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Anatomical, Histological, Histochemical study of the Esophagus andStomach of *Neurerguscrocatus*

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ABSTRACT

The Present study includes comparison between the anatomical, histological, histochemical features of esophagus, stomach in the Salamander. Anatomically the esophagus was long tube and was pouch in shape. Histologically, the wall of esophagus and stomach is composed of four basic layers called mucosa, submucosa, muscular externa and adventitia or serosa. The esophagus divided to two halves, upper, lower so the wall of esophagus contained esophageal folds. Also, the upper esophagus was absence of esophageal glands, whereas the lower esophagus was glandular. The esophageal glands showed strong positive reaction with PAS and AB. The stomach consisted from mucous ridges which differed in regions of the stomach separated between them grooves called gastric pits. The present study concluded that the structure of esophagus, stomach had anatomical, histological modulation conforms to the nature of this animal as well as the environment where it live.

Key words: crocatus Esophagus, Stomach, Salamander, Neurergus.

INTRODUCTION

Nutrition plays an important role in the daily life of living organisms. Food contains important nutrients, namely proteins, carbohydrates, fats, some vitamins, minerals so water. These all play a different role in maintaining the health of the body and building new cells [1]. The digestive tract in the vertebrates generally begins with the mouth and ends with the cloaca or anus. The gut wall consists of the four layers of Mucosa, Submucosa, Muscular externa, Serosa [2]. Most amphibians have almost no esophagus as in fish. The pharynx is opened in the stomach directly [3], in the amphibians containing the esophagus the esophagus is short up to the stomach but both areas are distinct. The epithelial layer may be single or double then contain goblet cells so a ciliated cell. The Muscular mucosa layer so the submucosa layer contains glands called esophageal gland, these glands are spread extensively [4].The stomach in amphibians consists of two parts Fundic and Pyloric, its wall histologically consisting of the four basic layers [5]. The mucous layer consists of a simple mucous columnar epithelial tissue with some of the goblet cells scattered where the mucous layer contains longitudinal folds [5,6]. The fundic part of the stomach contains glands called fundic glands [2,7]. The pyloric part contains glands called pyloric glands [7,8].

The muscular layer consists of two layers of internal, external muscles and is surrounded by the outside with the serosa layer [5]. After investigating in the information so scientific publications network so the thesis completed within the country, we did not find an anatomical, histological, histochemical study with in the country discuss the esophageal and stomach for the salamander so the current study is to investigate the anatomical, histological differences in the esophagus and stomach by the type of food and environment.

MATERIALS AND METHODS

The current study deals with the amphibious *Neurerguscrocatuscrocatus* (obtained from the Siba waterfall of Aqrah district). Ten samples of salamanders were collected into an appropriate basin and fed to thin slices of sheep liver until they were adapted and verified for their safety. The animal was anesthetized by ether then the esophagus and the stomach was removed carefullyafter the abdominal area was opened, the organs were removed so washed by a physiological solution and transferred to the dishes of Petri container to a physiological solution 0.64%. After completion of the dissection, the organs were left in a 10% formalin fixer for 18-24 hours.Histological section Preparation where prepared by the way of embedded by wax according to the following steps:

Wash organs with water to remove the effect of formalin. The dehydration by using progressive concentrationsof ethyl alcohol 50%, 70%, 90% for half an hour for each concentration. It was then transferred to concentration 100% of ethyl alcohol for one hour then repeat 100% again. Clearing by using xylene the organs were cleared twice by xylene for 10 minutes. Infiltration, the organs were mixed with wax and xylene by 1:1 in the oven, the temperature of 56ºCat half-hour. Then the wax and xylene were removed so the samples were placed in pure wax for one hour.

Embedding, when samples are transported by forceps and placed in iron molds filled with melted wax and then left to dry. Trimming and sectioning, the wax molds were finely trimmed then fitted to the cutting device (rotatory microtome) for lateral and longitudinal sections. Staining, was by using general histological stain are Delafield's Hematoxylin and Eosin stain (HE) been preparation and coloring depending on AL-Hajj,1998 [9], so Malory triple stain (M) been preparation and coloring depending on AL-Mukhtar*et al*.,1982 [10]. Using histochemical Staining technique, namely, Periodic Acid-Schiff technique(PAS)is used to discover carbohydrate so been preparation and coloring depending on AL-Kinani, 2013[11], Alcian Blue Stain (AB) pH1 is used to discover the sulfated mucous substance,Alcian Blue Stain (AB) pH 2.5is used to discover mucous material containing hyaluronic acid andSalic acid, been preparation and coloring depending on AL-Tyeb and Jarar, 1985[12].

The mounting,the slides were loaded container on the histological sections after coloring using the media ofDistendplasticizer xylene (D.P.X),then let the slides to dry at the laboratory temperature 25 ºC.The Measurement, tissue layers dimensions were measured using the ocular micrometer after calibration using the stage micrometer so the different magnification powers of the objective lenses to calculate the magnification of the image.The photocopyingcompleted using a digital camera type of, which is also attached to a laptop.

RESULTS

The Esophagus in the shape side, the esophagus appears in Salamander, such as the composing of sac, which connects the bucco-pharyngeal cavity of the top with the stomach from the bottom and has flexible walls that stretchy it extensibility and expand to absorb the amount of ingested food and have many longitudinal folds that increase its surface area,its long about 7 cm (Figure 1).

Fig 1: Photograph of the digestive tract of salamander (ESO)Esophagus (ST)Stomach.

The histological composition for the esophagus wall is different in the components density, so the number, length, as well as the thickness of the folds and the presence of the glands and their density between the cervical parts of the esophagus which is located near to the buccal cavity.

The thoracic part of the esophagus which is located near of the stomach, the mucous layer and submucosa of the upper esophagus appear to be projecting into interior to be esophageal folds, which increases the surface area of the esophagus (Figure 2, 3).

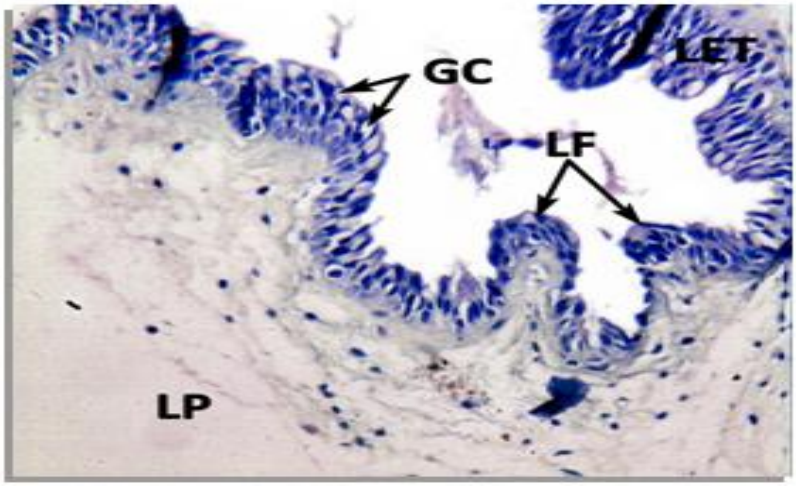

Fig 2: upper part of esophagus of salamander (LET) linning epithelial tissue (GC) Goblet Cell (LF) longitudinal fold, (LP)laminapropri. AB pH2.5\4X

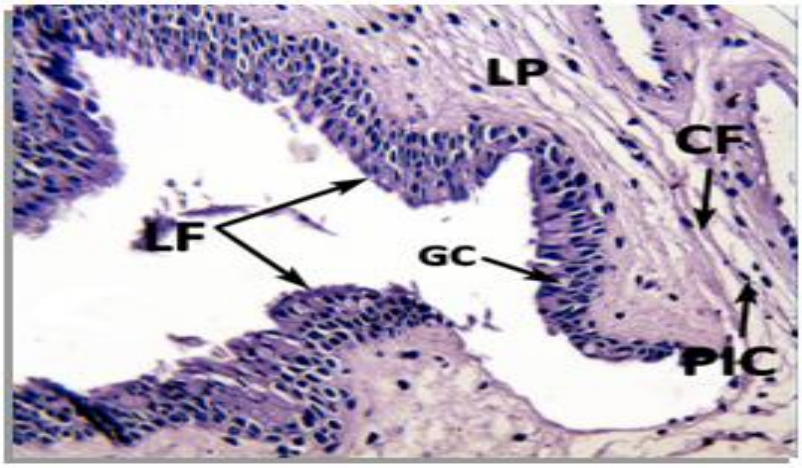

Fig 3: upper part of esophagus,(LET)lining epithelial tissue, (LF)longitudinal fold, (LP)lamina propria, (PIC)pigment cell,(CF) collagenous fiber.PAS\10X.

The mucous layer consists of a stratified columnar epithelial tissue, so the epithelial tissue is rich in the secretion cells called goblet cell, which discharge directly into the esophagus cavity (Figure 5), these cells appear under high magnification a container on the entire mucous secretion as the histochemical stains have shown as a PAS, AB and yet they have shown that the ingredients have a moderate positive harmony (Figure 4, 6).

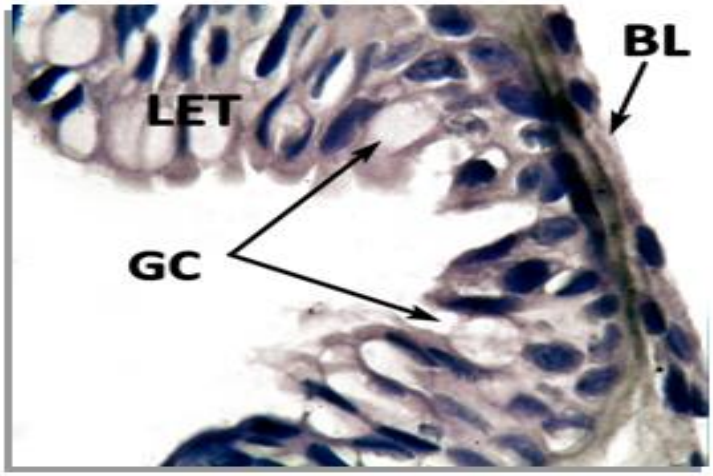

Fig 4: upper part of esophagus, (LET) lining epithelial tissue,(GC)goblet cell,(BL)basal lamina.ABpH.2.5\40x.

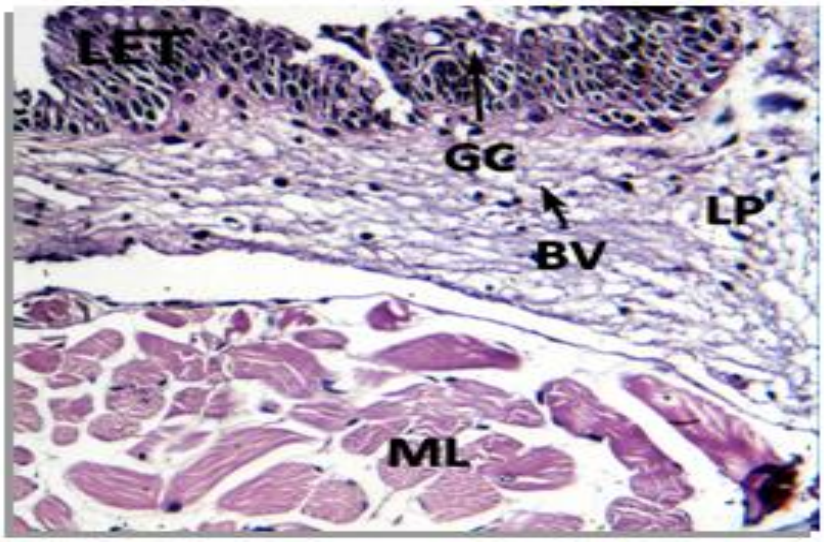

Fig 5: upper part of esophagus,(LET)lining epithelial tissue,(GC)goblet cell,(LP)lamina propria,(ML)muscle layer,(BV)blood vessel.HE\10X.

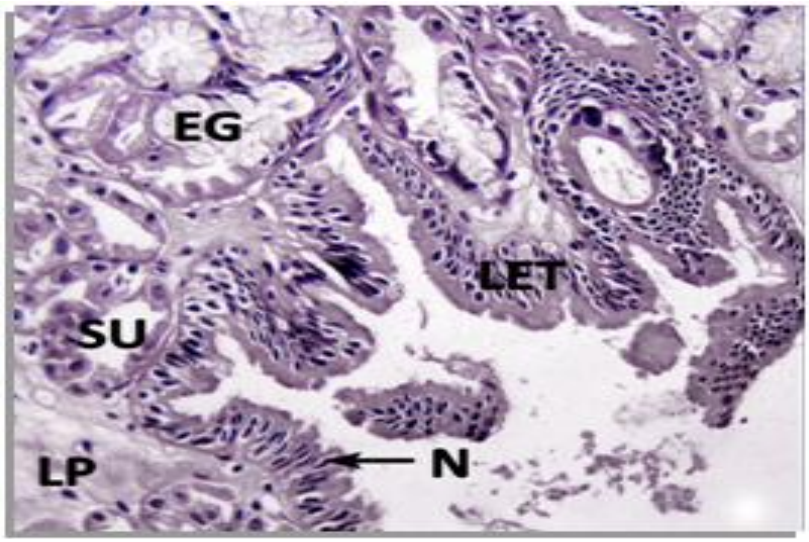

Fig 6: lower part of esophagus (EG)esophagus gland,(SU) secretory unit,(SC) secretory cell, (N)nucleus, (ML)muscular layer, (BV)blood vessel, (SUB) submucosa. PAS\10X.

The lamina propria of the mucous layer is composed of a rich connective tissue with blood vessels so collagenous fibers, while the muscular mucosa is composed of separated smooth muscle fibers. The submucosa layer is very thin and its components overlap with the components of the lamina propria (Figure 2, 3). One striking result is the absence of the esophageal glands in this part of the esophagus thatdid not appear in the mucous layer, not even in the submucosa layer.

The muscular externa layer consists of two layers of muscle, the inner layer of circular arrangementis relatively thick, has a thickness (±92.682 μm), either the outer layer of longitudinal arrangement with a thickness rate (±74.979 μm) (Figure 5). The adventitia layer consists of a connective tissue that encapsulates the esophagus from the outside. The lower esophagus in salamander appeared to be significantly different from the upper esophagus, as it was a big degree glandular. Contains a large number of glands known as the esophageal glands, which are mixed seromucous, as well as the goblet cells in epithelial tissue, the folds appeared more numerous than in the upper part of the esophagus it's had the length of these folds (±105.295 μm), its thickness (±55.323 μm) (Figure 6,7).

The mucous layer consists of a stratified columnar epithelial tissue that may appear in some areas ciliated also contain too many goblet cells which appears more number than its counterpart in the upper esophagus and the thickness rate of this layer is near to (±311.045 μm). PAS stain showed that the secretion of the goblet cells so the esophageal glands are very positive they show a strongly positive reaction to the color of AB. The components of the lamina propria are entered into the esophageal folds so form the structure support by these folds (Figure 7).

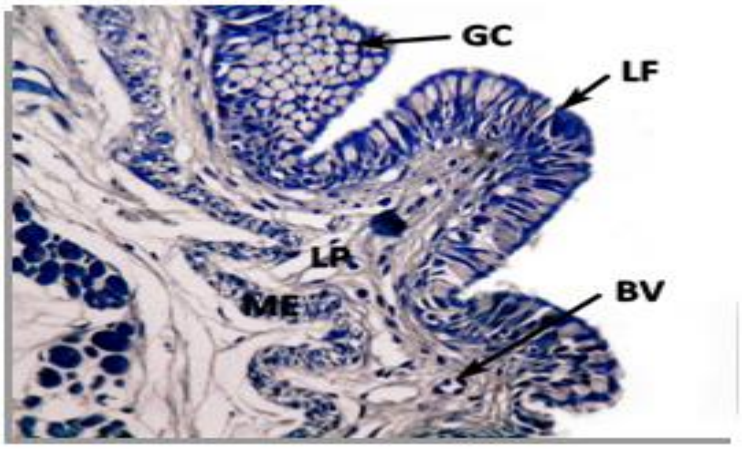

Fig 7: lower part of esophagus,(LF)longitudinal fold,(GC)goblet cell,(LP)lamina propria, (ME)muscular epithelia,(BV)blood vessel. AB pH2. 5\10X.

The muscular mucosa is similar to the upper esophagus as well as the mucous layer. This part of the esophagus is characterized by the spread of the esophageal glands in the lamina propria, these glands compound vesicular tubular glands, some secretory units are seromucous and some are mucous. The secretory units that the vesicles appeared in the form of concentrations of secretory cells, the inner structure of which cannot be distinguished and shown to be divided into units, separated by fiber from the connective tissue, which are surrounded by with a dense connective tissue the thickness rate of these units (±114.030 μm), these glands are very positive when treated with PAS and AB stains (Figure 9,8).

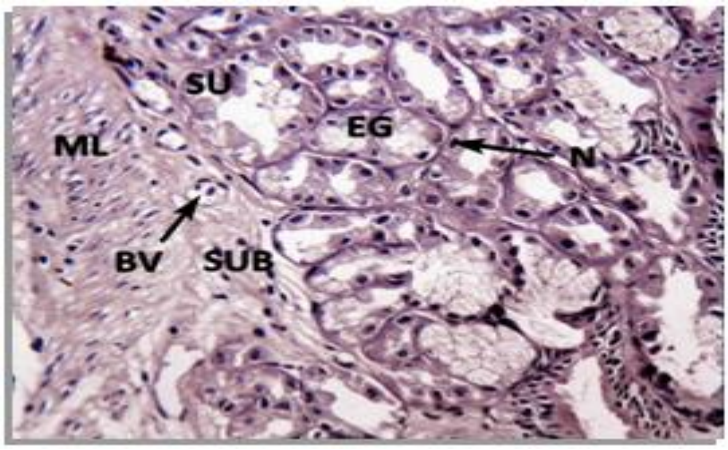

Fig 8: lower part of esophagus (EG)esophagus gland, (SU)secretory unite, (SC)secretory cell, (N)nucleus, (ML)muscular layer, (BV)blood vessel, (SUB)submucosa. PAS\10X.

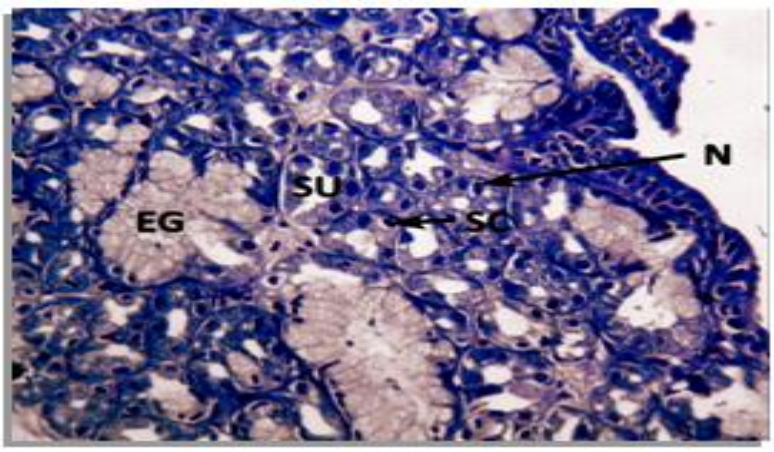

Fig 9: lower part of esophagus (EG)esophagus gland,(SU)secretory unite,(SC)secretory cell,(N)nucleus.AB pH2.5\10X.

The tubular units appeared more clearly structured and circular in the cross-section with a diameter (±50.949 μm) (Figure 9,8). An important result that has also appeared in this part of the esophagus is the lymphoid nodules, which are called the esophageal tonsils. As for the muscular externa layer, They are also composed of two layers of muscle so the diameter of the circular muscles is (±71.851 μm),thelength (±141.51 μm). The esophagus from the outside is surrounded by a serosa layer consisting of a dense connective tissue (Figure 10).

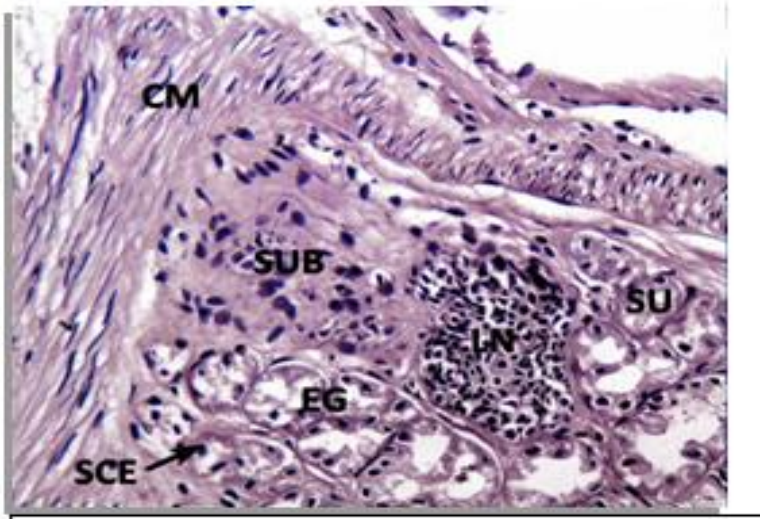

Fig 10: lower part of esophagus (LN) lymphoid nodules, (EG) esophagus gland,(SU)secretory unit,(SC)secretory cell,(SUB)submucosa,(CM)circular muscle.PAS\10X.

The stomach in Salamander appeared in the form of a muscular cystic composition that was stretched the base upwards so it's had an average length of approximately 1.5 cm, its inner wall contains many folds and grooves that increase the total area of the digest (Figure1).

The histological sections showed that the mucous layer of the stomach consists of projections or large folds, some of which are branched in the direction of the cavity the stomach called mucosal ridges separates the branches sulcus also know the Gastric Pits or the Foveala. These ridges appear to be more compactness and more in number in the cardiac region of the stomach compared to the body of the stomach, it's part of the pyloric region and the length of these grooves has reached in the front of the stomach (±216.148 μm) while its length in the back of the stomach (±106.753 μm) (Figure 11,12,13).

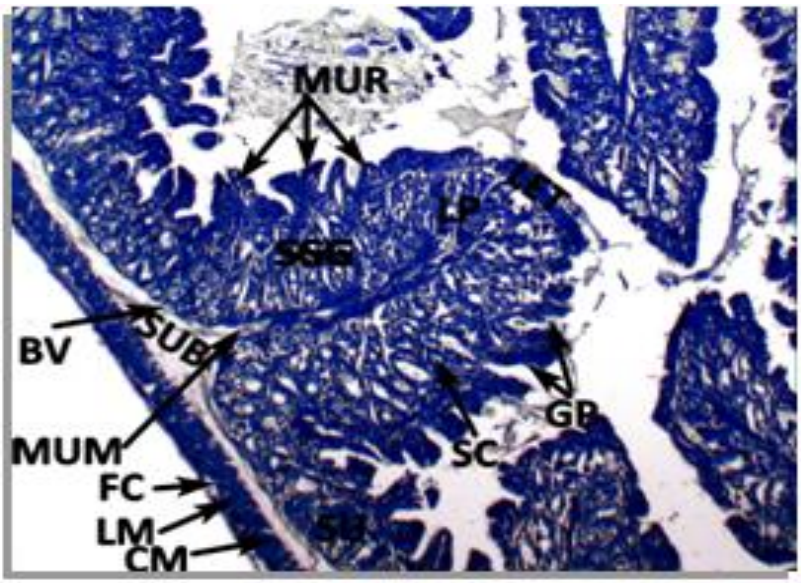

Fig 11: Front stomach,(SGG)superficial gastric gland, (SU)secretory unite, (SC)secretory cell, (MUR)mucosal ridges, (MUM)muscular mucosa, (LP)lamina propria, (CM)circular muscle, (LM)longitudinal muscle, (SUB)submucosa, (FC)fibroblast cells, (BV)blood vessel, (LET)lining epithelial tissue, (GP)gastric pit. ABpH 2.5\10X.

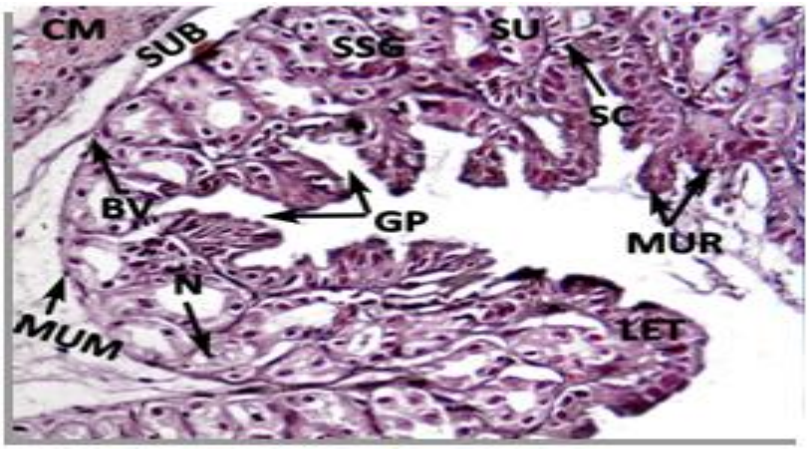

Fig 12: Front stomach, (SGG)superficial gastric gland, (SU)secretory unite, (SC)secretory cell, (MUR)mucosal ridges, (MUM)muscular mucosa, (CM)circular muscle, (LM) longitudinal muscle, (SUB)submucosa, (FC)fibroblast cells, (N)nucleus, (LET)lining epithelial tissue, (GP)gastric pit. HE\10X.

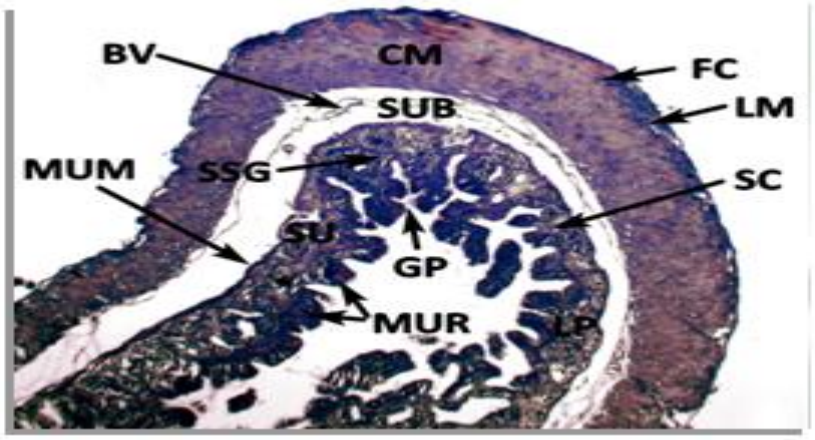

Fig 13: Posterior stomach,(SSG)superficial gastric gland,(SU)secretory unite,(SC)secretory cell,(MUR)mucosal ridges,(MUM)muscular mucosa,(CM)circular muscle,(LM)longitudinal muscle,(SUB)submucosa,(FC)fibroblast cells,(GP)gastric pit,(BV)blood vessel.ABpH 2.5\4X.

Some folds in the front of the stomach appear to be compound and contain many folds over one appearance (Figure 11). The surfaces of the folds or of the gastric grooves with a simple columnar epithelial tissue are compact, some cells in this area of the stomach take almost the cube shape (Figure 14,15,16).

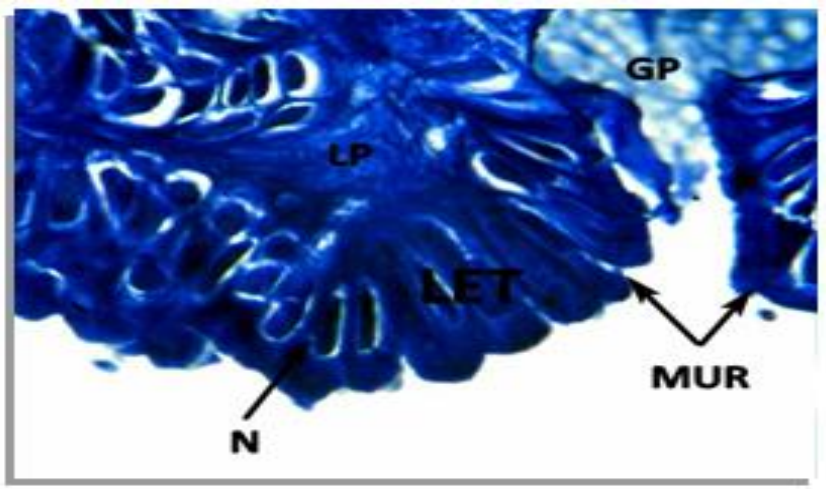

Fig 14: posterior stomach, (LET) lining epithelial tissue, (MUR) mucosal ridges, (LP) lamina propria, (N)nucleus, (GP)gastric pit . AB pH\40X.

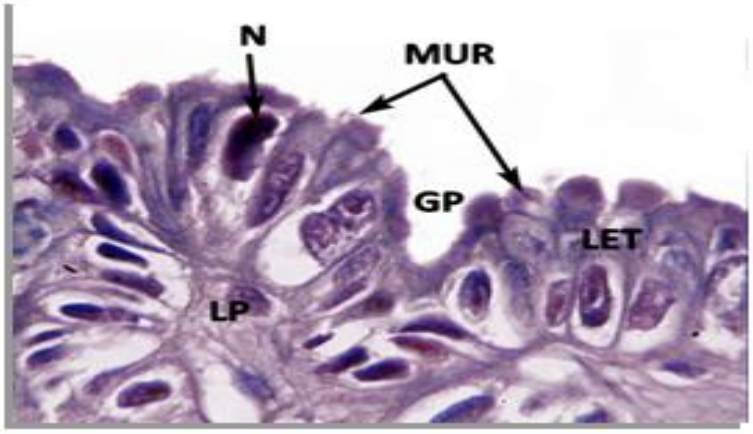

Fig 15: Posterior stomach, (LET) lining epithelial tissue, (MUR) mucosal ridges, (LP)lamina propria, (N)nucleus,(GP)gastric pit .M\40X.

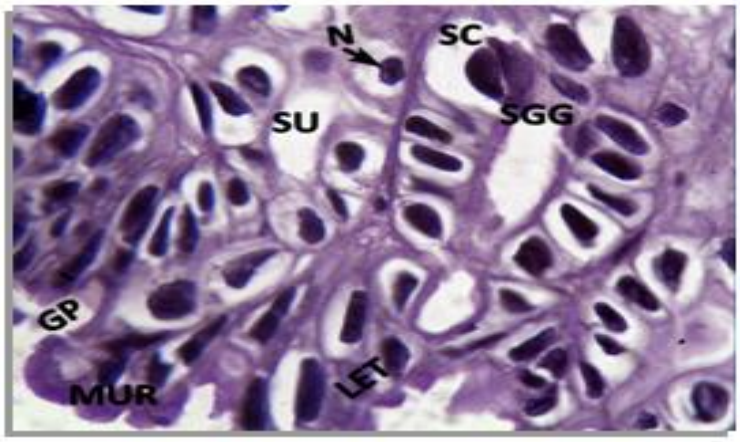

Fig 16: Posterior stomach, (LET)lining epithelial tissue, (MUR)mucosal ridges, (SSG)superficial gastric gland, (SU)secretory unite, (SC)secretory cell, (LP)lamina propria, (N)nucleus, (GP)gastricpit. PAS\40X.

The nuclei of these cells are of a dark color and a prolonged oval, while its oval or spherical shape in the behind part of the stomach, it has a diameter rate (±9.711 µm) (Figure 15,16). The histochemical stains have shown that the surfaces of these cells show moderate positive reaction with PAS, AB PH1 and AB ph2.5, which indicates that these cells are rich in secreting materials (Figure 14,16).

In the case of lamina propria, it has appeared in the form of a connective tissue that is rich in blood vessels, lymphoid vessels, collagenous fibers, muscle fibers (Figure 12). The lamina propria is characterized by its containment of many composite tubular glands that have its own spherical or oval units in its own sections and has the diameter rate (±43.672 µm), in the front part of the stomach (±79.092 µm), in the posterior part of the stomach (Figure 11). The number of these glands appears more in the front part of the stomach than in the posterior part of the stomach (Figure 12,13). These glands are called superficial gastric glands that open at the base of the gastric pits and these glands also show positive reaction with the histochemical stains (Figure 11,12,16). The cross histological section shows that these glands are in the form of longitudinal rows below the gastric pits, which are more in the front part of the stomach as previously reported.

The secretory units of these glands consist of a row of cubic cells, so sometimes they appear vertical the average length (±13.108 µm) with a centralized spherical nucleus of the location with a diameter rate (±10.155 µm). High magnification, using several stains, showed that these secreting units consisted of more than one type of cell, in which the parietal cell and the second type are the Chief cell (Figure 12,16,17). These glands also show moderate positive reaction with the histochemical stains PAS, AB pH1 and AB pH2.5. The muscular mucosa layer has appeared in the form of a smooth muscle fiber layer that is located along the inner edge of the lamina propria it's appeared scattered (Figure 12).

The submucosa layer is relatively thin in the various areas of the stomach;its contents are combined with the contents of the lamina propria, which consists of a loose connective tissue containing blood vessels, lymphoid vessels, collagenous fibers (Figure 13, 11).The thickness of the muscular externa layer varies in different stomach areas. This

layer appeared relatively thin in the front part of the stomach, and amount a thickness rate (±70.851 µm) while it was relatively thick in the posterior part of the stomach and had a thickness (±229.962 µm).

This layer consists of two secondary layers, the first circular arrangement in the front part of the stomach (±61.128 µm), in the posterior part (±176.407 µm), while the second layer is linear arrangement, the thickness rate in the front part of the stomach (±9.723 µm),so in the posterior part (±53.555 µm). On the other side, fibroblast cells have been observed with dense muscle fibers, blood vessels and connective tissue fibers between the two secondary layers of the muscular externa layer (Figure 11,13,18). The serosa layer consists of loose connective tissue containing blood and lymphoid vessels (Figure 18).

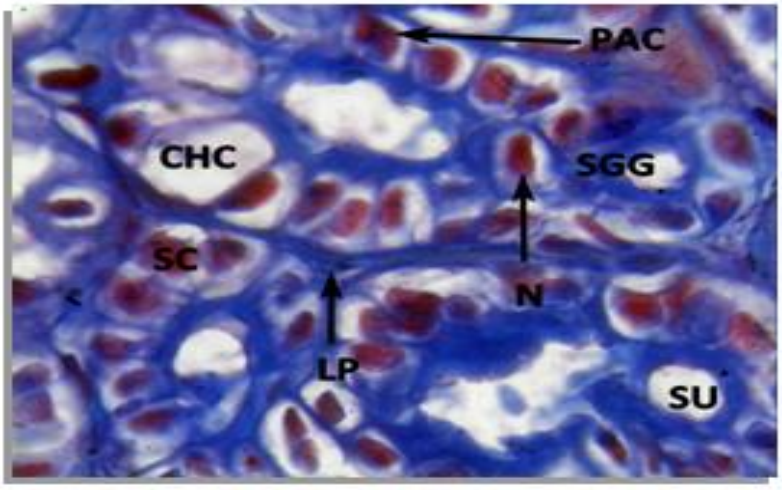

Fig 17: Front stomach, (SSG)superficial gastric gland, (SU)secretory unite, (SC)secretory cell, (LP)lamina propria, (N)nucleus, (CHC)chief cell, (PAC)partial cell. M\40X.

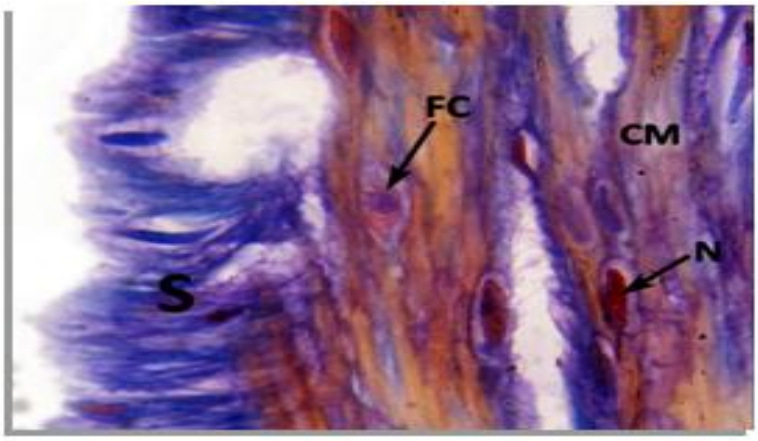

Fig 18: Front stomach, (S)serosa, (CM)circular muscle, (FC)fibroblast cell, (N)nucleus, (CHC)chief cell, (PAC) partial cell. M\40X.

DISCUSSION

The esophagus in the salamander was appear in the form of a cystic containing many folds that increase its surface area and be short [2] The esophagus in amphibians is a short tube between the stomach and pharynx containing folds. The esophagus appeared in the Bufoorientalis Frog as a short piece forming about 4% of the total length of the gastrointestinal tract [13].

Histologically in salamander the mucous layer of the upper esophagus appeared to be prominent in the front to be the esophageal folds that increase the area surface of the esophagus the mucous layer consists of a columnar epithelial tissue, so in Newt, SalamanderAndriasdavidanus[4, 14, 15]. The surface layers of the epithelial tissue were rich in the secrete cells (goblet cells) (4) that the goblet cells are positioned between the columnar cells of the epithelial tissue. The mucous layer appeared a very thin its size and its thickness varied according to the size of the goblet cells in the Chinese Frog [16], the results of the study were the emergence of the esophageal glands in muscular mucosa and

submucosa layer these glands are extensively and this differs with what it came to [4] in that the esophageal glands appeared in the mucous layer glands in the form of concentrations in the other species of salamanders.

The stomach is in salamander in the form of a conical enlarged muscle structure, which is agreed with what it came to [13]. The salamander's stomach is made up of three regions: the cardiac region, the fundic region of the stomach and the pyloric region, which is different from what appeared in the Trituruscarnifex. It is also different from what it appeared in the Rhinellaictericawhere the stomach appeared to be composed of two regions: the fundic region and the pyloric region [7,5]. In salamander, the mucous layer consists of folds that stand out in the direction of the stomach cavity called mucous ridges separated by grooves, namely, gastric pits these grooves so folds are covered with a simple columnar epithelial tissue this also appeared in the stomach of the Plophylaxberdriagae [17,18]. The surfaces of these cells show a positive reaction to the histochemical stains, which indicates that these cells are rich in secretory material and that's what appeared in T. carnifexas the surface epithelial cells of the produces mainly a neutral glycoprotein and this is what appeared in the Green Frog Bufoiridis [7, 8]. These folds appeared longer in the behind part than in the front part of the stomach in salamander it's similar to what appeared in the R. icterica [5].

The lamina propria consists of a loose connective tissue containing many of the compound glands called these glands in the gastric glands which are opened at the base of the gastric pits and that are what is shown in R. icterica. These glands in the salamander are complex tubular glands, which is different from that of R. icterica in that these glands are simple tubular glands,so may be branching out at the bottom of the gland [5].

The muscular mucosa have appeared in a smooth fiber layer whose located adjacent to the lamina propria, which is different with what it came to [5] that separates the mucosa from the submucosa by the muscular mucosa that is divided into two layers of the inner circular arrangement muscles, the outer longitudinal arrangement [18]. The submucosa layer consists of a connective tissue containing blood vessels, lymphatic vessels and collagenous fibers [5, 18, 19].

The muscular externa layer was made up of two secondary layers, the first circular so the second longitudinal arrangement, which appeared in the R. icterica [5, 18]. The fourth layer is the Serosa layer, which consists of a loose connective tissue containing blood vessels as well as lymphatic vessels [18, 19].

CONCLUSIONS

The study concluded that there are lymphoid nodules called esophageal tonsils in the lower esophagus. The stomach has appeared in the form of a cystic structure, in addition to the presence of glands in the lamina propria. There is a difference in the density and distribution of neutral and sulphated mucus in both esophagus and stomach.

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