CHAPTER THREE

O B S E R V A T I O N S

HISTOLOGY AND HISTOCHEMISTRY OF MUCO-SUBSTANCES IN BUCCAL MASS, ESOPHAGUS, STOMACH AND INTESTINE OF *O.Verraculatum*

A critical evaluation of the histological, histochemical studies published in the last two to three decades on the alimentary tract of vertebrates, significantly shows that metabolites such as mucosubstances, lipids, proteins and enzymes both intracellular and extracellular have been studied mainly in vertebrates. Very little attention has been paid to the alimentary tract of the invertebrates. This is particularly true for the mucosubstances in the alimentary tract of the mollusca. An extensive research project has therefore, been undertaken in this laboratory with a view to augment the understanding of mucosubstances elaborated and secreted in the various parts of the alimentary tract of the molluscan varieties. The present report concerns with the mucosubstances in the various organs, from buccal mass to intestine of Oncidium verraculatum . The results obtained in the present investigation on histology and histochemistry of mucosubstances in the various organs of the alimentary tract of O.verraculaum are presented hereafter. The histochemical results are recorded in tabulated form and histology and histochemical distribution of mucosubstances in the various organs are illustrated in numerous photomicrographs.

Observations :

1. Buccal mass

The mouth opened on the ventral surface of the head region between the foot and the head hyponotum which was bounded laterally by a pair of labial palps. Mouth leads within the buccal cavity along an oblique passage which was slightly eversible. The buccal region was continued behind into a large swollen bulb like muscular pharynx.

A) Histological observations

The radula when dissected and stretched out appears somewhat rectangular in form, and its anterior margin was conical. It was beset with transverse rows of teeth arranged on either side of a single median longitudinal row. In its usual position the posterior portion of the radula was rolled into a tube, with a dendrate surface forming its inner wall. The anterior portion of the radular plate was folded on itself, so that the dentrate surface in this region facing the pharyngeal wall. Anterior portion of the radula with its conical border, that remain exposed in the pharyngeal cavity and that operates in the rasping function when the food was taken in. The radular tube was filled with an elastic tissue

formed of fibres and cells, scattered and embedded in a homogenous non-cellular ground substance. The posterior end of the radular tube was inserted into what was known as the radular sac. The margin of folded anterior portion of radula was joined to the wall of the pharynx by means of a thin membrane which was capable of great flexion, which thus allowed the backward and forward movement of the radular apparatus.

B) Histochemical observations

The histochemical reactivities of various mucosubstances observed in the buccal cavity of O. verraculatum are recorded in Table No.1, according to the visually estimated intensity of staining and shade. The histochemical distribution of mucosubstances in the buccal mass is shown in photomicrographs (Figs. 1,2). The histochemical results requiring further description and considerations are presented hereafter.

I. Mucosa

a) Epithelial cells

The epithelial cells reacted moderately towards PAS (Fig 2). Their PAS reactivity remained unchanged after diastase digestion, moreover it was not abolished by prior phenylhydrazine treatment.

These cells exhibited moderate alcianophilia at both AB pH 1.0, and AB pH 2.5 . These results indicated the presence of sulfomucins which were predominant.

The conclusion that the epithelial cells contain sulfomucins was further substantiated by their purple-blue staining with AB pH 1.0-PAS, blue-purple staining with AF-AB pH 2.5 sequence, moderate metachromasia with azure A at lower pH, persistant alcianophilic staining in CEC technique in presence of 0.5 M Mg^{++} and blockade of their alcianophilia by active methylation which could not be restored completely. The sulfomucins were hyaluronidase resistant.

b) Radula

The radula exhibited in intense PAS reaction (Fig.1) which was partly abolished by phenylhydrazine pretreatment and was reduced by diastase digestion indicating the presence of neutral mucins and glycogen. Moreover, the radula showed weak alcianophilia at both AB pH 1.0 and AB pH 2.5 indicated the presence of sulfomucins. The presence of sulfomucins in it was also inferred from its weak reactivity with AF alone, as well as weak blue-purple staining with AF-AB pH 2.5 sequence, weak metachromasia with azure A at low pH, persistant alcianophilia in CEC technique upto 0.2 M Mg^{++} and complete

abolishing of its alcianophilia by active methylation and failure to restore it completely after saponification (it was restored partly). The sulfomucins were hyaluronidase resistant. Thus, the radular lining contain neutral mucins, trace glycogen and sulfomucins.

II. Connective tissue

The connective tissue exhibited weak staining with PAS which was diastase resistant but could completely be blocked by phenylhydrazine pretreatment, it exhibited only weak pink to magenta colouration with AB pH 1.0-PAS, and AB pH 2.5-PAS sequential staining procedures but remained unstained with AB pH 1.0, AB pH 2.5 and AF even after pepsin digestion. With azure A only blue orthochromatic staining was evident mainly at higher pH levels. These results indicated the presence of only neutral mucosubstances in the connective tissue.

III. Muscular layer

Muscular layer showed weak PAS staining reactivity, which could completely be blocked by phenylhydrazine pretreatment and abolished by diastase digestion indicating the presence of glycogen in them. The muscular layer remained

unstained with AB pH 1.0, AB pH 2.5 and AF. Moreover, it exhibited only blue orthochromatic staining with azure A thus, showing absence of acidic mucosubstances. The layer appeared only weak pink to magenta in sequential staining techniques such as AB pH 1.0-PAS, AB pH 2.5-PAS, indicating the presence of glycogen.

IV. Serosa

The serosa exhibited weak PAS reactivity (Fig. 1). Its PAS reactivity was resistant to diastase but could partly be blocked by phenylhydrazine pretreatment. It weakly stained with AB pH 1.0 and its staining intensity was not enhanced at AB pH 2.5 . These results indicated the presence of sulfomucins but absence of carboxymucins. The presence of sulfomucins in serosa was further substantiated from its poor to weak purple staining with AF alone or with AB pH 2.5, weak metachromasia with azure A at lower pH, persistant alcianophilia in CEC technique in presence of 0.2 M Mg^{++} and abolishing of its alcianophilia by active methylation which could not be restored after subsequent saponification. These results thus, revealed the presence of neutral mucosubstances and sulfomucins in the serosa.

2. Esophagus

The esophagus arises from the mid-dorsal region of the pharynx and turned posterio-ventrally over the pharyngeal bulb. After passing through the never ring it runs behind the floor of the buccal cavity and entered into a large swollen region of stomach (gizzard).

A. Histological observations

A transverse section of esophagus showed the inner wall thrown into the conspicuous folds. The esophageal wall was formed of three layers. The innermost layer was formed of columnar epithelium which secretes the inner chitinous lining. The middle layer was made up of loose connective tissue, containing blood vessels with open spaces, and nerve terminals. The third layer was the muscular layer which was made up of an inner circular and outer longitudinal muscle fibers. The outermost layer was very thin and mainly containing connective tissue and fibers, commonly called as a serosa.

B. Histochemical observations

The histochemical reactivities of various mucosubstances observed in the esophagus of O. verraculatum are recorded in Table No.2, according to the visually estimated intensity

Captions to figures

Fig. 1 T.S.buccal mass stained with PAS to show intense staining in radula and radular apparatus (arrow) weak staining in connective tisse(CT),muscular (ML) and serosa (S) X 20.

Fig. 2 T.S. of buccal mass stained with PAS to show moderate staining in epithelial cells (EC),weak staining in connective tissue (CT), muscular layer (ML) and serosa (S) X 60.

Fig. 3 T.S. of esophagus stained with PAS to show intense staining in chitinous lining (arrow),columaar epithelial cell(EC) and moderate staining in goblet cells(GC),weak staining in connective tissue (CT), muscular layer (ML) and serosa (S) X 200.

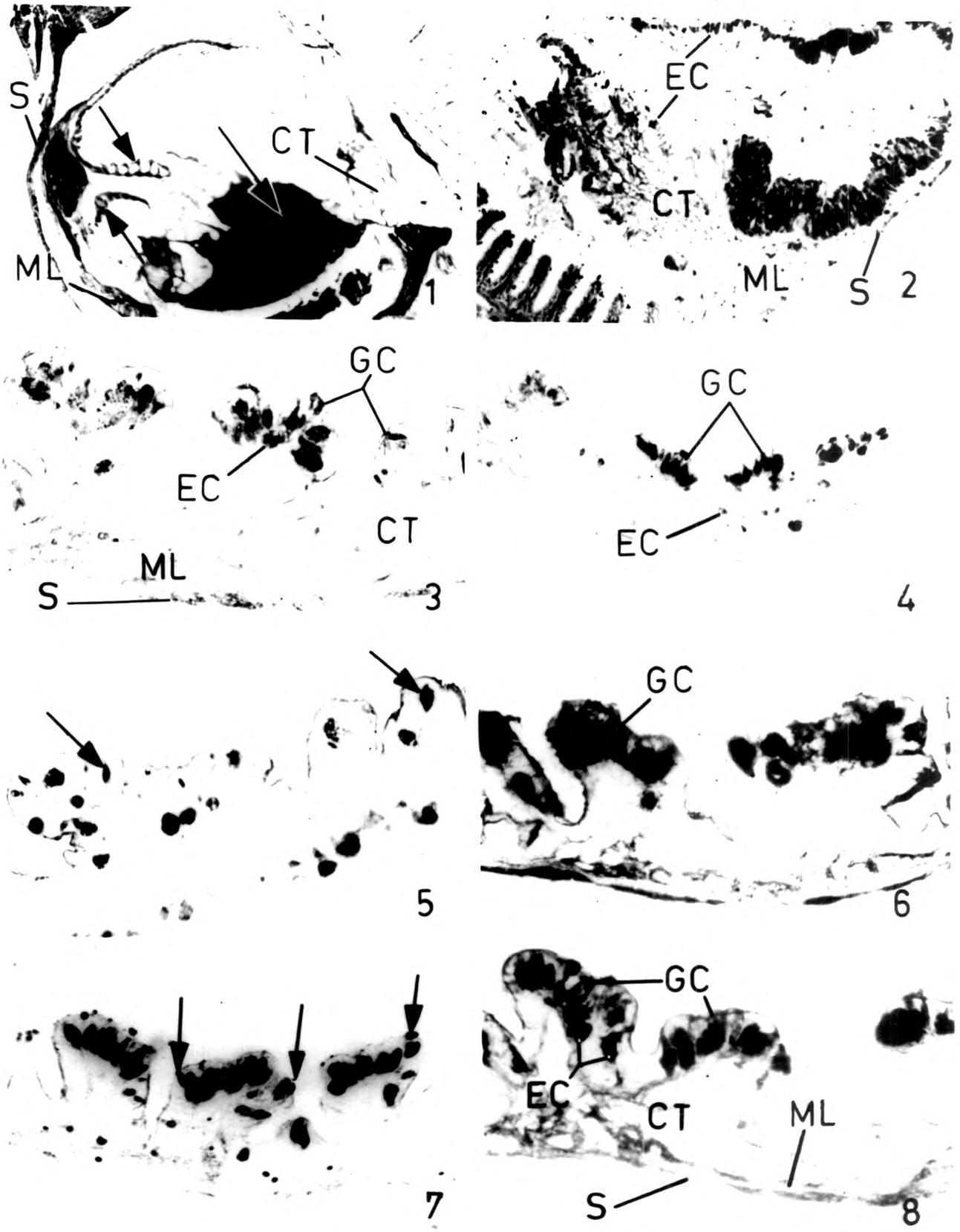
Fig. 4 T.S. of esophagus stained with AB pH 1.0-PAS to show moderate staining in goblet cells(GC)and weak staining in epithelial cells (EC) X 90.

Fig. 5 T.S. of esophagus stained with AB pH 1.0 to show moderate staining in goblet cells (arrows) X 200.

Fig. 6 T.S. of esophagus treated with mild methylation saponification AB pH 2.5, to show moderate staining in goblet cells (GC) X 200.

Fig. 7 T.S. of esophagus stained with AB pH 2.5, to show moderate to intense staining in goblet cells(arrows)X 90.

Fig. 8 T.S. of esophagus stained with AB pH 2.5-PAS to show moderate staining in goblet cells (GC),epithelial cells (EC) and weak staining in connective tissue (CT), mascular layer (MC) and serosa (S) X 90.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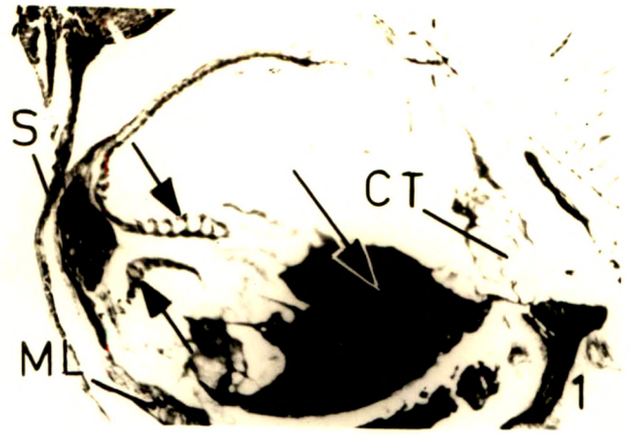

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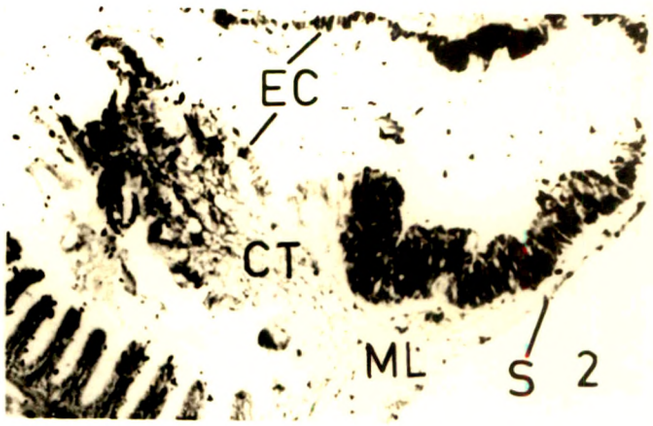

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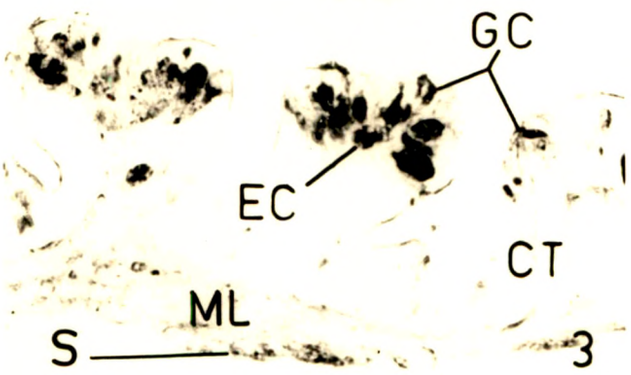

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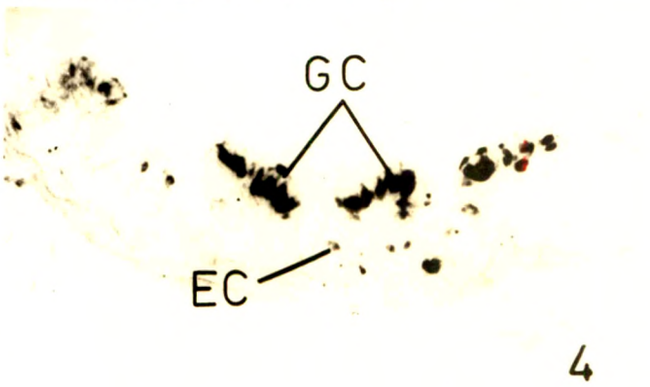

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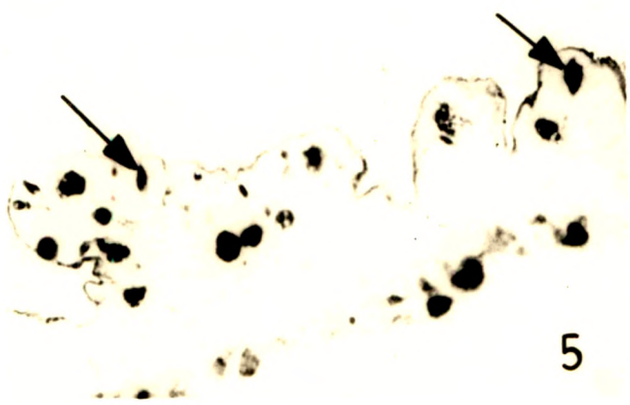
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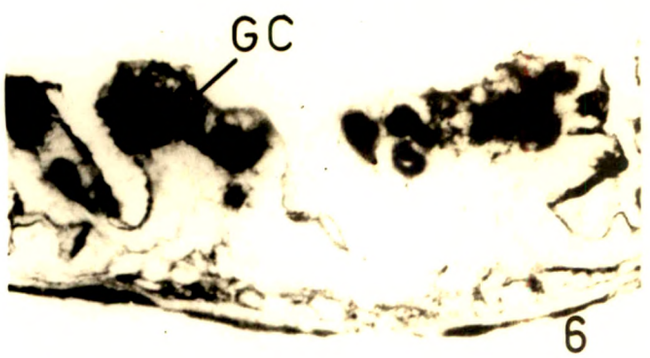

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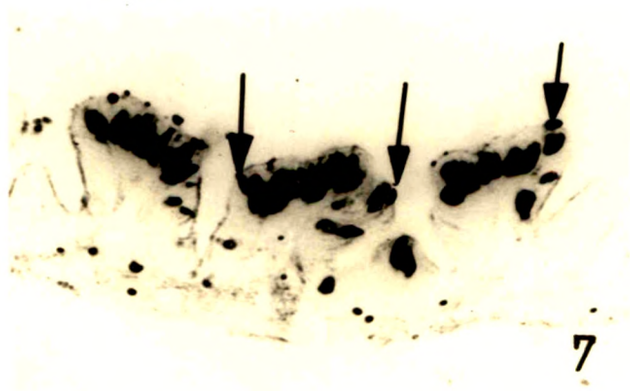
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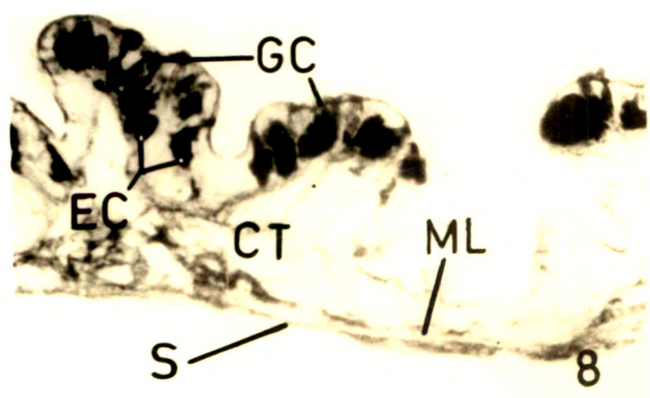

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of staining and shade. The histochemical distribution of mucosubstances in the esophagus is shown in photomicrographs (Figs. 3 to 8). The histochemical results requiring further description and considerations are presented hereafter.

I. Chitinous lining

The chitinous lining along the inner surface of the esophagus showed intense PAS reactivity (Fig.3) which was partly abolished by prior phenylhydrazine treatment and was reduced by diastase digestion indicating the presence of neutral mucins and glycogen. The chitinous lining showed weak alcianophilia at both AB pH 1.0 and AB pH 2.5 indicated presence of sulfomucins. With sequential staining procedures like AB (pH 1.0, 2.5)-PAS, which showed moderate to intense pink-blue colouration. Mild methylation was without any effect on alcianophilia at AB pH 2.5, but active methylation effected a complete and irreversible loss of alcianophilia. Acid hydrolysis failed to abolish alcianophilia at AB pH 2.5 . These staining reactions indicated the presence of sulfomucins. This was further confirmed by the studies on the extinction of the alcianophilia by the addition of the graded concentration of Mg^{++} to the staining solution. The alcianophilic reaction remained unaffected by addition of 0.M and 0.2 M Mg^{++} but any

further addition led to an extinction of the alcianophilia. Thus, chitinous lining contain neutral mucousubstances, trace of glycogen and sulfomucins.

II. Mucosa

a) Epithelial cells

The epithelial cells exhibited moderate to intense staining reaction with PAS (Fig.3), which was partly abolished by phenylhydrazine pretreatment and was slightly reduced by diastase digestion indicating the presence of neutral mucins and trace of glycogen. Moreover, the epithelial cells showed weak alcianophilia at both AB pH 1.0 (Fig.5) and AB pH 2.5 indicated the presence of weak sulfomucins. The presence of sulfomucins in the epithelial cells was also inferred from its weak reactivity with AF alone, as well as weak blue-purple staining with AF-AB pH 2.5 sequence, weak metachromasia with azure A at low pH, persistant alcianophilia in CEC technique upto 0.2 M Mg^{++} and complete abolishing of its alcianophilia by active methylation and failure to restore it completely after saponification. Sulfomucins were hyaluronidase resistant. Thus, the columnar epithelial cells contain neutral mucins, glycogen and sulfomucins.

b) Goblet cells :

These cells were concentrated at the apical region of the esophageal villi. These cells exhibited the moderate PAS reaction (Figs. 3,8). Their PAS reactivity was diastase resistant and it was not abolished by phenylhydrazine pretreatment indicating the absence of glycogen. Moreover, these cells exhibited moderate alcianophilia at AB pH 1.0 and at AB pH 2.5 (Fig.7) which indicated the presence of both sulfomucins (predominant) and corboxymucins (in less quantities). The presence of sulfomucins in them was also inferred from their moderate reactivity with AF alone, moderate blue-purple staining with AF-AB pH 2.5 sequence, moderate metachromasia with Azure A at low pH (pH 1.5) persistant alcianophilia in CEC technique upto 0.5 M Mg^{++} and complete abolishing of their alcianophilia by active methylation and failure to restore it completely after saponification. The sulfomucins were hyaluronidase resistant.

III. Connective tissue

The connective tissue showed weak PAS reactivity (Fig.3). The PAS reactivity was diastase resistant, but could be completely blocked by phenylhydrazine pretreatment. Moreover, the connective tissue remained unstained with AB pH 1.0 (Fig.5)

and AB pH 2.5 (Fig.7) and appeared slightly pink to magenta with AB pH 1.0-PAS sequence indicating the absence of sulfomucins in them. This conclusion was further substantiated from their purple staining with AB pH 2.5 PAS (Fig.8). In methylation-saponification, acid hydrolysis had no effect on staining reactivities. These results indicated the presence of neutral mucosubstances.

IV. Muscular layer

Both circular and longitudinal muscle layers exhibited weak PAS staining (Fig.3), which could be completely block by phenylhydrazine pretreatment and abolished by diastase digestion, indicating the presence of glycogen in them. The muscular layer remained unstained with AB pH 1.0 (Fig.5), AB pH 2.5 (Fig.7) and AF. The muscular layers was exhibited blue orthochromatic staining with azure A at higher pH level. The mascular layer appeared weak pink in sequential staining techniques such as AB pH 1.0-PAS, AB pH 2.5-PAS (Fig.8). The rest of the histochemical techniques exhibited negative reactivities, confirming the presence of glycogen in this layer.

V. Serosa

The serosa showed weak staining with PAS (Fig.3), which was diastase resistant but could be completely blocked by

phenylhydrazine pretreatment. This layer exhibited slightly pink colouration with AB pH 1.0-PAS, AB pH 2.5-PAS (Fig.8) sequential staining procedures. The serosa remained unstained with AB pH 1.0, AB pH 2.5 and AF even after pepsin digestion. These results indicated the presence of neutral mucosubstances in the serosa.

3. Stomach

The stomach proper was thin walled chamber, which was proximally in free communication with the crop and gizzard. A sagittal section through stomach region showed three different chambers of which anterolateral one on the right side is the crop, the posterior one was the gizzard and anterolateral one on left side was the stomach proper.

A) Histological observations

A stomach proper was a thin walled chamber, in cross section, it showed the dendritic branching of internal villi. The wall of the stomach was formed of three typical tissue layers, of which, the innermost was formed ciliated columnar epithelium. In between the columnar cells were also seen a few goblet cells of the secretory in nature. The second layer was formed of connective tissue. The third layer was made up of muscle and outermost layer was the serosa.

B) Histochemical observations

The results obtained with various histochemical staining techniques for the mucosubstances in various histological sites of the stomach are recorded in Table No.3 . The histochemical distribution of the mucosubstances in the various histological sites of the stomach is shown in photomicrographs (Figs. 9-14). The histochemical results requiring further description and considerations are presented hereafter.

I. Mucosa

a) Epithelial cells

The columnar epithelial cells in the mucosa of the stomach exhibited weak PAS reactivity (Fig.9). Their PAS reactivity was diastase resistant but could completely be blocked by phenylhydrazine pretreatment indicating the absence of glycogen in them but presence of only neutral mucosubstances. This was further substantiated by their only weak PAS reactivity with AB pH 1.0-PAS (Fig.11), AB pH 2.5-PAS sequential staining procedures. Moreover, the absence of acidic mucosubstances in these cells was inferred from their negative staining with AB at pH 1.0, AB at pH 2.5 and only blue orthochromasia with azure A at higher pH levels. These results could not be

Captions to figures

Fig. 9 T.S. of stomach stained with PAS to show weak staining in columnar epithelial cells (EC), connective tissue (CT), serosa (S) and moderate staining in goblet cells (GC) X 60.

Fig. 10 T.S. of stomach stained with AB pH 1.0 to show weak staining in goblet cells (arrows) X 50.

Fig. 11 T.S. passing through the junction of gizzard and stomach, stained with AB pH 1.0-PAS to show weak staining in epithelial cells (EC) moderate staining in goblet cells (GC) and weak staining in mascular layer (ML), serosa (S) X 60.

Fig. 12 T.S. passing through the junction of gizzard and stomach, stained with AB pH 2.5-PAS to show moderate to intense staining in goblet cells (arrows) X 60.

Fig. 13 T.S. of stomach stained with AF to show moderate staining in goblet cells (arrows) X 60.

Fig. 14 T.S. of stomach treated with mild methylation AB pH 2.5 showing moderate staining in goblet cells (GC) X 60.

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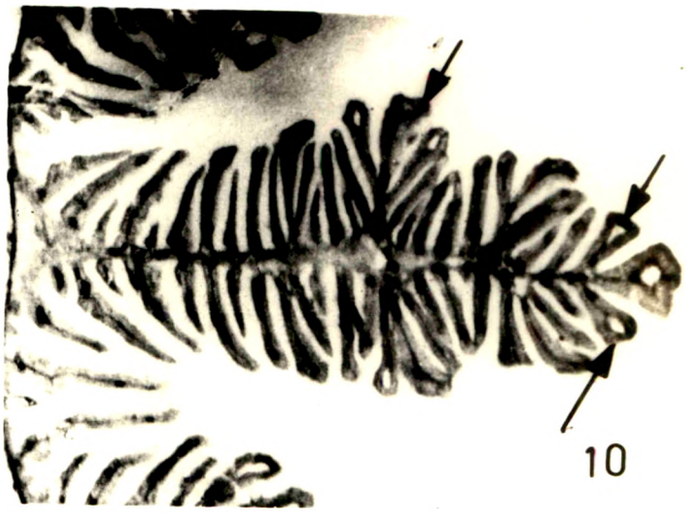

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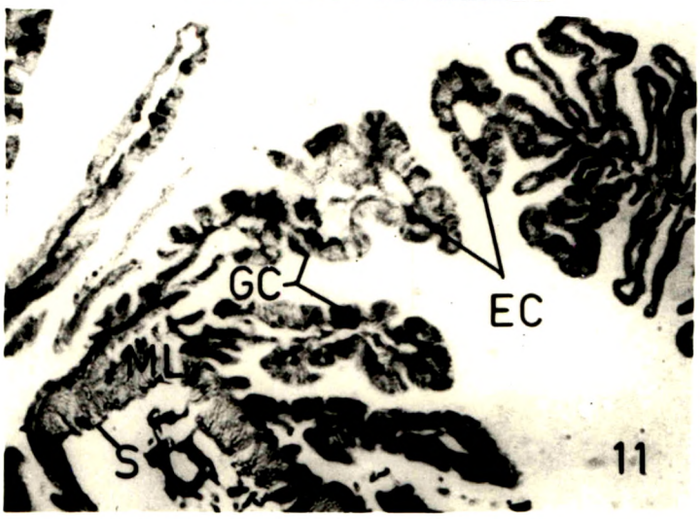

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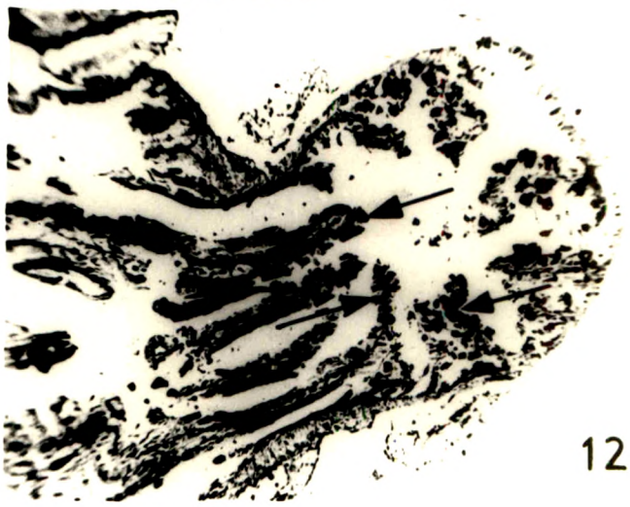

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altered by pepsin digestion. These results indicated the presence of neutral mucosubstances in the epithelial cells.

b) Goblet cells

The goblet cells in the mucosa of the stomach reacted moderately towards PAS (Fig.9). Their PAS reactivity remained unchanged after diastase digestion and it was not abolished by prior phenylhydrazine treatment. These cells exhibited weak alcianophilia at AB pH 1.0 indicated the presence of sulfomucins which were predominant. The enhance alcianophilia at AB pH 2.5 indicated the presence of carboxymucins also.

The conclusion that the goblet cells contain sulfomucins was further substantiated by their purple-blue staining with AB pH 1.0-PAS (Fig.11), blue-purple staining with AF-AB pH 2.5 sequence, moderate metachromasia with azure A at lower pH (pH 1.5), persistant alcianophilic staining in CEC technique in presence of 0.5 M Mg^{++} and blockade of their alcianophilia by active methylation which could not be restored completely. The sulfomucins were hyaluronidase resistant.

The enhanced alcianophilia at AB pH 2.5 than at AB pH 1.0 indicated the presence of carboxymucins in the goblet cells. This conclusion was further supported by their moderate reactivity blue staining with AB pH 2.5-PAS (Fig.12),

blue-purple staining in AF-AB pH 2.5 sequence, enhance meta chromasia with azure A at higher pH levels (pH 3.0 and above) and only partial restoration of their alcianophilia following saponification of the previously methylated (active methylation) sections. The carboxymucins were further identified as sialimucins since their alcianophilia was partly sensitive to acid hydrolysis and neuraminidase digestion. Thus, the aforementioned results indicated the presence sulfomucins (predominent) and sialomucins (less amount).

II. Connective tissue

Connective tissue showed weak PAS reactivity (Fig.9) which was diastase resistant but completely be blocked by phenylhydrazine pretreatment. Moreover, the connective tissue exhibited only weak pink to magenta colouration with AB pH 1.0-PAS, AB pH 2.5-PAS sequential staining procedures but remained unstained with AB pH 1.0, AB pH 2.5 and AF even after pepsin digestion. With azure A only blue orthochromatic staining was evident mainly at higher pH levels. These results indicated the presence of only neutral mucosubstances in the connective tissue.

III. Muscular layer

Muscular layer exhibited weak PAS staining which could

completely be blocked by phenylhydrazine pretreatment and abolished by diastase digestion indicating the presence of glycogen in them. The muscles remained unstained with AB pH 1.0, AB pH 2.5 and AF, whereas, it exhibited only blue orthochromatic staining with azure A thus, showing absence of acidic mucosubstances. The layer appeared only weak pink to magenta in sequential staining techniques such as AB pH 1.0-PAS (Fig.11), AB pH 2.5-PAS, indicating the presence of glycogen.

IV. Serosa

The serosa exhibited weak staining with PAS which was diastase resitant but could completely be blocked by phenylhydrazine pretreatment. This layer exhibited only weak pink to magenta colouration. With AB pH 1.0-PAS (Fig.11), AB pH 2.5-PAS sequential staining procedures but remained unstained with AB pH 1.0, AB pH 2.5 and AF even after pepsin digestion. With azure A only blue orthochromatic staining was evident mainly at higher pH levels. These results ineicated the presence of only neutral mucosubstances in the serosa.

4. Intestine

Intestine was a long narrow coiled tube, on one side

it communicates with stomach and other end opened to outside through the anus. It is the most important part of the digestive system from the digestive point of view.

A) Histological observations

The intestine was circular in cross section. It was comparatively thin walled. The mucousa was thrown into long and broad villi-like folds which projected into the lumen (Fig.15). The epithelium of the mucosa was single layered and contained columnar epithelial cells and goblet cells (Fig.15). Glands were found to be absent. The connective tissue was thin and extended in the villi-like folds to form the core of the folds. The outermost covering was the serosa.

B) Histochemical observations

The results obtained with histochemical techniques for the mucosubstances in the intestine are recorded in Table No.4 and the histochemical distribution is shown in photomicrographs (Figs. 16-20). The histochemical results requiring further description and considerations are presented hereafter.

I. Mucosa

a) Epithelial cells

Captions to figures

Fig. 15 T.S. of intestine stained with H-E to show goblet cells (GC), connective tissue (CT) mascularis (M) and serosa (S) X 200.

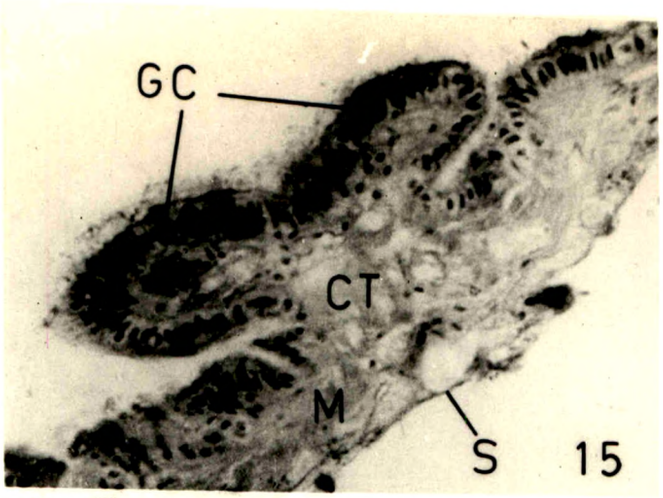
Fig. 16 T.S. of intestine stained with PAS to show moderate to intense staining in goblet cells (arrows), weak to moderate staining in epithelial cells (EC), serosa (S) and weak staining in connective tissue (CT) X 100.

Fig. 17 T.S. of intestine stained with AB pH 1.0 PAS showing moderate to intense staining in goblet cells (GC) weak to poor staining in epithelial cells (EC) and serosa (S) X 90.

Fig. 18 T.S. of intestine stained with AB pH 2.5 to show moderate staining in goblet cells (GC) and poor staining in columnar epithelial cells (EC) X 100.

Fig. 19 T.S. of intestine stained with AF showing moderate staining in goblet cells (arrows) X 90.

Fig. 20 T.S. of intestine stained with AF-AB pH 2.5 showing moderate staining in goblet cells (arrows) X 200.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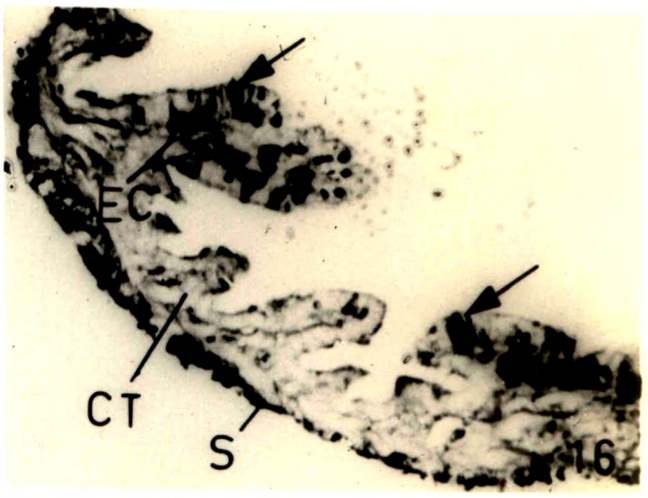
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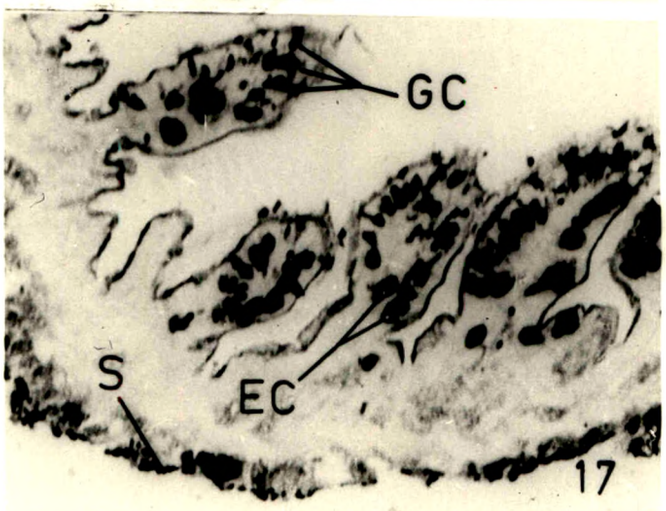
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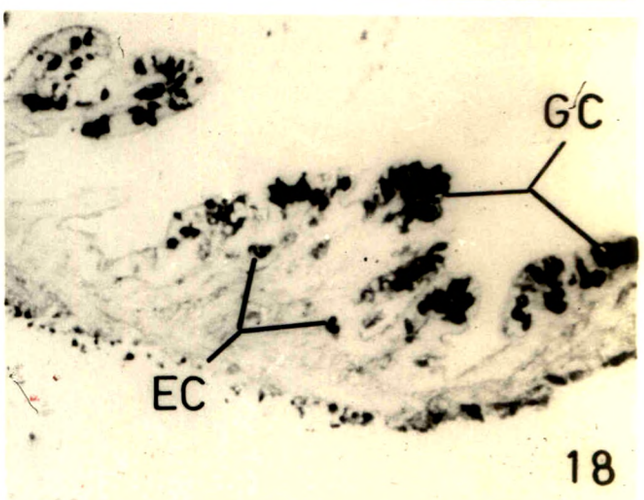
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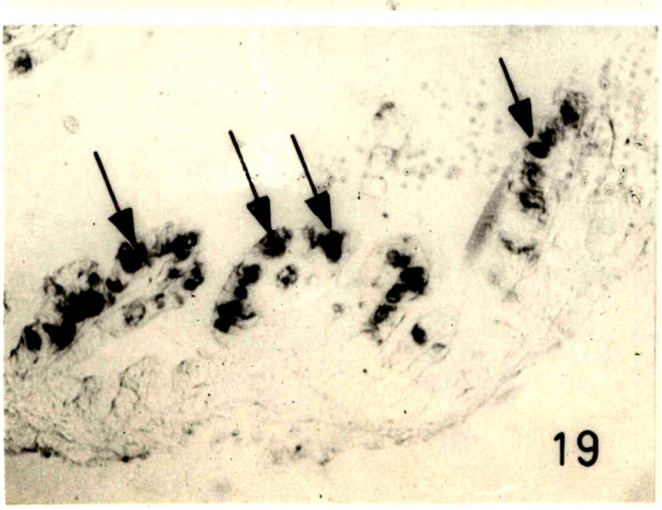
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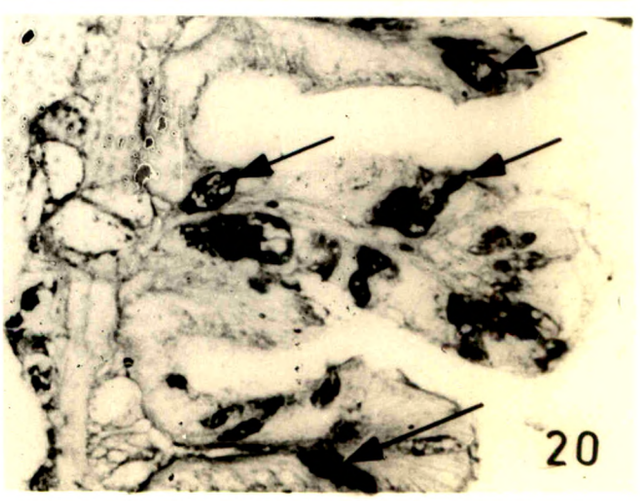
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The epithelial cells exhibited weak PAS reactivity (Fig. 16). Their PAS reactivity was resistant to diastase but could partly be blocked by phenylhydrazine pretreatment. The epithelial cells were poorly stained with AB pH 1.0 and their staining intensity was not enhanced at AB pH 2.5 (Fig. 18). These results indicated the presence of sulfomucins (traces) but absence of carboxymucins. The presence of sulfomucins in these cells was substantiated from their weak purple staining with AF alone or with AB pH 2.5 step afterwards. Weak metachromasia with azure A at lower pH (pH 1.5), persistant alcianophilia in CEC technique in presence of 0.2 M Mg^{++} and abolishing of their alcianophilia by active methylation which could not be restored after subsequent saponification. The sulfomucins were hyaluronidase resistant. These results thus, revealed the presence of neutral muco-substances and sulfomucins in the epithelial cells of the mucosa.

b) Goblet cells

The goblet cells reacted moderately towards PAS (Fig.16). Their PAS reactivity remained unchanged after diastase digestion and was not blocked by prior phenylhydrazine treatment, thus indicating the absence of glycogen but presence of neutral mucosubstances in them. Their moderate

alcianophilia at AB pH 1.0 indicated the presence of sulfomucins which were predominant. The alcianophilia at AB pH 2.5 (Fig. 18) remain unchanged indicated the absence of carboxymucins.

The conclusion that the goblet cells contain sulfomucins was further substantiated by their purple-blue staining with AB pH 1.0-PAS, blue-purple staining with AF-AB pH 2.5 sequence, moderate metachromasia with azure A at lower pH (pH 1.5), persistant alcianophilic staining in CEC technique in presence of 0.5 M Mg^{++} and blockade of their alcianophilia by active methylation which could not be restored completely. The sulfomucins were hyaluronidase resistant. Thus, the aforementioned results indicated the presence of neutral mucosubstances (less amount) and sulfomucins (predominant).

II. Connective tissue

The connective tissue exhibited weak staining with PAS which was diastase resistant but could completely be blocked by phenylhydrazine pretreatment. This layer showed only weak pink to magenta colouration with AB pH 1.0-PAS, AB pH 2.5 PAS sequential staining procedures but remained unstained with AB pH 1.0, AB pH 2.5 and AF even after pepsin digestion. With azure A only blue orthochromatic staining was evident mainly at higher pH levels. These results indicated the presence of only neutral mucosubstances in connective tissue.

III. Muscular layer

The muscular layer showed weak PAS staining which could completely be blocked by phenylhydrazine pretreatment and abolished by diastase digestion indicating the presence of glycogen in them. The muscular layer remained unstained with AB pH 1.0, AB pH 2.5 and AF and exhibited only blue orthochromatic staining with azure A thus, showing the absence of acidic mucosubstances. The muscles appeared only pink to magenta in sequential staining techniques such as AB pH 1.0 PAS, AB pH 2.5-PAS.

IV. Serosa

The serosa exhibited weak staining with PAS which was diastase resistant but could completely be blocked by phenylhydrazine pretreatment. This layer showed only weak pink to magenta colouration with AB pH 1.0-PAS, AB pH 2.5-PAS sequential staining procedures but remained unstained with AB pH 1.0, AB pH 2.5 and AF even after pepsin digestion. With azure A only blue orthochromatic staining was evident mainly at higher pH levels. These results indicated the presence of only neutral mucosubstances in the serosa.

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