the same result in other different

rodents (Wilczynska ,1999 ; Fairoze khattab ,2007; Ofusori & Caxton-Martins ,2008; Ali et al., 2008; Laila El - shall ,2010; Eman & Haider ,2012; Laakkonen et al., 2014; Jacklynn et al., 2014). The parietal cells are large pyramidal in shape with more acidophilic cytoplasm and central ovoid nuclei . these findings equal to the observation in other different rodents (Wilczynska ,1999 ; Fairoze khattab ,2007; Ofusori & Caxton-Martins ,2008; Ali et al., 2008; Laila El - shall ,2010; Eman & Haider ,2012; Jacklynn et al., 2014). The wall of the small intestine of lesser blind mole rat of this study is formed from four tunics, inward outward: mucosa, submucosa , muscularis and serosa . The mucosal folds contain finger-like projections (villi) The lamina epithelialis is simple columnar cells with goblet cells inbetween . The intestinal glands appear as crypts and open at the base of villi in the intervillar spaces. The same observations were recorded by (Wilczynska,1999; Ofusori et al., 2008; Nzalak ,2011) in rats . The large intestine of the lesser blind mole rat is characterized by absence of villi and the presence of numerous goblet cells and intestinal glands . this as that recorded by Nzalak (2011). The present study revealed that the liver of Egyptian blind mole rat is covered by thin dense fibrous connective tissue capsule.The same result is mentioned by (Abdel Aziz,1997; Boshra El- Salkh et al ., 2008; Hanaa Waer& Seham Helmy ,2012; Safea wally ,2013). The pancreas of the lesser blind mole rat of the present investigation is covered by connective tissue capsule that connected with the interlobular connective tissue septa that divide the pancreas into numerous pancreatic acini and small faint zone of endocrine part of pancreas (islets of langerhans) (Eman ,2008; Moslem and Arrak ,2009; Yasmine ,2011 and Hanaa & Seham ,2012).

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