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THE MICROSCOPIC STRUCTURE OF THE MUCOUS MEMBRANE AND GASTRIC GLANDS OF VERTEBRATES

BY

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ABSTRACT

The microscopic anatomy of the mucosal layer of the stomach is investigated in repr-esntatives of five vertebrate classes. The distribution of carbohydrates, mucin, total protein and histones are described. The pres-ent investigation shows that in lower verte-brates (Fishes, Amphipia, Reptelia and birds) the same type of cells in the gastric gland secrete both pepsin and hydrochloric acid, where in mammals the cells of the gastric gland are differented into chief and parietal cells, secreting pepsin and hydrochloric acid respectively. Therefore it is concluded that the evolutionary changes of vertebrate classes follow certain pattern.

INTRODUCTION

The review of the previous works concerned with the histological and histochemical studies of the alimentary canal of vertebrates, shows that they mainly deal with the adaptation of the alimentary tract to the different feeding habits (Al-Hussaini, 1946, !948 and 1953 ; Kapoor, 1953; Amer and Ismail, 1975 and 1976; Salem, 1978 and Martin and Blaber, 1984) or different habitats (Anwar and Mahmoud, 1975; Young, 1962 and Andrew and Hickman, 1974). However, the microscopic anatomy of the alimentary canal throughout the different vertebrate classes on evolutionary basis has not been given much attention.

The histological structure of the alimentary canal of fishes has been a subject of study by many authors (Al-Hussaini, 1948 and 1953; Barrington, 1957; Moshin, 1962; Kapoor et al., 1975; Al-Zahaby et al., 1979 and Martin and Blaber, 1984). The alimentary canal of amphibians and reptiles have been microscopically studied by Reese (1913); Botha (1958); El-Toubi and Bishai (1958); Bishai (1960 and 1961); Norris (1960); Gab (1971); Andrew and Hickman (1974); Amer and Ismail (1975 and 1976); Anwar and Mahmoud (1975) and Dehlawy and Zaher (1985). The hostology of the alimentary canal of birds and mammals have been reported by many investigations (Bradley, 1915; Browne, 1922; Fakete, 1956; Al-Hussaini and Ghorab, 1958; Romanoff, 1960; Sisson, 1962; Toner, 1964; Swarup et al., 1971 and Madkour et al., 1982).

The present investigation is carried out on the microscopic anatomy of the mucosal layer of the stomach of repesentatives of the different vertebrate classes on the hope

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to find out any evolutionary significance of the mucosal structure of the stomach and a special interest is given to the gastric glands.

MATERIAL AND METHODS

Adult alive specimens of Tilapia nilotica, Bufo regularis, Chalcides ocellatus, Streptopelia decaocto and albino rat were collected from different localities in Tanta, 90 Km. from Cairo. The specimens were anaesthetised,rapidly dissected and various parts of the stomach were rapidly washed and fixed in Carnoy's fluid and 10% neutral formalin . The material was dehydrated cleared, embedded and transversely sectioned at 6 µ thickness. The sections were stained with haematoxylin and Eosin for rautine microscopic examintion, periodic acid Schiff's reagent (PAS) after Hotchkiss (1948), alcian blue after Steedman (1950), mercury bromophenol blue after Mazia et al. (1953) and akaline fast green after Alfert and Geschwind (1953) for histochemical detection of carbohydrates, mucin, total protein and histones respectively. FitzGerald's method (1910) was employed for the detection of hydrochloric acid in the stomach cells of birds.

Tilapia nilotica

The stomach of Tilapia nilotica is differentiated into a cardiac and a pyloric portions; the cardiac portion is extended posteriorly to form a blind extension, the caecum.

The mucosa of the middle and posterior parts of the cardiac portion, as well as that of the anterior and middle parts of the pyloric portion is markedly thickned due to the presence of enormous numbers of gastric glands. The mucosa is thrown into simple, deep and broad folds. The epithelial lining of the mucosal folds and the necks of the gastric glands, is made up of simple columnar mucus- secreting cells. These cells have clear acidophilic cytoplasm and oval basal nuclei; they react positively with alcian blue.

The gastric glands are of the simple or branched tubular type and are more abundant in the cardiac portion (Fig. 1). They open into the lumen of the stomach by gastric pits. They are composed of one type of secretory cells which give a positive reaction with PAS, alcian blue and akaline fast green (Fig. 2). There is no differentiation into peptic and oxyntic cells.

On the other hand, the mucosa of the anterior part of the cardiac stomach, and that of the posterior part of the pyloric stomach is devoid of gastric glands. It is thrown into numerous deep and narrow villi which are lined with simple tall columnar cells. Goblet cells are frequently scattered among the epithelial lining of the cardiac villi but are absent in the pyloric ones (Fig. 3). They react positively with PAS, alcian blue and alkaline fast green (Fig. 4).

The mucosal folds are supported by highly vascular lamina proprea and consists of a dense connective tissue that extends into the glandular region.

A muscularis mucosa is present, it consists of a thin layer of circularly disposed unstriated muscle fibres.

Bufo regularis

The stomach of the toad is formed of the fore-stomach, fundic and pyloric regions. The gastric mucosa is thrown into deep and broad folds. In the fore-stomach region, the mucosal epithelium is composed of simple ciliated columnar epithelium containing numerous golet cells. A few glands are present, these are made up of two types of cells; the neck mucous and zymogenic cells. The zymogenic cells react negatively with PAS and alcian blue, whereas they react positively with bromophenol blue and alkaline fast green.

In the fundic region (Fig. 5) the epithelial lining of the folds is composed of simple columnar epitheium; goblet cells are found. Numerous fundic glands are present; these are of the simple or branched tubular type. They are packed close to each other and open into the lumen of the stomach by gastric pits. The glands consist of two types of cells i.e. neck mucous and acidophilic cells. The mucous cells possess oval, basally located nuclei and a vacuolated apical cytoplasm that stains greenish colour with alcian blue red with PAS (Fig. 6). The acidophilic cells constitute the major part of the gland, these possess oval, basal nuclei and granular esinophilic cytoplasm which reacts negatively with alcian blue and PAS stains (Fig. 6),but show a weak reaction with the alkaline fast green.

In the pyloric region, a few number of pyloric glands are present (Fig. 7). Acidophilic cells are entirely absent and the pyloric glands are mostely formed of mucous cells.

A muscularis mucosa is present, it is represented by a relatively thick layer of an outer longitudinal and an inner circular muscle layers; it is much thicker in the fundic region.

Chalcides ocellatus

The stomach is differentiated into a fundic and a pyloric portions. The mucosa is comparatively thick due to the presence of a large number of gastric glands. The mucous membrane is thrown into longitudinal folds made up of simple columnar cells with granular acidophilic cytoplasm and oval basal nuclei. These cells are filled with clear mucus, which stains positively with alcian blue and PAS (fig. 8).

The gastric glands are of the simple and simple-branched tubular type. They open into the lumen of the stomach by gastric pits. These glands are of two types ; fundic and pyloric glands. The fundic glands are present in the fundic region (Fig. 9), these possess wide lumina and are lined with colmar cells. The cells possess eosinophilic granular cytoplasm and large spheroidal basal nuclei. These cells give positive reaction with alcian blue and PAS. They also stain positively with bromophenol blue and alkaline fast green.

The pyloric glands are fewer in number than the fundic gland(Fig.10).They are present in the anterior pyloric portion and decrease in number towards pylorus . The glands have narrow lumina and consist of tall columnar mucus-secreting cells with clear cytoplasm and oval basal nuclei; the react positively with alcian blue and PAS.

The muscularis mucosa is thin, it is represented in the fundic region by an outer longitudinal and an inner circular muscle layer, whereas, in the pyloric region it is represented only by a thin circular muscle layer.

<u>Streptopelea decaocto</u>

The stomach consists of the proventriculus and gizzard. the mucous membrane of the proventriculus is thrown into narrow and somewhat deep folds. The mucosal folds are lined with simple columnar epithelial cells with numerous scattered goblet cells in between (Fig. 11). They react positively with alcian blue and PAS stains (Fig. 12).

The tunica propria is thin, highly vascular consists of dense connective tissue that extends between the gastric glands.

The gastric glands are multilobular;each lobule consists of a compound branched tubular gland (Fig. 13). The lobules are made up of cuboidal cells whose luminal ends are not in contract with each other, thus giving a serrated appearance. Such cells have large round central nuclei and vacuolated cytoplasm that reacts positively with bromophenol blue and alkaline fast green , but negatively with the mucus stains (Fig. 12). The cells react negatively with FitzGerald's histochemical method (1910) indicating the absence of hydrochloric acid. The ducts of the glands are lined with short columnar cells with lightly stained vacuolated cytoplasm and small basal nuclei.

The muscularis mucosa is thin, it is composed of an inner circular and an outer longitudinal muscle layers.

The mucous membrane of the gizzard is thrown into narrow deep folds that possess a thick horny layer at their inner surfaces (Fig. 14). The horny layer consists of more or less wavy threads lying parallel to the surface of the lumen and are nothing but accumulative layers of the glandular secretions of the gizzared. It stains red with eosin, yellowish with Mallory's triple stain and reacts positively with mucus stains. The mucosal folds are lined with cuboidal epithelial cells possessing large, rounded nuclei and granular cytoplasm. Simple tubular glands are found at the bottom of the folds; these are lined with cuboidal cells with rounded nuclei and granular cytoplasm that reacts positively to mucus stains.

The lamina propria is represented by a thin, richly vascular, layer of an areolar connective tissue rich in collagenous fibres. The muscularis mucosa is absent

Albino rat

The stomach of the rat consists of a nonglandular and a glandular regions.

The mucosa of the nonglandular region is thin due to the absence of glands(Fig. 15). It is thrown into low and somewhat broad folds made up of wavy keratinized stratified squamous epithelium. The outer suface of the epithelium stains positively with alcian blue and PAS (Fig. 16), but reacts negatively with bromophenol blue (Fig. 17). A thin lamina propria is present but a muscularis mucosa is absent.

The glandular region of the stomach consists of three portions; cardiac, fundic and pyloric. Cardiac glands form a narrow, ring-shaped area around the nonglandular stomach. Fundic glands lie in the fundus or through the main body of the stomach, whereas the pyloric glands occupy the distal part of the stomach.

The cardiac glands are of the simple or branched tubular type. They are bent, compact and possess shallow pits, that are lined with columnar mucus-secreting cells. Each cardiac gland is composed of three parts; a short neck, body and fundus. The neck is lined by low columnar mucous cells with clear cytoplasm and rounded basal nuclei. The cells of the pit and the neck are stained positively with alcian blue and PAS(Fig. 18). The body constitutes the main greater part of the cardiac gland and is followed by a more or less thinner basal portion, the fundus. Both the body and fundus are made up mainly of chief basophilic or zymogenic cells and acidophilic parietal cells. The chief cells (Fig. 19) are pyramidal in shape possess round basal nuclei and granular cytoplasm. The parietal cells are rounded in shape with round, central nuclei and clear cytoplasm. The cells of the body and fundus stain negatively with alcian blue and PAS (Figs. 18 and 21) but give a positive reaction with bromophenol blue and alkaline fast green.

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The structure of the fundic glands is more or less, the same as that of the cardiac ones. The neck of the gland is comparatively thick, the pits are also shallow, almost of the size as in the cardic glands. The parietal cells are more abundant (Fig. 20).

The pyloric are more branched, being coiled with wide lamina propria. The pits become deeper and extend to half the thickness of the mucosa. The parietal cells are numerous in the body of the glands, while they are very sparse in the fundus of them.

A thin muscularis mucosa is found. It is composed of a layer of circular muscle fibres.

SUMMARY AND CONCLUSIONS

The stomach of *Tilapia nilotica* is divided into cardiac and pyloric portions. The gastric glands are confined to the posterior two thirds of the cardiac portion and the anterior two thirds of the pyloric portion. A similar description has been given by Moshin (1962) in some Indian teleosts. However, Kapoor et al. (1975) mentioned that the gastric glands in teleost are confined to the cardiac part of the stomach.

The present study indicates that the gastric glands of *Tilapia nilotica* are composed of one type of secretory cells. This agrees with Al-Hussaini (1948 and 1953) and Barrington (1957) in teleosts.

The surface epithelium of the stomach of Tilapia nilotica stains positively for acid mucosubstances similar to those of many mammalian species such as cat, bison and baboon (Sheahan and Jervis, 1976).

The stomach of Bufo regularis is divided into three regions; fore, fundic and pyloric. Three types of cells are recognizec; zymogenic, acidophilic and mucous cells. Teh zymogenic cells are restricted to the glands of the fore-region, the acidophilic cells are found in those of the fundic region, while the mucous cells are distributed throughout the different regions of the stomach. This agrees with Norris (1960) in Rana pipiens. The acidophilic cells secrete hydrochloric acid and more or less, some pep-sinogen; therefore they probably correspond to both the parietal and cheif cells of the mammalian stomach. Andrew and Hickman (1974) suggested that these acidophilic cells may represent a type from which the parietal and cheif cells will be differentiated.

The stomach of reptiles as represented by Chalsides ocellatus, is divided into two portions; fundic and pyloric. The examintion of the fundic glands showed that their bodies are made up of low-columnar cells with eosinophilic granular cytoplasm. The positive reaction of these cells with alcian blue, PAS, bromophenol blue and alkaline fast green indicates the presence of a considerable amount of stored enzymatic protein in addition to the mucin in their secretion. Such cells have also been recorded by Farag (1982) in Uromastyx Philpyi, Ito (1967) in reptilia and Dehlawy and Zaher (1985) in Gecko pristurus and referred to by the latter two authors as oxyntico-peptic cells. The role ascribed to these cells was the dual prodустion of pepsin and hydrochloric acid secretions in some reptilian stomach (Luppa, 1977 and Smit, 1962).

The pyloric glands of Chalcides ocellatus are lined with columnar mucus-secreting cells. Similar glands have been recorded in Alligators (Reese, 1913 and Staley, 1925) in Uromastyx acgyptia (El-Toubi and Bishai, 1958) in Chamaeleon vulgaris and Varanus griseus (Bishai, 1960 and 1961) and in Mabuya quinauetaeniata and agama stellio (Amer and Ismail, 1975 and 1976).

In birds, the stomach is divided into two distinct parts; a glandular proventriculus and muscular gizzard. The cells of the proventricular glands are of one type; this agxees with Salem (1978 and 1984). These cells show a little difference from those of reptiles. Although, they are functionally homologous to the chief and parietal cells of mammals, producing both pepsin and hydrochloric acid respectively (Andrew and Hickman, 1974 and Yamade et al, 1979). However, the cells lining the central chamber and the duct of the glands have the same structure as the mucous neck and surface cells.

The gizzard has a thick layer formed by the secretions of the mucosal glands. These mucosal glands resemble in their nature those of the proventriculus. In contrast, Toner (1964) described three main types of cells in the epithelial lining of the glands of the gizzared in the fowl; these are chief, surface and basal cells. The first have a typical structure of protein-secreting cells. The surfac cells, may be in the form of mucoid cells, The basal cells, have distinctive microvilli.

The glandular provenlriculus may be compared with the fundic part of the stomach of other vertebrate classes, and a muscular gizzared, evidently derived from the pyloric part of the vertebrate stomach.

The stomach of the albino rat, as a mammalian representative, is divided into two portions; the nonglandular and glandular portions. There is an abrupt change of the nonglandular stratified squamous epithelium into columnar, mucus-secreting cells at the glandular part of stomach.

The same observation has been reported by many authors (Fakete, 1956 in mouse; Trautman and Fiebiger, 1960 and Sisson, 1962 in the domestic mammals).

The gastric glands, which occupy the largest area of the stomach, are made up of neck, chief and parietal cells. These cells secrete mucus, gastric enzymes and hydrochloric acid respeclively. In contrast, Agrawal and Gupta (1983) in Cynopterus Sphinx stated that the cardiac glands are mainly composed of mucin-producing cells and thus can be regarded as a source of lubricating substances needed for the food as it enters the stomach.

The pyloric glands of the distal part of the stomach of the investigated rat show deep pits extending to half the thickness of the mucosa. The parietal cells, though scanty, are found in these pyloric glands. This agrees with Swarup et al., (1971) in Rhinofome kinneari.

One should recall that in lower vertebrates and in birds the same type of cells of the gastric gland secrete both pepsin and hydrochloric acid, while in mammals the cells are differentiated into chief and parietal cells secreting pepsin and hydrochloric acid respectively.

The study of the structure of the mucosal layer of the different vertebrate stomachs suggests that the evolutionary changes of vertebrate poputations follow certain patterns.

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Fig. 1. T.S of the middle part of the cardiac stomach of _Tilapia nilotica_, F., Bouin; S., HE X 250

Fig. 2. T.S of the posterior part of the cardiac stomach of _Tilapia nilotica_, F., 10% n. formalin; S., alkaline fast green X 180

Fig. 3. T.S of the posterior part of the pyloric stomach of _Tilapia nilotica_, F., Bouin; S., HE X 230

Fig. 4. T.S of the anterior part of the cardiac stomach of _Tilapia nilotica_, F., 10% n. formalin; S., Alcian blue X 190

Fig. 5. T.S of the fundic part of the stomach of _Bufo regularis_, F., 10% n. formalin; S., HE X 230

Fig. 6. T.S of the fundic part of the stomach of _Bufo regularis_ F., 10% n. formalin; S., PAS. X 270

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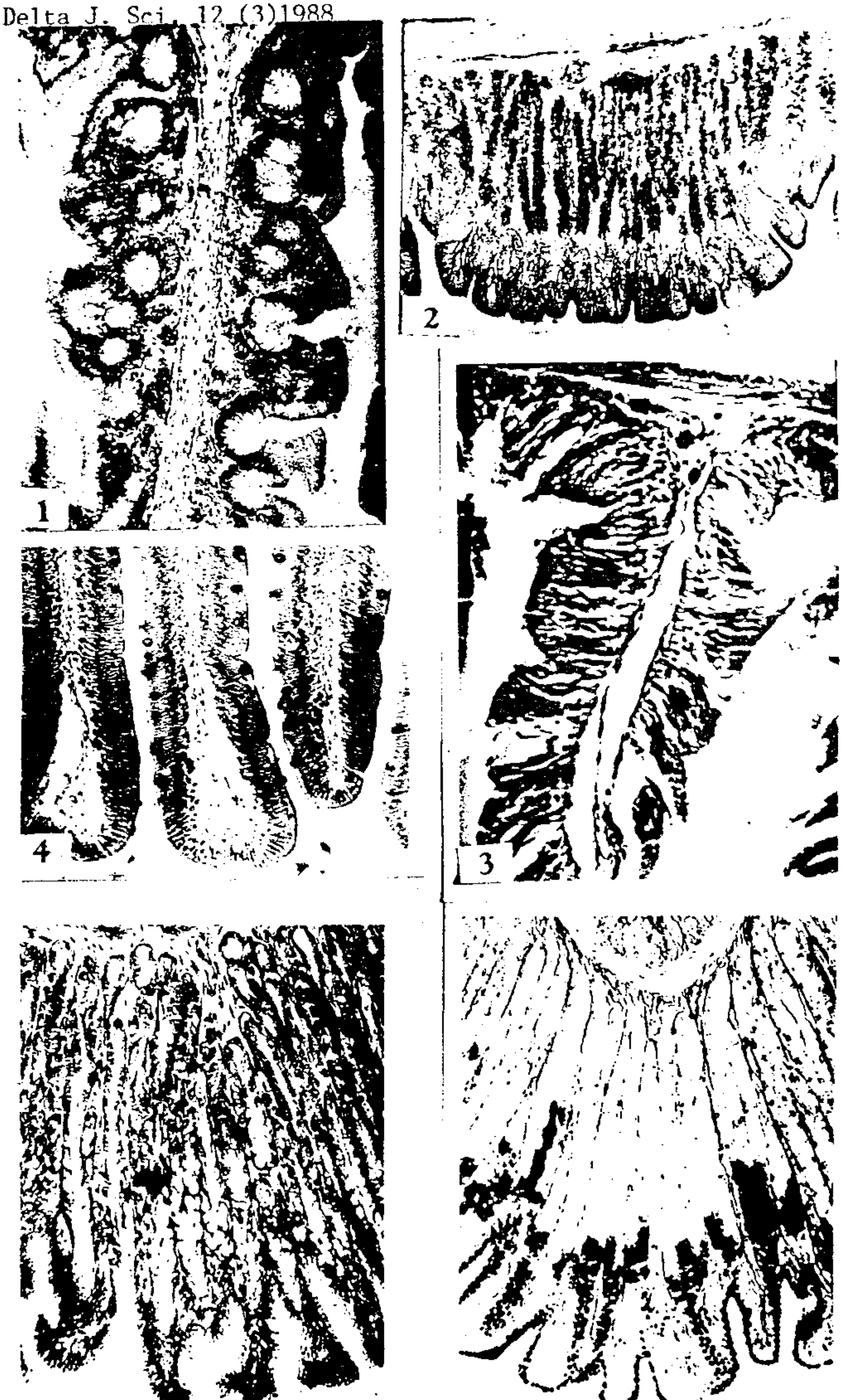

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Fig. 7. T.S of the pyloric part of the stomach of Bufo regularis F., 10% n. formalin; S., HE. X 230

Fig. 8. T.S of the fundic part of the stomach of Chalcides ocellatus, F., 10% n. formalin; S.PAS. X 250

Fig. 9. T.S of the fundic part of the stomach of chalcides ocellatus, F. Bouin; S., HE. X 190

Fig. 10. T.S of the pyloric part of the stomach of chalcides ocellatus, F. 10% n. formalin; S.HE. X 240

Fig. 11. Enlarged portion of the proventriculus of the pigeon. showing the mucosal folds.F., 10% n. formalin ; S., HE. X 300

Fig. 12. T.S of the proventriculus of the pigeon, F., 10% n. formalin S., Alcian blue. X 220

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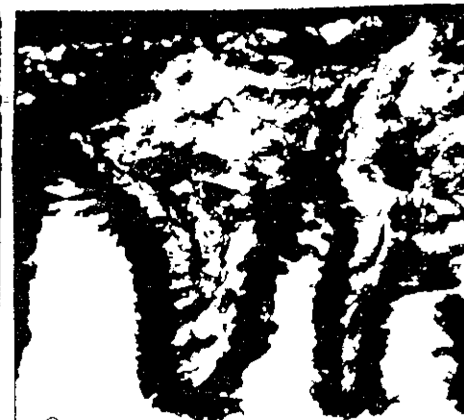

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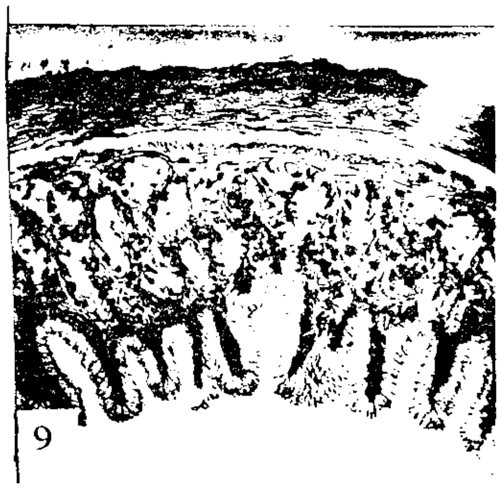

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Fig. 13. T.S of the proventriculus of the pigeon, F., 10% n.formalin; S., HE. X 200

Fig. 14. T.S of the mucosal part of the gizzard of the pigeon, F., 10% n. formalin; S. HE. X 350

Fig. 15. T.S cf the nonglandular stomach of the albino rat, F., carnoy ; S. HE. X 190

Fig. 16. T.S of the nouglandular stomach of the albino rat, F., carnoy; S. PAS. X 195

Fig. 17. T.S of the nonglandular stomch of the albino rat, F., 10% n. formalin; S. bromophenol blue X 220

Fig. 18. T.S of thecardiac portion of the glandular stomach of the albino rat, F., 10% n. formalin; S., Alcian blue X 240

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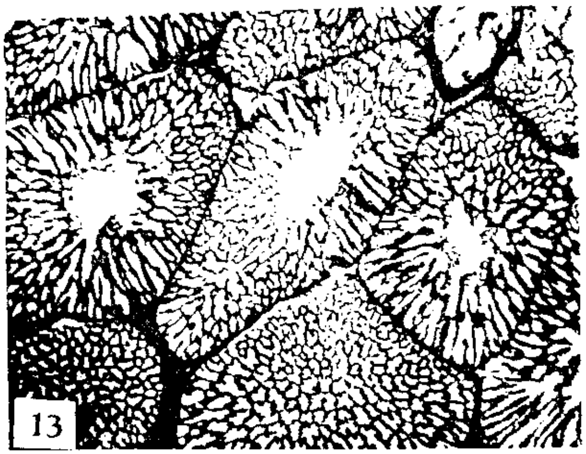
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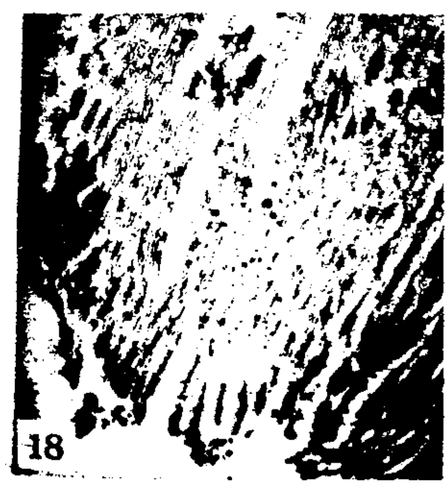
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Fig. 19. T.S of the cardiac portion of the glandular stomach of the albino rat, F., 10% n. formalin; HE. X 260

Fig. 20. T.S of the fundic part of the glandular stomach of the albino rat, F., 10% n. formalin; S. HE. X 260

Fig. 21. T.S of the fundic part of the glandular stomach of the albino rat, F., 10% n. formalin; S. PAS. X 240

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التركيب المجهرى للغشاء المخاطى والغدد المعدية فى الفقاريات

سهام بيومى سالم و فواد عفيفى أبو زيد

قسم علم الحيوان - كلية العلوم - جامعة طنطا - مصر

تم دراسة التركيب الهستولوجى للطبقة المخاطية والغدد المعدية فى خمس رتب من الفقاريات كما تم دراسة توزيع الكربوهيدرات والميوسين والبروتين والهستون فى تلك الطبقة • ولقد تبين أنه فى الفقاريات الدنيا (الاسماك ، البرمائيات ، الزواحف والطيور) يفرز كل من الببسين وحمض الهيدروكلوريك من نفس الخلايا بالغدة المعدية بينما تتخصص الخلايا لافراز كل نوع على حده فى حالة الثدييات• ويمكن استنتاج أن تلك التغيرات تتبع نظام تطورى ثابت حسب وضع الحيوان التصنيفى•