CHAPTER THREE

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

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON MUCOSUBSTANCES IN ESOPHAGUS, CARDIAC STOMACH, PYLORIC STOMACH, DUODENUM, SMALL INTESTINE AND LARGE INTESTINE OF THE FROG,

E. SYSTOMA.

A critical evaluation of the histological,histochemical and biochemical studies published in the last two to three decades on the alimentary tract of vertebrates, significantly shows that metabolites such as mucosubstances, lipids and proteins and enzymes both intracellular and extracellular have been studied mainly in the mammals. Very little attention has been paid to the alimentary tract of the lower vertebrates. This is particularly true for the mucosubstances in the alimentary tract of the Amphibians. 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 organs of the alimentary tract of the vertebrates from fishes to mammals. The present report concerns with the mucosubstances in the various organs from esophagus to large intestine of the frog, E.systoma. As the mucosubstances in the tongues (Nalavade,1975; Nalavade and Varute, 1971,1972 a; Gaikwad,1981) and pharynx (Nalavade, unpublished data) of amphibians including E.systoma. have already been studied, in the present investigation the mucosubstances have been studied in various organs from esophagus to large intestine. The results obtained in the present investigation on histology and histochemistry of mucosubstances in the various organs of the alimentary tract of E.systoma 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.

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

1. Esophagus

A) Histological observations

The esophagus was a thin walled tube leading from pharynx

towards the stomach. In cross section the esophagus was circular in outline. The mucosa was thrown into many longitudinal folds which projected into the lumen (Fig.1). The epithelium consisted of single layer of cuboidal to columnar epithelial cells which were numerically less but the goblet cells in the epithelium were predominant (Figs.1,2) Simple tubulo - alveolar glandular invaginations consisting of mucous cells were found at the base of the folds (Figs.1,2). The glands were more towards gastric region (Figs.4,8) but less in the proximal region (Figs.1,3,5,6). The lamina propria or connective tissue in the submucosa was thin and also extended in the center of each fold forming the core of the fold(Fig.1) but unlike toads lymphatic nodules were not observed. The muscularis was thin and consisted of inner circular muscles and outer longitudinal muscles,(Fig.1). The serosa consisted of mesothelium covering fibrous connective tissue together with normal complement of blood vessels and nerves. Histological observations revealed absence of sexual dimorphism in esophagus.

B) Histochemical observations

The histochemical reactivities of various mucosubstances observed in the esophagus of the frog are recorded in Table No.2 according to the visually estimated intensity 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) Mucosa

i) Epithelial cells: The epithelial cells exhibited poor PAS reactivity (Figs.3,4) which was diastase resistant but could completely be blocked by prior phenylhydrazine treatment (Fig.5). These

CAPTIONS TO FIGURES

Fig. 1. Section passing through the proximal part of the esophagus of the frog stained with H-E to show goblet cells (GC), connective tissue (CT), muscularis (M) and epithelial cells (E) X 240.

Fig. 2. Section passing through the distal part of the esophagus of the frog stained with H-E.

Note: epithelial cells (EC), goblet cells (GC) and esophageal glands (GL) X 180.

Fig. 3. T.S.of proximal part of esophagus stained with PAS to show intensely stained goblet cells (G) and poor to weak staining in the connective tissue (CT) X 240.

Fig. 4. T.S.of distal part of esophagus stained with PAS to show intense staining in the glands (GL) and goblet cells (G) CT = connective tissue X 240.

Fig. 5. T.S.of proximal part of esophagus stained with PAS after phenylhydrazine treatment.

Note: moderate staining in type-I goblet cells (G_1) and weak to moderate staining in type-II goblet cells (G_2) X 240.

Fig. 6. T.S. of proximal part of esophagus stained with AB pH 1.0 to show moderate alcianophilia in type-I cells (G_1) and unstained type-II cells (G_2) X 400.

Fig. 7. T.S.of distal part of esophagus stained with AB pH 1.Ø to show moderately stained type-I cells (G_1) and unstained type-II cells (G_2) in the glands X 400.

Fig. 8. T.S. of esophagus stained with AB pH 2.5 to show intense staining in goblet cells (GC) and gland cells (GL). CT = connective tissue X 240.

CAPTIONS TO FIGURES

Fig. 9. T.S.of cardiac stomach of the frog stained with H-E to show mucosal epithelium (E), mucous neck cells(MC) and gastric glands (G) X 240.

Fig. 10. T.S.of cardiac stomach of the frog stained with PAS showing poor staining in columnar epithelial cells (E), intense staining in goblet cells (GC) and mucous glands (MG). CT = connective tissue X 240.

Fig. 11. T.S.of cardiac stomach of the frog stained with AB pH 1.0

Note: unstained columnar epithelial cells(E),moderately stained goblet cells(GC) and poorly stained mucous glands (MG) X 240.

Fig. 12. T.S. of cardiac stomach of the frog stained with AB pH 2.5 to show unstained columnar epithelial cells(E), intensely stained goblet cells(GC) and poorly stained mucous glands (MG) X 240.

Fig. 13. T.S.of cardiac stomach of the frog stained with PAS.

Note: mostly goblet cells in the epithelium(E),intense staining in mucous glands (MG) and unstained deeper (oxyntic) cells of gastric glands(G). CT = connective tissue X 240.

Fig. 14. T.S. of cardiac stomach of the frog stained with PAS to show intense staining in mucous neck cells (MC),unstained oxyntic cells of gastric glands (G). CT = connective tissue, M = Muscularis X 144.

Fig. 15. T.S.of pyloric stomach of the frog stained with PAS showing intensely stained goblet cells (G) and pyloric glands (PG) and weakly stained connective tissue (CT). M = Muscularis X 400.

Fig. 16. A magnified view of Fig. No. 15 showing goblet cells(G), pyloric glands (PG) and connective tissue (CT) X 400.

CAPTIONS TO FIGURES

Fig. 17. T.S. of duodenum of the frog stained with PAS to show intense staining in goblet cells(G) moderate staining in connective tissue (CT) and weak staining in muscularis (M) X 400.

Fig. 18. T.S.of duodenum of the frog stained with PAS after phenylhydrazine treatment.

Note: absence of staining in columnar epithelial cells (E), intense staining in goblet cells(G), and unstained muscularis (M) X 400.

Fig. 19. T.S.of duodenum of the frog stained with AB pH 1.0.

Note: unstained columnar epithelial cells(E) and moderately stained goblet cells(G) X 400.

Fig. 20. T.S. of duodenum of the frog stained with AB pH 2.5.

Note: unstained muscularis (M) and intensely stained goblet cells (G) X 240.

Fig. 21. T.S. of duodenum of the frog stained with C.I. showing unstained columnar epithelial cells(E) and intensely stained goblet cells (G) X 400.

Fig. 22. T.S. of duodenum of the frog stained with AF to show unstained columnar epithelial cells(E) and intensely stained goblet cells(G) X 360.

CAPTIONS TO FIGURES

Fig. 23. T.S. of small intestine of frog stained with PAS showing poor to weak staining in columnar epithelial cells(CE) and intense staining in goblet cells (G) X 400.

Fig. 24. T.S.of small intestine of frog stained with AB pH 1.0.

Note: unstained muscularis (M), poorly stained connective tissue (CT) and moderately stained goblet cells (G) X 240.

Fig. 25. T.S. of small intestine of frog stained with AB pH 2.5.

Note: weak staining in connective tissue (CT) and intense staining in the goblet cells(G). Columnar epithelia cells(CE) are unstained X 800.

Fig. 26. T.S. of small intestine of frog stained with AF showing unstained columnar epithelial cells (E),weakly stained connective tissue (CT) and intensely stained goblet cells (G) X 480.

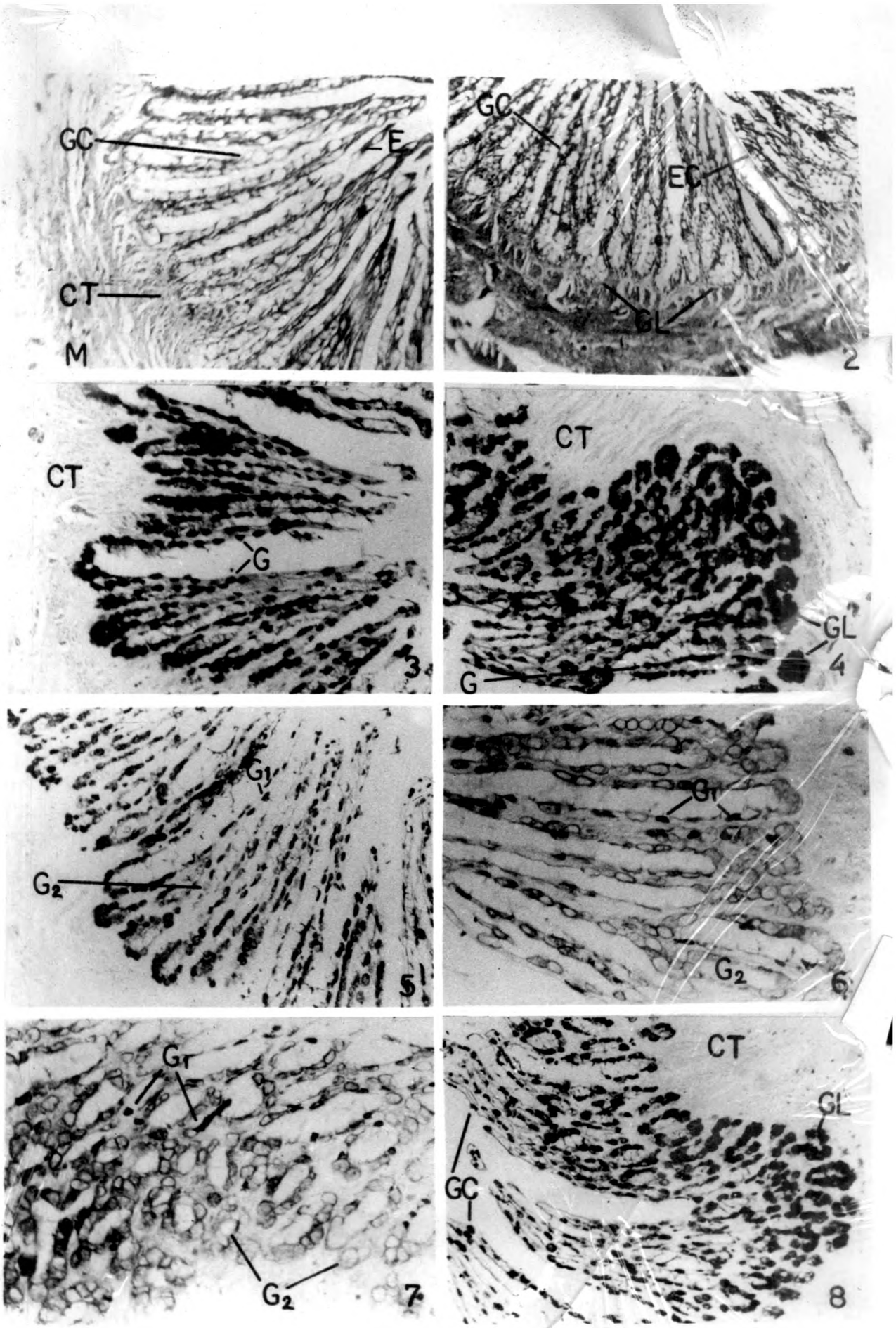
Fig. 27. T.S. of large intestine of frog stained with H-E to show goblet cells (G),connective tissue (CT) and muscularis (M) X 400.

Fig. 28. T.S. of large intestine of frog stained with PAS to show intense staining in goblet cells(G), poor to weak stainin in connective tissue (CT) and weak staining in musculari (M) X 400.

Fig. 29. T.S. of large intestine of frog stained with AB pH 1.0 showing moderately stained goblet cells(G) and unstained connective tissue (CT) and muscularis (M) x 400.

Fig. 30. T.S. of large intestine of frog stained with AB pH 2.5.

Note: intense staining in goblet cells (G), poorly stained connective tissue (CT) and unstained columnar epithelial cells (CE) X 400.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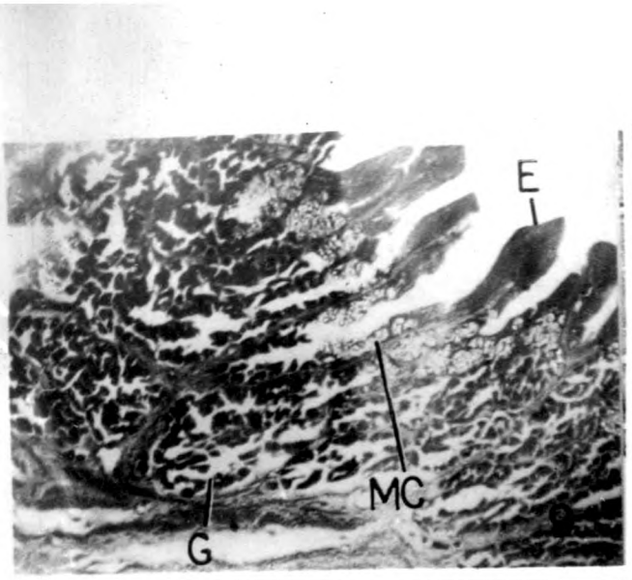
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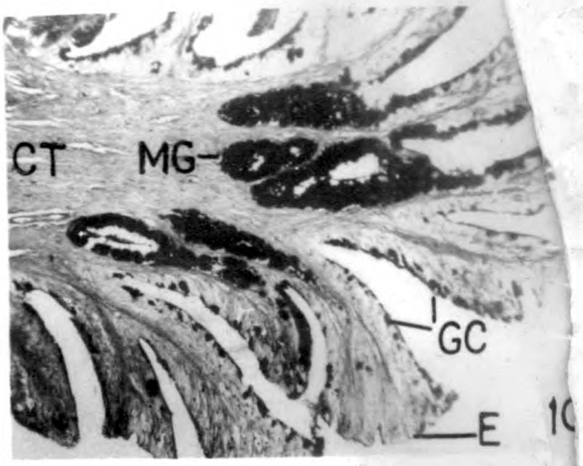
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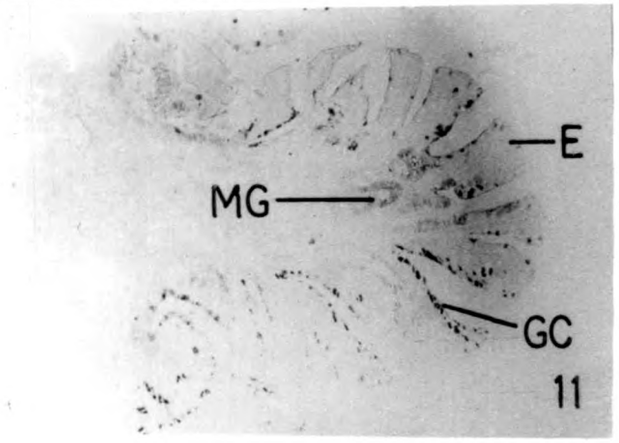
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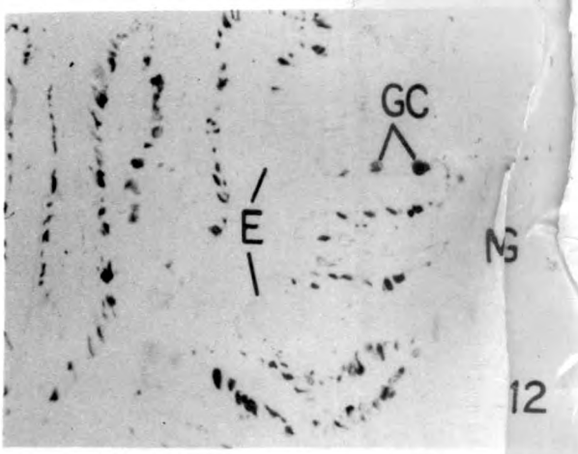
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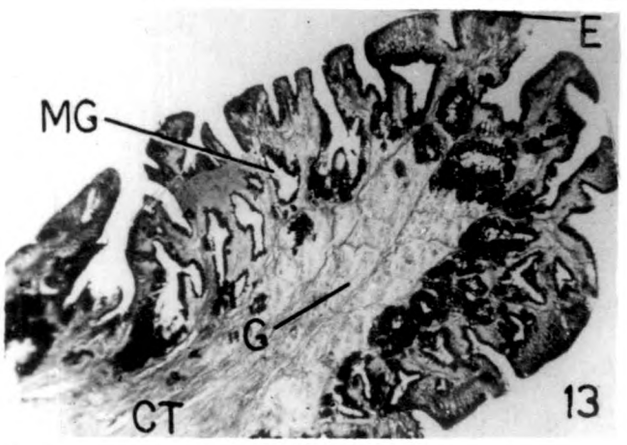
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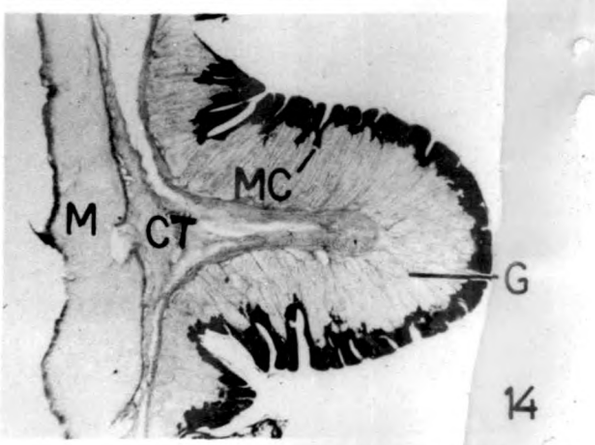
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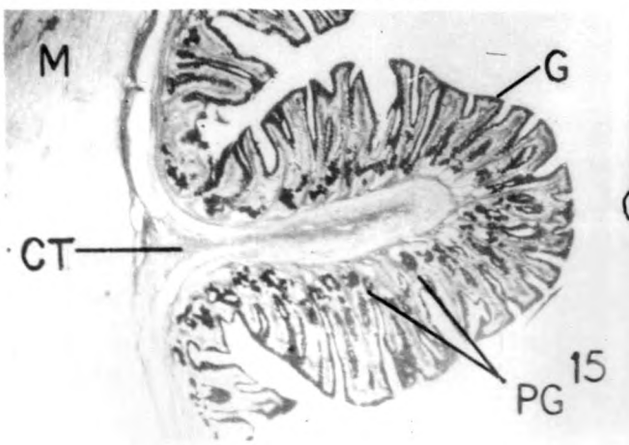
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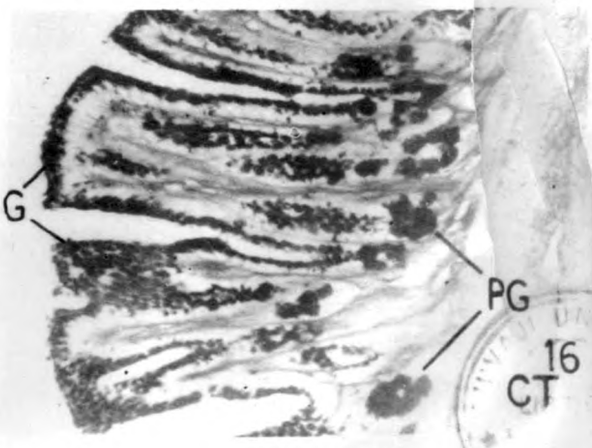
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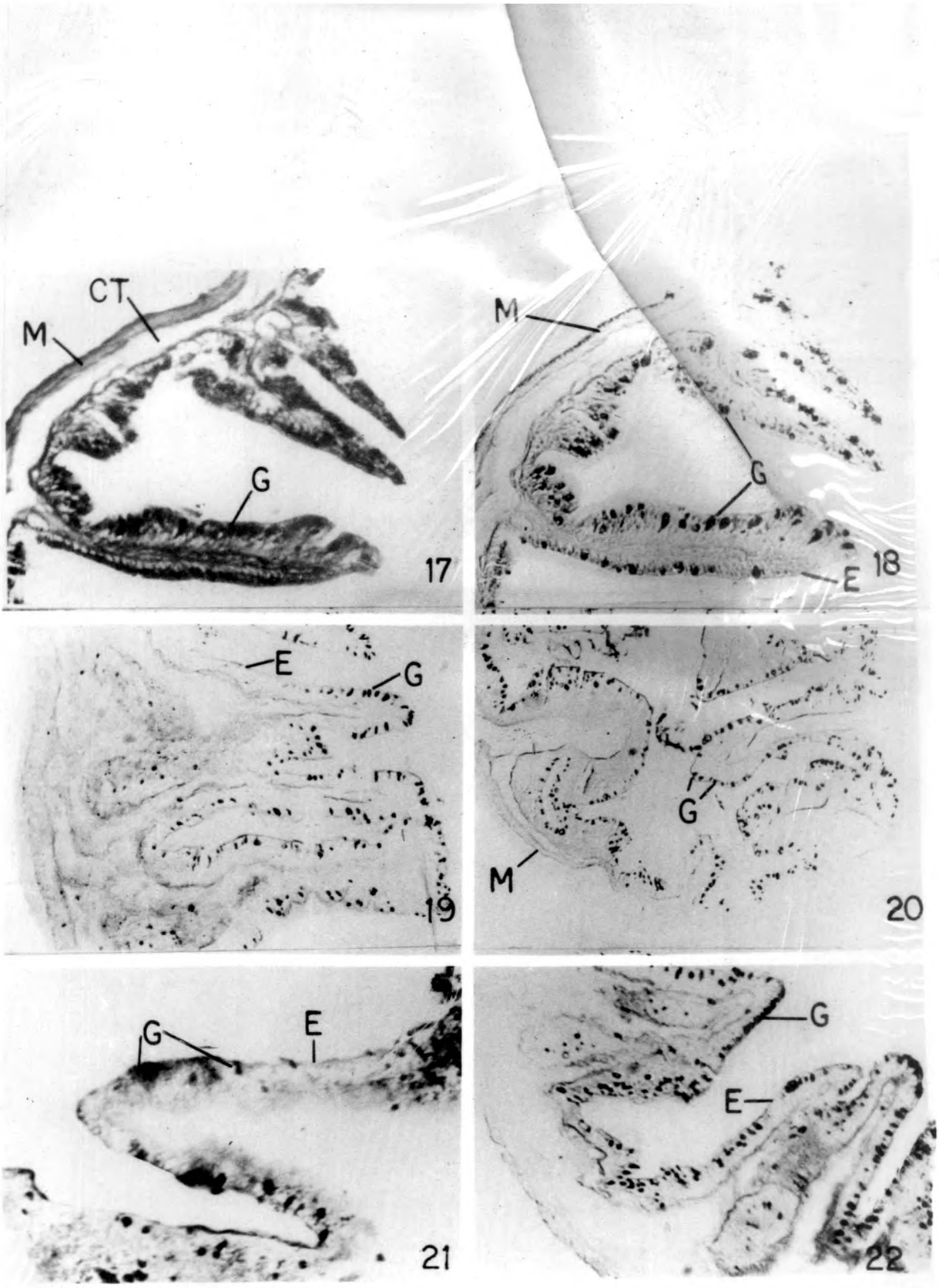

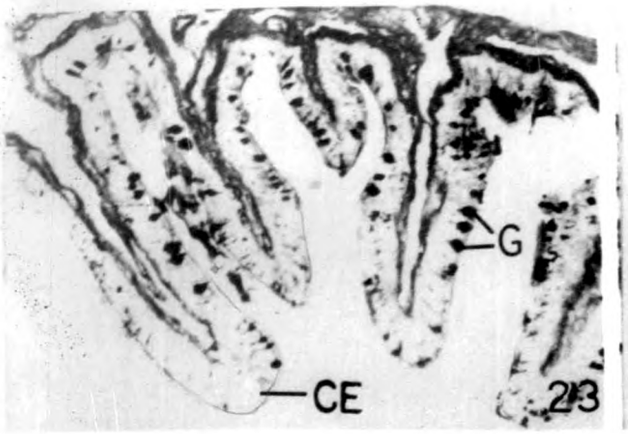
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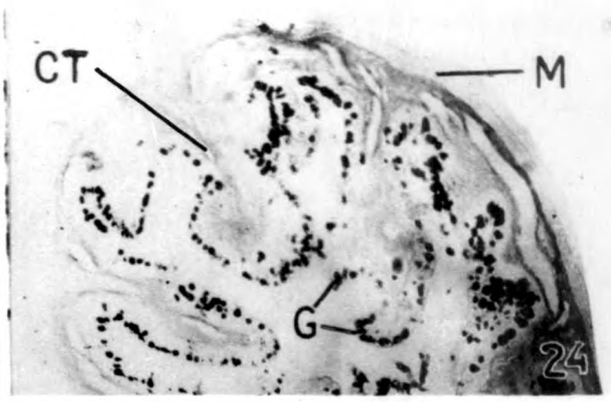
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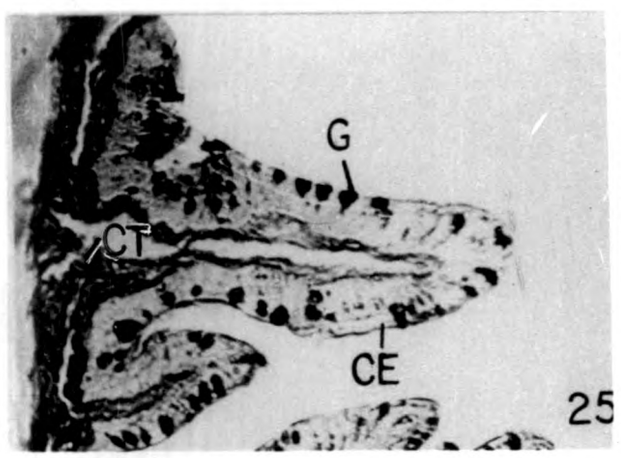
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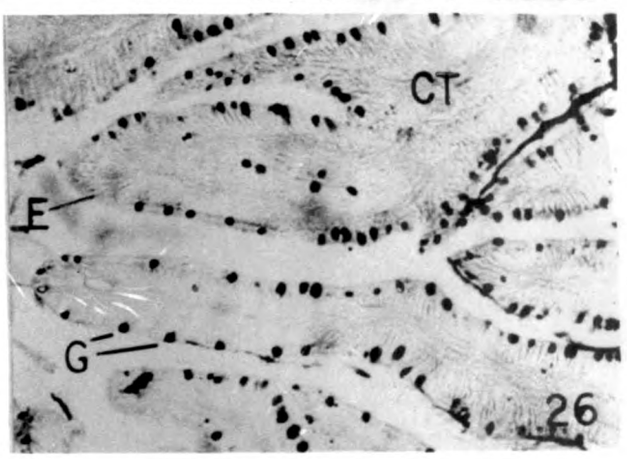
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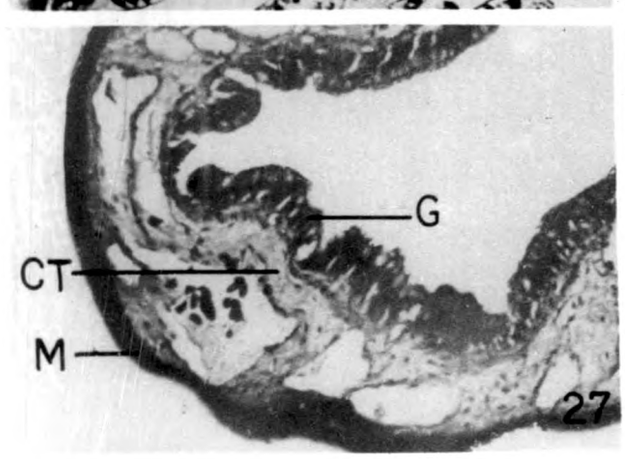
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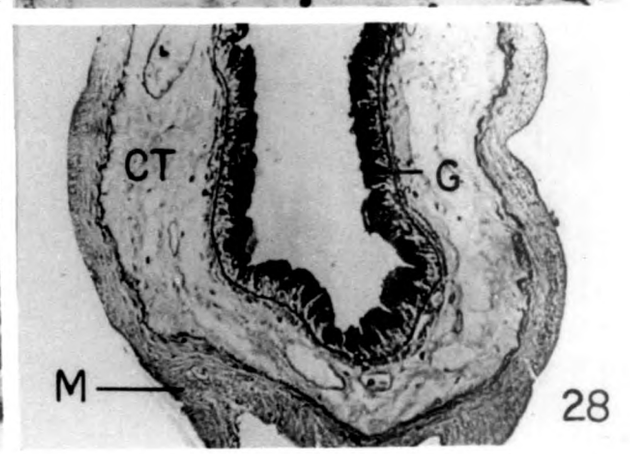
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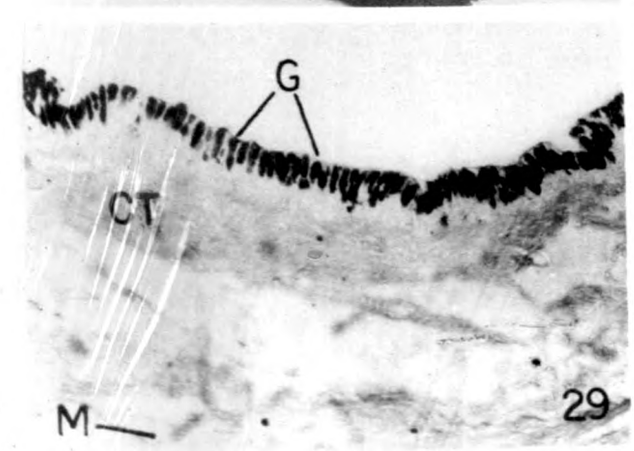
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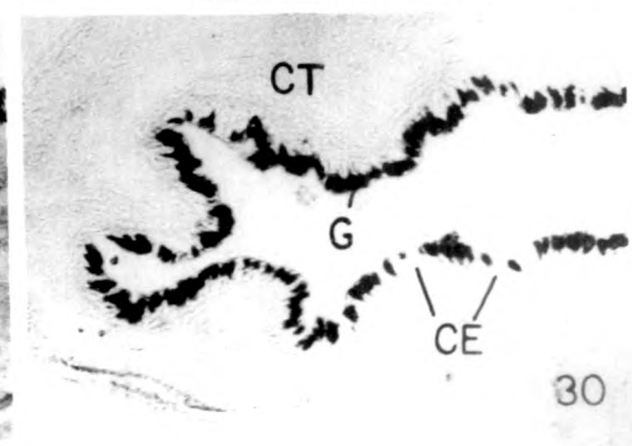
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initial staining reactivities indicated absence of glycogen but presence of neutral mucosubstances in them. These cells remained unstained with AB at pH 1.0 (Fig.6), AB pH 2.5 (Fig.8),C.I.and AF and exhibited only blue orthochromatic staining at higher pH levels. These histochemical results remained unaltered even after pepsin digestion. These histochemical results indicated the absence of acidic mucosubstances. The presence of neutral mucosubstances in these cells was also substantiated from their only PAS reactivity in sequential staining procedures such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. - PAS, there being no tinge of blue coloration.

ii) Goblet cells

Although the goblet cells appeared identical in H-E stained preparations, histochemically these cells could be distinguished into two types. Such a distinction was very clear in AB pH 1.0 stained preparations (Fig.6). The goblet cells which exhibited alcianophilia at pH 1.0 are referred to as type - I cells and those which remained unstained are referred to as type - II cells.With AB pH 1.0 - PAS staining procedure the type - I cells appeared blue and type - II cells pink or magenta.

a) Type - I goblet cells: These cells exhibited an intense PAS reaction (Figs.3,4). Their PAS reactivity was diastase resistant but could partly be blocked by phenylhydrazine pretreatment (Fig.5) indicating the absence of glycogen but presence of neutral muco-substances. Moreover, these cells exhibited moderate alcianophilia at pH 1.0 (Fig.6) and an intense alcianophilia at pH 2.5 (Fig.8) which indicated the presence of both sulfomucins (predominant)and carboxymucins(in less quantities).The presence of sulfomucins in them was also inferred from their moderate reactivity with AF

alone, intense 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 (it was restored but only partly). The sulfomucins were hyaluronidase resistant.

The presence of carboxymucins in these goblet cells was substantiated by their enhanced alcianophilia at pH 2.5 than at pH 1.0, intense C.I.reactivity, intense blue purple staining in AF - AB pH 2.5 sequence, enhanced metachromasia with azure A at pH 3.0 and above, and partial restoration of their alcianophilia after saponification of the previously methylated (active methylation) sections. The carboxymucins were identified as sialomucins since their alcianophilia was partly sensitive to acid hydrolysis and neuraminidase digestion. Hyaluronidase digestian and pepsin digestion had no effect on these tinctorial affinities. Thus the type - I cells contained neutral mucosubstances, sulfomucins and sialomucins.

b) Type - II goblet cells: These cells also exhibited an intense PAS reactivity (Figs.3,4) which could partly be blocked by prior phenylhydrazine treatment (Fig.5) but was unaffected by diastase digestion. These results indicated the presence of neutral mucosubstances but absence of glycogen in them. Moreover these cells remained unstained with AB at pH 1.0 (Fig.6) showing absence of sulfomucins in them. This conclusion was also supported by absence of metachromatic staining with azure A at low pH (pH 1.5),absence of alcianophilia in presence of 0.2 M Mg^{++} and reversible blockade of their alcianophilia in methylation - saponification techniques.

Their alcianophilia at pH 2.5 (Fig.8) indicated the presence of carboxymucins in them. The presence of carboxymucins in these goblet cells was also inferred from their intense blue staining with C.I.,purple-blue coloration with AB pH 2.5 - PAS and C.I. - PAS sequences, only blue staining in AF - AB pH 2.5 sequence, intense metachromasia with azure A at pH 3.0 and above,suppression of their alcianophilia in CEC technique by the addition of 0.1 M Mg^{++} and reversible blockade of their alcianophilia by mild methylation - saponification and active methylation - saponification procedures. The carboxymucins were further identified as sialomucins as acid hydrolysis and neuraminidase digestion completely abolished their alcianophilia. Hyaluronidase and pepsin digestion had no effect on the aforementioned histochemical results. Thus the type - II goblet cells elaborated neutral mucosubstances and sialomucins.

iii) Mucous glands

In the esophageal mucous glands also histologically all the cells appeared identical (Figs.1,2) but the histochemical studies revealed the presence of two types of cells. Such a distinction was very clear in the AB pH 1.0 stained preparations,wherein type - I goblet cells exhibited moderate alcianophilia but the type - II cells remained unstained (Fig.7).

a) Type I cells - These cells in the esophageal glands (Figs. 3,4,5,7,8) exhibited histochemical reactivities similar to those described for type - I goblet cells in the mucosal epithelium indicating the presence of neutral mucosubstances,sulfomucins and sialomucins.

b) Type - II cells - These cells in the esophageal glands

(Figs.3,4,5,7,8) exhibited tinctorial affinities very similar to the type - II goblet cells in the epithelium and thus indicated the presence of neutral mucosubstances (less amount) and sialomucins (predominant).

II)Connective tissue in submucosa :

The connective tissue showed poor to weak PAS reactivity (Figs.3,4). Their PAS reactivity was resistant to diastase but could partly be blocked by phenylhydrazine pretreatment.Moreover the connective tissue remained unstained with AB at pH 1.0 and appeared only pink to magenta in AB pH 1.0 - PAS sequence indicating the absence of sulfomucins in them. Their alcianophilia at pH 2.5 and C.I.reactivity revealed the presence of carboxymucins in the connective tissue.

This conclusion was further substantiated from their purple - blue staining with AB pH 2.5 - PAS, and C.I. - PAS sequences, only blue staining with AF - AB pH 2.5 sequence,suppression of their alcianophilia in CEC technique by the addition of 0.1 M Mg^{++} and reversible blockade of their alcianophilia in methylation-saponification techniques. Loss of alcianophilia of connective tissue following hyaluronidase digestion indicated the presence of hyaluronic acid. Metachromasia with azure A at and above pH 4.5 also indicated the presence of hyaluronic acid in the connective tissue. Acid hydrolysis, neuraminidase and pepsin digestions had no effect on these staining reactivities. These results indicated the presence of neutral mucosubstances and hyaluronic acid in the connective tissue.

III) Muscularis

Both circular and longitudinal muscles 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, C.I. and AF and exhibited only blue orthochromatic staining with azure A thus, showing the absence of acidic mucosubstances. The muscles appeared only weak pink to magenta in sequential staining techniques such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I.- PAS.

IV) Serosa

The serosa exhibited weak staining with PAS which was diastase resistant but could completely be blocked by phenylhydrazine pretreatment. This layer exhibited only weak pink to magenta coloration with AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. -PAS sequential staining procedures but remained unstained with AB pH 1.0, AB pH 2.5, C.I. 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.

Histochemical observations also indicated absence of sexual dimorphism in the esophagus.

2. Cardiac stomach

The stomach in the frog under present investigation appeared as a simple sac-like dilation of the alimentary tract. The first part of the stomach next to the esophagus was the cardiac stomach. The cardiac stomach was more wider than the pyloric stomach, the distal part of the stomach which opens into the duodenum. The stomach was relatively thin walled.

A) Histological observations

The cardiac stomach was relatively thin walled and consisted of strongly glandular mucosa, thin submucosa and well organized muscular coat (Fig.14). In cross section the cardiac stomach was circular in outline. The mucosa of the cardiac stomach was thrown into broad, longigudiual and branched folds. These are also called as gastric rugae (Figs.10,13,14). The single layered epithelium of the gastric mucosa consisted of non-ciliated columnar epithelial cells and goblet cells (Figs.9,10,11,12). The goblet cells increased in number towards the pyloric region and the epithelium appeared to be formed entirely of goblet cells(Figs.13,14).

In the proximal part (next to the esophagus) of the cardiac stomach contained mucous glands, wherein all the cells appeared mucous in nature(Fig.10). This region appeared transitional between the esophagus and the main glandular stomach. In middle region of the cardiac stomach the superficial mucous glands were observed and gastric glands in the deeper region (Fig.13). The region near the pyloric stomach contained the so-called gastric glands with mucous neck cells near gastric pit region and cells in the deeper region of the gastric glands(Figs.9,14). These cells in the deeper region are comparable to the cells described in the gastric glands of the other amphibians which secrete pepsinogen and acid (HCl). Some investigators consider these cells as zymogen cells.

The connective tissue in the submucosa was thin (Fig.14)which also appeared to form the central core of the rugae. The muscularis consisted of comparatively thick circular muscle layer and some what thinner longitudinal muscles. The outermost layer consisted of a typical serosa.

Sexual dimorphism was not evident in the cardiac stomach.

B) Histochemical observations

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

I) Mucosa

i) Epithelial cells: The simple cuboidal to columnar epithelial cells in the mucosa of the cardiac stomach exhibited poor PAS reactivity (Fig.10). 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, AB pH 2.5 - PAS and C.I.- 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 (Fig.11), AB at pH 2.5 (Fig.12), C.I., AF and only blue orthochromasia with azure A at higher pH levels. These results could not be altered by pepsin digestion. These results indicated the presence of neutral mucosubstances in the epithelial cells.

ii) Goblet cells

The goblet cells in the mucosa of the cardiac stomach reacted intensely towards PAS (Figs.10,13,14). Their PAS reactivity remained

unchanged after diastase digestion but was partly blocked by prior phenylhydrazine treatment, thus indicating the absence of glycogen but presence of neutral mucosubstances in them.

Unlike the esophagus all the goblet cells in the cardiac stomach were identical. Their moderate alcianophilia at pH 1.0 (Fig.11) indicated the presence of sulfomucins which were predominant. The enhanced alcianophilia at pH 2.5 (Fig.12) 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, 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 (it was restored but partly). The sulfomucins were hyaluronidase resistant.

The enhanced alcianophilia at pH 2.5 than at pH 1.0 indicated the presence of carboxymucins in the goblet cells. This conclusion was substantiated by their intense C.I.reactivity,purple-blue staining with AB pH 2.5 - PAS and C.I. - PAS sequences,blue-purple staining in AF - AB pH 2.5 sequence, enhanced metachromasia 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 sialomucins since their alcianophilia was partly sensitive to acid hydrolysis and neuraminidase digestion. Thus, the aforementioned results indicated the presence

of neutral mucosubstances (less amount), sulfomucins (predominant) and sialomucins (less amount).

iii) Mucous glands : The cells in the mucous glands in the cardiac stomach exhibited intense PAS reactivity (Figs.10,13) which was unaffected by diastase digestion but could partly be blocked by prior phenylhydrazine treatment. After phenylhydrazine treatment only poor PAS reactivity was evident in these glandular cells which indicated the presence of neutral mucosubstances (predominant) but absence of glycogen.

The cells in the mucous glands were poorly stained with AB at pH 1.0(Fig.11) and their staining intensity was not enhanced at pH 2.5(Fig.12). 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 mucosubstances(predominant) and sulfomucins(traces) in the glandular cells of the mucous glands.

iv) Gastric glands

a) Mucous neck cells

The cells which are present near the gastric pit of the glands - the mucous neck cells - exhibited diastase resistant intense PAS reactivity (Fig.14) which was completely blocked by

phenylhydrazine pretreatment. These initial staining reactivities revealed the presence of only neutral mucosubstances rich in vic - glycols but absence of glycogen in them. Moreover, these cells exhibited only pink-magenta coloration with AB pH 1.0 - PAS, AB pH 2.5 -PAS and C.I.-PAS sequences and remained unstained with AB pH 1.0, AB pH 2.5, C.I. and AF. These cells showed only blue orthochromatic staining with azure A at higher pH(pH 3.0 and above). These results could not be modified following pepsin digestion. Thus the mucous neck cells of the gastric glands contained only neutral mucosubstances.

b) The cells in deeper region of the gastric glands (zymogen cells)

These cells remained unstained with all the histochemical techniques including PAS (Figs.13,14) indicating the absence of mucosubstances both neutral and acidic.

II) Connective tissue in submucosa

The connective tissue in the cardiac stomach (Figs.10 to 14) contained neutral mucosubstances and hyaluronic acid. This conclusion was based on the staining reactivities of the connective tissue which were practically identical to those described for the connective tissue in the esophagus.

III) Muscularis

The circular and longitudinal muscles in the wall of the cardiac stomach (Fig.14) contained glycogen but not acidic or other neutral mucosubstances. Their histochemical reactivities were similar to those exhibited by the esophageal circular and longitudinal muscles.

IV) Serosa

The serosa of the cardiac stomach (Fig.14) contained only diastase resistant PAS reactive neutral mucosubstances. The tinctorial affinities of the serosa in cardiac stomach were identical to those exhibited by the esophageal serosa.

Results obtained with histochemical techniques also indicated absence of sexual dimorphism in cardiac stomach.

3. Pyloric stomach

A) Histological observations

The pyloric stomach was relatively short, thin walled and circular in cross section. The pyloric stomach was narrow in diameter than the cardiac stomach. The pyloric stomach was also consisted of strongly glandular mucosa, thin submucosa,muscular coat and serosa. The mucosa was thrown into broad folds which projected into the lumen (Fig.15). The folds were branched and were continuous with the folds or rugue in the cardiac stomach. The single layered epithelium of the pyloric mucosa contained mainly goblet cells (Figs.15,16). The non-ciliated columnar or cuboidal cells could not be distinguished even in histologically stained preparations. Pyloric glands were well organized. The cells of these glands were mucoid and histologically resembled to the goblet cells in the surface epithelium except the cells were bigger in size(Fig.16). The connective tissue in the submucosa was thin and also extended into the rugae or folds (Fig.15). As in the cardiac stomach the muscularis consisted of thick circular muscle layer towards inner side and relatively thinner

layer of outer longitudinal muscles. The outer-most layer of the pyloric stomach consisted of a typical serosa. Similar histology was also seen for pyloric stomach of males and females.

B) Histochemical observations

The results obtained with various histochemical staining techniques for pyloric mucosubstances of the frog are recorded in Table No.4 and the histochemical distribution of mucosubstances is shown in photomicrographs(Figs.15,16).

I) Mucosa

a) Goblet cells

The goblet cells in the surface epithelium of the pyloric mucosa exhibited an intense PAS reactivity (Figs.15,16). Their PAS reactivity could completely be blocked by phenylhydrazine pretreatment but remained unaffected after diastase digestion indicating the absence of glycogen in them but the presence of only neutral mucosubstances rich in vic - glycols.The absence of acidic mucosubstances in them was inferred from their negative reactivity towards AB at pH 1.0, AB at pH 2.5, C.I.and AF and only blue arthochromatic staining with azure A at pH 3.0 and above. The conclusion that the goblet cells contain only neutral mucosubstances was further supported from their only pink to magenta coloration with sequential staining procedures such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I.- PAS. Similar results were also obtained even after pepsin digestion. These aforementioned histochemical results indicated the presence of only neutral mucosubstances in the goblet cells of the surface epithelium of pyloric stomach.

b) Pyloric glands

The secretary cells in the pyloric glands also exhibited an intense PAS reactivity (Figs.15,16),the intensity of which could not be reduced following diastase digestion but their PAS reactivity could completely be blocked by prior phenylhydrazine treatment. These preliminery observations revealed the presence of neutral mucosubstances but absence of glycogen in the pyloric gland cells. The negative staining with AB pH 1.0, AB pH 2.5,C.I.and AF and only blue orthochromasia with azure A at pH 3.0 and above confirmed the absence of acidic mucosubstances. Identical results even after pepsin digestion substantiated this conclusion. The presence of only neutral mucosubstances in pyloric gland cells was further supported by their only pink to magenta staining with sequential staining procedures such as AB pH 1.0 - PAS, AB pH 2.5 -PAS and C.I.- PAS. Thus the pyloric gland cells also contained only neutral mucosubstances as the goblet cells in the surface epithelium of the pyloric mucosa.

II) Connective tissue in submucosa

The connective tissue in the pyloric stomach (Figs.15,16) also contained neutral mucosubstances and hyaluronic acid. This conclusion was derived from their histochemical reactivities towards different histochemical techniques which were identical to those described for esophageal connective tissue and the connective tissue in the cardiac stomach.

III) Muscularis

The circular and longitudinal muscle layers of the pyloric stomach also contained glycogen. This conclusion was based on their

staining affinities towards different dyes which were practically identical to those exhibited by the muscle layers of esophagus and cardiac stomach.

IV) Serosa

The histochemical reactivities of the serosa of the pyloric stomach were very much identical to those exhibited by the serosa of the esophagus or cardiac stomach. Thus as in the esophagus and cardiac stomach, the serosa of the pyloric stomach contained only diastase resistant and PAS reactive neutral mucosubstances rich in vic - glycols.

Histochemical results also showed absence of sexual dimorphism in pyloric stomach.

4) Duodenum

A) Histological observations

The small intestine is long,narrow and coiled tube. The first part of the intestine is called as duodenum. The part of the small intestine which immediately follows the stomach is called as duodenum. It is here that the ducts from liver and pancreas open. The duodenum was tubular and circular in transverse section. The mucosa was thrown into elongated finger like projections or leaf like ridges known as villi which projected into the lumen (Figs.17,18). The mucosa consisted of single layered cells which could be distinguished into the columnar epithelial cells and the goblet cells (Figs.17 to 22). Crypts in between the villi and the glands were found to be absent in the duodenum of this frog. The submucosa containing the connective tissue was very thin(Figs.17,18). The muscularis was also comparatively thinner (Figs.17,18) and

consisted of inner circular muscle layer and outer longitudinal muscle layer. As in esophagus and stomach a typical serosa formed the outermost layer of the duodenum. Histological observations revealed absence of sexual dimorphism in duodenum.

B) Histochemical observations

The results obtained with various histochemical techniques for the duodenal mucosubstances are recorded in Table No.5 and the histochemical distribution of mucosubstances is shown in photomicrographs (Figs.17 to 22).

I) Mucosa

i) Epithelial cells

The columnar epithelial cells in the mucosa of the duodenum showed weak to moderate PAS staining (Fig.17). Their PAS reactivity resisted diastase digestion but could completely be blocked by phenylhydrazine pretreatment. These initial staining reactivities revealed the presence of only neutral mucosubstances rich in vic - glycols but absence of glycogen in the epithelial cells.Moreover these cells exhibited only PAS reactivity in sequential procedures such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I.-PAS.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, C.I.and AF and only blue orthochromatic staining with azure A at pH 3.0 and above. These results could not be altered by pepsin digestion. These results indicated the presence of only neutral mucosubstances in the columnar epithelial cells.

ii) Goblet cells

The goblet cells in this region differed in their staining

reactivities as compared to the goblet cells in the previous regions. The goblet cells in this region were intensely stained with PAS (Fig.17) even after phenylhydrazine pretreatment (Fig.18) and diastase digestion. These preliminery observations indicated the absence of glycogen and neutral mucosubstances in them. Moreover their moderate alcianophilia at pH 1.0(Fig.19) and intense alcianophilia at pH 2.5 (Fig.20) indicated the presence of both sulfomucins and carboxymucins in them.

The presence of sulfomucins in them was inferred from their purple-blue staining with AB pH 1.0 - PAS purple staining with AF (Fig.22) blue purple staining with AF - AB pH 2.5 sequence, persistant alcianophilia in CEC technique in presence of 0.5 M Mg^{++} and,partial loss of the alcianophilia after active methylation which was failed to restore (it was restored but only partly).The sulfomucins were hyaluronidase resistant.

The carboxymucins in them was inferred from their enhanced alcianophilia at pH 2.5,C.I.reactivity of the same intensity (Fig.21),only blue staining with AB pH 2.5 - PAS and C.I. -PAS, enhanced metachromasia with azure A at and above pH 3.0 and partial restoration of their alcianophilia following saponification of the previously methylated (active methylation) sections. Partial loss of their alcianophilia following acid hydrolysis and neuraminidase digestion indicated the presence of sialic acid in them. These results indicated the simultaneous occurrence of sulfomucins and sialomucins in the goblet cells.

II) Connective tissue in submucosa

The connective tissue in the submucosa of the duodenum

reacted moderately with PAS (Fig.17). Their PAS reactivity was diastase resistant but could partly be blocked by phenylhydrazine pretreatment(Fig.18). These initial staining reactivities indicated the presence of neutral mucosubstances but absence of the glycogen in the connective tissue.

Moreover its poor alcianophilia at pH 1.0(Fig.19) revealed the presence of sulfomucins. The presence of the sulfomucins in this layer was also inferred from blue purple staining with AB pH 1.0 - PAS sequence, purple staining with AF (Fig.22),weak purple-blue staining with AF-AB pH 2.5 sequence, poor metachromasia with azure A at lower pH (pH 1.5), persistant poor alcianophilia in CEC technique in presence of 0.2 M Mg^{++},and abolishing of their alcianophilia by active methylation which could not be restored completely following subsequent saponification (alcianophilia was restored but only partly).

Enhanced alcianophilia at pH 2.5 than at pH 1.0 indicated the presence of carboxymucins in the connective tissue. This conclusion was further substantiated by their purple-blue staining with AF - AB pH 2.5 sequence, enhanced metachromasia with azure A at and above pH 4.5 and partial restoration of their alcianophilia after saponification of the previously methylated sections. Hyaluronidase digestion partly reduced the alcianophilia in the connective tissue which indicated the presence of hyaluronic acid. These aforementioned histochemical results indicated the presence of neutral mucosubstances, sulfomucins and hyaluronic acid.

III) Muscularis

As in esophagus and stomach(cardiac and pyloric),the

circular and longitudinal muscle layers in the duodenum (Figs.17,18) also contained glycogen. The muscle layers exhibited histochemical reactivities which resembled to those described for muscle layers in esophagus and stomach.

IV) Serosa

The tinctorial affinities of the serosa of the duodenum (Figs.17,18,20,22) were practically identical to those exhibited by the serosa of the esophagus,cardiac stomach and pyloric stomach. Thus it was concluded that the serosa in the deodenum contained diastase resistant and PAS reactive neutral mucosubstances rich in vic - glycols.

Histochemical results also showed absence of sexual domorphism in duodenum.

5. Small intestine (ileum)

Small intestine was a long, narrow coiled tube. This region behind the duodenum is also called as ileum. It is the most important part of the digestive tract because the bulk of digestive activities are carried to conclusion in it.

A) Histological observations

The small intestine or ileum was circular in cross section (Figs.23,24). It was comparatively thin walled. The mucosa was thrown into long and broad villi-like folds which projected into the lumen(Figs.23,24). The epithelium of the mucosa was single layered and contained columnar epithelial cells and goblet cells (Figs.23 to 26). Glands were found to be absent in the small intestine.The connective tissue in the submucosa was thin (Figs.23 to 26) and it also extended in the villi-like folds to form the core of

the folds. The submucosa was surrounded by circular and longitudinal muscle layers forming the muscularis. Surrounding the muscularis was a typical serosa. Histology of the small intestine was identical both in males and females.

B) Histochemical observations

The results obtained with various histochemical techniques for the mucosubstances in the small intestine are recorded in Table No.6 and the histochemical distribution is shown in photomicrographs (Figs.23 to 26).

I) Mucosa

i) Epithelial cells

The columnar epithelial cells in the mucosa of the small intestine exhibited poor to weak PAS reactivity (Fig.23). Their PAS reactivity could completely be blocked by prior phenylhydrazine treatment but was diastase resistant indicating the absence of glycogen but presence of neutral mucosubstances in them. This conclusion was further supported by their only pink to magenta coloration with sequential staining techniques such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. - PAS, and only blue orthochromatic staining with azure A at higher pH levels. The absence of acidic mucosubstances in the columnar cells was inferred from their negative reactivity with AB pH 1.0 (Fig.24), AB pH 2.5 (Fig.25), C.I. and AF (Fig.26). Pepsin digestion had no effect on these staining reactivities.

ii) Goblet cells

The goblet cells in the intestinal mucosa showed intense PAS reaction (Fig.23).Their PAS reactivity was resistant to both

phenylhydrazine pretreatment and diastase digestion which indicated the absence of both glycogen and neutral mucosubstances in them.

Their moderate alcianophilia at pH 1.0 (Fig.24) indicated the presence of sulfomucins and intense alcianophilia at pH 2.5 (Fig.25) indicated the presence of carboxymucins in them. The presence of sulfomucins in the intestinal goblet cells was supported from their intense purple staining with AF (Fig.26),blue-purple staining with AF - AB pH 2.5 sequence, moderate metachromasia with azure A at lower pH (pH 1.5),persistant weak alcianophilia in CEC technique in the presence of 0.5 M Mg^{++} and abolishing of their alcianophilia by active methylation which could not be restored completely after subsequent saponification (it was restored but only partly). The sulfomucins were hyaluronidase resistant.

The conclusion that the intestinal goblet cells also contain carboxymucins was based on their intense blue staining with C.I., only blue staining with AB pH 2.5 - PAS and C.I.-PAS sequences, blue-purple staining with AF - AB - pH 2.5 sequence,enhanced metachromasia with azure A at and above pH 3.0 and partial restoration of their alcianophilia after saponification of the previously methylated sections; Partial reduction of their alcianophilia following acid hydrolysis and neuraminidase digestion indicated the presence of sialomucins in them. These results revealed the simultaneous occurrence of sulfomucins and sialomucins in the goblet cells of the small intestine.

II) Connective tissue in submucosa

The connective tissue in the submucosa of the small intestine

(Figs.23 to 26) contained neutral mucosubstances, sulfomucins and hyaluronic acid. This conclusion is based on the staining reactivities of the connective tissue which resembled to that described for the connective tissue of the duodenum.

III) Muscularis

The circular and longitudinal muscles in the wall of small intestine contained glycogen but not acidic and other neutral mucosubstances. Their histochemical reactivities were similar to those described for muscularis of esophagus,stomach and duodenum.

IV) Serosa

The staining reactivities of the serosa of the small intestine were identical to those described for serosa of esophagus,cardiac stomach,pyloric stomach and duodenum. Hence it was concluded that the serosa in intestine also contained only diastase resistant PAS reactive neutral mucosubstances.

The aforementioned results were identical in the small intestine of males and females.

6. Large intestine

A) Histological observations

The large intestine had slightly larger diameter than the small intestine. It was also tubular and was shorter in length. Histologically it could be distinguished from the small intestine by the lack of high slender folds(Figs.27 to 30) short broad folds may be present but often they are very low(Figs.28,30). The epithelial lining of the mucosa consisted of abundant goblet cells. (Figs.27 to 30) with occasional columnar cells scattered through-

out. The connective tissue layer in the submucosa was comparatively thick (Figs.28 to 30). The muscularis was thin (Figs.27,28).Surrounding the muscularis was a typical thin serosa.Histology of the large intestine was similar in male and female frogs.

B) Histochemical observations

The results obtained with various histochemical techniques for the mucosubstances in the large intestine are recorded in Table No.7 and the histochemical distribution is shown in photomicrographs (Figs.27 to 30).

I) Mucosa

i) Epithelial cells

The columnar epithelial cells in the mucosa of the large intestine showed poor to weak PAS staining (Fig.28). Their PAS staining was resistant to diastase digestion but could completely be blocked by prior phenylhydrazine treatment, thus showing the absence of glycogen but presence of only neutral mucosubstances rich in vic - glycols.This conclusion was substantiated by their only pink to magenta staining with sequential staining procedures such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. - PAS and only blue orthochromatic staining with azure A at pH 3.0 and above.

These cells remained unstained with AB at pH 1.0 (Fig.29), AB at pH 2.5 (Fig.30), C.I. and AF even after pepsin digestion which indicated absence of acidic mucosubstances in them.

ii) Goblet cells

The goblet cells in the mucosa of the large intestine exhibited intense PAS reactivity (Fig.28) which was diastase

resistant but could only partly be blocked by phenylhydrazine pretreatment. These initial staining results showed the presence of neutral mucosubstances (traces) but absence of glycogen.

Moreover, these goblet cells exhibited moderate to intense alcianophilia at pH 1.0 (Fig.29) and there was no enhancement in alcianophilia at pH 2.5 (Fig.30) which indicated the presence of only sulfomucins (predominant) and absence of carboxymucins.The presence of sulfomucins was also concluded from their intense purple-blue staining with AB pH 1.0 - PAS, moderate to intense purple staining with AF alone or with AB pH 2.5 step afterwards,metachromasia with lower pH (pH 1.5), persistant alcianophilia in CEC technique in the presence of 0.5 M Mg^{++} and irreversible loss of their alcianophilia with methylation(active) - saponification procedure. The sulfomucins were hyaluronidase resistant. Thus, the goblet cells in the large intestine contained neutral mucosubstances (less concentration) and sulfomucins(predominant).

II) Connective tissue in submucosa

The connective tissue in the large intestine (Figs.28 to 30) contained neutral mucosubstances and hyaluronic acid. This conclusion was based on the staining reactivities of the connective tissue which were practically identical to those described for the connective tissue in the esophagus,cardiac stomach and pyloric stomach.

III) Muscularis

The circular and longitudinal muscles in the wall of the large intestine (Fig.28) contained glycogen. Their tinctorial affinities were identical to those exhibited by the muscle layers

in esophagus,stomach,duodenum and small intestine.

IV) Serosa

The histochemical reactivities of the serosa of the large intestine (Figs.28 to 30) were practically similar to the serosa of esophagus,stomach,duodenum and intestine. Hence it was concluded that the serosa contained diastase resistant PAS reactive neutral mucosubstances.

Histochemical results revealed the absence of sexual dimorphism in large intestine of the frogs investigated.

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