Anatomical, histological and histochemical adaptations of the avian alimentary canal to their food habits: *I-Coturnix coturnix*

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Abstract: The present work is the first in a series of studies aiming at establishing a connection between the food habits of aves and the anatomical, histological and histochemical structures of their alimentary canal. In this study the gross anatomy, histology and histochemistry of the alimentary canal of common quail, *Coturnix coturnix,* a granivorous bird, have been investigated. This study revealed that, the oesophagus is not ably long with a well developed crop; thus stomach is differentiated into a glandular proventriculus and a muscular ventritculus or gizzard. The gizzard is much more developed having a thick hard cuticle, its wall consists of two strong smooth muscles, the small intestine is divided into duodenum, jejunum and ileum and the transition from the jejunum to ileum is indicated by the vitelline (Meckel's) diverticulum, and the ileum was the longest part of the small intestine. The large intestine consists of paired well developed caeca and a short rectum. The present histological studies revealed that the alimentary tract showed the usual four laminae: serosa, musculosa, submucosa and mucosa. The oesophageal mucosa of the quail was thrown into numerous longitudinal folds. The mucosa of oesophagus is lined with stratified squamous epithelium. The proventricular glands are simple tubular to simple branched tubular glands. The mucosal surface of the ventriculus is indented by deep, broad crypts into which simple to branched tubular gastric glands open. A thick gastric keratinoid material covers the mucosa of the ventriculus. The intestinal mucosa is thrown into intestinal villi which show a marked variation in density, shape and size in the different regions of the intestine. The goblet cells gradually increase in frequency from the duodenum to the rectum. Also, the histochemical study revealed the existence of a high amount of mucopolysaccharides in the oesophageal glands, PAS and Alcian blue positive mucin granules as well (neutral and acid mucin, respectively). The ventriculus mucosa is covered by a thick keratinized laminated layer of koilin membrane which is formed of proteinous material similar to keratin and stained positive for PAS and Alcian blue indicating the presence of neutral and acid mucin within its contents. The proventriculus mucosa shows folds lined by simple columnar cells containing PAS and Alcian blue positive mucin granules. The goblet cells and crypts of Lieberkühn have acid and neutral mucopolysaccharide secretions and the luminal surface of the columnar cells and the lamina propria of the intestine contains proteins.
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1. Introduction

Several studies have been conducted to examine how dietary habits have shaped the physiological and the morphological features of the digestive system (Penry and Jumars, 1968; Martı´nez del Rio and Karasov, 1990; Martı´nez del Rio *et al*., 1992; Lopez-Calleja *et al*. 1997; Hume, 1998; Caviedes-Vidal and Karasov, 2001; Sabat and Veloso, 2003; Sassi *et al.*, 2007; Naya *et al.*, 2008).

El-Banhawy *et al*. (1993 a) studied the comparative histochemistry of the proventriculus and ileum of the piscivorous bird, the black headed gull *Larusridi bundus*, as well as those of granivorous bird, the palm dove *Strepto peliasene galensis.* The authors found that the proventriculus of both birds have a moderate polysaccharides content. The brush borders as well as the goblet cells of the ileum have a high amount of that material.

Stevens & Hume (1995) added that the proventriculus function is the secretion of gastric juice during the passage of food into the gizzard.

Klasing (1999) studied the avian gastrointestinal anatomy and found that the avian gastrointestinal tract is a double-ended open tube (as seen in mammals) that begins at the beak and finishes at the vent. In sequential order it is composed of a mouth, oesophagus, crop, proventriculus, ventriculus (gizzard), intestine, caeca, rectum and cloaca. Some of these structures may be vestigial or even lost during the evolution of some species. The author found that the oesophagus extends down the neck into the thoracic cavity and terminates in the proventriculus. The peristaltic contraction of the inner circular and the outer longitudinal muscles in the musculosa propels the food posteriorly through the oesophagus. Also, the author mentioned that the proventriculus of granivorous bird is elongated and relatively small in diameter in relation to the gizzard. The author found that the duodenal loop of the intestine encircles the pancreas and receives the pancreatic and hepatic ducts. The epithelium of the intestine contains villi and intestinal crypts. The author added that the liver has two lobes and the pancreas has three pancreatic lobes.The progress of food through the tract follows a specific digestive sequence including premoistening and softening, acidifying, grinding, hydrolyzing, emulsifying and propulsion of the end products.

Denbow (2000) and Taylor (2000) stated that, in birds that eat hard food items, the proventriculus is relatively thin-walled and glandular. The ventriculus is muscular, thick-walled and powerful. The intermediate zone connects the two.

Taylor (2000) stated that, in general, the jejunum is thought to begin just after the ascending duodenal loop begins to turn back on itself, where the jejunal branches of the cranial mesenteric artery begin. The ileum is thought to begin at the vitelline (Meckel’s) diverticulum and end at therecto-caecal junction.

Vukicevic *et al*. (2004) mentioned that the small intestine of Ratites is differentiated into duodenum, jejunum and ileum.

Kadhim *et al*. (2011) studied the histomorphology of the stomach, proventriculus and ventriculus of the Red jungle fowl. They found that both chambers presented folds of the mucosa lined by a simple prismatic epithelium that was positive for neutral mucin. Simple tubular glands occupied the lamina propria of both chambers; in the ventriculus of older birds, they showed a coiled base. These ventricular glands were lined by simple cuboidal cells represented by the chief cells and a few large basal cells. The luminal and tubular koilin rodlets and folds of the ventriculus were positive to periodic acid Schiff (PAS) stain. The proventricular glands were situated between the inner and outer layers of the muscularis mucosae. Cells lining the tubulo-alveolar units of the proventricular glands showed a dentate appearance. Vacuoles were not observed, and the cells were negative for Alcian–PAS stain. The submucosa was very thin in the proventricular wall. In the ventriculus, it was not separated from the lamina propria owing to the absence of any muscularis mucosae. The musculosa of the proventriculus was formed by a thick inner layer of circular smooth muscle fibres and a thin outer layer of longitudinal fibres. In addition to these layers, oblique muscle fibres formed the most internal layer of the musculosa in the ventriculus.

With the purpose of correlating the molophology of the alimentary tract and the feeding habits of birds in general, we carried out histological and histochemical studies of the alimentary tract of the common quail, *Coturnix coturnix* .

2. Material and Methods

Coturnix coturnix (Order: Galliformes, Family: Phasianidae), was used in the present investigation. Healthy ten specimens of common quail were trapped alive from quail farms; Egypt. It was used as a model of granivorous birds (Fig.1). The specimens were anaesthetized by chloroform, and then they were carefully dissected for studying the gross anatomy of the alimentary canal (Fig.2). In addition, in two specimens, the alimentary canal was cut longitudinally to describe the structure of the internal surface as the folds, the villi and valves. For the general histological studies, the contents of the alimentary canal were drained by saline solution; small pieces of the various segments; the oesophagus, the stomach (proventriculus, ventriculus), small intestine (duodenum, ileum), and large intestine (caecum, rectum). Were fixed rapidly in alcoholic Bouin's solution or 10% neutral formalin for 24 hours and then kept in a mixture of 70% ethyl alcohol.

After fixation, the different parts of the alimentary tract were dehydrated through ascending grades of alcohol, cleared in cedar wood oil and finally embedded in parablast. Transverse sections of the different studied samples were cut at thickness of 7 microns. Sections were stained with differential double stained haematoxylin and eosin (Castro and Camargo, 1951) for general histological structures. For the histochemical studies, the following techniques were implied:

1- General carbohydrates were demonstrated by using the periodic acid-schiff (PAS) technique (Pearse, 1968). In this procedure, sections were placed in 0.5% periodic acid for the liberation of aldehydes, and treated with Schiff's regent for 2 minutes. A positive reaction is indicated by the appearance of magenta colouration resulting from the reaction between aldehydes and the decolourized solution (leucofuchsin) of Schiff's regent.
2- Acid and neutral mucopolysaccharides were demonstrated by using the Alcian blue- PAS method according to Mowry(1956). By this method, acid mucins exhibit blue stainabilities where as neutral mucins take a reddish colouration, and the mixture of both mucins acquire a purple stainability.
3- For the detection of total proteins, the mercury bromophenol blue method (Mazia *et al*., 1953) was employed. The existence of a dark blue

stainability denotes the occurrence of total proteins.

4- Nucleic acids (DNA and RNA) were demonstrated by using the methyl green-pyronin method (Kurnick, 1955).While the application of Feulgen reaction was used for demonstration of DNA only (Stowel, 1945).

Photomicrographs were taken to illustrate the histological, histochemical structure of the different studied samples; oesophagus, proventriculus, ventriculus, small intestine and large intestine.

3. Results

The Gross Anatomy

The alimentary tract consists of a buccal cavity, pharynx, oesophagus, proventriculus, ventriculus, small intestine (consisting of duodenum, jejunum and ileum), large intestine (consisting of paired caeca and a rectum) and cloaca, which opens to the outside by the cloacal opening (Fig.3).

The oesophagus:

The oesophagus is a thin walled distensible tube. It lies dorsal to the trachea in the anterior regions of the neck and then runs along the right side. The oesophagus transports the food from the pharynx to the gastric region allowing birds to swallow their food whole. Thus, it contains a number of longitudinal folds which provide distensibility. It is distinguished into three distinct anatomical portions: the cervical part, the crop and the thoracic part. The cervical part of the esophagus is considerably larger than the thoracic part.

The oesophagus has a single outpouching to form the crop; it is located just outside the body cavity in the neck region. It is a storage place for food. From here food passes through the lower oesophagus into the proventriculusand it contains longitudinal folds on the inner surface thus making it distensible. Beyond the crop, the oesophagus continues as the thoracic part connects with the proventriculus.

The stomach

The stomach is formed of two distinct parts; the glandular portion, gastric proventriculus or true stomach (pars glandularis) which is caudal to the oesophagus and the muscular portion, gastric ventriculus or gizzard (pars muscularis) which is located caudal to the proventriculus. The two parts of the stomach are connected together by an intermediate zone. The proventriculus secretes HCL and pepsin which is needed for protein digestion. The proventriculus being buff while the gizzard bluish-red.

The proventriculus

The proventriculus is a spindle shaped organ located between the oesophagus and the ventriculus. It arises from the oesophagus without adistinct demarcation, the wall was thicker than that of the oeosphagus, and the caudal extent of the proventriculus was marked by a constriction, the isthmus gastris. The mucosal surface of the proventriculus revealed the presence of raised papillae, papillae proventriculus, over its entire surface.

The ventriculus

The gizzard is a small spheroid organ; the ventriculus lies in the left dorsal and ventral regions of the thoracoabdominal cavity, placed partly between the lobes and partly behind the left lobe of the liver. It is much larger and more muscular than the proventriculus. It is consists of two pairs of opposing muscles. The caudoventral and craniodorsalthin muscles line the caudal and cranial sac of the ventriculus, respectively. The cranioventral and caudodorsal thick muscles are responsible for the powerful grinding contractions seen in the ventriculus. The asymmetrical arrangement of these four muscles provides mixing and grinding actions during contraction. The ventriculus is lined by the koilin, a cuticle layer, which acts as a grinding surface and protects the underlying mucosa from the acid and pepsin produced by the proventriculus, which frequently appeared green or yellow in colour due to the regurgitation of bile.

The pyloric region of the stomach opens distally into the duodenum by pyloric orifice which is guarded by a small pyloric valve. This lies at the right angle of the pyloric orifice.

The small intestine

The small intestine extends from the pyloric end of the stomach to the junction of the small intestine, caeca and colon. It is long and consists of a coiled mass forming a series of loops and lies within the abdominal cavity. It is distinguished into three main parts, the duodenum, jejunum and ileum.

The duodenum begins at the end of ventriculus and forms an elongated loop about 20 cm long. The pancreas lies between the arms of the loop and being attached to each arm of the duodenum actually holds the two arms together. The jejunum and the ileum are very long and coiled, commence at the caudal end of the duodenum where the bile and the pancreatic ducts are located and terminate at the ileo-caecal-colic junction. This junction is where the small intestine, the two caeca and the colon all meet. The external diameter of which is roughly uniform, so the transition between them is anatomically unrecognizable, but, we have found that Meckel's diverticulum marks the end of the jejunum and the start of the ileum. Meckel's diverticulum appears as a small projection on the outer surface of jejunum, the projection is where the yolk sac was attached during the development of the embryo.

The large intestine

The rectum of the *Coturnix coturnix* is extending from the end of the small intestine until it opens distally in the cloaca in the form of a short and straight tube. Also, the large intestine contains a pair of outpocketing caeca that project from the proximal part of the rectum at its junction with the small intestine. Internally, the mucous membrane of the rectum is thrown into numerous distinct longitudinal folds.

These caeca, the right and left caeca arise from the lateral walls of the rectum, close to the junction with ileum, they are well developed. Each caecum could be divided externally into a short proximal neck, a long middle thin-walled body, and a short apex.

The cloaca

The cloaca extends from the end of the rectum to the cloacal opening.It is divided, as it is generally the case in birds, into three chambers namely, coprodaeum, uerodaeum and proctodeum.

The liver:

The liver was dark red–brown in colour. Placed in its natural position. It consists of two lobes, namely; the right and the left lobes, the former is much larger than the left one and the latter is sub divided into two lobes. The right and the left lobes are united together antero-dorsally by a thin isthmus. It is connected to both the diaphragm and the ventral body wall by means of the falciform ligament.

The gall bladder is partially embedded in the right lobe of the liver at the posterior surface. It is somewhat elongated and oval-shaped thin –walled sac, dark green in colour. Two bile ducts emerge from the right lobe. One of these originates from the gall bladder and the second provides a direct connection from the liver to the small intestine.

The pancreas:

The quail pancreas, which is a pale-yellow organ with a finely lobulated surface located between the ascending and descending loops of the duodenum, was determined to be composed of the dorsal, ventral, third and splenic lobes united together by a broad median bridge (Fig .1).

Three pancreatic ducts opening into the duodenum proceed directly from the dorsal, ventral and third lobes, respectively.

Histological studies of alimentary canal of the common quail (*Coturnix coturnix*).

The oesophagus:

The oesophageal wall consists of the usual layers of a tubular digestive organs, i.e. mucosa, submucosa, musculosa and adventitia or serosa (Fig.4a).

The mucosa is formed of stratified squamous epithelium of about seven to eight longitudinal folds of different shapes. These folds are shallow and narrow 7-10 cells thick. The lamina propria is formed of loose connective tissue. Fine connective tissue fibers, fibroblasts and areas of lymphocyte infiltration can be seen in this layer. This layer is considered as an extension of the submucosa. The muscularis mucosa, which is a very well developed continuous layer, is formed of smooth muscle fibers. The submucosais composed of loose connective tissue with larger blood vessels, lymphatics and nerve fibers. The simple branched mucus glands called oesophageal glands, scattered throughout the submucosa, are present in both the cervical and the thoracic parts of oesophagus. The glands are less developed in the cervical part in comparison to the thoracic part of the organ (Figs.4a& b). An important characteristic feature of the oesophagus in common quail is the presence of mucous glands in the crop (Fig.4c). The musculosa of the oesophagus is composed only of smooth muscle and is composed of two distinct layers: an inner circular and an outer longitudinal layer. The circular layer is thicker than the longitudinal layer. The two layers are separated by connective tissue fibers in which runs a nerve plexus (Fig.4d). Peristaltic contractions of the inner circular and the outer longitudinal muscles propel food posteriorly through the oesophagus. The adventitia is composed of loosely arranged connective tissue which binds the organ to the surrounding tissues. Nerve fibers and blood vessels can be found in this layer. The thoracic part of the oesophagus that extends below the diaphragm is covered by a serosa.

Stomach

Microscopically, the wall of the stomach (proventriculus and gizzard) consists of four distinct functional tunicae namely, mucosa, submucosa, musculosa and outermost serosa.

The proventriculus

The proventriculus mucosa, unlike that of the oesophagus, is thrown into folds with varying heights; the folds were lined by a simple columnar epithelium. Lamina propria is occupying the center of the mucosal folds. This layer is dense irregular connective tissue with collagen fibers, fibroblasts and lymphoid infiltration. Delicate smooth muscle fibers are scattered in the deepest part of this layer and between the proventricular glands. That extends to hold two types of gastric glands, the deep and the superficial gastric glands (Fig. 5a).

The superficial gastric glands are of simple tubular type appearing in the form of numerous folds of the mucosal epithelium. The walls of these glands are composed of columnar mucus-secreting epithelial cells with centrally located nuclei and a cytoplasm filled with translucent mucous secretion (Fig.5b).The submucosais a narrow connective tissue layer sandwiched between the circular layer of the musculosa and the main mass of the muscularis mucosae.

Compound tubulo-alveolar proventricular glands formed the greatest thickness of the proventricular wall. The proventricular glands are distributed throughout the entire organ. The glands are composed of rounded, oval, hexagonal or polymorphic lobules separated from each other by thin perilobular connective tissue sheath containing fibroblasts and few smooth muscle fibers. The wall of each lobule is formed of numerous secretory alveoli or tubules opening together into a wide central cavity, from which a wide duct originates. Ducts from several lobules joined together to form a short main duct which is connected to the apex of the raised mucosal papillae and open into the narrow lumen of the proventriculus. The duct system of the proventricular glands is lined with tall columnar epithelium with oval or vesicular nuclei which are located sometimes at different levels giving the epithelium pseudostratified appearance. The proventricular gland alveoli were formed from one cell type (oxynticopeptic cell), the proventriculus cells secrete hydrochloric acid & proteolytic enzymes. The proventricular tubules are composed of cuboidal cells. Their nuclei were nearly rounded and located near to the basement membrane. The secretory cells are oriented obliquely to the long axis of the glandular tubules of the proventriculus and are separated from each other by relatively narrow spaces giving the epithelial cells a serrated appearance (Figs.5c).

The muscularis mucosa of the proventriculusis formed of two small muscle fibers layers; inner isolated muscular bands arranged in a longitudinal manner and outer band arranged in a circular manner. The deep proventricular glands are located between the inner and the outer layers of the muscularis mucosa. The musculosa is moderately thick and consists of an inner circular and an outer longitudinal muscle layer. The inner layer is two times thick as the outer layer. Serosa was constituted by connective tissue, containing many blood vessels and nerves, lined by mesothelium.

The ventriculus

The mucous membrane of the gizzard presents low folds which are lined by columnar epithelial cells possessing generally rounded nuclei. Over the mucous membrane, a thick cuticle is disposed. The lamina propria is constituted by a dense connective tissue and it is occupied by numerous deep simple tubular glands which expand in the base of the fold, partially located between them. Those glands are lined by a simple columnar epithelium, which is lower in the base of the glands and higher in their upper portion in the interior of the glands. There are crypts in the base of the folds (Fig.6a). We observed eosinophilic secretion fillets continuous with the cuticle. Glandular tubes are narrower, while others are wider (Fig.6b). No muscularis mucosa is present making no partitions between the lamina propria and the submucosa. The musculosa is well developed forming the main bulk of the gizzard wall and represented by smooth muscle fibers arranged mostly in a circular manner. The muscular bundles are interspersed with bands of connective tissue. The musculosa is very thick to support the mechanical force of grinding.

The serosa, which is constituted by connective tissue lined by methelium is rich in blood vessels and nerve endings. It is followed by a subserosal layer (Fig.6b).

The small intestine

The small intestine is conveniently divided into three main regions namely, the duodenum, the jejunum and the ileum. All the three divisions show the usual tunicae namely; mucosa, submucosa, musculosa and serosa. The mucosa of the intestine is thrown into villi which show a marked variation in density, shape and size in the different regions of the intestine. Intestinal villi gradually decrease in length and size moving from the duodenum to the ileum.

The mucosa is built up of a lamina propria of loose connective tissue supporting the mucosal membrane which is thrown into deep, narrow finger-like villi in the duodenum while the villi are relatively short, somewhat broad and numerous in the ileum (Figs. 7a.8a).

The mucosa consists of a simple columnar epithelium and a tunica propria. The muscularis mucosa is represented by a narrow part of longitudinally arranged smooth muscle fibers towards the side of the submucosa, but on the side of the lamina propria, it is represented by vertically arranged smooth muscle fiber strands. The columnar cells possess elongated nuclei and a clear cytoplasm.

Goblet cells frequently occur amongst the columnar or absorptive cells. Each cell is rounded or oval in shape. The goblet cells are more numerous in the ileum than in the duodenum (Figs. 7b. 8b). The goblet cells increase from the duodenum towards the rectum. The goblet cells are positive to the stains specific for mucus. Lymphocytes are scattered amongst the bases of the columnar epithelial cells. They are small more or less spherical and their nuclei are rounded and darkly stained. Crypts of Lieberkühn, in the form of simple tubular stands, occur at the bases of the villi, being more numerous and too crowded in the duodenum. They are built of cells similar in structure to those of the mucosal epithelium.

The cores of the villi are formed of the areolar connective tissue of the tunica propria. They contain blood vessels and capillaries, lymph vessels and numerous darkly stained lymphocytes.

The submucosa is thin, narrow and hardly distinguished in some regions. The submucosa connective tissue holds few blood vessels. The muscularis mucosais composed of thin layer of longitudinal muscle fibers which merges gradually into the submucosa and extends into the core of the villi. The musculosa consists of two smooth muscle layers; outer longitudinal layer and a thick circular muscle layer. All muscle fibres are of the unstriated type. Two muscle layers surround the intestine, the inner circular and outer longitudinal layers that allow mixing and propulsion of the digesta through the intestinal tract. The serosa is made up of flattened simple squamous epithelium

The rectal caeca

Each rectal caecum is distinguished into three main regions: proximal, middle and distal. Its mucous membrane is raised into simple villi. These show a gradual change in their depth and width from end to end. Thus, the height of the villi increased as the villi neared the proximal caecum. Conversely, the villi height decreased as the position moved far from the proximal caecum. The villi are lined by a simple columnar epithelium whose cells resemble those of the small intestine. villi were found and had globet cells and crypts, and the muscle layer was thicker (Fig.9). No villi in the middle zone caecum was found. The muscularis mucosa is composed of thin layer of longitudinal muscle fibers. Accordingly, the narrow and thin submucosa connective tissue layer merges into that at the lamina propria. The musculosa consists of two layers of unstriated muscle fibres: an outer longitudinal layer and inner circular one. These muscle layers are in the proximal part of the caecum, continuous with those of the ileum and of almost the same thickness. In the middle and distal parts, the outer longitudinal muscle layers are quite thin. In the middle zone caecum the muscle layer was thin with saw-shaped or parallel ridged tract lumen. The distal caecal muscle layer was thinner than that in the proximal caecum and its surface was saw-shaped and no parallel ridges or villi were found. The caecum is covered externally with a thin serosal layer; formed of simple squamous epithelial cells which possess flattened nuclei.

The rectum

Apart from minor differences such as the thickness of the various coats and the shape of the villi, the musculosa and the serosa are similar to those described above for the small intestine. The wall of the rectum is made up of serosa, musculosa, submucosa, muscularis mucosa and mucosa. The serosa is a thin layer composed of simple squamous epithelium with flattened nuclei. The musculosa is made up of two muscle layers; an outer thin longitudinal and thick circular one. The submucosa consists of loose connective tissue holding blood vessels. The muscularis mucosa is composed of longitudinal muscle fibers. This layer extends inside the mucosal folds as vertical muscle fiber strands (Fig.10a). The mucosa is thrown up into numerous leaf-like villi, all covered by simple columnar epithelium containing goblet cells. The goblet cells are numerous in number and open into the lumen. At the base of the mucosal folds, rectal glands (simple tubular) are noticed. These glands are crypts as in the small intestine, lined with simple columnar epithelium and goblet cells (Fig. 10b).

Histochemical studies of the alimentary canal of the common quail (*Coturnix coturnix)*

Carbohydrates (PAS-positive material):

The oesophagus:

The esophageal glands are composed of typical alveoli .These glands react positively with the periodic acid Schiff (PAS) stain. These glands were loaded with positively stained material (Fig. 11).

The stomach

In the proventriculus, The cells of the surface lining epithelium of the mucosal folds, showed PAS- positive mucin granules occupying the supra nuclear area of the cells . The ductular cells that lined the ducts of the proventricular glands showed PAS positive reaction in their apical ends. The secretory cells were negatively stained with PAS stain (Fig. 12). The luminal and tubular koilin rodlets and folds of the ventriculus were positive to PAS stain (Fig.13).

The small intestine (the duodenum and the ileum)

Application of PAS method indicated that, the mucosa of the small intestine revealed a strong magenta colouration in the goblet cells of both the villi and the crypts of Leiberkühn as well as the apical plasma membranes of the columnar epithelial (absorptive) cells. However, the ground cytoplasm of the columnar epithelial cells exhibits moderate PAS-reactivity (Figs.14&15).

The rectum

General carbohydrates are localized in the rectal glands, goblet cells and in the surface mucous epithelium with PAS method in the form of magenta colouration (Fig.16).

Mucopolysaccharides:

The oesophagus

The Alcian blue - PAS method, showed that the nature of the oesophageal glands of quail in the form of acid mucopolysaccharides (the blue colour, Fig. 17).

The stomach

Neutral mucopolysaccharides are abundantly found in the gastric glands. On the other hand, the superficial glands secrete acid and neutral mucopolysaccharides since they give blue and red colour with Alcian- PAS stain. Cells lining the tubulo-alveolar units of the proventricular glands showed a negative reaction for Alcian-PAS stain (Fig.18).

In the ventriculus, the mucosal epithelium was PAS and Alcian blue positive especially at the apical portion of the cells indicating the presence of both neutral and acid mucin, similar to the proventriculus. The ventriculus mucosa covered by a thick keratinized laminated layer of koilin membrane which is formed of proteinous material similar to keratin and stained positive for PAS and Alcian blue indicating the presence of neutral and acidic mucin within its contents (Fig.19).

The small intestine

The goblet cells and crypts of Leiberkühn have acid and neutral mucopolysaccharide secretions (Figs.20&21)

The rectum

Application of Alcian blue PAS method revealed that the rectal glands contain acid and neutral mucopolysaccharides. Also, the columnar cells contain neutral mucopolysaccharides while the goblet cells contain acid mucopolysaccharides (Fig. 22)

Total proteins:

The oesophagus

Application of mercuric bromophenol blue method on the oesophagus of the quail proved an exaggerated amount of proteinic elements in the cytoplasm of its stratified squamous epithelium. On the other hand, the oesophageal glands showed a negative response to the above mentioned method (Fig.23)

The stomach

In the proventriculus, the cytoplasm of cells of the surface lining epithelium of the mucosal folds and the ductular cells that lined the ducts of the proventricular glands show weak reaction with bromophenol blue method (Fig. 24).The luminal and tubular koilin rodlets of the ventriculus showed a strong response to the bromophenol blue method, while a weak reaction was noticed in the cell lining folds.(Fig. 25).

The small intestine and the rectum

In the small intestine and the rectum of the *Coturnix coturnix,* the bromophenol blue stain reacts positively with the absorptive columnar cells. A similar feature is also noted in the lamina propria.While a weak reaction was noticed in the cytoplasm of the goblet cells (Figs. 26&27).

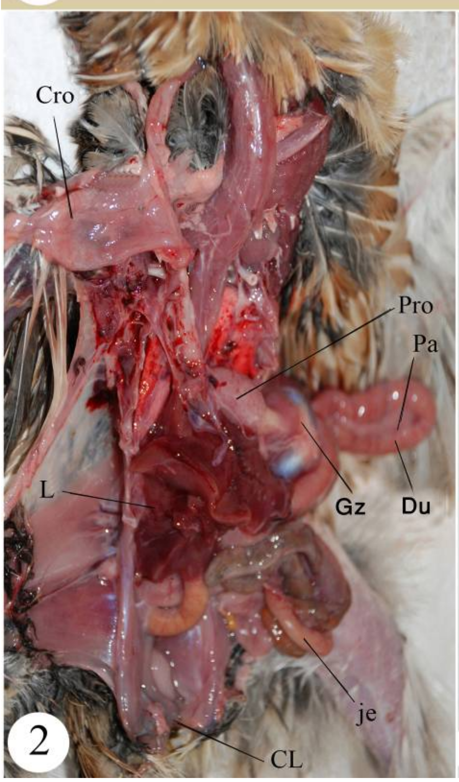
Nucleic acids:

Histochemical demonstration of DNA revealed the appearance of adense product in the nuclei of the oesophageal, gastric and intestinal mucosal cells. Such A positive staining product is present in the place of the chromatin substances containing DNA (Figs.28-30). Application of methyl green pyronin methods proved the existence of a considerable amount of RNA inside the cytoplasm of the columnar epithelial cells in the different gut regions of quail (Figs.31-35).

4. Discussion

The anatomical observation of the alimentary canal of *Coturnix coturnix* detects that the oesophagus of *Coturnix coturnix*is a long tube since, it is characterized by three main parts; the upper part starts at the end of the pharynx, the next part is the crop which represent a musculo membranous pouch and the lower part which is connected to the proventriculus. These results are in agreement with those obtained by Klasing (1999).In *Coturnix coturnix*, the presence of the crop serves as a storage receptacle for the swallowed grains since this bird has to take a large quantity of is food in a fast and quick manner similar finding were observed by Wallace (1961).The stomach of *Coturnix coturnix* is a smooth muscular organ, located between the esophagus and the intestine, and it is constituted of two different portions: glandular and muscular stomach and its gastric ventriculus is constituted by four semi-autonomous muscles, two thick and dark colored, the caudodorsalis and the cranioventralis, and two with fine thickness and clear colored, the craniodorsalis and the caudoventralis, that are responsible to crush the victuals ingested. This observation is similar to that of Suganuma *et al*., 1981; Dyce *et al*., 1996; Bailey *et al*., 1997; Bacha & Bacha, 2000. Anatomically, the present study revealed that, the ventriculus of *Coturnix coturnix*, was thick-walled and powerful. The intermediate zone connects the two .This observation is similar to that of Denbow (2000) and Taylor (2000). The differences in proventriculus structure may also be related to diet. The large walled quail proventriculus allows it to accommodate dry bulky food, since both the proventriculus and the gizzard contain rocks.

In the present results the small intestine of *Coturnix coturnix*, is differentiated into the duodenum, jejunum and ileum. These results agree with Taylor (2000) and Vukicevic *et al*. (2004). The present study revealed that, the rectum of the *Coturnix coturnix* extends from the end of the small intestine until it opens distally in the cloaca in the form of a short and straight tube. Similar observation had been pointed out in *Bubo bubo* (Abo-Shaeir, 2001). The rectal villi are shorter than that of the small intestine. These results agree with Klasing (1999). Quail are granivorous and have a pair of well developed caeca. This agrees with Chen *et al*.(2002) in geese.



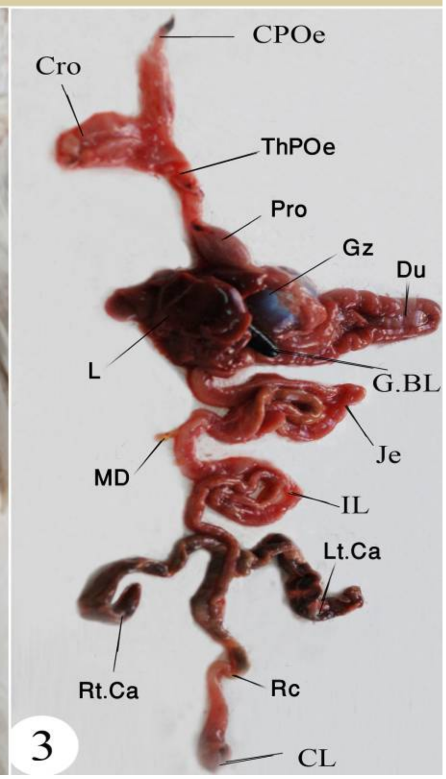


Fig. (1): Photograph of the common quail, *Coturnix coturnix*.

Fig. (2): Photograph of the dissection of the alimentary tract of *Coturnix coturnix* showing crop (Cro), proventriculus (Pro), Gizzard (Gz), duodenum (Du), jejunum (je), cloaca (CL), liver (L), and pancreas (pa)

Fig. (3): Photograph of a fresh isolated alimentary tract of *Coturnix coturnix* showing the cervical part of oeosphagus (CPOe), thoracic part of oesophagus (ThPOe), crop (Cro), proventrculus (Pro), Gizzard (Gz), duodenum (Du), jejunum (je), ileum (il), Caecum (Ca), liver (L), Mechel's deverticulum (MD), rectum (Rc) , and cloaca (CL).

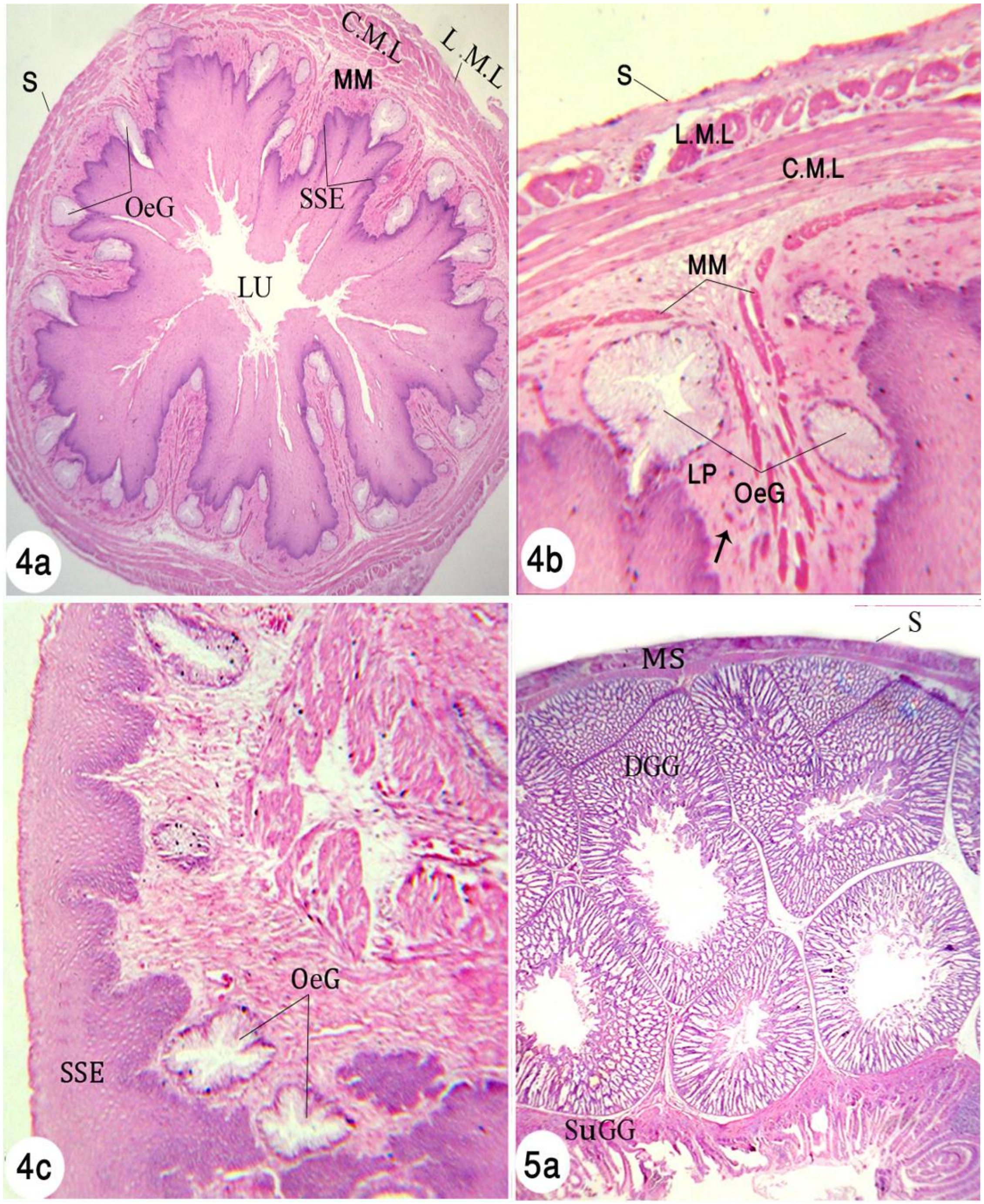
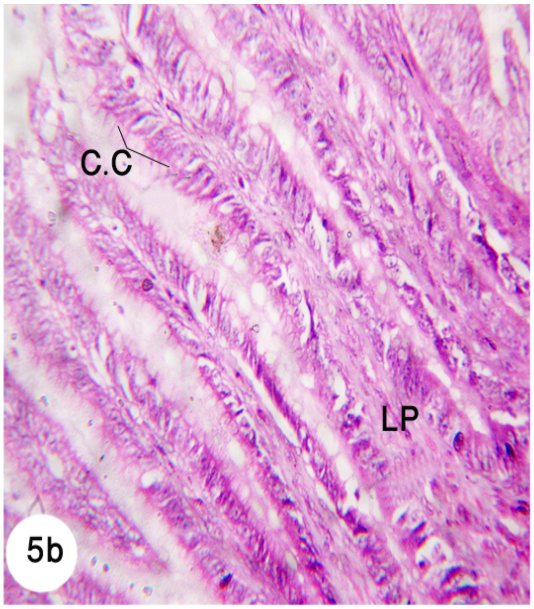


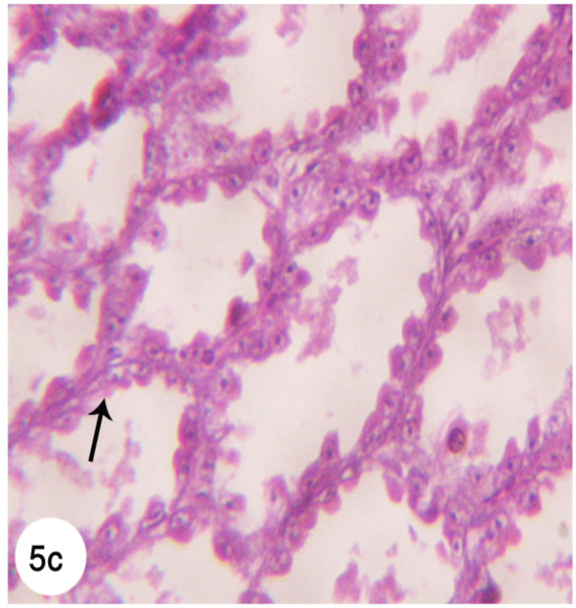
Fig. (4a): Photomicrograph of a transverse section of the thoracic part of oesophagus in the *Coturnix coturnix* showing the lumen(LU), oesophageal gland(OeG),stratified squamous epithelium(SSE), mucosa(Mu), muscularis mucosa (M.M) musculosa (circular and longitudinal layers(CML&LML) and serosa(S). H&E stain, X56.

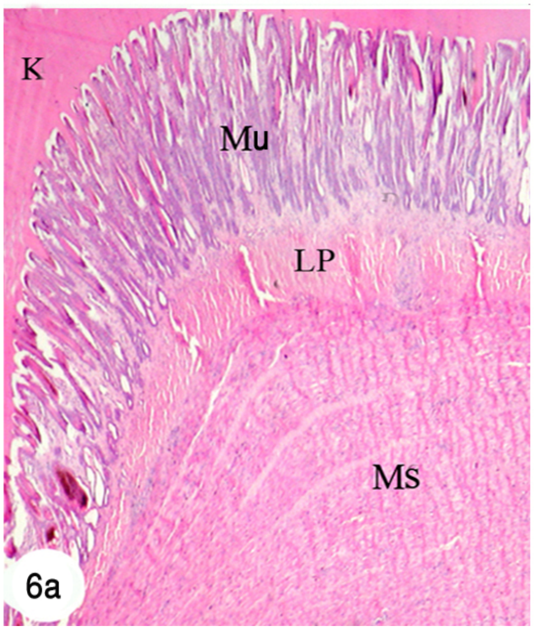
Fig. (4b): Photomicrograph of the enlarged portion of the cervical part of the oesophagus in the Coturnix coturnix showing the fibro blasts and areas of lymphocyte in filtration in the lamina propria (arrow), H&X 182.

Fig. (4c): Photomicrograph of a transverse section of the crop of *Coturnix coturnix*. Showing the oesophageal gland (OeG), stratified squamous epithelium (SSE), H&E stain, X100.

Fig. (5a): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix*. Showing the serosa(S), muscularosa (Ms), deep gastric gland (DGG), and superficial gastric gland (SuGG). H&E *stain*, X 40.







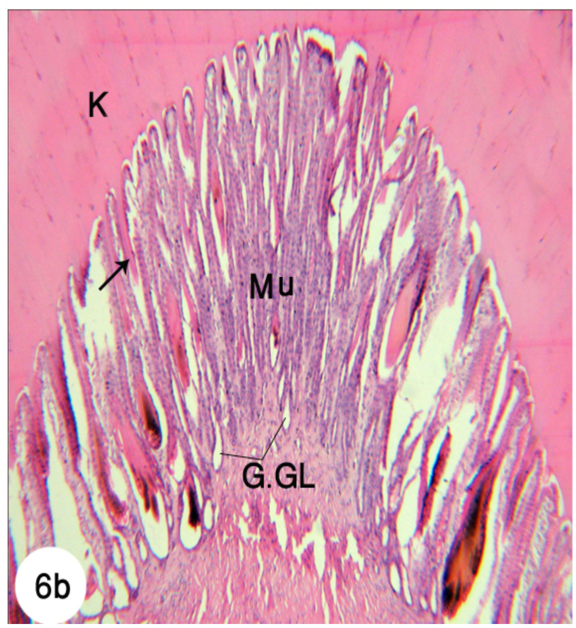


Fig. (5b): Photomicrograph of enlarged portion of the proventriculus of *Coturnix coturnix*. Showing lamina propria (Lp) and columnar cells (C.C) lining the superficial glands H&E *stain*, X 400.

Fig(5c): Photomicrograph of enlarged portion of the proventriculus of *Coturnix coturnix*. Showing serrated appearance of the glandular tubules and cuboidal cells lining the tubules(arrow). H&E *stain*, X 600.

Fig. (6a): Photomicrograph of a transverse section of the ventriculus of *Coturnix coturnix*. Showing the mucosa (Mu), muscularosa(Ms), lamina propira(LP)and koilin(K). H&E stain, X100.

Fig. (6b): Photomicrograph of enlarged portion of the ventriculus of *Coturnix coturnix*. Showing the secretion fillets continuous with the cuticle (arrow). and glandular tubes, gastric gland (G.Gl) are narrower, while others are wider. H&E stain, X150.

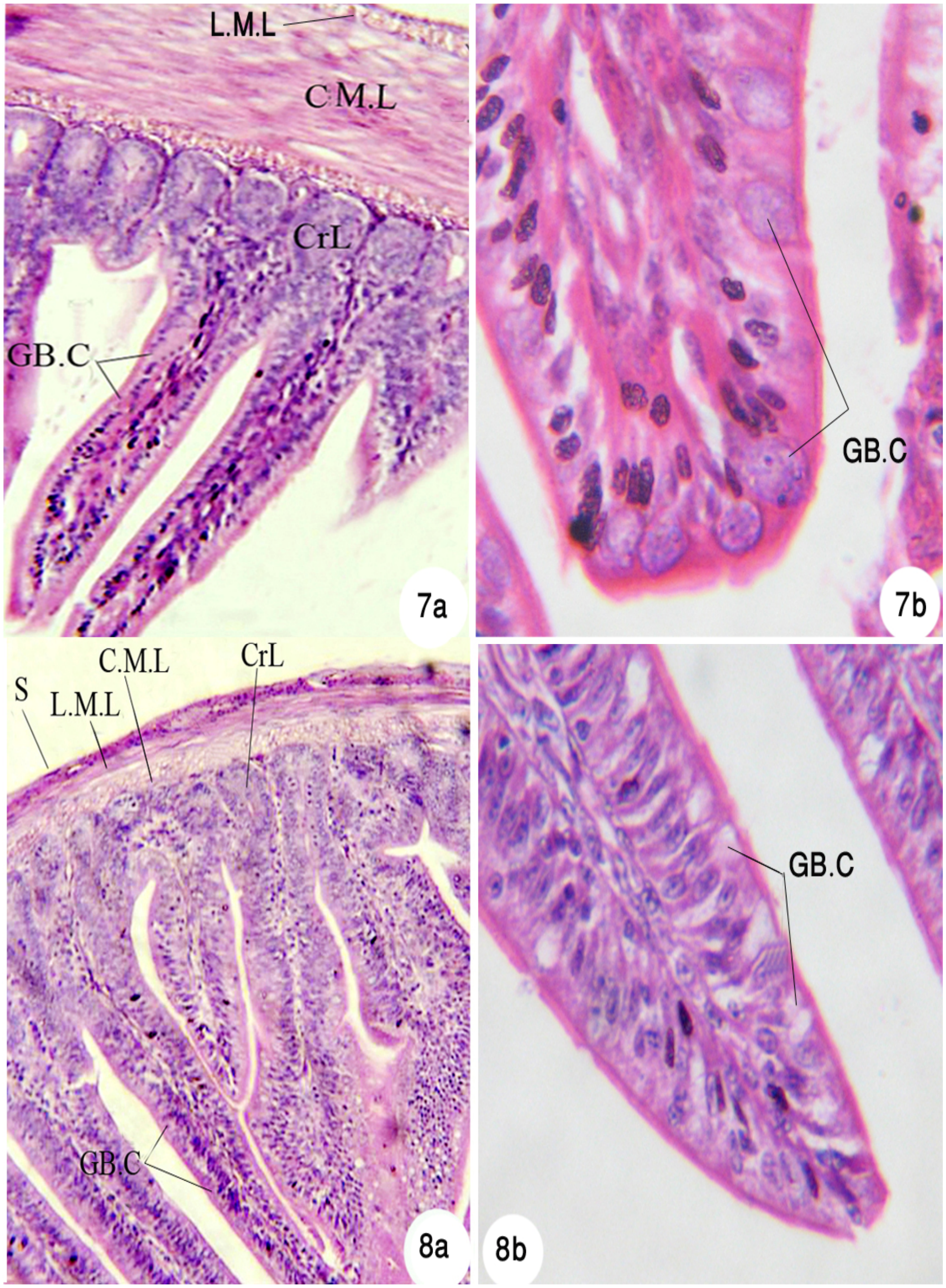


Fig. (7a): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix* showing the musculosa (circular and longitudinal layers (CML&LML), goblet cell (GB.C) and crypts of leiberkühn(CrL) H&E stain, X60.

Fig. (7b): Photomicrograph of enlarged portion of duodenum of *Coturnix coturnix* in the mucosal layer showing goblet cells (GB.C). H&E stain, X400.

Fig. (8a): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* showing the musculosa, circular and longitudinal layers (CML&LML), serosa(S), goblet cell (GB.C). H&E stain, X100.

Fig. (8b): Photomicrograph of enlarged portion of ileum of *Coturnix coturnix*, showing goblet cells (GB.C), in the mucosal layer. H&E stain, X400.

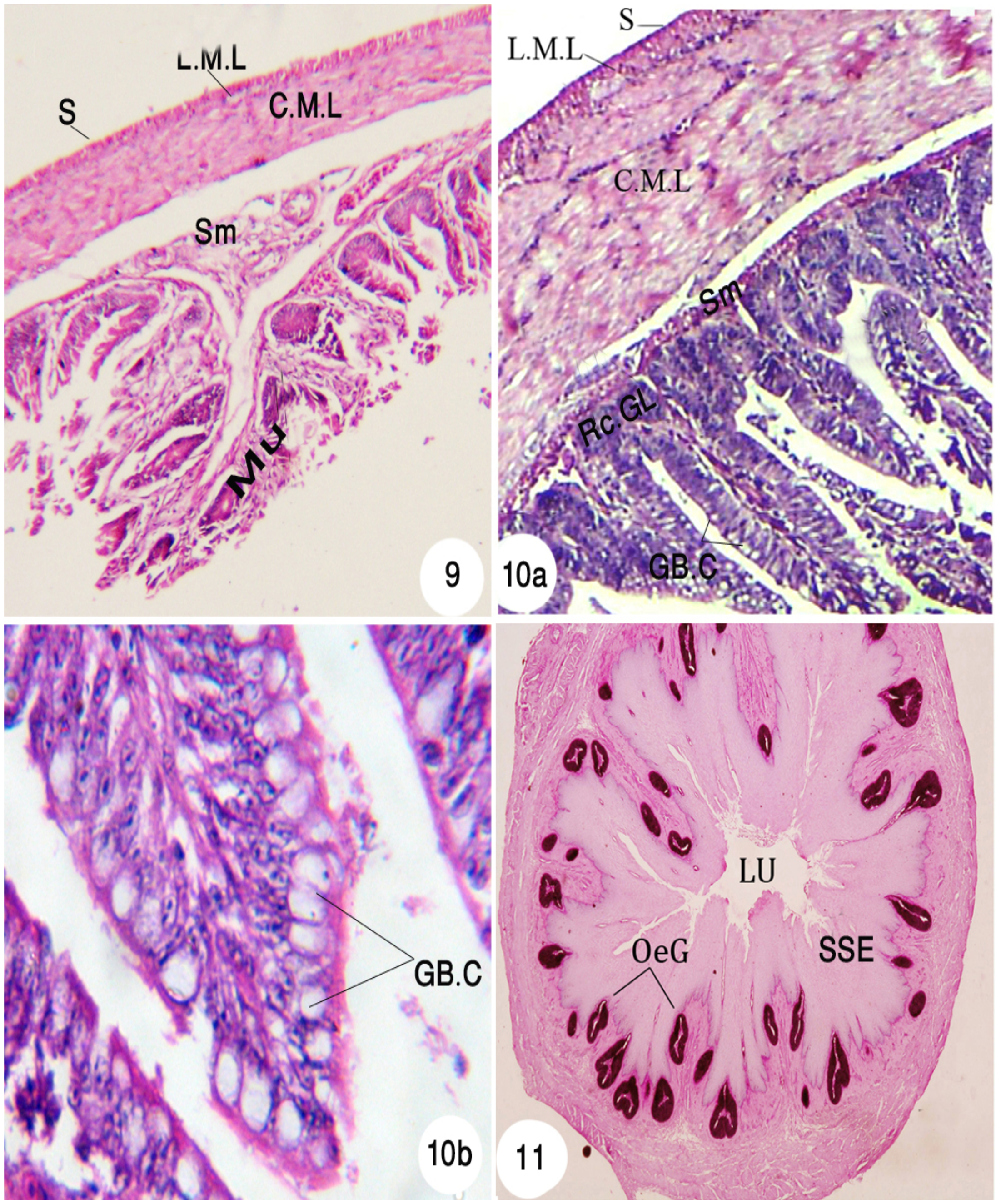


Fig. (9): Photomicrograph of a transverse section of the proximal caecum of *Coturnix coturnix.* Showing the serosa (S) muscularosa (circular and longitudinal layers CML&LML), (H&E stain, X100.

Fig. (10a): Photomicrograph of a transverse section of the proximal caeca of *Coturnix coturnix.* Showing the serosa (S) muscularosa (circular and longitudinal layers (CML&LML), and mucosa (Mu) H&E stain, X100.

Fig. (10b): Photomicrograph of enlarged portion of rectum of *Coturnix coturnix* in the mucosal layer showing goblet cells (GB.C) H&E stain, X400.

Fig. (11): Photomicrograph of a transverse section of the oesophagus of *Coturnix coturnix*. Showing the carbohydrate content (PAS-positive stain) X40.

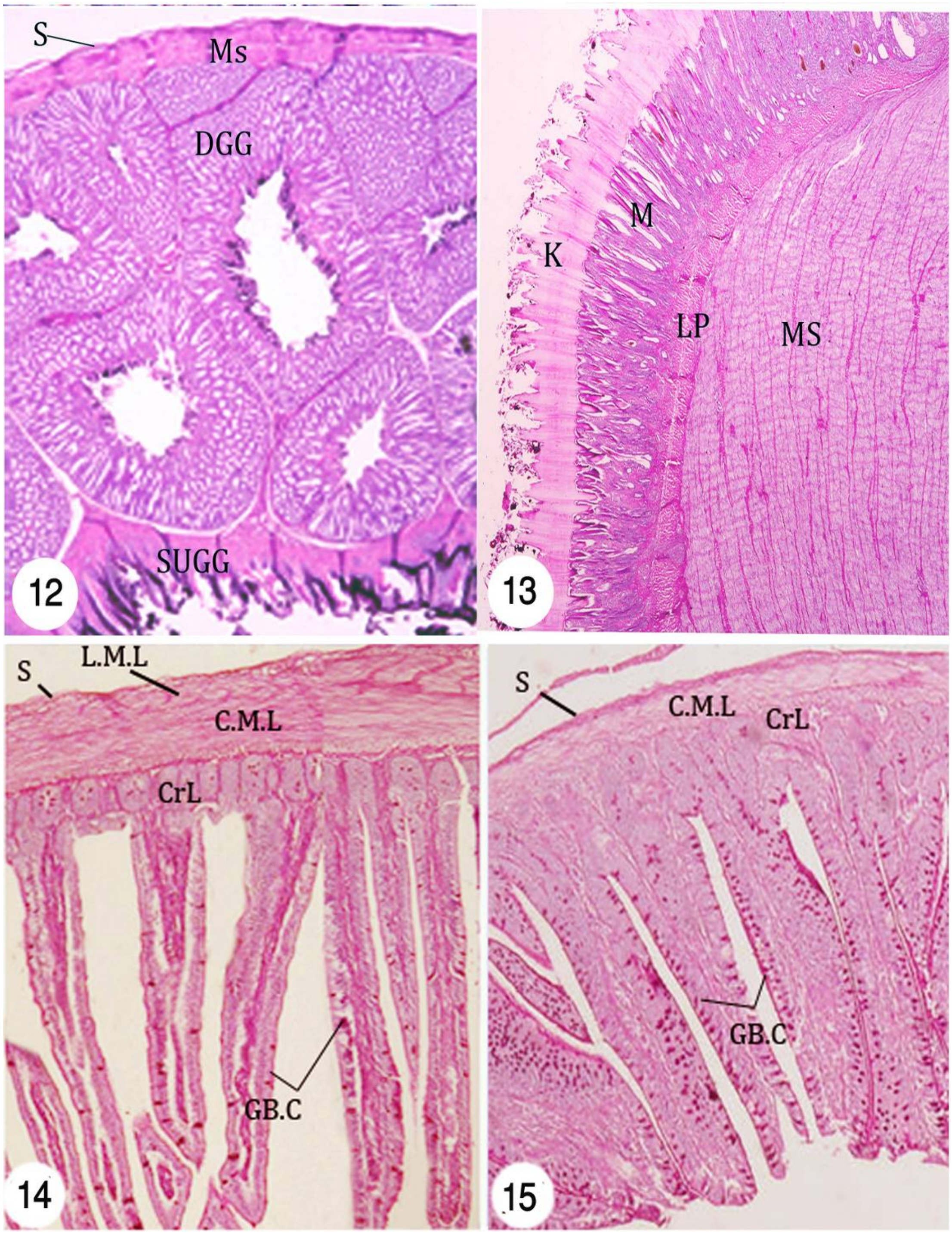


Fig. (12): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix*. Showing the carbohydrate content: (PAS-positive stain) X40.

Fig. (13): Photomicrograph of a transverse section of the ventriculus of *Coturnix coturnix*. Showing the carbohy drate content. (PAS-positive stain) X656.

Fig. (14): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix*. Showing the carbohydrate content. (PAS- positive stain) X164.

Fig. (15): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix*. Showing the carbohydrate content. (PAS- positive stain) X 656.

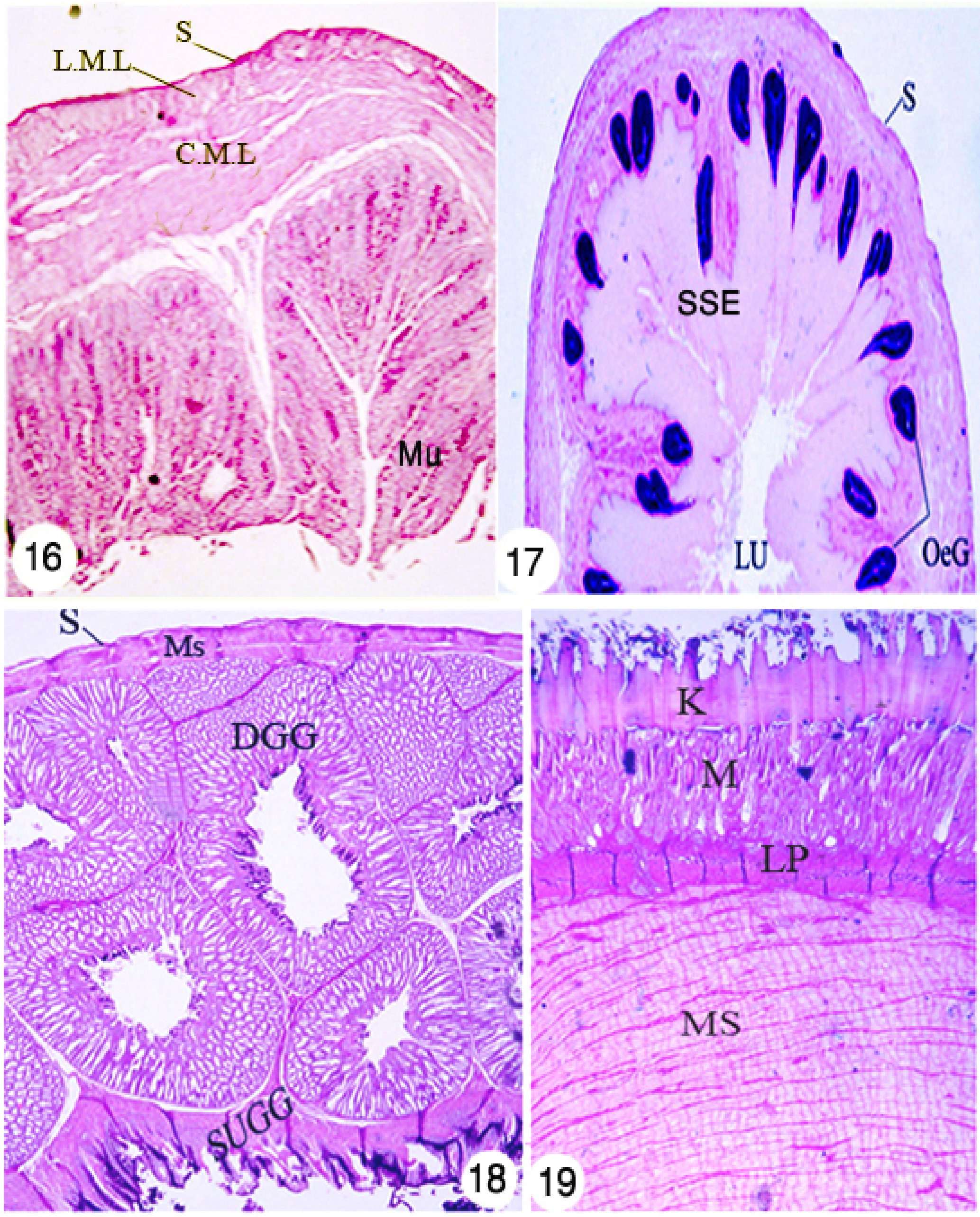


Fig. (16): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix*. Showing the carbohydrate content (PAS- positive stain) X600.

Fig. (17): Photomicrograph of a transverse section of the oesophagus of *Coturnix coturnix*. Showing the mucopolysaccharide content (PAS- Alcian blue stain) X 600.

Fig. (18): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix* showing the mucopolysaccharide content (PAS-Alcian blue stain) X 164.

Fig. (19): Photomicrograph of a transverse section of the ventriculus of *Coturnix coturnix* showing the mucopolysaccharide content (PAS-Alcian blue stain) X 600.

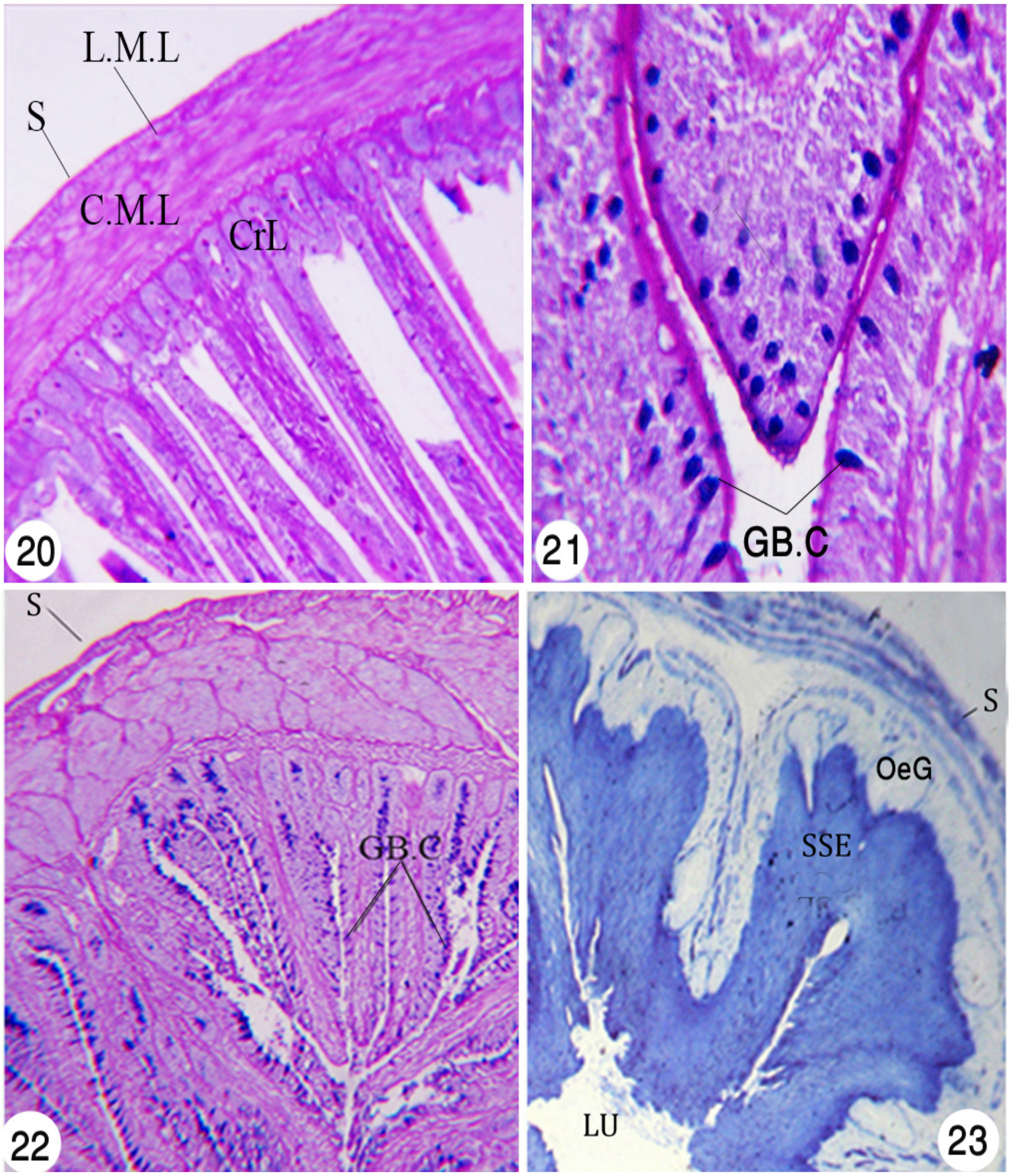
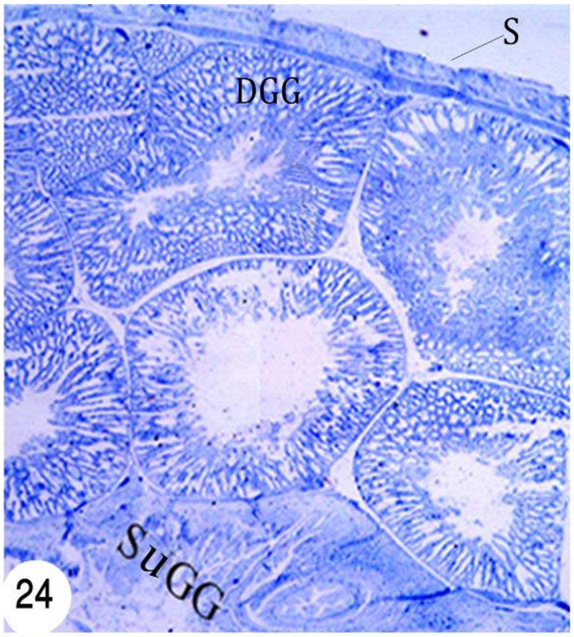


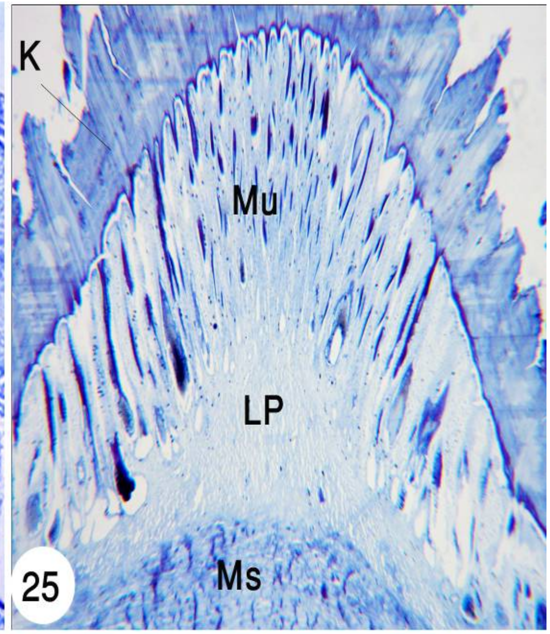
Fig. (20): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix* showing the mucopolysaccharide content. (PAS-Alcian blue stain) X 140.

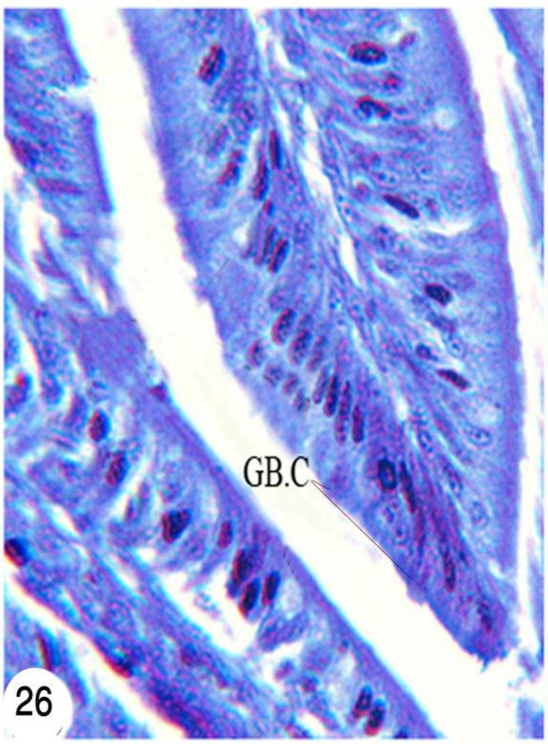
Fig. (21): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* showing the mucopolysaccharide content. (PAS-Alcian blue stain) X 560.

Fig. (22): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix* showing the mucopolysaccharide content (PAS-Alcian blue stain) X560.

Fig. (23): Photomicrograph of a transverse section of the oesophagus of *Coturnix coturnix* showing the protein content (Bromophenol blue stain) X 600.







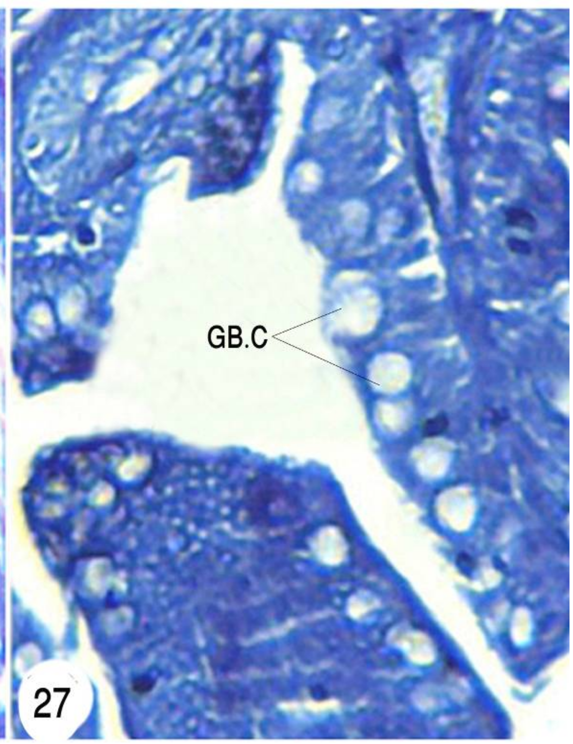


Fig. (24): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix*, showing the protein content (Bromophenol blue stain) X 600.

Fig. (25): Photomicrograph of a transverse section of the ventriculus of *Coturnix coturnix* showing the protein content (Bromophenol blue stain) X 600.

Fig. (26): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* showing the protein content (Bromophenol blue stain) X 600.

Fig. (27): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix* showing the protein content (Bromophenol blue stain) X 600.

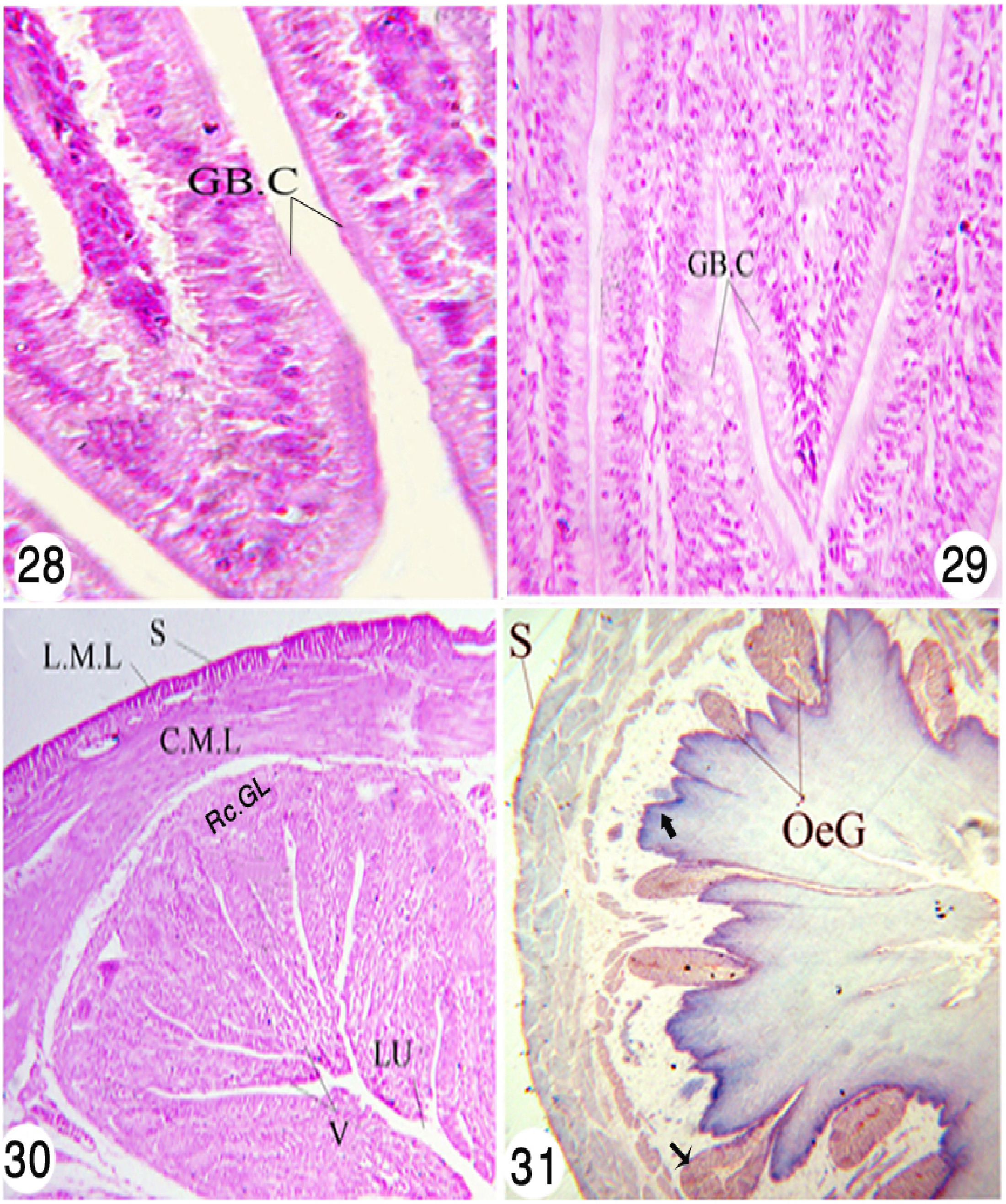


Fig. (28): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix* showing the DNA content. (Feulgen technique) X 560

Fig. (29): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* showing the DNA content. (Feulgen technique) X 560

Fig. (30): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix* showing the DNA content. (Feulgen technique) X 560

Fig. (31): Photomicrograph of a transverse section of the oesophagus of *Coturnix coturnix* indicating the DNA (small arrow) and RNA content (large arrow) (Methyl green-pyronin stain) X560.

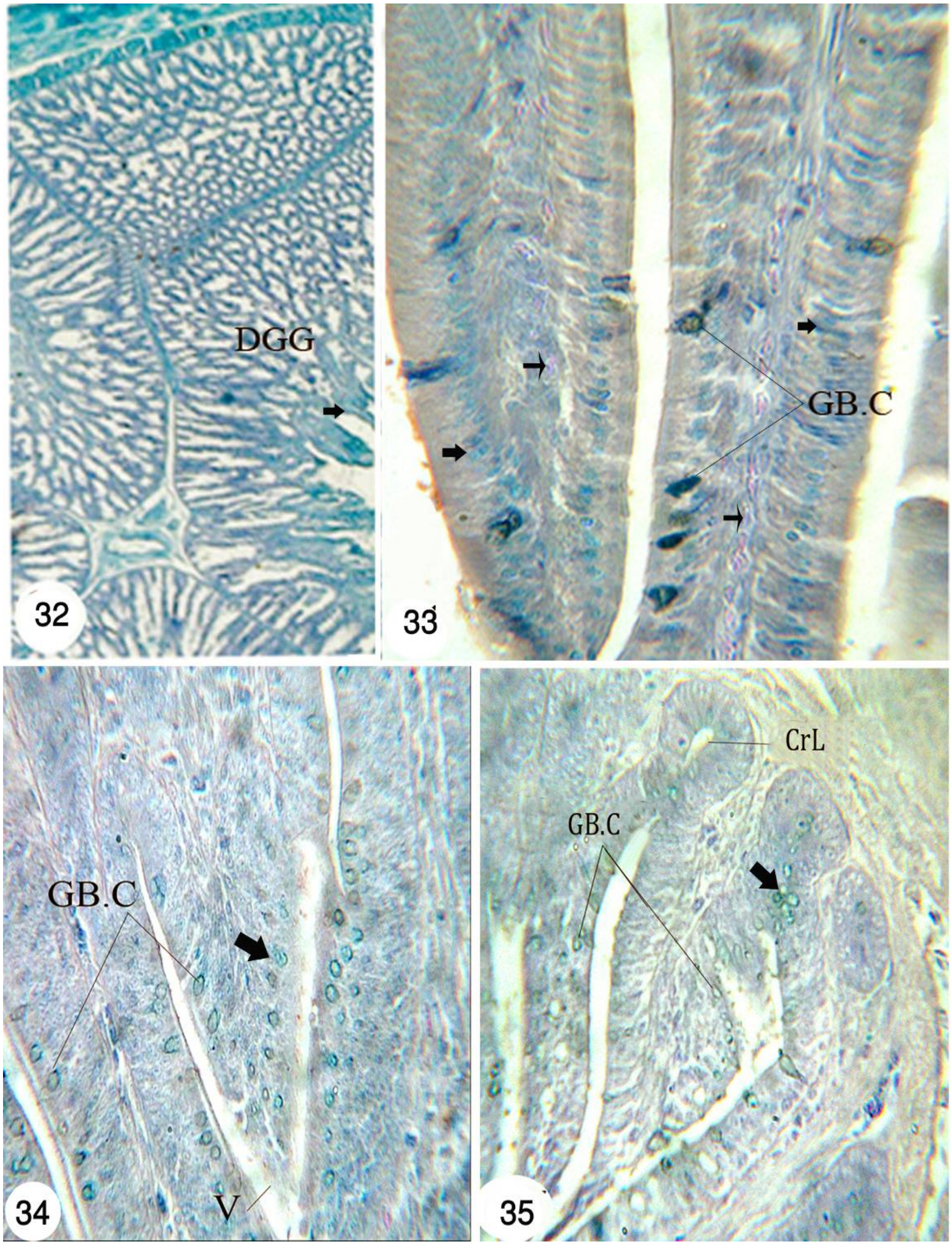


Fig. (32): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix* indicating the DNA content (small arrow) (Methyl green-pyronin stain) X560.

Fig. (33): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix* indicating the DNA (small arrow) and RNA content (large arrow) (Methyl green-pyronin stain) X560.

Fig. (34): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* indicating the DNA (small arrow) and RNA content (large arrow) (Methyl green-pyronin stain) X560.

Fig. (35): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix* indicating the DNA (small arrow) and RNA content (large arrow) (Methyl green-pyronin stain) X560.

Our results indicate that well-developed caeca also occur in omnivorous and some granivorous species. However, this is due to the inclusion in both categories of Galliform species from Leopold's data (1953) in which he designates quails, partridges, and pheasants as "seed"-eating species (granivorous) that also consume greens, fruits, and insects (omnivorous). Never the less, a common component of both diets is the insoluble carbohydrate, cellulose. Several studies have demonstrated that as the amount of cellulose in the diet increases, whether in natural or commercial diets, so do the lengths of the caeca (Lewin 1963; Moss 1972). If caecal length is an indicator of ingested cellulose, then species consuming the cell walls of higher plants would be expected to have well-developed caeca, and those species consuming nectar, fruits, and animal proteins would be expected to have less caecal development because these foods are easily digested by endogenous lipases, proteases, and carbohydrases Duke (1986).

The caeca and rectum may provide area for use symbiotic bacteria to aid in the digestion of fibrous components of food. This agrees with Klasing (1999).

The liver of *Coturnix coturnix* is bilobed organ. It is composed of two lobes right and left. It contains the gall bladder which is a spherical greenish sac. These results are in agreement with those observed by Leake (1975), Klasing (1999) and Abo-Shaeir (2001).In the present work, the quail pancreas is composed of 4 lobes (dorsal, ventral and splenic lobes and a third lobe) as described in chicken Rawdon (1998), in geese Gulmez (2003) and in quail (Baumel *et al*., 1993), the pancreas fills the gap between the duodenal limbs, while in the duck and goose, the pancreas is too short to reach the end of these limbs (Getty, 1975; Nickel *et al*., 1977). The shortest pancreas is found in bustards, which consist of two lobes (Bailey *et al*., 1997). According to suggestions by Gussekloo (2006) on chicken and other birds that feed on grains and seed, they need more enzymatic activity to compensate for their lack of teeth and hydrolytic enzymes in their saliva. Our data agreed with Nickel *et al*. (1977) who reported that there are three efferent pancreatic ducts in the fowl and pigeon, two of which arise from the ventral and one from the dorsal lobe. These ducts enter the proximal loop of the ascending duodenum, there are only two ducts in goose (Gulmez, 2003) and duck, in addition to the first pancreatic duct from the dorsal pancreatic lobe, which enters the duodenum loop between its descending and ascending limb (Liu *et al*. 1998).

The histological studies of the present investigation revealed that, the alimentary canal wall of *Coturnix coturnix*is differentiated into the same basic four layers of other birds. These layers are; serosa, musculosa, submucosa, and mucosa. Similar findings were achieved by Chikilian & De Speroni (1996) and Abou-Dief & El-Akkad (1999). Histologically, the wall of the oesophagus in *Coturnix coturnix* is formed from the same layers; serosa, musculosa, submucosa, and mucosa. This result agrees with that mentioned by Mclelland (1979).The serosa is a thin layer formed of simple squamous epithelium. It is present only in the lower part of the oesophagus while a fibrous layer surrounds the upper part. The musculosa consists of two distinct muscle layers; circular and longitudinal. This result agrees with that mentioned by Salem (1984). Also, Klasing (1999) added that the peristaltic contraction of inner circular and outer longitudinal muscles propels food posteriorly through the oesophagus. The mucosal folds are lined by stratified squamous epithelium interrupted by the ducts of the mucosal glands. This observation is similar to that of the fowl Bradley (1915) and Calhoun(1933) and in *Tyto alba* (Ismail,2000). In *Coturnix coturnix*, mucosa contains oesophageal glands which are tubulo-alveolar type. Similar observations were reported by Abd El- Aziz (1984), Salem (1984), El-Bahrawy *et al.,* (1989), El-Banhawy *et al.,* (1993b) and El-Sayyad (1995) and the presence of these mucous glands could be considered as another kind of oesophageal adaptation with the nature of food items.

The secretion of the crop mucous glands may serve to moisten seeds for a certain degree which allows the bird to avoid the unpleasant feeling of swallowing rough and dry seeds (Leznicka,1971). Similarly, the mucosal glands of the lower oesophagus may serve to lubricate the moisten food reducing its tackiness ability which may cause difficulty in the process of swallowing. In agreement with Selvan *et al*.(2008), the current study showed that the wall organization of the proventriculus is according to the general pattern that specified most of the digestive organs; tunica mucosa, tunica submucosa, tunica muscularosa and tunica serosa. In agreement with Okamoto & Yamada(1981),Prasad & Kakade (1990),Imai *et al*. (1991) and Liman *et al*. (2010) the current study showed that the proventricular folded mucosal surface is not smooth but it is covered by several projections or papillae. The proventricular glands which form the most thickness of the proventricular wall open at the apex of this papilla by ducts elaborating pepsinogen, hydrochloric acid and mucus discharge into the stomach lumen. In agreement with Langlois (2003) and Rahman *et al*.(2003), the current study showed that the surface lining epithelium of the proventriculus is of a simple columnar type however the glandular epithelium is formed by only one principle exocrine cells of a simple cuboidal type; oxyntico-peptic cells.

The occurrence of two types of gastric glands in proventriculus of the bird under investigation was previously reported by Calhoun (1933) in *Gallus domesticus* and Langlois (2003). The glands located between the inner and outer layers of the muscularis mucosa. The location of the proventricular glands is a matter of controversy between the authors. Some authors reported that the proventricular glands present in the lamina propria of duck proventriculus Calhoun (1954) in similar to our results, however many studies suggested the glands in other species to be submucosal Farner (1960) contradicting our results. These differences in the location of the glands may due to species variation but may be also due to developmental stages differences. In agreement with Prasad and Kakade (1990) in duck and Rahman *et al*. (2003) in chickens, the current study showed that lymphocytes and lymphatic nodules were seen in the lamina propria of the quail proventricular surface mucosa. That is likely, indicates a sort of participation of the proventriculus in the quail immune response. In our results, the musculosa was represented by a thick inner circular layer and a thin external longitudinal layer of smooth muscle fibres. These data are in agreement with those of Hodges (1974) but differ from the results of Catroxo *et al*. (1997) and Ogunkoya & Cook (2009), who described the musculosa of the proventriculus in three layers, viz. an inner longitudinal, a middle circular and an external longitudinal layer of smooth muscles. In contrast to our findings, however, Rossi *et al*., (2005) mentioned only two layers, i.e. the inner longitudinal and outer circular layers. In agreement to other reports, the current study showed that the ventriculus wall in *Coturnix coturnix* made by mucosa, submucosa, muscularosa and serosa (Gabella ,1985 and Bailey *et al*., 1997).The cuticagastrica in *Coturnix coturnix* is injunction with stones taken into the ventriculus with food forms an effective surface for hard food. These findings agree with Leake (1975) who added that the cuticagastrica produced by the gastric tubular glands. The absence of lamina muscularis mucosae of ventriculus in this study is in agreement with the report of Catroxo *et al*. (1997) but does not support the findings of Rocha and De Lima (1998). The observation of the submucosa beneath the ventricular glands in the current study does not agree with the report of Chikilian and De Speroni (1996), Catroxo *et al*. (1997), and Rocha and De Lima (1998). The observation that the tunica musculosa is made of two layers coincides with the report of Gabella (1985), Catroxo *et al*. (1997) and Rocha and De Lima (1998). And contrasts with the report of Hodges (1974) who observed three layers with different directions of muscle fibres.

The histological studies showed that, the ileal wall is built up of the same layers; serosa, musculosa, submucosa, muscularis mucosa, and mucosa. The serosa is the outermost layer made up of flattened simple squamous epithelium. The musculosa consists of two layers of muscle fibres; outer longitudinal and inner circular muscle layers. This finding is accordance with that of Abd El-Aziz (1984) in *ardeola ibis ibis*. The submucosa is a thin loose connective tissue containing a number of blood capillaries. The muscularis mucosa is represented by a narrow part of longitudinally arranged smooth muscle fibers. The mucosa is in vaginated at the bases of the villi into straight tubular glands (crypts of Leiberkühn) which are continuous with the columnar epithelium linning the villi. The same condition has been found in *Strothio* Bezuidenhout& Van Aswegen (1990) in *Larusridi bundus&Strepto peliasen egalensis* El-Banhawy *et al.* (1993a) and in *Cattle egret* Abou-Dief & El-Akkad (1999).

Also, goblet cells in the ileum which have slender bases containing elongated nuclei secrete mucous that protect the intestinal epithelium**.** Leznicka (1971) mentioned that these cells (goblet cells) are greatly correlated with the consistency of the bird's food items.

The mucosa of the rectum is thrown up into numerous leaf-like villi, all covered by simple columnar epithelium containing goblet cells. The goblet cells are numerous in number and open into the lumen. This agrees with Abd El-Aziz (1984). Rectal glands are noticed at the base of the mucosal folds. This observation was recorded by Abo-Shaeir (2001) in *Tyto alba*.

The histochemical study revealed the existence of a high amount of mucopolysaccharides in the oesophageal glands. These results agree with El-Bahrawy *et al.* (1989) and El- Banhawy*et al.* (1993a). Moreover, Leznicka (1971) reported that food composed of green plants or food of high content of starch had a stimulating effect on increasing the number of the oesophageal mucous glands and consequently the amount of secreted substances. On the other hand, Leznicka (1971) added that the size of the oesophageal mucous glands also, changed depending on the kind of food and the amount of water in it. The proventriculus mucosa shows folds lined by simple columnar cells containing PAS and Alcian blue positive mucin granules (neutral and acid mucin, respectively) as reported by Hodges (1974) in domestic fowl. The presence of neutral and acid mucin acts as a barrier to protect the proventricular mucosal surface Mogil'naia *et al*. (1978). The secretory cells, oxyntico-peptic cells are separated from each other by narrow spaces giving the cells a serrated appearance, due to the presence of cell junctions at the lower lateral portions of the cells but not at the upper ones. The cells stained negatively for PAS and Alcian blue indicating that these cells may not be of a mucous secretory function, but certainly secrete HCL and pepsin analogues to that of mammalian stomach secretion Hodges (1974). The gizzard mucosa is covered by a thick layer of koilin membrane which is formed of proteinous material similar to keratin and stained positive for PAS and Alcian blue indicating the presence of neutral and acidic mucin within its contents; in agreement with reports of other authors (Selvan *et al*., 2008). The goblet cells and the crypts of Leiberkühn have acid and neutral mucopolysaccharide secretions and the lamina propria of the intestine contains proteins, these findings are in agreement with El-Banhawy *et al*.(1993 a) and El-Sayyad (1995).

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