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Histological Studies on the Alimentary Tract of the Colubrid Snake *Coluber florulentus* (Family Colubridae)

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ABSTRACT. The histological structure of the alimentary tract of the snake *Coluber florulentus* was described and compared with that of other examined reptiles. The wall of the oesophagus, stomach, small intestine and large intestine is built up of the following layers from outside inwards; serosa, muscularis, submucosa and mucosa, while in the wall of the stomach there is a new layer, subserosa which follows serosa.

The oesophageal mucosa consists of simple columnar ciliated epithelial and goblet cells. The oesophageal glands were found to be completely absent. The gastric mucosa consists of simple columnar cells. There are two types of gastric glands. These are the fundic and pyloric glands (granular and light-celled types). The mucosa of the small intestine consists of three types of cells; the absorptive, the goblet and the endocrine cells. The intestinal glands as well as crypts of Lieberkhun are absent. The caecal mucosa consists of simple columnar epithelial and goblet cells. The mucosal epithelium of the colon consists of three types of cells; simple columnar, goblet and endocrine cells. The rectal mucosa is only represented by simple columnar epithelial cells.

Introduction

A review of the histological studies on the alimentary tract of the members of the order Squamata revealed that most of these studies were dealt with the members of the suborder Lacertilia[1-11]. On the other hand, a little attention has been paid to the members of the suborder Ophidia. Moreover, most of the available works on the latter suborder were dealt with certain parts of the tract[12-21]. However, the histological studies of the different regions of the Ophidian tract, in general, were carried out, only, by a few number of workers[22,23&9]. This indicated that the present subject did not receive the necessary attention. This stimulated the present authors to carry out some work in this field on some members of order Ophidia.

The present article is the first in the series. It deals with the general histology of the different regions of the alimentary tract of the colubrid snake *Coluber florulentus*.

Material and Methods

The animal used in this work is the colubrid snake *C. florulentus* (Family Colubridae). It was caught by hand from different localities in Arafat region, 20 km from Makkah, Saudi Arabia.

Animals were dissected and the different regions of the alimentary canal; oesophagus, stomach, small and large intestine were fixed in Bouin's fluid and were subsequently processed for sectioning. Section 8 μ-thick were stained with haematoxylin and eosin as well as with Mallory's triple stain.

Observations

Oesophagus

Microscopic examination of the oesophagus showed that its wall consists of serosa, muscularis, submucosa and mucosa (Fig. 1).

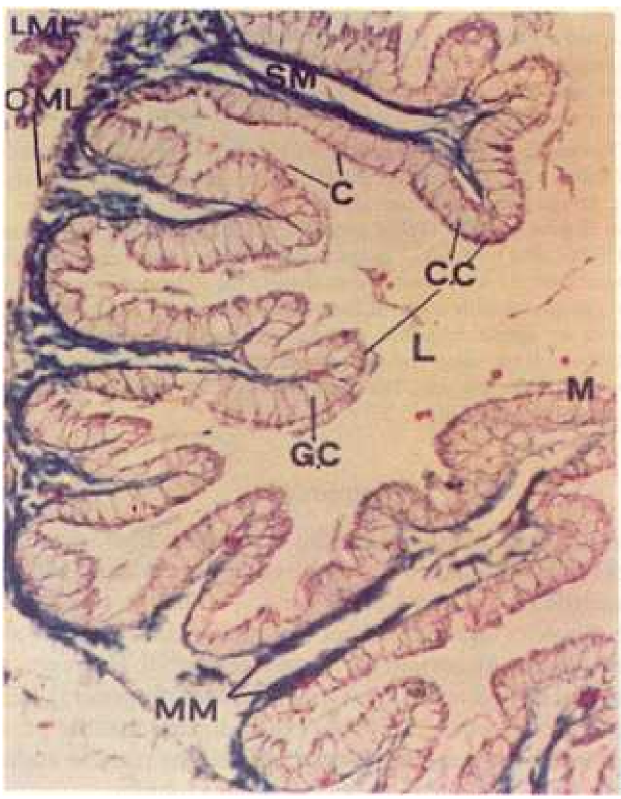


FIG. 1. T.S. in the oesophagus of the snake *C. florulentus*. × 82.5

The serosa is represented by a thin layer of squamous epithelium which may be ruptured during the preparation of the sections. The muscularis has an outer layer of longitudinal smooth muscle fibres and an inner one of smooth circular muscle fibres. It is uniform in thickness through the total length of the oesophageal region. The submucosa is well developed throughout the whole length of the oesophagus and extends into folds. The muscularis mucosa is weakly developed and is represented by a thin continuous striated layer of an outer longitudinal and an inner circular muscle fibres.

The oesophageal mucosa is thrown into many folds with variable lengths and wavy appearance. They surround a relatively wide lumen. The number and distribution of the folds is nearly constant through the total length of the oesophagus. The oesophageal mucosa comprises the lamina propria of connective tissue, and the epithelium which lines the lumen. The former propria extends in the well developed oesophageal folds.

The mucosal epithelium is simple and formed of two types of cells; the elongated thin ciliated epithelial cells and large goblet cells (Fig. 2). The goblet cells are mar-

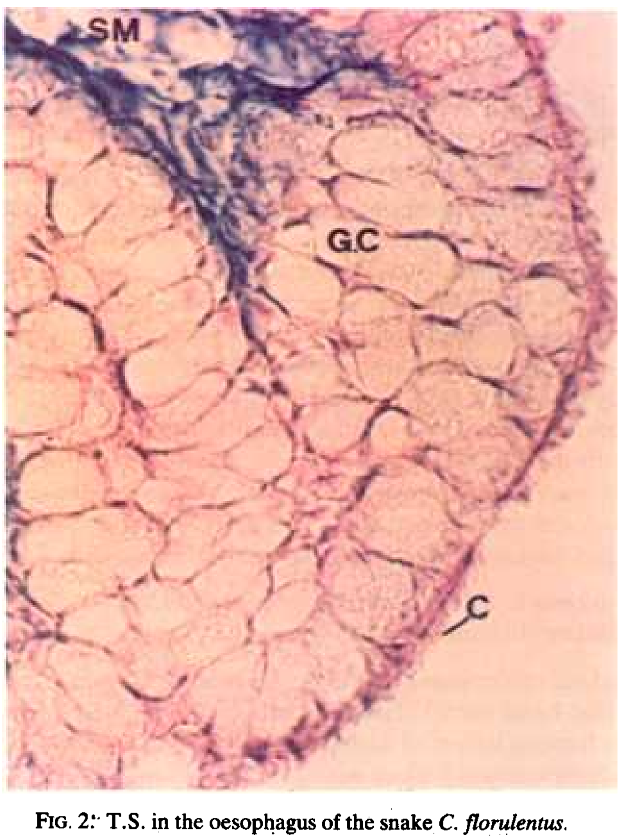


FIG. 2: T.S. in the oesophagus of the snake *C. florulentus.*
× 330

kedly numerous at the bottom of the oesophageal folds. They could not be considered as glands because they are identical to the surface epithelial cells. The mucous cells are large and club-shaped. Each consists of an inner small basal part and an outer large hyaline portion. The basal part contains a flat nucleus which is intensely stained and is embedded into the cytoplasm (Fig. 2). The outer large hyaline portion is stained faint blue with Mallory stain. This means that the mucin occupies most of the mucous cells. The openings of the mucous cells in the oesophageal lumen are wide and sometimes mucous plugs were observed outside the cells.

In the present study, the ciliated epithelial cells of the oesophageal mucosa are found scattered between the numerous goblet cells (Fig. 2). They are relatively fewer in number as compared with those of the latter cells. Their cilia are found arranged along the outer border of the mucosal epithelium. Each columnar cell is provided with an oval nucleus rests near its base. It is stained dark red with the Mallory stain. The cytoplasm is provided with fine granules which are also stained dark red with the same stain.

Microscopic examination of the oesophagus showed that its mucosa is totally devoid of glands.

Stomach

The stomach of *C. florulentus* can be divided into an anterior fundic portion following the oesophagus and a posterior pyloric portion which ends with the pylorus and leads to the duodenum, (Fig. 3,4,5 & 6).

The serosa of the stomach consists of a thin layer of spindle-shaped simple aquamous epithelial cells (Fig. 3). A layer of subserosa is present with scattered longitudinal muscle fibres. It has a uniform thickness through the whole length of the stomach (Fig. 5). The muscularies is composed essentially of smooth circular muscle fibres. It is more or less continuous, thick in the anterior region and increases in thickness towards the middle region and reaches its maximum thickness in the posterior region where it is highly developed (Fig. 5). The submucosa is well developed in both anterior and posterior regions of the stomach (fundic and pyloric portions), (Fig. 4 & 6). The muscularis mucosa is composed of two smooth continuous layers; an outer longitudinal and inner circular muscle layers. The latter is relatively thin while the longitudinal one is more developed and forms a thicker layer. Such a pattern is continuous in both pyloric and cardiac portions of the stomach (Fig. 4 & 6).

The gastric mucosa is thrown into longitudinal folds of varying height and is formed of mucosal epithelium, lamina propria and gastric glands.

The gastric surface epithelium is of the simple columnar type. Its cells are cylindrical with ovoid and basal nuclei. Each nucleus contains one or two nucleoli. The cytoplasm of the luminal halves of such cells is filled with dark red stained granules. Their inner portions contain a clear non-granular cytoplasm (Fig. 4). The lamina propria is represented by the connective tissue found between the muscularis mucosa and the mucosal epithelium. It extends into the gastric folds. In the lamina propria,

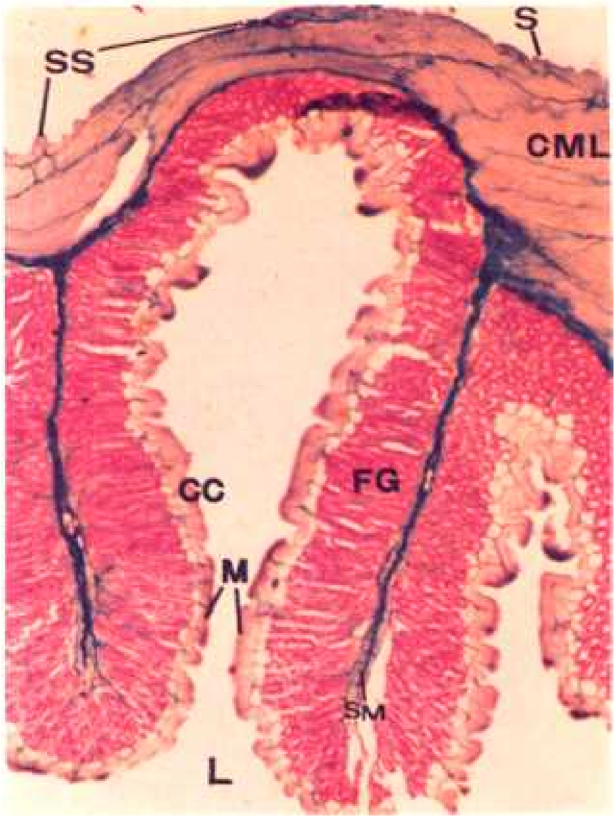


FIG. 3. T.S. in the fundic portion of the stomach.
× 82.5

bodies of the gastric glands are embedded where they open into the lumen between the gastric folds through the gastric pits (Fig. 4). The gastric glands are numerous in the anterior region of the stomach (fundic region) and comparatively fewer in the posterior region (pyloric region), (Fig. 4 & 6).

Microscopic examination of the stomach revealed the presence of two types of gastric glands; the granular type and the light-celled one. The fundic region contains both types of glands (Fig. 4), while the pyloric region contains only the light-celled one (Fig. 6). In the fundic region, the light-celled glands were found to be arranged along the periphery of the lamina propria, while those of the granular type are found scattered in the areolar connective tissue of the lamina propria.

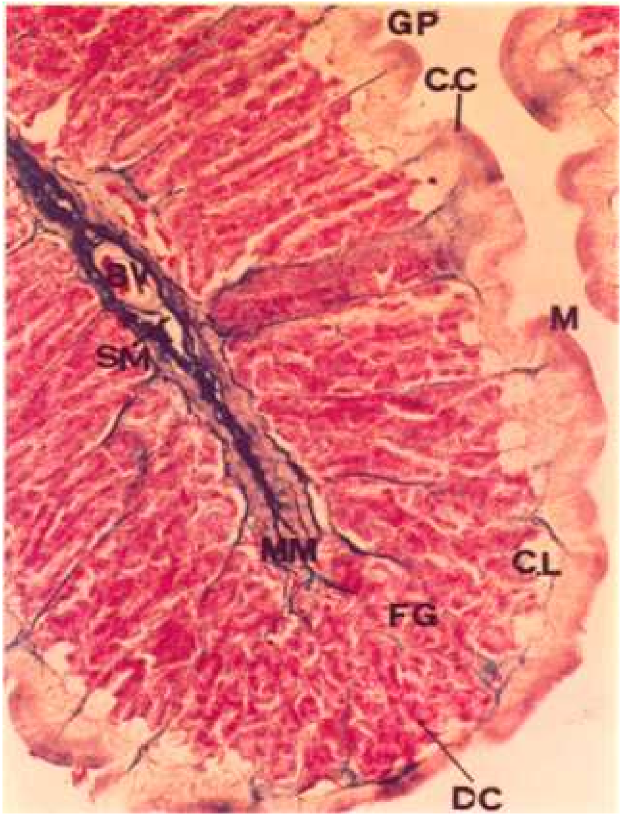


FIG. 4. T.S. in the fundic portion of the stomach
× 330

The granular glands are mainly of simple tubular type. They open by their pits into the gastric lumen. Their bodies consist of dome-shaped granular cells. They are full of dark granules and have central round nuclei. They stain violet with Mallory (Fig. 4). The light-celled glands, on the other hand, were found to be of the simple tubular or simple branched tubular type. Their bodies consist also of dome-shaped cells. The cytoplasm is pale and contains indistinct granules. The nucleus is round or flat and is scattered against the base. The cytoplasm of these cells is stained faint blue with Mallory and red with eosin-haematoxylin. This means that they show positive reaction with mucous stain (Fig. 6).

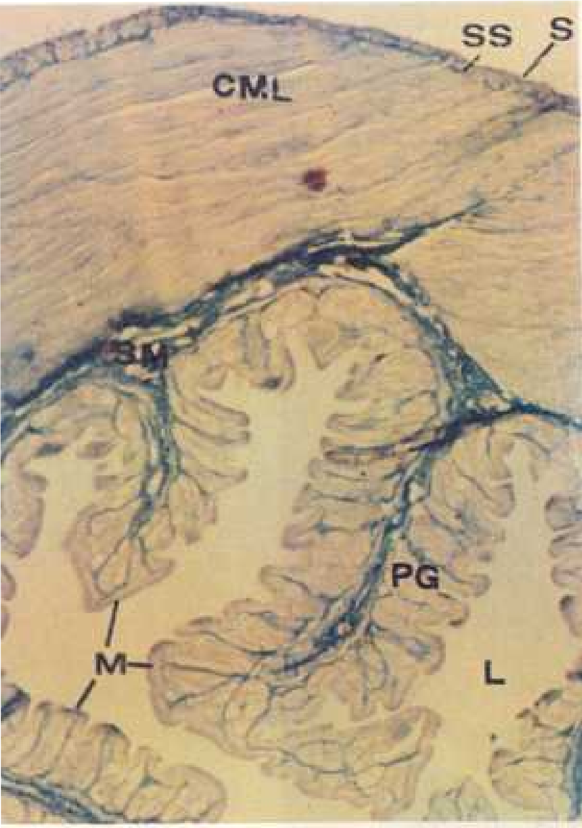
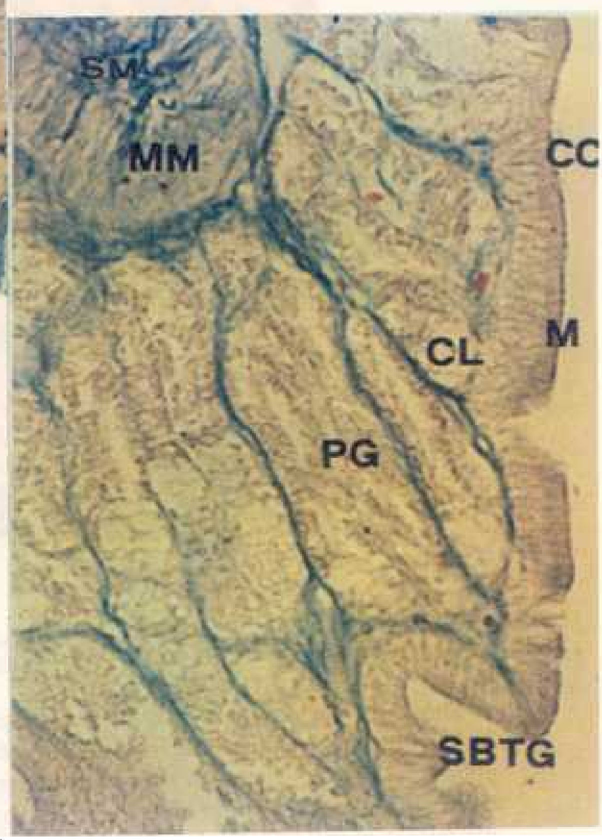


FIG. 5. T.S. in the pyloric portion of the stomach.
× 82.5



in the pyloric portion of the stomach

Small Intestine

The lumen of the small intestine is more or less narrow due to the presence of extremely long and coiled villi (Fig. 7).

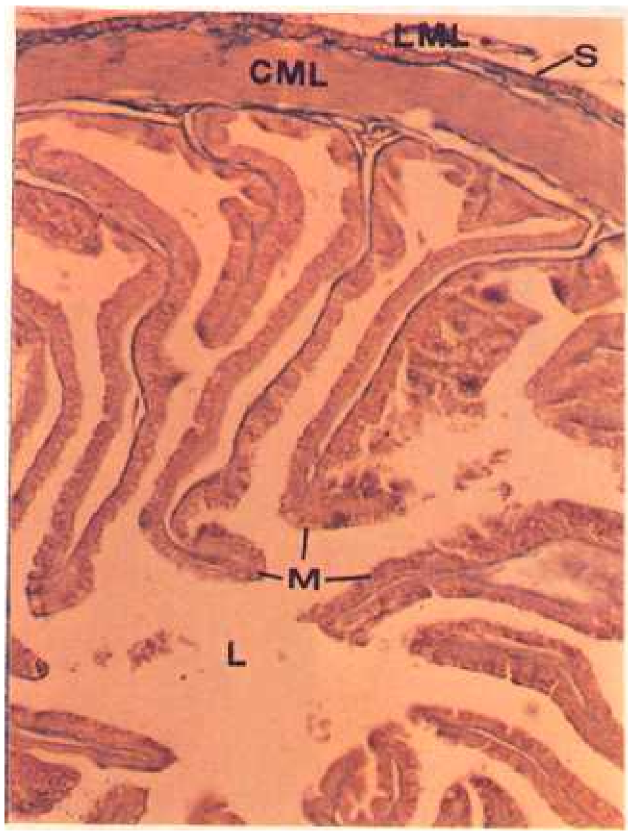


FIG. 7. T.S. in the ileum.
× 82.5

The serosa is composed of simple squamous epithelial cells. It represents the outer-most layer of the small intestine (the duodenum and ileum), (Fig. 7). The muscularis, which is formed of smooth muscles, is arranged into an outer longitudinal layer and an inner circular layer (Fig. 7). The submucosa is vascular and enters in the formation of the intestinal villi, whereas the muscularis mucosa is totally absent.

In the duodenum and ileum, the mucosal lining of the intestinal villi is uniform throughout. It is composed of three types of cells; simple columnar epithelial cells, goblet cells and endocrine cells (Fig. 8). The columnar cells have large elongated nuclei situated at the base of the cells. The coarsely granular cytoplasm is deeply stained with Mallory. The goblet cells are fewer in number as compared with the columnar ones. They are provided with a short basal part with a faint nucleus, and an upper flask shaped mucous part that has a wide opening into the lumen (Fig. 8). The endocrine cells are small in size, limited in number and have a clear cytoplasm in which spherical and central nuclei were located (Fig. 8).

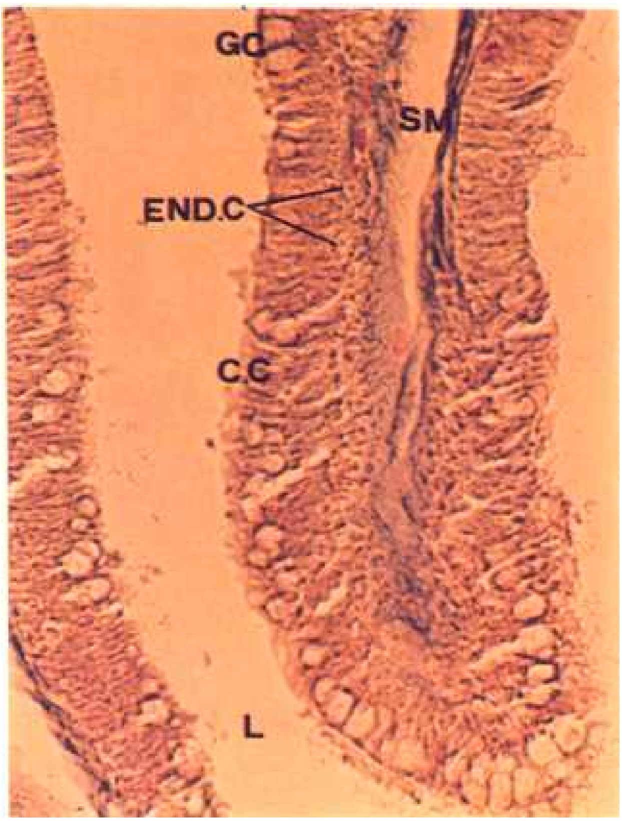


FIG. 8. T.S. in the ileum.
× 330

The intestinal glands as well as the crypts are totally absent from the submucosa of the duodenum as well as of the ileum.

Large Intestine

The large intestine of *C. florulentus* is in the form of a relatively short straight muscular tube extending from the end of the ileum till the cloaca. The large intestine is of three parts; the caecum, the colon and the rectum (Fig. 9 to 13).

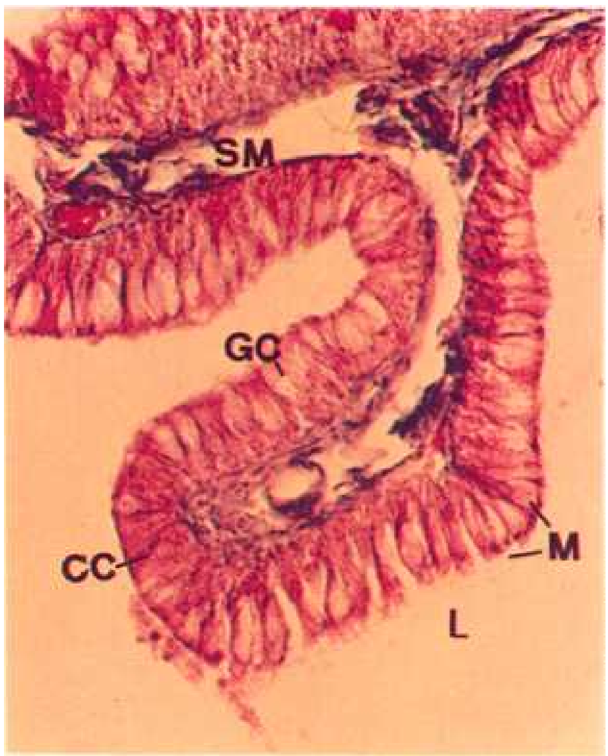


FIG. 9. T.S. in the caecum.
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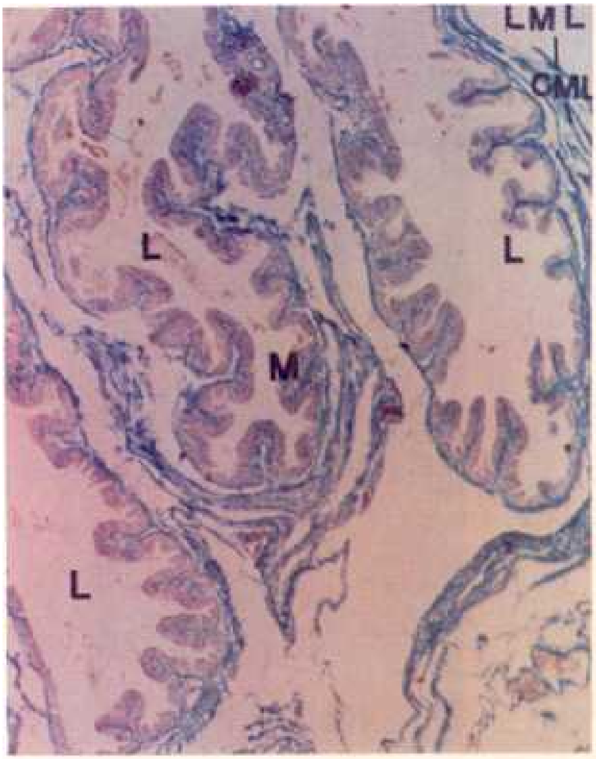

FIG. 10. T.S. in the colon.
× 82.5

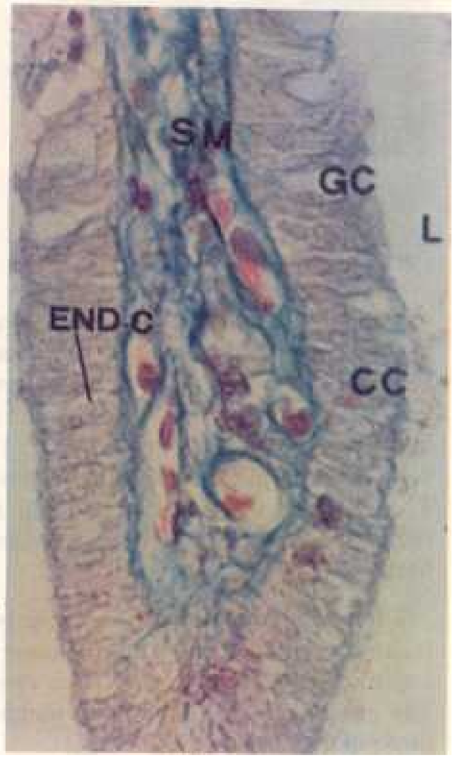

FIG. 11. T.S. in the colon
× 330

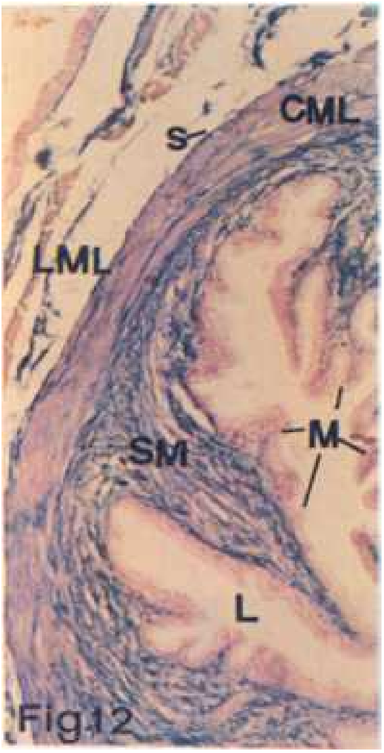

FIG. 12. T.S. in the rectum.
× 82.5

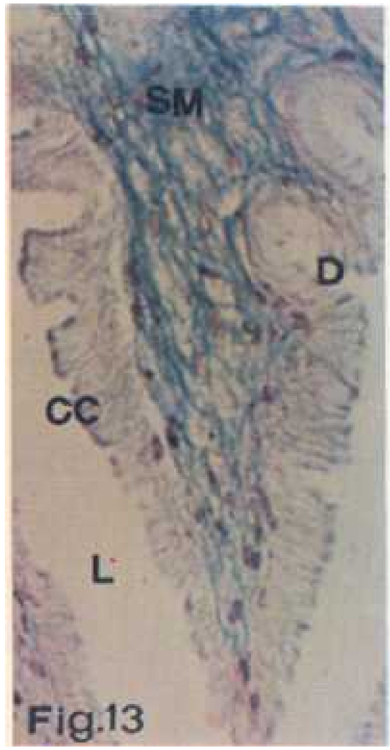

FIG. 13. T.S. in the rectum.
× 330

The smooth muscles of the muscularis are in the form of an outer longitudinal muscle layer and inner broader circular one (Fig. 12). A thick well-developed submucosa follows muscularis and enter in the formation of the high folds of the mucosa (Fig. 9). The caecal mucosa consists of simple columnar cells as well as the goblet cells. The latter cells are present in abundant. Their apical part forms most of the body of the cell. Their basal part is short and contains a faint basal nucleus with an oval outline. The columnar cells are thin and tall. They have spindle-shaped nuclei situated near the middle of the cells. Their cytoplasm is coarsely granular and deeply stained with Mallory stain (Fig. 9).

The epithelium of the colon consists of three kinds of cells; simple columnar, goblet and endocrine cells. The columnar cells are present in a large number. They have a spherical and basal nuclei. Their cytoplasm is heavily granular and deeply stained. The goblet cells have a limited number and have the same shape and structure of those of the caecum (Fig. 11).

The rectal mucosa, which is provided with several depressions, consists only of simple columnar epithelial cells, *i.e.* the goblet cells are completely absent. Here, the columnar cells contain heavily granular and deeply stained cytoplasm. Their oval basal nuclei were difficult to identify due to the heavy granularity of the cytoplasm (Fig. 13).

Discussion

Microscopic examination of the oesophageal mucosa of several investigated species of reptiles showed a considerable variation in their histological structure. The present study revealed that the oesophageal mucosa of the colubrid snake *C. florulentus* is represented by simple columnar ciliated and goblet cells. Such a structure is similar to the observations obtained in *Mauremys caspica*[24], *Cnalcides levitoni*[25] and *Acanthodactylus boskianus*[10]. However, in *Uromastyx aegyptia*[3], *Chameleon vulgaris*[4] and in *Uromastyx philbyi*[26] only the anterior region of the oesophageal mucosa assumes identical to the present observation, while the posterior region was found to consist of a layer of ciliated columnar and goblet cells. This latter layer is followed by two or three layers of replacing cells.

In *Lacerta agilis,* the oesophageal mucosa is only represented by goblet cells[9]. Moreover, in the snake *Natrix natrix,* and *Vipera berus*[9], the ciliated cylindrical cells occurring in the anterior portion of the oesophagus are replaced posteriorly by more cubical cells devoid of cilia. In many snakes' species, the posterior portion of the oesophagus is devoid of ciliated cells[9,27-31].

The presence of oesophageal glands in reptiles, in general, was a matter of great dispute between several authors. The present study revealed the absence of such glands as in many previous examined reptiles, *e.g.* in Alligators[32], *Ablephorius pannonicus*[1], *Scincus officinalis*[2], *Typhlops vermicularis*[23], *Agama stellio*[6], the colubrid snake *N. natrix* and *V. berus*[9], *C. levitoni*[25], *M. caspica*[24], *A. boskianus*[10] and *Pristurus rupestris*[11]. The presence of oesophageal glands have been observed into *Uromastyx acanthinurus*[33], *U. aegyptia*[3], *C. vulgaris*[4], *Chrysemus picta*[34] and *Diplometopon zarudnyi*[35].

A review to the published works on the alimentary tract of reptiles shows a considerable controversy on the nature of the granular cells forming the glandular bodies of the fundic glands of the reptilian stomach. Thus, many authors[36-38] reported that these cells play a role in the secretion of pepsin and hydrochloric acid and they termed them as oxyntico-peptic cells. However, the same cells were termed as oxyntic cells in the herbivorous *U. aegyptia*[3] and in the insectivorous *C. vulgaris*[4], whereas in *Varanus griseus*[39], which is purely carnivorous, and *Mabuya quinquetaeniata*[5], which is purely insectivorous, they were termed as peptic cells.

In the present work, granular and light-celled glands were observed in the stomach. The granules of the first type were found to react in the same way as do oxyntic cells, since they stain violet with Mallory. This proves that these glands are oxyntic. On the other hand, the cells of the light-celled glands show a positive reaction with mucus stains, since they stain faint blue with Mallory (analine blue).

On the other hand, the cells of the light-celled glands show a positive reaction with mucus stains, since they stain faint blue with Mallory (aniline blue). This means that these glands may be mucus secreting in function. Thus, it can be concluded that the gastric glands of the present snake are either oxyntic glands (granular type) or mucus secreting glands (light-celled type). This is identical to what was observed into the stomachs of the snakes *Casarca dussumieri*[20], *Natrix naura, Typhlops braminus* and *Viper aspis*[40].

In the present study, the mucosal epithelium of the small intestine contains endocrine cells. Such cells were observed into the snakes *T. vermicularis*[23], *N. natrix, V. berus*[9], *U. phylbyi*[26], *A. boskianus*[10] and *P. rupestris*[11]. However, the endocrine cells were not recorded into the small intestine of other investigated reptiles such as *U. aegyptia*[3], *V. griseus*[39], *C. vulgaris*[4] and *M. quinquetaentiata*[5].

The absence of intestinal glands or the crypts of Lieberkhun in the small intestine was noticed in the present work. Similar data has been observed into *M. quinquetaentiata*[5], *A. boskianus*[10], *V. aspis*[22], *N. natrix* and *V. berus*[9]. It is to be mentioned that the Lieberkhun crypts was found to be present in some other reptilian species such as *U. aegyptia*[3], the amphisbaenian *Diplometopon zarudnyi*[35] and *P. rupestris*[11].

The present study revealed the absence of Paneth cells. This is confirmed by the provided observations recorded in *A. stellio*[6], *M. quinquetaentiata*[5], *P. rupestris*[11], *N. natrix.* and *V. berus*[9].

Microscopic examination of the intestinal mucosa indicated the presence of extremely long and coiled villi to compensate for the shortness of the small intestine. Such histological feature may allow efficient absorption to take place.

Examination of the mucosa of the large intestine proved that it is free from the glandular crypts, while the rectal mucosa was found to be provided with several depressions. Such a feature may be an adaptation for increasing the surface for water absorption.

Finally, we have to refer here to a histological feature which is apparently characteristic to the gut of the colubride snake *C. florulentus,* represented by the predominance of mucus cells in the mucosal epithelium. Such foundation in the oesophagus may agree with its basic task which conveys food from the buccal cavity to the stomach, while the presence of a large number of goblet cells in the large intestine may be an adaptation for water absorption. Moreover, the presence of mucus in the colon is normally interpreted as a lubricant to facilitate the passage of faeces.

In conclusion, it seems that the histology of the alimentary tract in *C. florulentus* shows certain specific features as a reflection of the mode of life of the animal. Further physiological as well as histochemical studies seem to reveal certain points of significance which are worthy of study.

Acknowledgement

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List of Abbreviations

B.V.	= Blood Vessel
C.	= Cilia
C.C.	= Columnar Cell
C.M.L.	= Circular Muscle Layer
Cl.C.	= Clear Cell
D.	= Depression
D.C.	= Dark Cell
END. C.	= Endocrine Cell
F.	= Fold
F.G.	= Fundic Gland
G.C.	= Goblet Cell
G.P.	= Gastric Pit

GR.G.	=	Granular Gland
L.	=	Lumen
L.C.G.	=	Light-Celled Gland
L.M.L.	=	Longitudinal Muscle Layer
LY.	=	Lymphocytes
M.	=	Mucosa
M.M.	=	Mascularis Mucosa
P.G.	=	Pyloric Gland
S.	=	Serosa
S.B.T.G.	=	Simple Branched Tubular Gland
S.M.	=	Submucosa
S.S.	=	Sub-serosa
S.T.G.	=	Simple Tubular Gland
VI	=	Villus

دراسات هستولوجية على القناة الهضمية لثعبان الأزرود (عائلة الثعابين العديمة الأنياب)

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قام الباحثان بدراسة التركيب النسيجي للقناة الهضمية لثعبان عديم الأنياب وهو الأزرود . وقد قورنت نتائج هذه الدراسة بما سبقها من نتائج في مجال الدراسات الهستولوجية (النسيجية) للقناة الهضمية لمختلف الزواحف المدروسة من قبل . هذا وقد سجلت في هذا البحث الصفات الهستولوجية العامة للأجزاء المختلفة للقناة الهضمية لعائلة الثعابين عديمة الأنياب . لوحظ أن جدار القناة الهضمية في المناطق المختلفة يتكون من الخارج إلى الداخل من المصلية ، العضلية ، تحت المخاطية ، والمخاطية . . ذلك إلى جانب الطبقة تحت المصلية التي وجدت في المعدة ، وتلي الطبقة المصلية .

تتكون مخاطية المرىء في ثعبان الأزرود من خلايا عمادية مهدبة بسيطة وخلايا كأسية ، ولا توجد غدد في المرىء على الإطلاق . تتكون المخاطية المعدية من خلايا عمادية بسيطة ، وتحتوى المعدة على نوعين من الغدد هما الفؤادية والبوابية ، تتكون مخاطية الأمعاء الدقيقة من ثلاثة أنواع من الخلايا : الخلايا الامتصاصية ، الخلايا الغدية ذات الإفراز الداخلي والخلايا الكأسية . لم يلاحظ وجود خباءات ليبركين أو الغدد المعوية . تتكون طلائية مخاطية الأعور من خلايا عمادية بسيطة وخلايا كأسية ، في حين تتكون مخاطية القولون من ثلاثة أنواع من الخلايا : خلايا عمادية بسيطة ، خلايا كأسية ، خلايا غدية ذات إفراز داخلي . أما مخاطية المستقيم فتتكون من خلايا طلائية عمادية بسيطة فقط .