قسم : علم الحيوان .
كلية : العلوم - جامعة أسيوط .
رئيس القسم : أ.د / محمد خليل النفار .

دراسات هستوكيميائيه وسيتولوجيه على الجهاز الهضمي لسمكة الشال الشامي

عزت يواقيم ، بثينة خضر

يمكن تلخيص أهم نتائج هذا البحث كما يلي :

١- توجد مواد مخاطيه حامضيه عديدة التسكر في الخلايا المخاطية الموجودة في بطانيه التجويفين الفمي والبلعومي ومخاطيه المرئ وكذلك في قمة الخلايا العماديه المبطنه لمخاطيه المعدة والخلايا الكأسيه والسطح الفرشوى في الأمعاء.

٢- يوجد الجليكوجين بوفرة في خلايا الكبد وبدرجة قليله في خلايا الجزء القنوى للبنكرياس.

٣- هناك تناسب طردى بين كمية حمض ر.ن.أ وكمية البروتين في الخلايا المفرزه لمواد منذرة وخلايا الغدد الهضميه بالمعدة وخلايا الجزء القنوى للبنكرياس.

٤- أوضحت دراسة كمية أجسام جولجى وتوزيعها في الخلايا المفرزه لمواد منذرة ، وخلايا الغدد الهضميه في المعدة ، والخلايا العمادية في مخاطيه الأمعاء ، وخلايا الكبد ، وخلايا الجزء القنوى للبنكرياس أن هناك علاقة بين أجسام جولجى والنشاط الافرازى للخلايا السابقة .

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HISTOCHEMICAL AND CYTOLOGICAL STUDIES ON THE DIGESTIVE SYSTEM OF THE NILE CATFISH CHRYSICHTHYS AURATUS

(With 29 Figs.)

By
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(Received at 12/7/1982)

SUMMARY

Acid mucopolysaccharides were localized in the mucous cells of the lips, buccopharyngeal cavity and oesophagus. They were also demonstrated in the apical ends of the columnar surface epithelial cells of the stomach and the goblet cells and brush border of the intestinal epithelium. Glycogen was pronouncedly demonstrated in the hepatic cells and feebly so in the exocrine pancreatic cells. A parallel between the amount of RNA and proteins in the gastric gland cells, exocrine pancreatic cells and alarm substance cells was revealed. The pattern and amount of Golgi bodies of the gastric gland cells, hepatic cells and exocrine pancreatic cells were found to vary according to the secretory activity of the cell.

INTRODUCTION

BARRINGTON (1957) and HARDER (1975) nicely reviewed the literature concerning the histology of the digestive system of fishes. Among the few histochemical studies on the piscine digestive system one may mention those of BISHOP and ODENSE (1966), JIRGE (1970), SHAFI (1974) and REIFEL and TRAVILL (1978). KHIDR (1981) studied the anatomy and histology of the digestive system of the Nile catfish, Chrysichthys auratus. In the present investigation, an account of the histochemistry and cytology of such a system of the same fish will be given.

MATERIAL and METHODS

A total of 50 live specimens (8-16 cm in standard length) of Chrysichthys auratus were collected from the River Nile at the Barrage of Assiut. Immediately after decapitation, they were dissected and selected portions of the digestive system were fixed in aqueous Bouin's, Zenker's formol, Carnoy's or 10% formalin fluids. All tissues were paraffin embedded and sectioned at 5-7 um.

The histochemical procedures employed included the aqueous PAS technique for the demonstration of the 1:2 glycol linkage of carbohydrates. Control sections were incubated in human saliva at 37°C for one hour prior to PAS staining. The absence of stained material from such sections was taken as evidence for the presence of glycogen. Glycogen was also demonstrated by Best's carmine; control slides were treated in the same way as in PAS technique. Acid mucopolysaccharides were revealed by alcian blue technique and by their metachromatic reaction with 0.1% toluidine blue in acetate buffer at pH 4.5. Mercury bromophenol blue was used for the detection of

general proteins. A 0.5% solution of Sudan black in 70% ethyl alcohol was used for the demonstration of general lipids in frozen sections (20 um thick). RNA was revealed by the Brachet's pyronin-methyl green procedure. Aoyama's and Da Fano's silver impregnation techniques were used for the detection of Golgi bodies. All the aforementioned histochemical procedures were carried out according to PEARSE (1972).

RESULTS

A. CARBOHYDRATES

Mucous Cells

The mucous cells of the lips, buccal cavity, pharynx and oesophagus reacted positively with PAS before and after digestion in saliva and there were no significant differences between the two sets of sections (Figs 1 and 2). Those cells gave a negative reaction with Best's carmine. They also reacted positively towards alcian blue and stained metachromically with toluidine blue at pH 4.5. Those results indicated the absence of glycogen and that the main carbohydrate content was an acid mucopolysaccharide.

Alarm Substance Cells

The alarm substance cells of the lips and the buccopharyngeal cavity reacted negatively with PAS, indicating the absence of carbohydrates (Fig. 1).

Stomach

The apical cytoplasm of the columnar surface epithelial cells in both the corpus ventriculi and pars pylorica gave a positive, saliva resistant PAS reaction (Fig. 3). Such cytoplasm was alcianophilic and gave a metachromatic reaction with pH 4.5 toluidine blue. These results indicated the presence of acid mucopolysaccharides. By contrast, the gastric glands reacted negatively towards PAS, indicating the absence of carbohydrates (Fig. 3).

Intestine

The reaction of the brush border and the goblet cells of the intestinal epithelium towards the histochemical tests of carbohydrate detection considered in the present investigation simulated that of the mucous cells of the lips and buccopharyngeal cavity (Fig. 4). Accordingly, the main carbohydrate content in such sites was an acid mucopolysaccharide.

Liver

The hepatic cells reacted positively with PAS and Best's carmine tests and the carbohydrate contents were in the form of coarse granules (Fig. 5). The reaction was abolished in control sections. The hepatic cells revealed a negative reaction with alcian blue and did not stain metachromically with pH 4.5 toluidine blue. These results indicated that the carbohydrate content of the hepatic cells was mainly glycogen.

Exocrine Pancreas

With PAS and Best's carmine tests, the carbohydrate contents of the exocrine pancreas were revealed in the form of few deeply stained fine granules (Fig. 5). The reaction was abolished on the pretreatment of parallel sections with saliva. The exocrine pancreatic cells were faintly alcianophilic (Fig. 6). Such results signified the presence of little amounts of glycogen and acid mucopolysaccharides in those cells.

B. GENERAL PROTEINS

The intensity of reaction of a cell with mercury bromophenol blue was considered as a criterion for the amount of the general protein content of such a cell. In the following description, th terms weak, moderate and strong were used to denote the amount of general proteins present. Thus, weak referred to the least amount and strong to the greatest amount.

Mucous Cells

The mucous cells of the lips, buccal cavity, pharynx and oesophagus revealed a weak reaction (Fig. 7).

Alarm Substance Cells

The cytoplasm of the vacuolated alarm substance cells gave a moderate reaction, whereas that of the non-vacuolated ones showed a strong reaction. The proteinaceous material was in the form of fine granules in the perinuclear cytoplasm, while it was homogeneously diffused throughout the remainder of the cytoplasm (Fig. 7).

Stomach

The greater bulk of the cytoplasm of the columnar surface epithelial cells stained moderately, but the apical ends of those cells showed a strong stain. On the other hand, the cytoplasm of the gastric gland cells revealed a strong reaction. The proteinaceous material of these cells was in the form of coarse granules,whereas that of the surface columnar epithelial cells was revealed as fine granules (Fig. 8).

Intestine

The greater bulk of the cytoplasm of the columnar cells stained moderately, whereas the brush border of such cells stained strongly. In the goblet cells, protein granules were moderately stained and restricted to their basal parts (Fig. 9).

Liver and Exocrine Pancreas

The hepatic and exocrine pancreatic cells revealed a strong reaction. The proteinaceous material was in the form of coarse granules distributed through the cytoplasm (Fig. 10).

C. RIBONUCLEIC ACID (RNA)

The cytoplasm of the alarm substance cells,gastric gland cells and exocrine pancreatic cells reacted strongly with pyronin. The greater bulk of the cytoplasm of the columnar surface epithelial cells of the stomach and the columnar cells of the intestine showed a moderate reaction with pyronin. On the other hand, the hepatic cells, luminal ends of the surface columnar epithelial cells of the stomach and brush border of the intestine revealed a weak reaction with pyronin (Figs. 11-14).

D. GENERAL LIPIDS

Mucous Cells

Mucous cells reacted negatively towards the Sudan black stain.

Alarm Substance Cells

In most alarm substance cells, the sudanophilic material was revealed as large, deeply stained

patches in the peripheral cytoplasm and as small, faintly stained patches in the remainder of the cytoplasm. The peripheral sudanophilic patches varied in size and in many cases they coalesced together forming a continuous mass (Fig. 15).

Stomach

The columnar surface epithelial cells were sudanophobic. On the other hand, the cytoplasm of the gastric gland cells contained moderately stained, medium sized sudanophilic granules (Fig. 16).

Intestine

The supranuclear cytoplasm of the columnar epithelial cells was deeply sudanophilic, whereas their infranuclear cytoplasm contained coarse feebly sudanophilic granules. Generally, the epithelial cells lying at the tips of the folds had more lipid contents than those lying at their bases. Goblet cells were sudanophobic (Fig. 17a and 17b).

Liver

The great majority of hepatic cells were strongly sudanophilic and their lipid contents were homogeneously diffused throughout the cytoplasm. Some hepatic cells had their lipid material in the form of coarse sudanophilic granules. The nuclear membrane and the nucleolus were sudanophilic. Frequently, some lipid granules were seen adhering to the nuclear membrane (Fig. 18).

Exocrine Pancreas

Few coarse sudanophilic granules were revealed in the cytoplasm of the exocrine pancreatic cells (Fig. 19).

E. GOLGI BODIES

Alarm Substance Cells

In the non-vacuolated alarm substance cells, few Golgi bodies were revealed perinuclearly, many of which were adherent to the nuclear membrane. Such bodies were either in the form of small rodlets or thread-like structures showing thickenings at irregular intervals (Fig. 20). On the other hand, in the vacuolated alarm substance cells, a marked increase of the Golgi bodies was observed. Many of those bodies were located perinuclearly or juxtanuclearly, some of them were seen diffused through the cytoplasm and still some others were associated with the vacuoles (Figs. 21 and 22).

Gastric Glands

Generally, the Golgi bodies of the gastric gland cells were in the form of small rodlets. In gastric gland cells which lacked zymogen granules,the Golgi bodies were located perinuclearly. On the other hand, gastric gland cells whose secretory activity was revealed by the presence of zymogen granules showed another pattern of arrangement for the Golgi bodies. In those cells, most of the Golgi bodies were diffused through the cytoplasm, esecially in the regions of zymogen granules; however, some Golgi bodies were in a juxtanuclear position (Fig. 23).

Epithelial Cells of the Intestine

Generally, Golgi bodies of the columnar and goblet cells were in the form of coarse granules or small rodlets. In the columnar cells, most of the Golgi bodies were located in the apical part of the cytoplasm and their densities varied in different cells (Fig. 24). In the goblet cells, most of the Golgi bodies were concentrated supranuclearly and few of them were associated with the secretory vacuoles (Fig. 25).

Liver

The Golgi bodies were thread like. In the non-vacuolated hepatic cells, most of the Golgi bodies were arranged perinuclearly (Fig. 26). In the vacuolated hepatic cells, however, Golgi bodies were diffused through the cytoplasm and some of them were associated with the secretory vacuoles (Fig. 25).

Exocrine Pancreas

The Golgi bodies were thread like. The exocrine pancreatic cells which lacked zymogen granules had their Golgi bodies disposed perinuclearly (Fig. 28). On the other hand, those cells which contained zymogen granules revealed Golgi bodies lying between those granules and the nucleus. However, few Golgi bodies were associated with the zymogen granules (Fig. 29).

DISCUSSION

In the present investigation, proteins were demonstrated in the cytoplasm of the alarm substance cells, but carbohydrates were totally missing. YOAKIM and GRIZZLE (1982) reported simular results for the alarm substance cells in the epidermis of the channel catfish, Ictalurus punctatus. They discussed the different carbohydrate content of the alarm substance cell cytoplasm and the possible role of the proteinaceous material of such cytoplasm in various ostariophysian fish.

Different types of carbohydrates have been localized in the apical cytoplasm of the teleostean columnar surface epithelial cells of the stomach. Such carbohydrates were found to be acid mucopolysaccharides and glycogen in Glarias batrachus (SHAFI, 1974) and neutral mocopolysaccharides in Ictalurus nebulosus and Perca flavescens (REIFEL and TRAVILL, 1978). In Esox americanus vermiculatus and Esox lucius (REIFEL and TRAVILL, 1978) as well as in Chrysichthys auratus of the present investigation, the apical cytoplasm of the columnar surface epithelial cells of the stomach contained acid mucopolysaccharides.

Species variations in the carbohydrate content of the apical ends of the teleostean columnar surface epithelial cells of the stomach were considered by JIRGE (1970) to be correlated with the mode of feeding. According to him, acid mucopolysaccharides were much more numerous in herbivorous fishes than they were in the omnivorous and carnivorous ones. Also, carnivorous fish contained more neutral mucopolysaccharides than herbivorous fish. However, REIFEL and TRAVILL (1978) opposed Jirge's conclusion since they localized neutral mucopolysaccharides in Micropterus salmoids and Perca flavescens and acid mucopolysaccharides in Esox americanus vermiculatus and Esox lucius; all of them were piscivorous.

In the present investigation, the carbohydrate content of the apical cytoplasm of the columnar surface epithelial cells of the stomach was similar to that of the brush border of intestinal columnar epithelial cells. This result may be considered as an evidence for an absorptive role of the teleostean stomach. Several authors (BISHOP and ODENSE, 1966; WESTERN, 1969; SHAFI, 1974 and REIFEL and TRAVILL, 1978) were in favour of that role of the teleostean stomachs, especially for fats.

In the present investigation, the secretion of the goblet cells, throughout the whole length of the intestine, contained acid mucopolysaccharides. Similar conclusions were arrived at by BULLOCK (1963) in some salmonids and by REIFEL and TRAVILL (1979) in Ambloplites rupestris, Lepomis macrochirus, Micropterus salmoides and Pomoxis nigromaculatus. However, acid mucopolysaccharides and glycogen were localized by SHAFI (1974) in the goblet cells of the intestinal epithelium of

Clarias batrachus. The carbohydrate content of the intestinal goblet cell secretion was found by REIFEL and TRAVILL (1979) to vary in the proximal and distal intestines in fish species having an intestino-rectal valve; Perca flavescens was an exceptional case in this connection.

Several authors mentioned that mucus secreted by the teleostean columnar surface epithelial cells of the stomch and intestinal goblet cells provides lubrication to the ingested food and protects the mucosal lining of the gastrointestinal tract from mechanical injury and autodigestion (AL-HUSSAINI, 1949 a&b; WESTERN, 1969 and SHAFI, 1974). FREEMAN (1966) and SPEICER et al. (1957) reported similar functions to the mucus secreted in the gastrointestinal tracts of other vertebrates. Such functions were considered by the latter authors to be related to the presence of acid muco-polysaccharides in the mucous secretion. In Chrysichthys auratus of the present investigation, the mucous secretion in the gastrointestinal tract contained acid mucopolysaccharides; accordingly, it may play the same aforementioned functions.

It is now a well established fact that the Golgi bodies are associated with the production of secretion in various exocrine glands. Such a fact has been documented histologically (MOUSSA and KHATAB, 1957) as well as ultrastructurally (BLOOM and FAWCETT, 1975). In the present investigation, the pattern and/or the amount of Golgi bodies of the gastric gland cells, hepatic cells and exocrine pancreatic cells were found to vary with the variation of the secretory activity of the cell. Also, the Golgi bodies of the alarm substance cells which are known to be engaged in the secretion of alarm substances (YOAKIM and GRIZZLE, 1982) revealed similar variations. LUTFY (1960) reported a similar conclusion for the alarm substance cells of Synodontis schall.

A parallel between the amount of RNA and proteins in active protein synthesizing cells was reported by BROWN and BERTHE (1974). In the present investigation, a similar relation between RNA and proteins was revealed in the gastric gland cells, exocrine pancreatic cells and the alarm substance cells, all of which are considered, according to the nature of their secretions, as active protein synthesizers.

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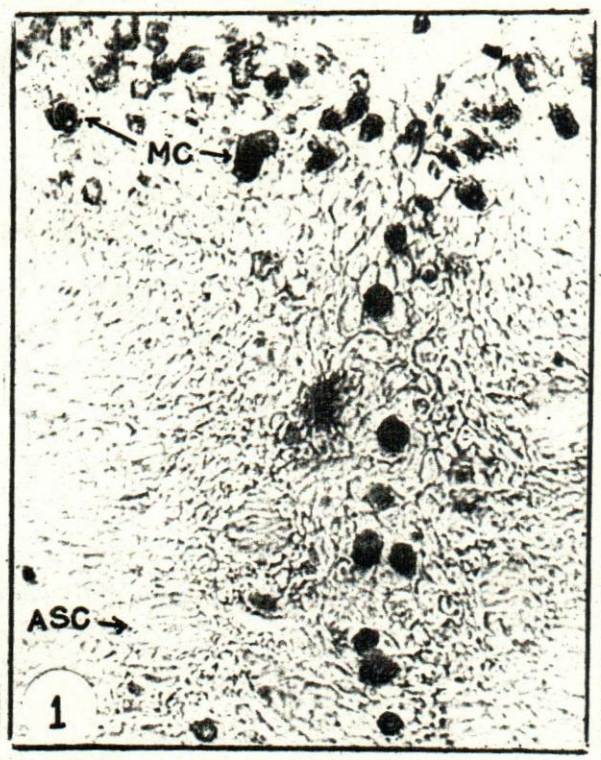
EXPLANATION OF FIGURES

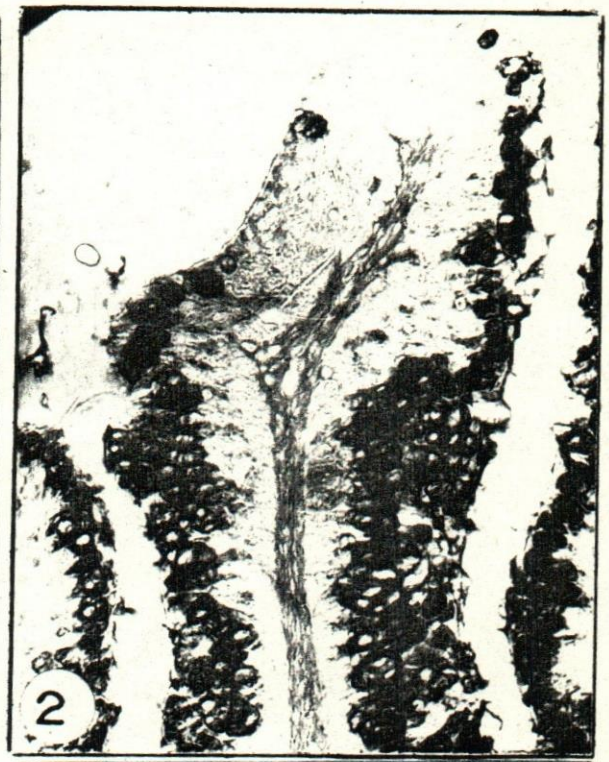
Fig. (1): T.S. of the upper lip. PAS X 420.
Fig. (2): T.S. of the oesophagus. PAS. X 280.
Fig. (3): T.S. of the stomach (corpus ventriculi). PAS. X 280.
Fig. (4): T.S. of the intestine. PAS. X 280.
Fig. (5): Section of the the liver. Best's carmine. X 420.
Fig. (6): Section of the liver. Alcian blue. X 420.
Fig. (7): T.S. of the epithelial lining of the buccal cavity. Mercuyry bromophenol blue. X 420.
Fig. (8): T.S. of the stomach (corpus ventriculi). Mercury bromophenol blue. X 280.
Fig. (9): T.S. of the intestine. Mercury bromophenol blue. X 420.
Fig. (10): Section of the liver. Mercury bromophenol blue. X 280.
Fig (11): T.S. of the epithelial lining of the buccal cavity. Pyronin-methyl green. X 280.
Fig. (12): Section of the liver. Pyronin-methyl green. X 280.
Fig. (13): T.S. of the intestine. Pyronin-methyl green. X 280.
Fig. (14): T.S. of the stomach (corpus ventriculi). Pyronin-methyl green. X 280.
Fig. (15): T.S. of the epithelial lining of the buccal cavity. Sudan black. X 420.
Fig. (16): T.S. of the stomach (corpus ventriculi). Sudan black. X 420.
Fig. (17a): T.S. of the intestine. Sudan black. X 100.
Fig. (17b): Magnified portion of the intestinal epithelium. Sudan black. X 420.
Fig. (18): Section of the liver. Sudan black. X 420.
Fig. (19): Section of the liver, showing the reaction of exocrine pancreatic cells towards Sudan black. X 420.
Fig. (20): T.S. of the epithelial lining of the buccal cavity, showing the Golgi bodies of a non-vacuolated alarm substance cell (arrowed). Da Fano. X 1000.
Fig. (21): T.S. of the epithelial lining of the buccal cavity, showing the Golgi bodies of a vacuolated alarm substance cell (arrowed). Da Fano. X 1000.
Fig. (22): T.S. of the epithelial lining of the buccal cavity, showing the Golgi bodies of a vacuolated alarm substance cell (arrowed) mostly diffused through the cytoplasm. Da Fano. X 1000.
Fig. (23): T.S. of the stomach, showing the Golgi bodies of a gastric gland cell without zymogen granules (a) and of another cell having zymogen granules (b). Aoyama. X 1000.
Fig. (24): T.S. of the intestine, showing the Golgi bodies of the columnar epithelial cells. Aoyama. X 1000.
Fig. (25): T.S. of the intestine, showing the Golgi bodies of the goblet cells. Aoyama. X 1000.
Fig. (26): Section of the liver, showing the Golgi bodies of a non-vacuolated hepatic cell (arrowed). Da Fano. X 1000.
Fig. (27): Section of the liver, showing the Golgi bodies of a vacuolated hepatic cell (arrowed). Da Fano. X 1000.
Fig. (28): Section of the exocrine pancreas showing the Golgi bodies of a cell without zymogen granules (arrowed). Da Fano. X 1000.
Fig. (29): Section of the exocrine pancreas, showing the Golgi bodies of a cell having zymogen granules. Da Fano. X 1000.

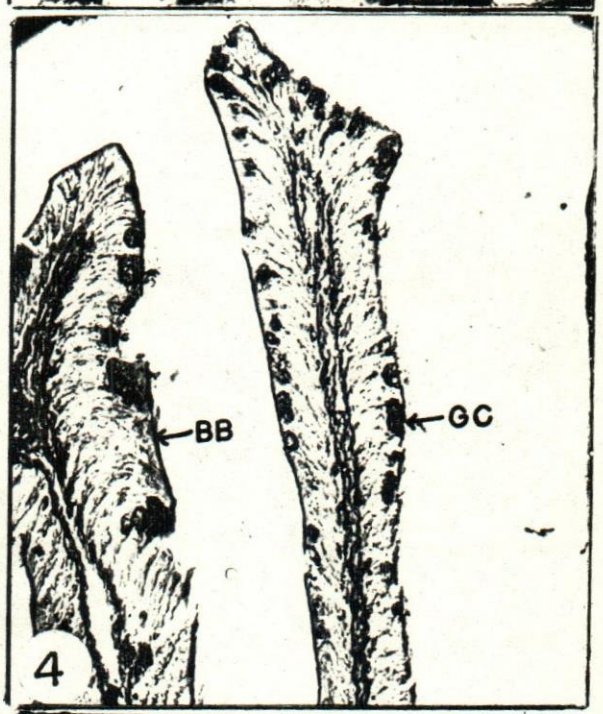
LETTERING OF FIGURES

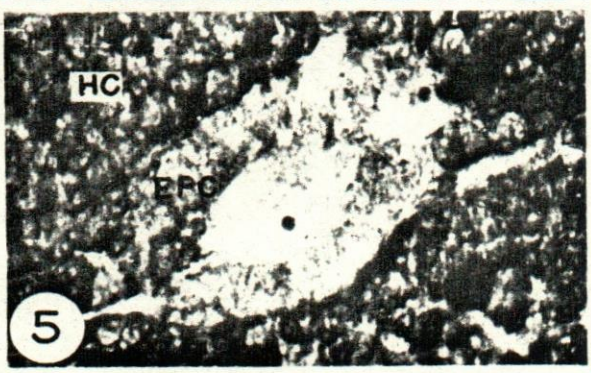
ASC, alarm substance cell; BB, brush border; CEC, columnar epithelial cell; EPC, exocrine pancreatic cell; GC, goblet cell; GGC, gastric gland cell; HC, hepatic cell; MC, mucous cell.

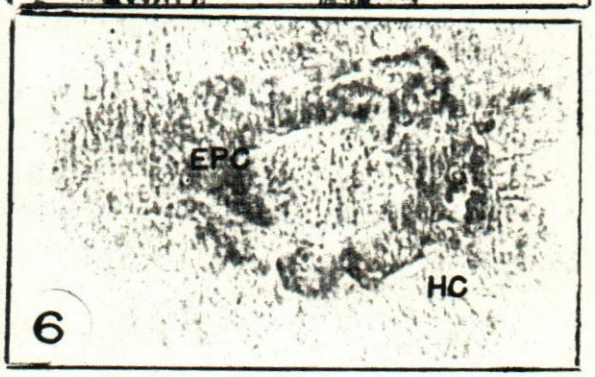
PLATE I











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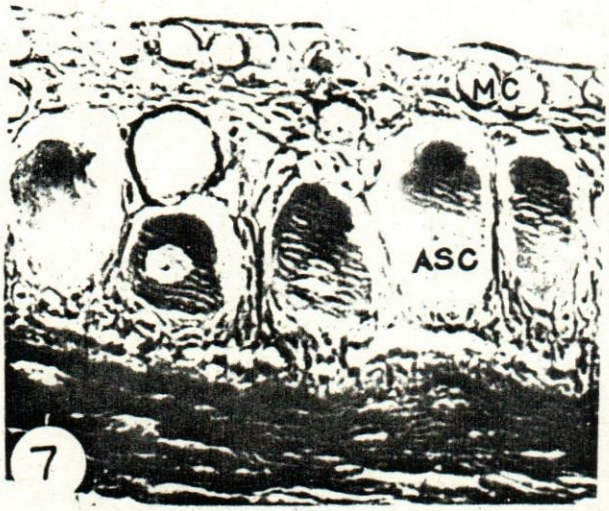

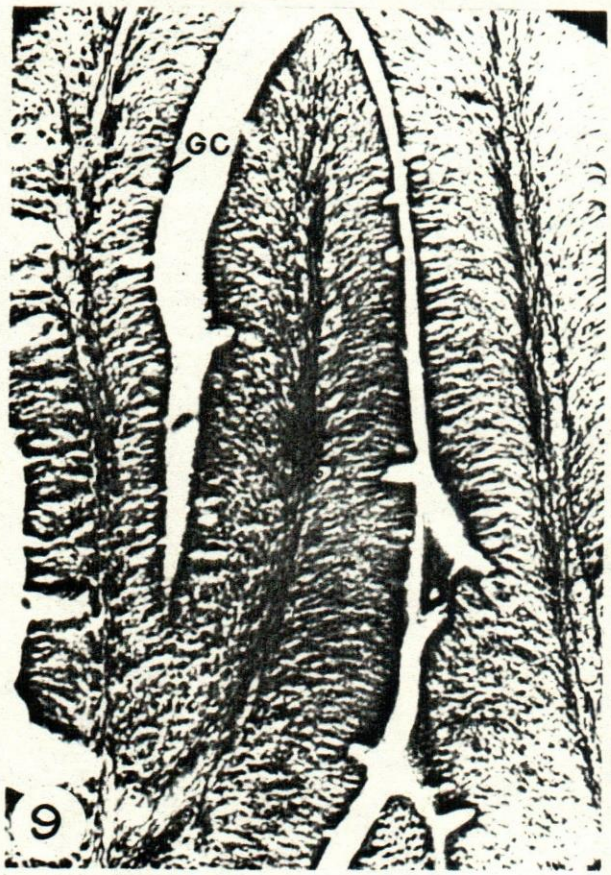

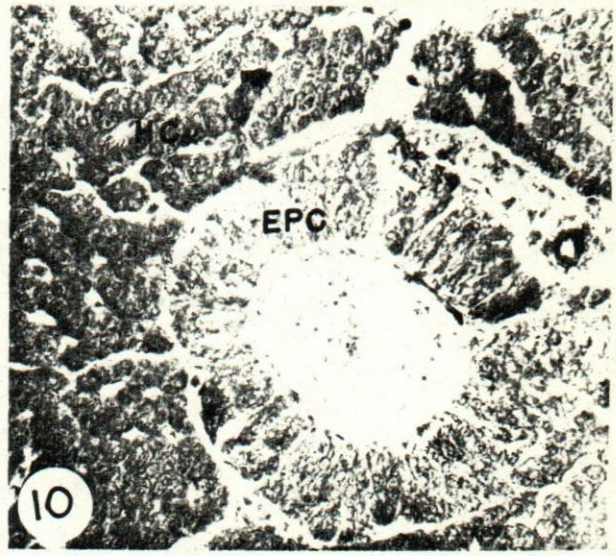

PLATE III

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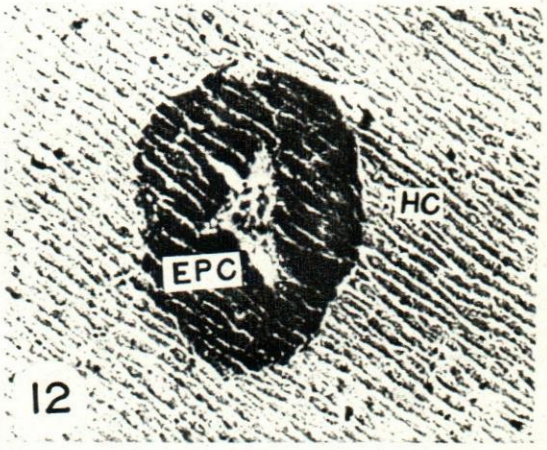

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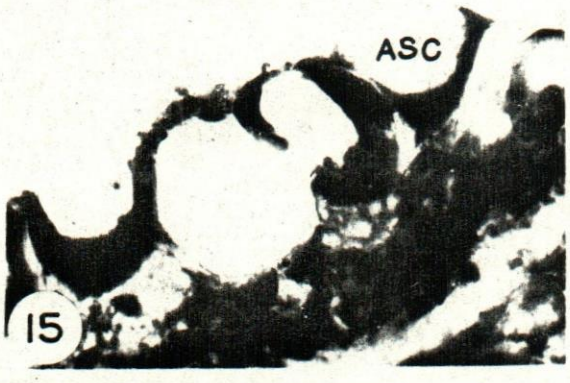
PLATE IV



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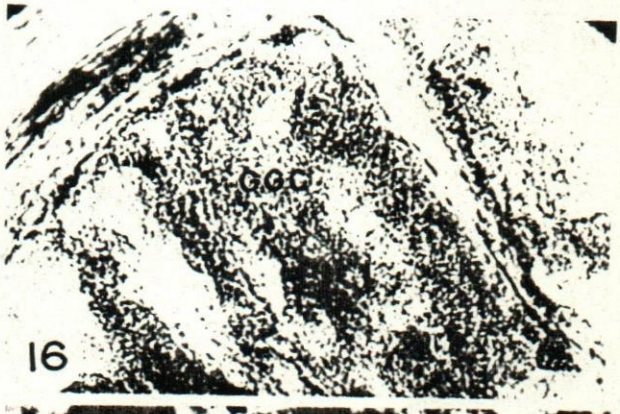

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17a

17b

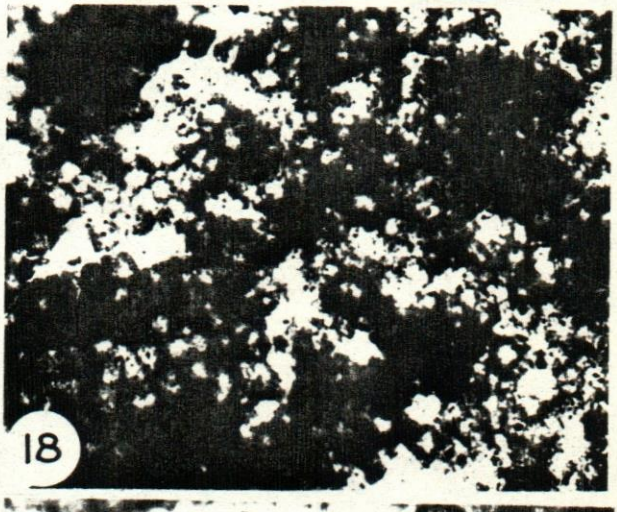
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PLATE V


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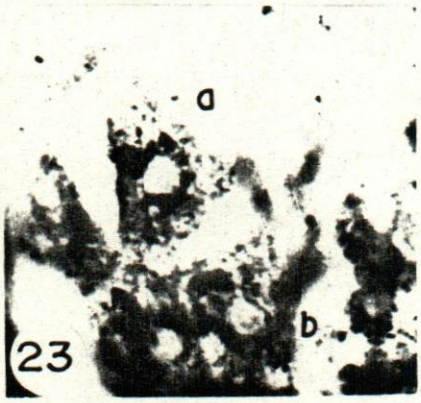

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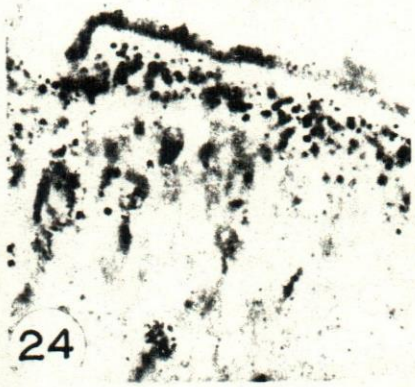
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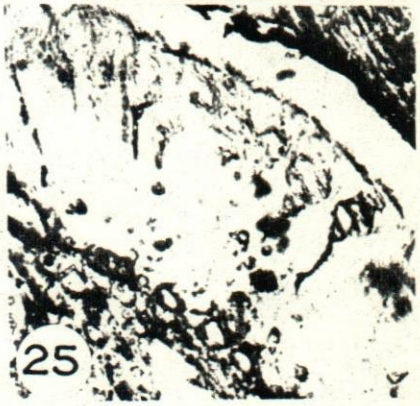
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