Comparative Histological and Ultrastructural Studies on the Stomach of *Hemiechinus auritus*, *Cavia porcellus* and *Mustela nivalis* in Relation to Their Diet

Hanaa R. Aboelwafa*, Fatma M.A. Taki-El-Deen and Samia M.I. Sakr

Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt
hanaa_aboelwafa@yahoo.com, hanaarezk@edu.asu.edu.eg

Abstract: Background: The present study aimed to provide a comprehensive histological and ultrastructural comparison of the stomach of three mammalian animals namely; *Hemiechinus auritus*, *Cavia porcellus,* and *Mustela nivalis* in relation to their diet. **Materials & Methods:** Six animals from each species were collected from different localities representing their natural habitats in Egypt. The cardiac portions of their stomachs were excised, cut into small pieces, fixed in the appropriate fixatives and processed for light and electron microscopic investigations. **Results:** Although some basic structural similarities existed in the stomach of these animals, marked differences were noticed. The histological results revealed that the mucosa appeared thicker in *Hemiechinus auritus* than that in the other two animals, the presence of well-developed peptic and parietal cells in both *Hemiechinus auritus* and *Mustela nivalis* than those found in *Cavia porcellus*, and the muscularis appeared thicker in *Hemiechinus auritus* than in the other two animals. Ultrastructurally, certain cytological differences were detected in the gastric mucosae of the examined animals. The surface epithelial cells showed numerous and denser mucous secretory granules in *Hemiechinus auritus* and *Cavia porcellus* than in *Mustela nivalis.* The peptic cells in *Mustela nivalis* displayed more abundantly secretory zymogen granules, mitochondria, rough and smooth endoplasmic reticula than those in *Hemiechinus auritus* and *Cavia porcellus.* Whereas, the parietal cells of *Hemiechinus auritus* and *Mustela nivalis* showed more discriminated intracellular canaliculi and tubule-vesicles among their cytoplasm than in *Cavia porcellus*. **Conclusion**: The present study showed marked differences in the histological and ultrastructural features of the stomachs of the examined mammalian animals, *Hemiechinus auritus, Cavia porcellus* and *Mustela nivalis* which may be correlated to the nature of the consumed food.

[Hanaa R. Aboelwafa, Fatma M.A. Taki-El-Deen and Samia M.I. Sakr. **Comparative Histological and Ultrastructural Studies on the Stomach of *Hemiechinus auritus*, *Cavia porcellus* and *Mustela nivalis* in Relation to Their Diet.** *Life Sci J* 2017; 14(6):1-13]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 1. doi:10.7537/marslsj140617.01.

Key words: Stomach; gastric mucosa, mammals; histology; ultrastructure.

1. Introduction

The stomach is an important organ of the gastrointestinal tract which implements the function of storage and churning of food into a semi-liquid substance known as chyme. The gross morphology of the stomach differs considerably across mammalian species; however, it reveals some basic structural similarities **(Ghoshal and Bal, 1989)**. The same author mentioned that the stomach morphology is greatly affected by the nature of feed, duration and need for food storage, frequency of food intake and adaptation, as well as body shape and size. Also, **Smith *et al.* (2000)** reported that mammals possess the most varied stomachs of any of the vertebrate classes, and the spacious variety of sizes and shapes of their stomachs is mostly due to the great difference of habitats they occupy, as well as to the wide range of food stuff they ingest.

Although several studies on different mammalian species are available, there is a lack of basic information concerning the detailed microscopic anatomy of the stomach of some mammals in the literature. Also, the ultrastructural features of the stomachs of several mammals were subjected to little inspection. Along the last decade, few studies were conducted on such topics which when evaluated were proven to be not complete and did not adequately cover such important biological subjects. In this regard, many mammalian animals have been previously studied such as Babirysa (*Babyrousa babyrussa*) **(Leus *et al.*, 2004)**; rat (*Rattus norvegicus*), pangolin (*Manis tricuspis*) and bat (*Eidolon helvum*) **(Ofusori and Caxton-Martins, 2008)**; different species of bats **(Santos *et al.*, 2008; Machado-Santos *et al.*, 2009; Sakr, 2010)**; Malayan pangolin (*Manis javanica*) **(Nisa *et al.*, 2010)**, guinea pig **(Berghes *et al.*, 2011)**; camel (*Camelus dromedarius*) **(Raji, 2011)**; mouse **(Dare *et al.*, 2012)**; Muong indigenous and Vietnamese wild pigs **(Tranget *al.*, 2012)**; Southern African Spiny mouse (*Acomys spinosissimus*), Reddish-grey Musk shrew (*Crocidura cyanea*), and Hottentot Golden mole (*Amblysomus hottentotus*) **(Boonzaier *et al.*, 2013)**; rabbit and chinchilla **(Stan, 2013)**; Laotian rock rat (*Laonastes aenigmamus*) **(Scopin *et al.*, 2015)**; Western grey kangaroo (*Macropus fuliginosus*)

Shoeib *et al.,* 2015) and the African rope squirrel (*Funisciurus anerythrus*) **(Igbokwe and Obinna, 2016)**.

The long-eared hedgehog, guinea pig, and least weasel are mammalian animals that ingest different diets. The long-eared hedgehog (*Hemiechinus auritus*) is one of the smallest Middle Eastern hedgehogs belonging to the family Erinaceidae **(Nowak and Paradiso, 1983; Hutterer *et al.,* 2005)**. It is an insectivorous animal that activates in the early evening looking for insects, myriapods, amphibians, gastropods and small vertebrates, as well as plants **(Hutterer, 2005; Stubbe *et al.,* 2008; Poddar-Sarkar *et al.,* 2011)**. While, guinea pig (*Cavia porcellus*) is a species of rodent belonging to the family Caviidae **(Wagner, 1976; Banks, 1989)**. It is also known as the cavy and is a strict herbivore which is often fed lettuce, cabbage and various types of grasses and fruits **(Sachser, 1998; Vanderlip, 2003)**. On the other hand, least weasel (*Mustela nivalis*) is the smallest and quickest carnivorous predator in the world belonging to the family Mustelidae **(Nowak, 1991; Wilson *et al.,* 2005)**. It is a nocturnal, widespread and abundant animal throughout the Northern hemisphere. Least weasel has a reputation for killing prey much larger than them. It is highly specialized rodent predators. When rodents are scarce, it eats also birds` eggs, lizards, amphibians, small fish, and invertebrates **(Sheffeld and King, 1994; King and Powell, 2007; Tikhonov *et al.,* 2013)**.

From the previous introductory remarks, we felt praiseworthy that the histological and ultrastructural studies on the stomachs of these animals are very limited to give a clear picture in order to make a comparison between them in correlation with their dietary habits and life styles. So, this investigation aims at comparatively examining the histological and ultrastructural features of the stomachs of the three mammals; the long-eared hedgehog, guinea pig, and least weasel to categorize any differences between them in relation to their feeding habits.

2. Materials and Methods

2.1. Experimental Animals

The present investigation concentrates mainly on three different mammalian animals; *Hemiechinus auritus* (long-eared hedgehog), *Cavia porcellus* (guinea pig) and *Mustela nivalis* (least weasel). Six animals from each species were collected from different localities representing their natural habitats in Egypt; *Hemiechinus auritus* is lived in burrows in the dry steppes, semi-deserts and deserts in the north of Egypt, guinea pig is lived in Nile Delta, and least weasel is restricted to the lower Nile Valley, from Alexandria in the west to Port Said in the east and from the Delta south to Beni Suef.

After collecting the animals, they were housed in separate cages and left for 12 h exempted from any food administration for the purpose of evacuating their stomachs. All animal studies were performed under protocols approved by the local Institutional Animal Ethics Committee of Ain Shams University.

2.2. Histological Preparations

Three animals from each species were anaesthetized under chloroform inhalation and their stomachs were harvested following midline-abdominal incision. The cardiac portions of their stomachs were cut into small pieces, fixed rapidly in aqueous Bouin`s fixative, dehydrated in ascending series of ethyl alcohol, cleared in terpineol and embedded in paraffin wax. After routine processing, 4-6μm sections were cut and stained with heamatoxylin and eosin (H & E) **(Bancroft and Gamble, 2002)**, then they were examined by light microscope and photographed as required.

2.3. Ultrastructural Preparations

For ultrastructural examination as described previously by **Dykstra *et al.* (2002)**, freshly excised cardiac portions of the stomachs from three animals of each species were cut into small pieces, fixed for 24 h in cold 4F1G (4% formalin + 1% glutaraldehyde adjusted at pH 2.2), then post-fixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.3). After that, they were dehydrated in ethanolic series culminating in 100% acetone and infiltrated with epoxide resin overnight at 60^{o}C. Semithin sections (0.5 μm) were cut, stained with toluidine blue and examined with a light microscope. Areas of gastric mucosa were selected and the blocks were trimmed accordingly, then ultrathin sections (80-90 nm) were cut, mounted on 200 mesh copper grids, and stained with uranyl acetate and lead citrate. The stained grids were examined and photographed by JEOL. JEM-1400-EX-ELECTRON MICROSCOPE at the Electron Microscopy Department of Theodor Bilharz Research Institute, El-Giza, Egypt.

3. Results

3.1. Histological Results

The histological investigation of the stomachs of the three mammals revealed a basic pattern of gastric structure with differences in the organization.

3.1.1. The stomach of *Hemiechinus auritus*

The stomach of *Hemiechinus auritus* consists of four consecutive layers arranged from inside to outside as; the mucosa, submucosa, muscularis, and serosa (Fig. 1A). The mucosa comprises of an epithelial lining, a supporting lamina propria of loose connective tissue rich in blood and lymph vessels and a thin smooth muscle layer, the muscularis mucosa. As obviously seen in Figures (1A & 1B), the muscularis mucosa is well developed consisting mostly of a thin inner circular fibers layer and an outer longitudinal fibers layer separating the mucosa from the submucosa. The gastric mucosa is the thickest part of the gastric layers and is thrown into prominent folds or rugae consisting of gastric glands which extend from the level of the muscularis mucosa to open into the stomach lumen via gastric pits which being numerous and deep (Figs. 1A & 1B). At the luminal part of the tubular mucosa, the surface epithelium formed of distinct tall columnar cells having large basally oval nuclei and eosinophilic cytoplasm. The gastric glands contain a mixed population of cells of three main types as clearly seen in Figures (1C & 1D). The first type is the surface mucus-secreting cells which cover the luminal surface of the stomach and line the gastric pits into which the gastric glands open; the cytoplasmic mucigen granules which pack these cells are stained poorly with H&E. Another type of mucus-secreting cells; the neck mucus cells are found in the necks and isthmus portions of the gastric glands (Fig. 1C). The second type is the acid-secreting cells, called parietal or oxyntic cells, are distributed along the length of the glands being most numerous in the middle portion or isthmus of the glands, and sometimes are intermingled with the neck cells towards the gastric pits. These cells are large round or cuboidal in shape having centrally located nuclei and cytoplasm which appears pinkish and clear with the stain (Figs. 1C & 1D). The third type is the pepsin-secreting cells, called peptic cells, are located towards the bases of the gastric glands. Peptic cells are characterized by their condensed, basally located nuclei and strongly basophilic granular cytoplasm as obviously illustrated in micrograph (1D).

As designated in Figure (1D), thin strands of the muscularis mucosa extend between the gastric glands from the base; contraction of this muscle expels gastric secretions into the stomach lumen. The submucosa is well defined constituting of loose connective tissue which contains blood vessels and lymphatics (Figs. 1A & 1B). The muscularis comprises a very thick layer of inner circular muscle fibers which is reinforced by a further inner oblique layer and a relatively thick layer of outer longitudinal muscle fibers. The coating layer, serosa which covers the peritoneal surface is thin consisting of simple squamous epithelium (Fig. 1A).

3.1.2. The stomach of *Cavia porcellus*

Examination of the histological sections of the stomach of *Cavia porcellus* revealed that its wall composes likewise of four distinct layers; the mucosa, submucosa, muscularis, and serosa (Figs. 2A & 2B). The mucosal layer reveals the arrangement of compound tubular glands which opened via less numerous gastric pits (Fig. 2B). The outer surface of these tubular glands forms of short columnar epithelial cells possessing narrow, weakly eosinophilic cytoplasm and small basally located nuclei. The surface mucous-secreting cells are numerous (Figs. 2C & 2D). Towards the base of these gastric glands, a considerable number of large parietal cells having highly eosinophilic cytoplasm and centrally located less weakly basophilic nuclei are present. These parietal cells extended up to the neck of the glands. At the border of the gastric glands, small numbers of these parietal cells are localized. Also, few numbers of peptic cells which are rounded in shape having conspicuously condensed centrally located nuclei and basophilic cytoplasm are present (Figs. 2C & 2D).

The muscularis mucosa lying immediately beneath the base of the gastric glands is thin and ill-developed as seen in Figure (2B). The submucosa is well represented consisting of loose connective tissue which embodies few small blood vessels. The muscularis constructs of a relatively moderate inner layer of circular muscle fibers and a thin outer layer of longitudinal muscle fibers (Figs. 2A & 2B). Thin serosa invests the tunica muscularis comprises of thin squamous epithelial cells is also illustrated in Figure (2A).

3.1.3. The stomach of *Mustela nivalis*

As illustrated in Figures (3A & 3B), the wall of the stomach of *Mustela nivalis* similarly consists of the mucosa, submucosa, muscularis, and serosa. The mucosa is in-folded into rugae built up of long closely packed tubular glands that composed of surface epithelium of tall columnar cells having large condensed oval nuclei and eosinophilic cytoplasm. These tubular glands appear provided with numerous surface mucous-secreting cells (Fig. 3C). Numerous large rounded parietal cells with highly eosinophilic cytoplasm and darkly stained spherical nuclei are found towards the neck of the gastric glands; small dark-blue stained peptic cells are intermingled with them (Fig. 3C). At the base of the tubular glands increased number of peptic cells possessing darkly stained nuclei and basophilic cytoplasm is clearly noticed (Fig. 3D). The supporting lamina propria is also illustrated in Figures (3C & 3D). The gastric pits are relatively numerous, deep and well defined. The muscularis mucosa is well developed (Figs. 3A & 3B). Also, the same figures illustrate the submucosa forms of loose connective tissue provided with few small blood vessels, the muscularis consists of a conspicuously thick inner circular muscle fibers layer intermingled with an oblique one and a relatively thin outer layer of longitudinal muscle fibers and the serosa formed of simple squamous epithelium.

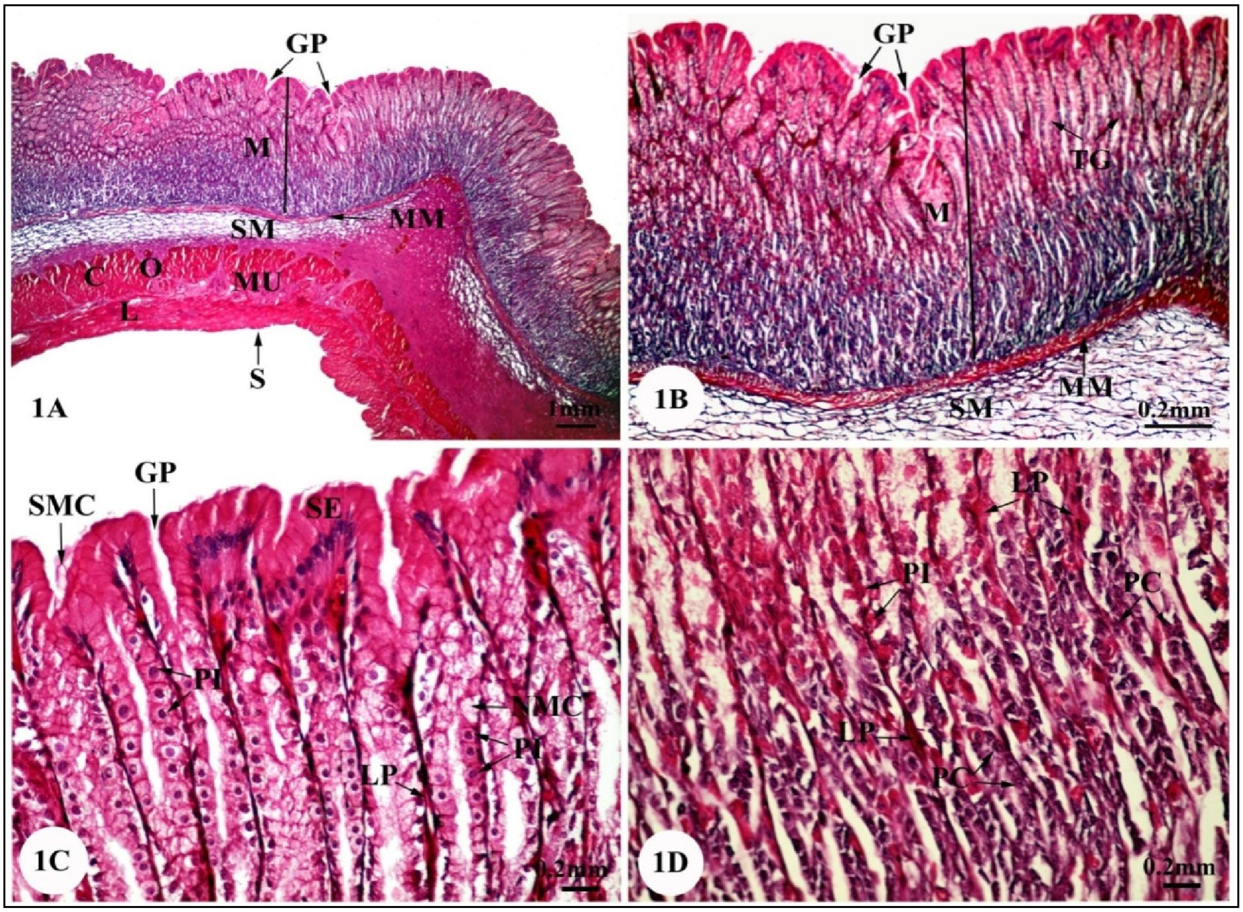

Figure 1. Light photomicrographs (A-D) of transverse sections of the cardiac region of *Hemiechinus auritus* stomach stained with H & E showing A: the mucosa (M), muscularis mucosa (MM), submucosa (SM), muscularis (MU) comprises an inner circular (C), an oblique (O) and an outer longitudinal (L) muscle fibers layers, as well as the serosa (S). **B:** The gastric mucosa (M) composes of straight tubular glands (TG) which open into the stomach lumen via deep gastric pits (GP). Note, the muscularis mucosa (MM) and the submucosa (SM) are well-developed. **C:** Highly magnified part of the gastric tubular glands revealing tall columnar surface epithelial cells (SE), surface mucous-secreting cell (SMC), neck-mucous cells (NMC) and parietal cells (PI). The supporting lamina propria (LB) is also seen. **D:** The bases of the gastric tubular glands are occupied almost by peptic cells (PC), as well as spindle-shaped parietal cells (PI). Lamina propria (LP) is seen.

3.2. Ultrastructural Results

Transmission electron microscopical examination of the gastric mucosae of stomachs of the three examined mammalian animals showed three distinct cell types, i.e., the surface epithelial cells, zymogenic or peptic cells and parietal or oxyntic cells.

3.2.1. The gastric mucosa of *Hemiechinus auritus*

3.2.1.1. The surface epithelial cells

The examined surface epithelial cells of *Hemiechinus auritus* are tall columnar in shape with distinct plasma membranes which exhibit extensions of short microvilli on their apical surfaces (Fig. 4A). The apical areas of the cytoplasm of these cells are loaded with numerous mucous granules of considerable sizes, being spherical or discoid in shape and showing electron density. Few stacks of rough endoplasmic reticulum are seen in the cytoplasm near the nuclei which are located towards the base of the cells being irregular in shape, surrounded by irregular double-layered nuclear envelopes and having dense masses of heterochromatin that are mainly concentrated in the inner aspect of the nuclear envelopes and interrupted by small areas of euchromatin (Figs. 4A & 4B).

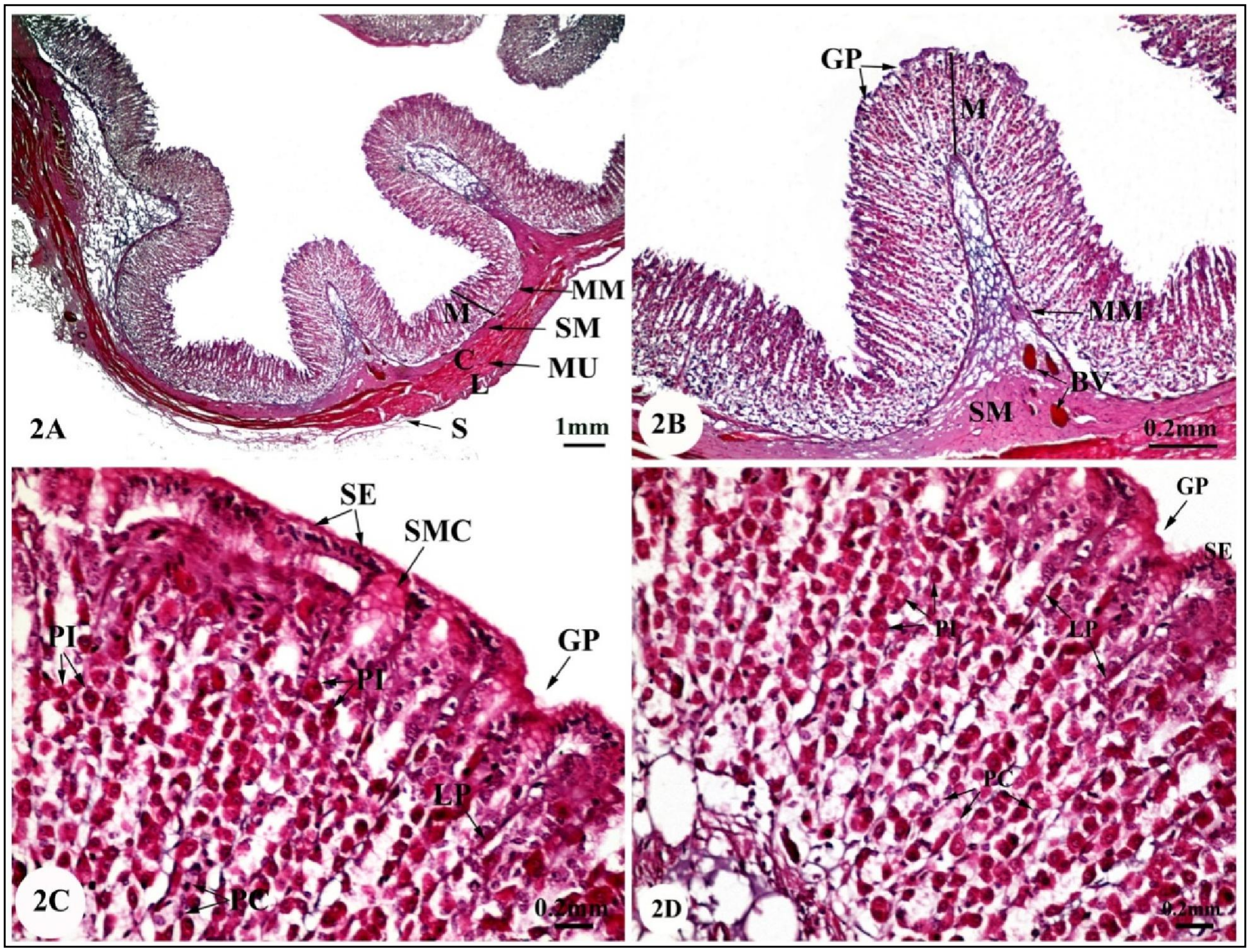

Figure 2. Light photomicrographs (A-D) of transverse sections of the cardiac region of *Cavia porcellus* stomach stained with H & E showing A: the four consecutive layers; the mucosa (M), muscularis mucosa (MM), submucosa (SM), muscularis (MU) comprises an inner circular (C) and an outer longitudinal (L) fibers layers, and serosa (S). **B:** The mucosa (M) throws into straight tubular glands (TG) opening via gastric pits (GP), ill-developed muscularis mucosa (MM) and well-developed submucosa (SM) embodying small blood vessels (BV) are seen. **C:** Magnified part of the gastric tubular glands shows less numerous gastric pits (GP), short columnar surface epithelial cells (SE), surface mucous-secreting cells (SMC), parietal cells (PI), and few numbers of peptic cells (PC). The lamina propria (LP) is seen. **D:** Another part of the gastric tubular glands showing a considerable number of large parietal cells (PI) present towards the base of the glands and extend up to the neck of the gland and few blue stained peptic cells (PC). The lamina propria (LP) is also observed.

3.2.1.2. The peptic cells

Examination of the peptic cells shows that these cells are pyramidal in shape with highly distinguished plasmalemma. As illustrated in Figures (4C & 4D), the cytoplasm is characterized by the aggregation of numerous spherical electron-dense zymogen granules at the apical secretory surfaces of these cells. Small numbers of spherical or oval mitochondria and cisternae of rough endoplasmic reticulum in the form of short tubular element are seen at the basal part of the cytoplasm. Free ribosomes are also seen distributed all over the cytoplasm. The nuclei of the peptic cells display somewhat irregular outlines and having prominent nucleoli, electron dense heterochromatin, and finely euchromatin.

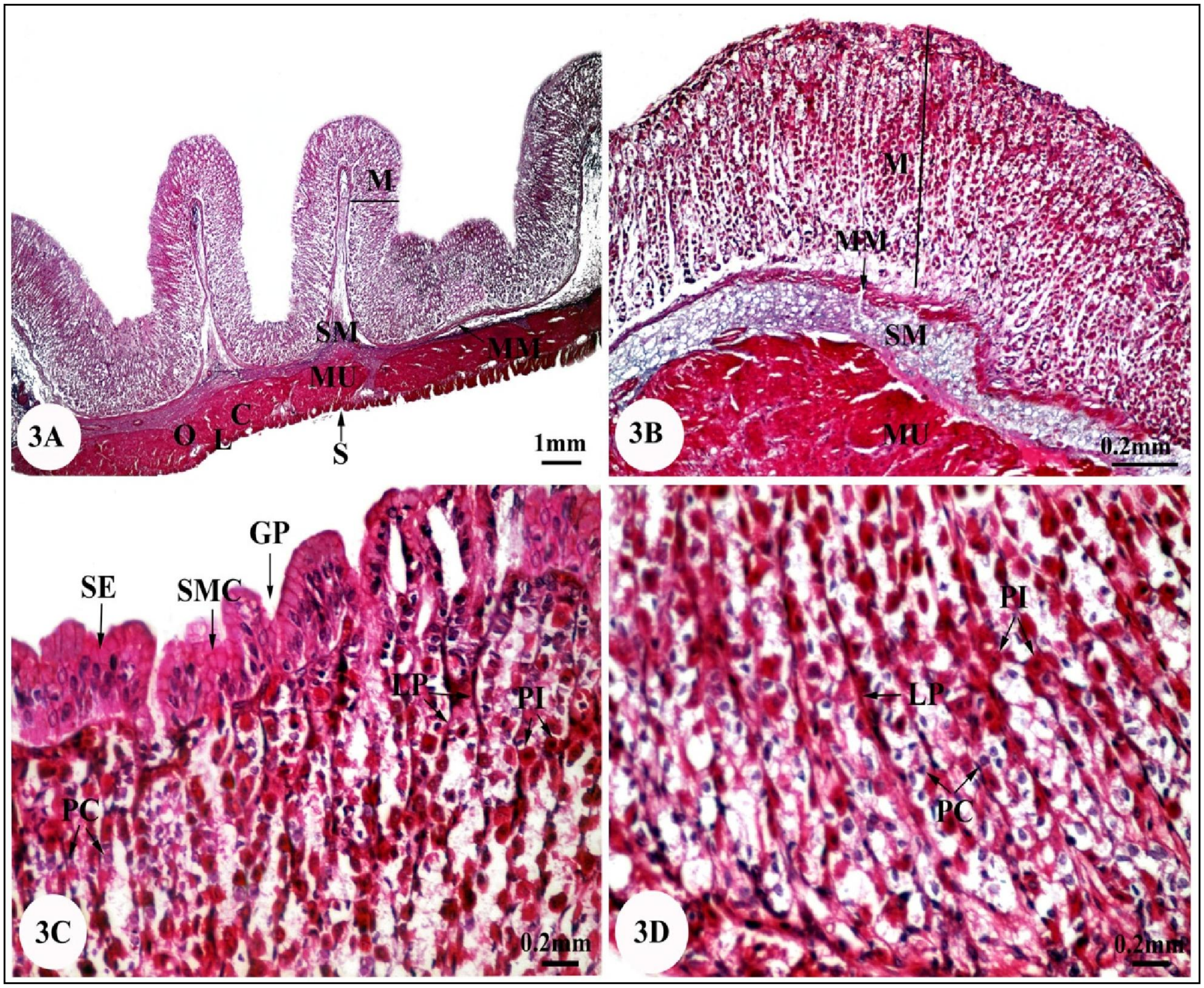

Figure 3. Light photomicrographs (A-D) of transverse sections of the cardiac region of *Mustela nivalis* stomach stained with H & E showing A: the stomach wall layers; the mucosa (M), muscularis mucosa (MM), submucosa (SM), muscularis (MU) distinguished into an inner circular (C), an oblique (O) and an outer longitudinal (L) muscle fibers layers and serosa (S). **B:** The mucosa (M) forming of straight tubular gastric glands (TG), the well-developed muscularis mucosa (MM), the submucosa (SM) and the muscularis (MU) are seen. **C:** Magnified part of the gastric tubular glands that open with deep gastric pits (GP) and contain tall columnar surface epithelial cells (SE), numerous parietal cells (PI) and spherical peptic cells (PC) situated in between them. **D:** Another enlarged part from the bases of the tubular glands showing increased number of peptic cells (PC) and parietal cells (PI) situated towards the neck of the glands. The lamina propria (LP) is seen.

3.2.1.3. The parietal cells

The parietal cells are pyramidal or oval in shape with their apices directed towards the lumen of the stomach. Numerous intercellular canaliculi, as well as many tubule-vesicles are randomly scattered in the cytoplasm as clearly observed in the electron micrographs (4E & 4F). The cytoplasm of the parietal cell is also crowded with large oval mitochondria, some of them are arranged around the nucleus and the remainders are closely packed in the cytoplasm peripheral to the canaliculi. The same figures display that the cytoplasmic matrix contains small tubular stacks of rough endoplasmic reticulum as well as free ribosomes. The nuclei of the parietal cells appear oval in shape with irregular envelopes. They are basally-located and their nucleoplasm contained aggregations of euchromatin as well as numerous heterochromatin particles.

3.2.2. The gastric mucosa of *Cavia porcellus*

3.2.2.1. The surface epithelial cells

As revealed in Figures (5A & 5B), the surface epithelial cells are rather cuboidal to columnar in shape with distinct plasma membrane which exhibits extensions of few short microvilli on their apical cell surfaces. The cytoplasm is heavily loaded with mucous granules of variable sizes and electron-densities, being localized at the apical secretory poles of these cells. Also, the cytoplasm contains scant mitochondria that are randomly distributed. The nucleus is located towards the base of the cell and being oval or irregular in shape having a double-layered nuclear envelope, homogeneous distribution of euchromatin as well as dense masses of heterochromatin.

3.2.2.2 The peptic cells

The peptic cells are columnar or pyramidal in shape with their apical borders covered with small microvilli. The apical secretory part of the cytoplasm contains numerous variable-sized electron dense zymogen granules as clearly observed in Figure (5C). Cisternae of the rough endoplasmic reticulum are present near the nucleus with their outer surfaces are studded with ribosomes (Fig. 5D). The nuclei of peptic cells display somewhat irregular outlines, covered with double-layered nuclear envelopes and having electron dense heterochromatin which are mainly concentrated on the inner aspect of the nuclear envelopes as well as dense finely euchromatin (Figs. 5C & 5D).

3.2.2.3. The parietal cells

As designated in the electron micrographs (5E & 5F), the parietal cells are oval in outline having cytoplasm showing numerous tubule-vesicles being randomly scattered. The cytoplasm also reveals numerous mitochondria, being ovoid or spherical in shape with rather distinguished mitochondrial ridges which are perpendicularly oriented along their membranes (Fig. 5E). Small parallel cisternae of the rough endoplasmic reticulum are also illustrated in Figure (5E). The nuclei of the parietal cells are centrally-located showing distinct nuclear membranes, aggregations of euchromatin and numerous heterochromatin particles (Figs. 5E & 5F).

3.2.3. The gastric mucosa of *Mustela nivalis*

3.2.3.1. The surface epithelial cells

The surface epithelial cells of the gastric mucosa of *Mustela nivalis* are columnar in shape with distinct plasma membranes (Figs. 6A & 6B). The same electron micrographs revealed that the apical areas of the cytoplasm are laden with numerous mucous granules of considerable sizes, as well as small sized mitochondria and few stacks of rough endoplasmic reticulum are randomly scattered all over the cytoplasm. The nuclei are oval in shape situated at the base of the cells and having a homogeneous distribution of chromatin materials.

3.2.3.2. The peptic cells

The peptic cells as illustrated in the electron micrographs (6C & 6D) are elongated or pyramidal in shape. Their cytoplasm is characterized by the aggregation of numerous spherical electron-dense zymogen granules at the apical secretory surfaces of these cells. Large numbers of spherical or oval mitochondria are seen in the cytoplasm. Also, cisternae of rough endoplasmic reticulum, as well as vacuoles of smooth endoplasmic reticulum are seen more abundantly in the peptic cells of this animal (Figs. 6C & 6D). Similarly, the nuclei of these cells possess irregular nuclear envelopes, nucleoli, aggregations of euchromatin, as well as numerous heterochromatin particles (Figs. 6C & 6D).

3.2.3.3. The parietal cells

Electron microscopical examination of the parietal cells showed that these cells are somewhat oval to pyramidal in shape with their apices directed towards the lumen of the stomach (Figs. 6E & 6F). The cytoplasm rich in tubular stacks of rough endoplasmic reticulum, free ribosomes, and oval shaped mitochondria. Intracellular canaliculi, as well as tubule-vesicles are seen in the cytoplasm. The nuclei of the parietal cells appear oval in shape possessing prominent nucleoli, aggregation of euchromatin as well as dense clumps of heterochromatin particles (Figs. 6E & 6F).

4. Discussion

The present study aimed to illustrate the differences in the histological and ultrastructural characteristics of the stomach of three mammalian animals which live in different localities in Egypt and ingest almost different types of food namely; *Hemiechinus auritus*, *Cavia porcellus* and *Mustela nivalis*, in order to correlate such differences with the nature of the consumed food by these animals.

The histological results obtained in the present study showed marked differences between the three mammalian species. The first dissimilarity was in the mucosa which was thick in the insectivore *Hemiechinus auritus*, while in the herbivore *Cavia porcellus* it appeared thinner than that present in the other two mammalian species. Also, the gastric pits between the villi of the mucosa in *Hemiechinus auritus* and *Mustela nivalis* appeared deep and numerous, while these pits appeared shallower in the mucosa of *Cavia porcellus*. In addition, the presence of well-developed peptic cells in both the insectivore *Hemiechinus auritus* and the carnivore *Mustela nivalis* may be due to that these animals feed on the flesh of their preys, thus they need the secretions of proteolytic enzymes by the peptic cells to digest the proteinic components of their food. Similarly, the current results showed the presence of numerous parietal cells in both *Hemiechinus auritus* and *Mustela* *nivalis* than those found in *Cavia porcellus*. These cells facilitate the process of digestion through their secretion of HCl which regulates the acidic medium to become suitable for the proteolytic enzymes. The second difference was in the muscularis which appeared thicker in *Hemiechinus auritus* than in the other two mammalian animals.

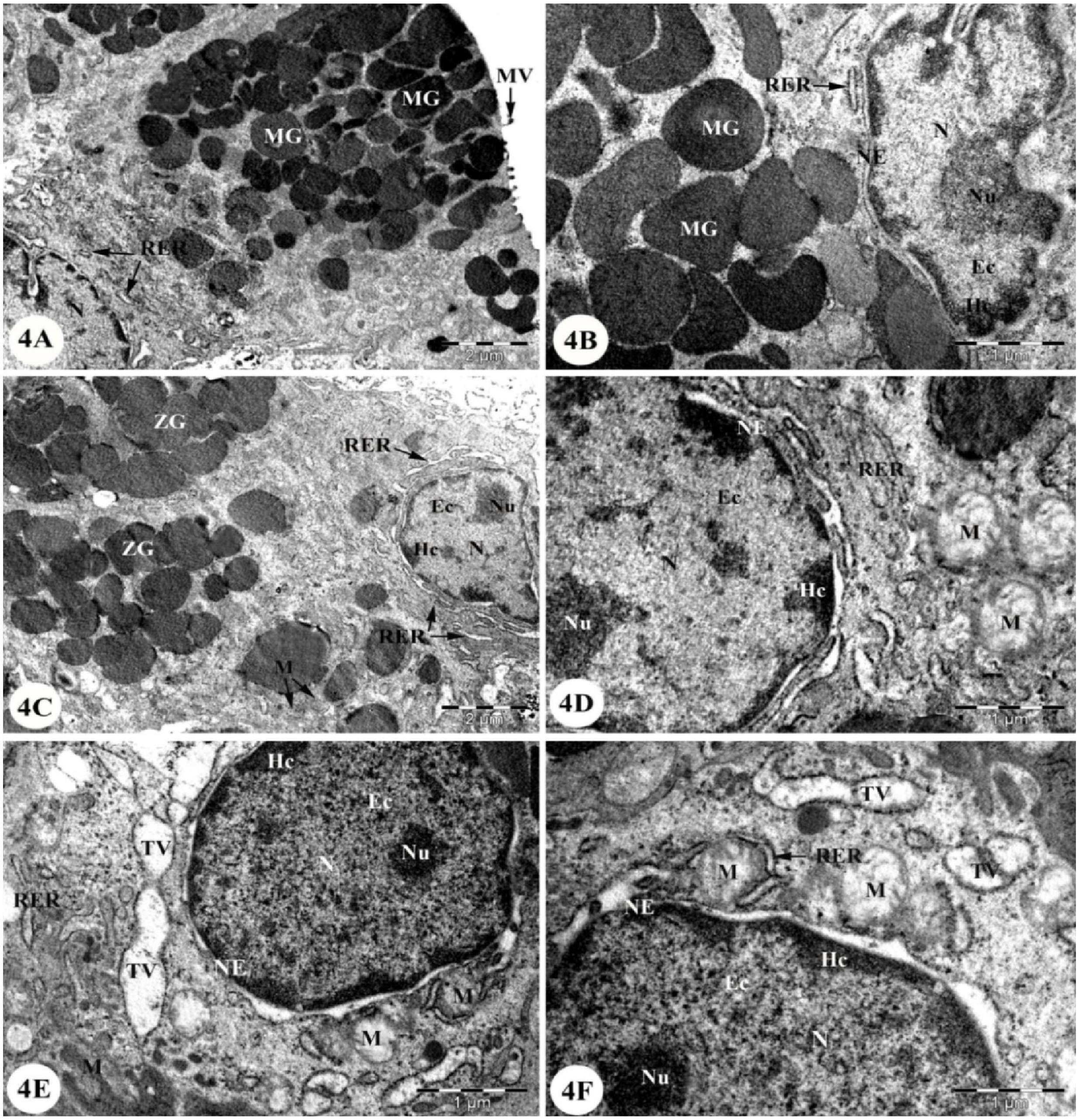

Figure 4. Electron micrographs (A-F) of the gastric mucosa of *Hemiechinus auritus* showing A & B: tall columnar surface epithelial cell having distinct microvilli (MV) on the apical surface, cytoplasm with numerous darkly stained mucous granules (MG) and few stacks of rough endoplasmic reticulum (RER), as well as nucleus (N) with irregular nuclear envelope (NE), prominent nucleolus (Nu), dense masses of heterochromatin (Hc) interrupted by euchromatin (Ec). **C & D:** Peptic cell possesses numerous electron-dense zymogen granules (ZG), oval mitochondria (M), cisternae of rough endoplasmic reticulum (RER), and nucleus (N) having prominent nucleolus (Nu), electron dense heterochromatin (Hc) and finely euchromatin (Ec). **E & F:** Parietal cell contains numerous tubule-vesicles (TV), large oval mitochondria (M), few stacks of rough endoplasmic reticulum (RER), and nucleus (N) contained aggregations of heterochromatin (HC) and euchromatin (EC).

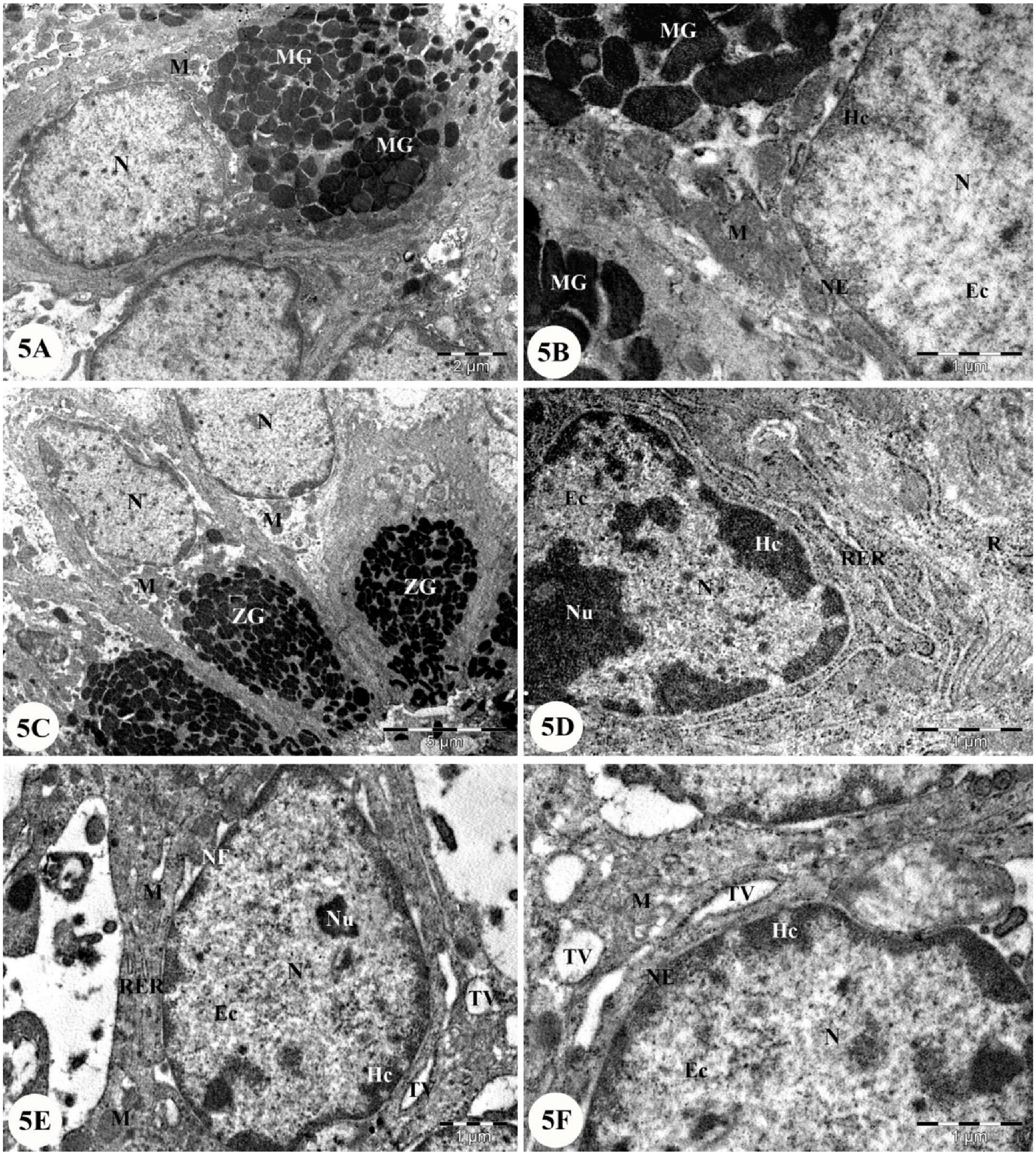

Figure 5. Electron micrographs (A-F) of the mucosa of *Cavia porcellus* showing A & B: the surface epithelial cell which is rather cuboidal or columnar in shape with its cytoplasm contains electron dense mucous granules (MG) and scant mitochondria (M). The nucleus (N) with its nuclear envelope (NE) and homogenous distribution of heterochromatin (Hc) and euchromatin (Ec) is also seen. **C & D:** Columnar peptic cell possesses electron dense zymogen granules (ZG), cisternae of rough endoplasmic reticulum (RER), free ribosomes (R) and mitochondria (M), besides nucleus (N) with irregular nuclear envelope (NE), electron dense heterochromatin (Hc) and euchromatin (Ec). **E & F:** Oval shaped parietal cell has centrally-located nucleus (N) with nuclear envelope (NE), dense heterochromatin (Hc) and euchromatin (EC). The cytoplasm reveals numerous tubule-vesicles (TV), mitochondria (M) and rough endoplasmic reticulum (RER).

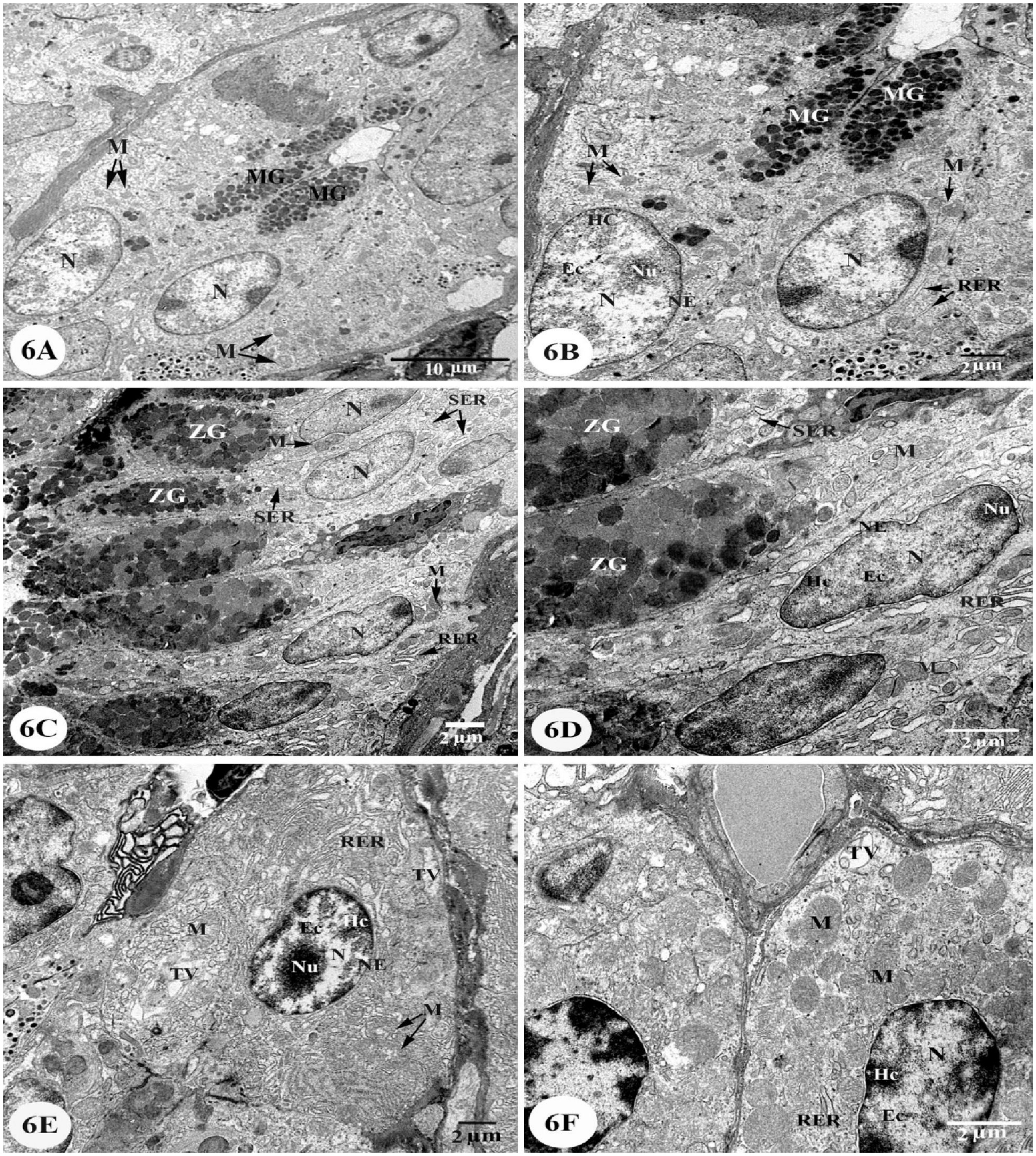

Figure 6. Electron micrographs (A-F) of the mucosa of *Mustela nivalis* showing A & B: columnar shaped surface epithelial cells with their cytoplasm loaded with numerous mucous granules (MG), small sized mitochondria (M) and few stacks of rough endoplasmic reticulum (RER). Their nuclei (N) are oval in shape having homogeneous distribution of chromatin materials. **C & D:** Elongated peptic cells contain aggregation of spherical electron-dense zymogen granules (ZG), mitochondria (M), rough (RER) and smooth (SER) endoplasmic reticula, as well as nuclei (N) showing regular structure. **E & F:** Oval to pyramidal shaped parietal cells with their cytoplasm rich in rough endoplasmic reticulum (RER), mitochondria (M) and tubule-vesicles (TV), and their nuclei (N) possessing prominent nucleoli (Nu), aggregation of euchromatin (Ec) as well as dense clumps of heterochromatin (Hc).

Mucous-producing and surface epithelial cells prevail the surfaces of the gastric glands of the stomachs of the three examined animals. The dominance of the epithelial cells on the surface of the entire gastric mucosa could be bound with their function of absorption of some food materials. The abundance of mucous cells in the cardiac region of the gastric mucosa indicates that large quantities of mucous were produced in this region to neutralize the damaging corrosive effect of hydrochloric acid (HCl) produced by the parietal cells in the stomach. These observations are in accordance with those reported by **Rindi *et al.* (2002)** and **Dare *et al.* (2012)** who illustrated that the mucous cells were the dominant cell type in both cardiac and pyloric region of the stomach of man and other mammals.

The present study showed that such differences in the histological structure of the gastric layers of the three Egyptian mammalian species may be connected either with the nature of their habitats or environmental adaptation or response to a particular kind of food present in the localities occupied by these animals or deprivation of another kind of food or the types of the preys trapped by the animal. Such suggestion agrees with the findings of some investigators. In this concern, **Hume (2002)** displayed that the gastrointestinal tracts of the insectivores showed similarities to the relatively short and simple digestive tracts of carnivores. The simple gastrointestinal morphology of carnivores generally correlates with the high digestibility of their food and this may be the case in the insect-eating species studied here.

Also, **Ofusori and Caxton-Martins (2008)** in their comparative study on the stomach of the rat (*Rattus norvegicus*), the frugivorous bat (*Eidolon helvum*) and the pangolin (*Manis tricuspis*) in relation to their diet illustrated that the cellular diameter of zymogenic and parietal cells are significantly different in the three animals. Also, histological examination showed slight differences in the pattern of organization and distribution of the connective tissue fibers. They related such different patterns in the stomach of the three mammals to their respective diet.

The current ultrastructural results showed that the surface epithelial and the mucous-secreting cells of the three studied animals; *Hemiechinus auritus*, *Cavia porcellus,* and *Mustela nivalis* displayed the presence of well-developed discoid electron-dense secretory mucous granules that localized at the apical surfaces of these cells. These granules are more numerous in *Hemiechinus auritus* and *Cavia porcellus* than in *Mustela nivalis.* Also, these cells contain few mitochondria as well as poor elements or stacks of rough endoplasmic reticulum in *Hemiechinus auritus* and *Mustela nivalis* when compared with *Cavia porcellus.* The cytoplasm of the peptic cells in the carnivorous *Mustela nivalis* displayed more abundantly secretory zymogen granules, mitochondria, well-developed stacks of rough endoplasmic reticulum and vacuoles of smooth endoplasmic reticulum than those in the *Hemiechinus auritus* and *Cavia porcellus.* Such differences may be due to the different feeding habits of the three animals since the peptic cells are very important in the digestion of the proteinic components by their secretory zymogen granules synthesized by the endoplasmic reticulum, as well as the mitochondria are important in this concern for energy production that supports the process of digestion. Such opinion was supported by the studies carried out by **Junqueria *et al.* (1995)**, **Guyton and Hall (2006)**, **Khattab (2007)** and **Dare *et al.* (2012)** who pointed out the importance of the secretory zymogen granules of these cells in the stomach in order to carry out their digestive function since they are considered as the originator of the proteolytic enzymes.

Also, the current ultrastructural results revealed the presence of intracellular canaliculi and tubule-vesicles among the components of the cytoplasm of the gastric parietal cells of all examined mammalian animals since they play an important role in the biosynthesis of HCl, but it is more discriminating in both *Hemiechinus auritus* and *Mustela nivalis* than in *Cavia porcellus* due to the differences in the nature of consumed food since the first two species depend on the proteinic components of their preys, while the last one is herbivorous in feeding. **Ramadan (2001)** showed that the intracellular canaliculi of the gastric parietal cells of the stomach are considered as the main site of the protein pump mechanism by which the HCl is liberated. Such postulation was confirmed by the studies carried out by **Ramadan (2006)**, **Guytan and Hall (2006)** and **Khattab (2007)** in the gastric mucosa of mammalian representatives.

In conclusion, the current study revealed marked differences in the histological and ultrastructural features of the stomachs of the three Egyptian mammalian animals; *Hemiechinus auritus*, *Cavia porcellus* and *Mustela nivalis* which may be correlated with the nature of their consumed food. These dissimilarities are mainly concentrated in the muscularis and mucosal layers; the surface mucous glands, peptic cells and parietal cells.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Corresponding Author:
Name: Dr. Hanaa R. Aboelwafa

Address: Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt.
E-mail: hanaa_aboelwafa@yahoo.com

References

2. Bancroft, J.D., Gamble, M. (2002). Theory and practice of histological techniques. 5th ed. London, New York, Philadelphia: Churchill, Livingstone.
3. Banks, R. (1989). The Guinea Pig: biology, care, identification, nomenclature, breeding and genetics. US-AMRIID Seminar Series. Accessed February 02, 2010 at http://netvet.wust1.edu/species/guinea/guinpig.txt.
4. Berghes, C., Tanase, P., Parvu, M., Dinu, C., Cuca, D. (2011). Contributions to the study of the esophagus and stomach morphology in guinea pig. Anim. Sci. Biotech. 44(2), 150-154.
5. Boonzaier, J., Van der Merwe, E.L., Bennett, N.C., Kotze, S.H. (2013). Comparative gastrointestinal morphology of three small mammalian insectivores: Acomys spinosissimus (Rodentia), Crocidura cyanea (Eulipotyphla), and Amblysomus hottentotus (Afrosoricida). J. Morph. 274(6), 615-626.
6. Dare, W.N., Oyinbo, C.A., Izunya, A.M. (2012). Study of the structure of the gastric mucosa in the mouse (cell population). J. Life Sci. Biomed. 2(5), 182-186.
7. Dykstra, M.J., Mann, P.C., Eiwell, M.R., Ching, S.V. (2002). Suggested standard operating procedures (SOPs) for the preparation of electron microscopy samples for toxicology/pathology studies in a GLP environment. Toxicol. Pathol. 30(6), 735-743.
8. Ghoshal, N.G., Bal, H.S. (1989). Comparative morphology of the stomach of some laboratory mammals. Lab. Anim. 23, 21-29.
9. Guytan, M.D., Hall, J.E. (2006). Text-book of medical physiology, 11th ed. Elsevier Saunders, Inc. Library of Congress Cataloging, New York, pp. 1962-1968.
10. Hume, I.D. (2002). Digestive strategies of mammals. Acta Zool. Sinica. 48,1-9.
11. Hutterer, R., Wilson, D.E., Reeder, D.M., eds. (2005). Mammal species of the world: A taxonomic and Geographic Reference, 3rd ed. Johns Hopkins University Press. pp. 215.
12. Igbokwe, C.O., Obinna, S.J. (2016). Oesophageal and gastric morphology of the African Rope Squirrel *Funisciurus anerythrus* (Thomas, 1890). J. Appl. Life Sci. Inter. 4(2), 1-9.
13. Junqueira, L.C., Carneiro, J., Kelley, R.O. (1995). Basic histology, 8th ed. Appleton and Lange Publication, Norwalk, California.
14. Khattab, F.K.I. (2007). Histological and ultrastructural studies on the gastric mucosa of rat after treatment with Ethylene glycol. Aust. J. Basic Appl. Sci. 1(3), 157-168.
15. King, C., Powell, R. (2007). The natural history of Weasels and Stoats, 2nd ed. New York, Oxford University Press.
16. Leus, K., Macdonald, A.A., Goodall, G., Veitch, D., Mitchell, S., Bauwena, L. (2004). Light and scanning electron microscopy of the cardiac glandregion of the stomach of the Babirusa (*Babyrousa babyrussa* - Suidae, Mammalia). CR. Biologies. 327, 735–743.
17. Machado-Santos, C., Nascimento, A.A., Peracchi, A.L., Mikalauskas, J.S., Rocha, A.P., Sales, A. (2009). Distribution of the endocrine cells in the gastrointestinal tract of nectarivorous and sanguivorous bats: a comparative immunocytochemical study. Tissue Cell. 41(3), 222-229.
18. Nisa, C., Agungpriyono, S., Kitamura, N., Sasaki, M., Yamada, J., Sigit, K. (2010). Morphological features of the stomach of Malayan pangolin, *Manis javanica*. Ant. Histol. Embryol. 39, 432-439.
19. Nowak, R. (1991). Carnivora: family Mustelidae, In: Walker's Mammals of the World, Vol. 2, 5th ed. Baltimore: Johns Hopkins University Press. pp. 1104-1105.
20. Nowak, R.M., Paradiso, J.L. (1983). Walker`s Mammals of the world. 4th ed. John Hopkins University Press, Baltimore, MD.
21. Ofusori, D.A., Caxton-Martins, E.A. (2008). A comparative histomorphometric study of the stomach of rat (*Rattus norvegicus)*, bat (*Eidolon helvum)* and the pangolin (*Manis tricuspis)* in relation to diet. Int. J. Morphol. 26(3), 669-674.
22. Poddar-Sarkar, M., Raha, P., Bhar, R., Chakraborty, A., Brahmachary, R. (2011). Ultrastructure and lipid chemistry of specialized epidermal structure of Indian porcupines and hedgehog. Acta Zool. 92(2), 134.
23. Raji, A.R. (2011). Morphological and histochemical investigation of the camel (*Camelus dromedarius*) abomasal mucous membrane by light and scanning electron microscopy (SEM). Iran J. Vet. Res. 12(4), 304-308.
24. Ramadan, R.A. (2001). Electron microscopical study of the effectiveness of Ranitidine (Histamine-H2-Antagonist Receptor) drug for regeneration of the gastric mucosal cells of mice

post–treated with indomethacin. J. Egypt Ger. Soc. Zool. 34(C), 301-319.
25. Ramadan, R.A. (2006). Ultrastructural study of the gastric parietal cells of albino mice treated with the anti-ulcer drug Lansoprozole (Zollipak). J. Egypt Ger. Soc. Zool. 49(C), 43-58.
26. Rindi, G., Necchi, V., Savio, A., Torsello, A., Zoli, M., Locatelli, V., Raimondo, F., Cocchi, D., Solcia, E. (2002). Characterisation of gastric ghrelin cells in man and other mammals: studies in adult and fetal tissues. Histoch. Cell Biol. 117(6), 511-519.
27. Sachser, N. (1998). Of domestic and wild guinea pigs: studies in sociophysiology, domestication, and social evolution. Naturwissenschaften.85(7), 307-317.
28. Sakr, S.M.I. (2010). Ultrastructural studies on the gastric mucosal cells of some Egyptian bat species. J. Egypt Ger. Soc. Zool. 61(C), 25-49.
29. Santos, C.M., Nascimento, A.A., Peracchi, A.L., Sales, A., Mikalauskas, J.S., Gouveia, S.F. (2008). Immunocytochemical study of gastrointestinal endocrine cells in insectivorous bat (Mammalia: Chiroptera). Braz.J. Biol. 68(3), 663-669.
30. Scopin, A.E., Gashkova, I.V., Saveljev, A.P., Abramov, A.V. (2015). Histologic features of the gastrointestinal tract of *Laonastes aenigmamus* (Rodentia: Diatomyidae). Vertebrate Zool. 65(1), 151-163.
31. Sheffeld, J., King, C. (1994). Mustela nivalis mammalian species. Am. Soc. Mammalogists. 454, 1-10.
32. Shoeib, M.B., Hassanin, A., Elnasharty, M. (2015). Morphological and morphometric characteristics of gastric mucosa in western grey kangaroo (*Macropus fuliginosus*). J. Adv. Vet. Anim. Res. 2(1), 40- 48.
33. Smith, D.M., Grasty, R.C., Theodosiou, N.A., Tabin, C.J., Nascone-Yoder, N.M. (2000). Evolutionary relationships between the amphibian, avian, and mammalian stomachs. Evol. Dev. 2 (6), 348-459.
34. Stan, F. (2013). Comparative study of the stomach morphology in rabbit and chinchilla. Agro Life Sci. J. 2(2), 73-78.
35. Stubbe, M., Samiya, R., Ariunbold, J., Buuveibaatar, V., Dorjderem, S., Monkhzul, T.S., Otgonbaatar, M., Tsogbadrakh, M., Zagorodniuk, I., Amori, G., Hutterer, R., Kryštufek, B., Yigit, N., Mitsain, G., Muñoz, L.J.P. (2008). *Hemiechinus auritus*. In: IUCN 2008. IUCN Red List of Threatened Species. Retrieved 2009-04-01.
36. Tikhonov, A., Cavallini, P., Maran, T., Kranz, A., Herrero, J., Giannatos, G., Stubbe, M., Conroy, J., Kryštufek, B., Abramov, A., Wozencraft,C., Reid, F., McDonald, R. (2013). "Mustela nivalis" (On-line). IUCN Red List of Threatened Species.
37. Trang, P.H., Ooi, P.T., Zuki, A.Z., Noordin, M.M. (2012). Comparative gastric morphometry of Muong Indigenous and Vietnamese Wild Pigs. Sci. World J. Volume 2012, Article ID 894952, 1-9.
38. Vanderlip, S. (2003). The guinea pig handbook. China; Barron`s Education Series. ISBN 0-7641-2288-6.
39. Wagner, J.E. (1976). Introduction and taxonomy. In the biology of the guinea pig_eds., J.E. Wagner and P.J. Manning, 1-2, New York: Academic Press, Inc.
40. Wilson, D.E., Reeder D.M., eds. (2005). Mammal Species of the World: A Taxonomic and Geographic Reference, 3rd ed., Vols. 1 & 2. 2142.

5/28/2017