

observations

CHAPTER THREE

OBSERVATIONS

HISTOLOGY AND HISTOCHEMISTRY OF MICOSUBSTANCES IN -

- A. OESOPHAGUS
 - B. PROVENTRICULUS
 - C. GIZZARD
 - D. DUODENUM
 - E. SMALL AND LARGE INTESTINE
 - ⁸
F. OF A.phoenicurus phoenicurus
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Since the aim of the investigation undertaken was to find out the nature and distribution of mucosubstances in various organs of alimentary tract of A.phoenicurus phoenicurus, the most popular, atleast most frequently used system of histochemical quantitation was employed which involves grading the strengths of the reactions somewhat subjectively during visual examination. A system of plusses was used to represent the strengths of the reactions, for e.g., +++ describes very intense reaction, + describes poor reaction while - is assigned to a negative reaction. This must surely be the crude but most widely used method of histochemical quantitation, and it requires no special instrumentation but special practice.

The histochemical results are recorded in tabulated forms while histological sites and histochemical distribution of mucosubstances in the various organs are illustrated in microphotographs. The observations are taken organwise from oesophagus to large intestine and also layerwise viz. mucosa (innermost) to serosa (outermost), specific tissue-components especially in mucosa are considered separately in the observation tables. Alimentary tract of A.phoenicurus phoenicurus → Anatomy in brief - It essentially consists of a tubular duct varying in morphology, histology and physiology. It extends from the beak anterior to the external orifice of the cloaca posterior. The pharynx can also be considered as a part of respiratory system and cloaca is shared with urinary and reproductive systems. The alimentary tract, in turn, can be

subdivided into several regions like beak, mouth, buccal cavity, tongue, pharynx, oesophagus, crop, proventriculus (glandular stomach), ventriculus (grinding stomach), small intestine, caeca and large intestine ending into a cloaca which is a common chamber for digestive, excretory and reproductive tract. The alimentary tract has a similar basic pattern of structure to which all organs therein conform to a greater or lesser extent.

The entire length of the alimentary tract from mouth to cloaca measured 130 cms., which in pond heron (A. grayii) was reported to be 113-120 cms (Malvadkar, 1985). Beak was long, stout and suitably modified to capture living/non-living food from aquatic/semi-aquatic habitat. The tongue was long, less massive narrow and triangular in shape. Oesophagus was a long, narrow, muscular, highly distensible tube extending from glottis at the posterior end of pharynx through the neck and thorax to join with the proventriculus. Gastric apparatus, as in other birds, had dual structure viz. anterior (proximal) proventriculus and posterior (distal) ventriculus (gizzard). Proventriculus was glandular, narrow proximally but considerably dilating towards distal end. Ventriculus was stout, highly muscular structure resembling flattened sphere. Intestine was noticeably long, narrow coiled tube comprising small intestine extending from ventriculus upto origin of caeca and large intestine extending from the point where caeca open into intestine upto the external orifice (anus). Small intestine intum consisted of proximal most a few cms of duodenum very next to ventriculus

and ileum as distal remaining part (no external demarkation). Ileum in distal region had thin nature (muscularis less massive). Paired caeca were narrow, moderately developed originating at the junction of small and large intestine. Large intestine was more muscular as compared to small intestine. The cloaca was posterior most continuation of the large intestine.

For each organ, the observations were taken from three points of views, viz. I) histology, II) histochemistry, III) Sexual dimorphism.

A) OESOPHAGUS :

I) HISTOLOGICAL OBSERVATIONS :

It was a narrow, muscular highly distensible tube. In its normal contracted stage, the tube appeared narrow reducing the lumen furthermore. In fixed condition, epithelial lining and its underlying layers became highly folded. Histologically, as usual, it had four tunics viz. mucosa (mucous membrane), submucosa, muscularis (muscular coat) and serosa (Fig.No. 9), The mucosa was composed of thick stratified epithelium (squamous) and mucous glands, having openings into the lumen by simple ducts passing through epithelium lining. The submucosa was prominent and extended into mucosal folds forming core. Muscularis as usual showed inner circular and outer longitudinal muscles. Serosa exhibited a layer of loose adventitial connective tissue with blood vessels and nerve fibers (tunica adventitia).

II) HISTOCHEMICAL OBSERVATIONS :

The histochemical reactivities of various mucosubstances in the oesophagus of A.phoenicurus phoenicurus are recorded in Table No. 2 according to the visually estimated intensities of stains and shades, while the distribution of mucosubstances is shown in microphotographs. Some of the observations are described in details.

1) MUCOSA :

i) STRATIFIED EPITHELIUM :

The cells showed poor PAS staining (Fig.No. ?), which was diastase labile and could be blocked by Ph-PAS techniques, thus indicating presence of glycogen therein. The cells remained unstained with AB-pH 1, AB-pH 2.5 and AF and these results remained unchanged even after pepsin digestion, thus indicating absence of acidic mucins. The cells exhibited blue orthochromatic staining in trace with Azure A-pH 5.

ii) MUCOSAL GLANDS :

The glandular epithelial cells reacted intensely with PAS (Fig.No. ?) and PAS activity was found to be resistant to D-PAS, but slightly labile to Ph-PAS. These initial results indicated presence of neutral mucins. The glands exhibited weak alcianophilic at pH 1.0, which became enhanced at pH 2.5 (intense staining), thus indicating presence of both sulfo and carboxymucins (Fig.No. ? and ?). The glands revealed purple-blue staining with AF-AB (pH 2.5) inferring presence of sulfomucins

supported by other results, like weak to moderate metachromasia with Azure A, graded decrease in alcianophilia in CaC technique and loss of alcianophilia after active methylation. The sulfomucins were hyaluronidase resistant.

The presence of carboxymucins was inferred from their purple blue colouration with AF - AB (pH 2.5), enhanced metachromasia with Azure A (weak to moderate), and partial restoration of alcianophilia after saponification of methylated sections. The alcianophilia (AB pH 2.5) was partially sensitive to acid hydrolysis and neuraminidase suggesting presence of carboxymucins in the form of sialomucins. Hyaluronidase and pepsin digestion had no effect on the staining intensities. To summarise, mucosal glands exhibited presence of neutral, sulfo and sialomucins.

2) SUBMUCOSA :

The connective tissue in submucosa exhibited weak reactivity with PAS, which was diastase resistant but Ph-PAS treatment blocked it. The layer remained unstained with AB-pH 1, AB-pH 2.5 and AF also, however it showed weak pink to magenta colouration with sequential staining techniques (AB pH 1 - PAS, AB pH 2.5 - PAS) (Fig.No. 7). So also with Azure A, at high pH level (viz.), orthochromatic staining was evident. These results indicated presence of only neutral mucosubstances in submucosa.

3) MUSCULAR COAT :

Both circular and longitudinal muscles showed weak/poor

Table No. 2 : Histochemical staining reactivities of mucosubstances in the Oesophagus of A.phoenicurus phoenicurus.

	Histochemical techniques	M U C O S A					Submucosa	Muscularis	Serosa
		Stratified epithelium	Glandular epithelium						
1.	PAS	+P	+++P	++P	++P	++P	++P	+P	
2.	Ph-PAS	-	++P	-	-	-	-	-	
3.	D-PAS	-	+++P	++P	++P	++P	++P	+P	
4.	AB pH 1	-	++B	-	-	-	-	-	
5.	AB pH 1 - PAS	+P	+++BP	++P	++P	++P	++P	+P	
6.	AB pH 2.5	-	+++B	-	-	-	-	-	
7.	AB pH 2.5 - PAS	+P	+++BP	++P	++P	++P	++P	+P	
8.	AF	-	+++PL	-	-	-	-	-	
9.	AF - AB pH 2.5	-	+++PS	-	-	-	-	-	
10.	Azure A pH 1.5	-	++M	-	-	-	-	-	
11.	Azure A pH 3.0	-	++M	-	-	-	-	-	
12.	Azure A pH 5.0	+O	+++M	++O	++O	++O	++O	+O	
13.	AB pH 5.6 - .1 M Mg ⁺⁺	-	+++B	-	-	-	-	-	
14.	AB pH 5.6 - .2 M Mg ⁺⁺	-	++B	-	-	-	-	-	
15.	AB pH 5.6 - .4 M Mg ⁺⁺	-	++B	-	-	-	-	-	
16.	AB pH 5.6 - .5 M Mg ⁺⁺	-	++B	-	-	-	-	-	
17.	MM - AB pH 2.5	-	++B	-	-	-	-	-	
18.	MM - Saponf. - AB pH 2.5	-	+++B	-	-	-	-	-	

19.	AM - AB pH 2.5	-	-	-	-
20.	AM - Saponi. - AB pH 2.5	-	++B	-	-
21.	AM - AB pH 2.5	-	++B	-	-
22.	Stalidase - AB pH 2.5	-	++B	-	-
23.	Eyel. - AB pH 2.5	-	++B	-	-
24.	Pepsin - AB pH 2.5	-	+++B	-	-

PAS staining which was diastase labile, indicating presence of glycogen therein. The layer remained unstained with AB pH 1, AB pH 2.5, AF and exhibited poor orthochromatic staining with sequential staining techniques like AB pH 1 - PAS and AB pH 2.5 - PAS. These results supported that acidic mucins were lacking in muscularis.

4) SEROSA :

It showed weak staining with PAS which was diastase resistant but totally labile to pH - PAS treatment. It remained unstained with AB pH 1, AB pH 2.5 and AF even after pepsin digestion. The layer exhibited poor pink colouration with sequential staining techniques, with Azure A poor blue orthochromatic reactivity was seen at enhanced pH level. These all evidences suggested presence of only neutral mucins in serosa of oesophagus.

III) SEXUAL DIMORPHISM :

Histological as well as histochemical results obtained in oesophagi of male and female waterhens studied were identical indicating no sexual dimorphism among the mucosubstances in oesophagus.

8) PROVENTRICULUS (GLANDULAR STOMACH) :

I) HISTOLOGICAL OBSERVATIONS :

The shape of the organ was circular in proximal region which became flattened in the distal part. H-E stained section showed glandular mucosa, submucosa, muscularis and serosa as

usual (Fig.No.). Mucosa exhibited longitudinal folds of varying heights and intervening depressions. Mucosal epithelium consisted of goblet cells. In this organ, basic pattern of tissue layers was considerably altered by the gross development of proventricular glands, reduction of submucosa and spreading of muscularis mucosa. The proventricular glands were tubulo-alveolar compound type which extended upto the submucosa and had openings in the region of mucosal folds, via their own collecting ducts. Each gland exhibited collecting chamber lined by duct cells comparable to mucous neck cells in glandular stomach of other vertebrates.

II) HISTOCHEMICAL OBSERVATIONS :

The observations are tabulated in Table No. 3 and distribution of mucosubstances is illustrated in microphotographs.

1) MUCOSA :

1) SURFACE GOBLET CELLS :

The cells reacted similar to glandular cells in oesophagus. Hence it was concluded that these cells contained a mixture of neutral, sulfo and sialomucins (Fig.No.).

11) GLANDULAR DUCT CELLS :

These cells exhibited an intense PAS reactivity (Fig. No.), which was diastase resistant but partially labile to Ph-PAS treatment. This indicated absence of glycogen but presence of neutral mucins (poor quantities). Their presence was further inferred from positive reactivity with sequential

staining techniques (Fig.No.).

The cells exhibited moderate alcianophilia with AB pH 1, which was found to be slightly enhanced at pH 2.5, indicating presence of sulfo as well as carboxymucins (Fig.No. and). The sulfomucins in duct cells were also characterised by staining reactivity with AF, AF-AB pH 2.5, metachromasia with Azure A even at low pH level, graded decrease in alcianophilia in CEC technique from 0.1 M Mg^{++} to 0.5 M Mg^{++} and above, loss of alcianophilia following active methylation and acid hydrolysis. While carboxymucins were identified by blue-purple staining with AF AB-pH 2.5, restoration of alcianophilia with saponification treatment.

111) GLANDULAR SECRETARY CELLS :

These cells exhibited moderate PAS reactivity. The same was observed in sequential staining techniques, the PAS staining was found to be diastase resistant but totally labile to Ph-PAS treatment. The cells showed no response to AB pH 1, AB pH 2.5 (Fig. and) as well as AF. With Azure A, only at higher pH level, poor reactivity was observed. These observations indicated presence of only neutral mucosubstances in glandular secretary cells.

2) SUBMUCOSA :

The results were identical to oesophageal submucosa, thus suggesting presence of only neutral mucins therein. Hyaluronic acid was absent in this zone.

Table No. 3 : Histochemical staining reactivities of mucosubstances in the Proventriculus of A.phenicurus phoenicurus.

Histochemical Techniques	M U C O S					Submucosa	Muscularis	Serosa
	Surface goblet cells	Glandular duct cells	A		Glandular Secretory cells			
1. PAS	++++P	++++P	++P		++P	++P	++P	++P
2. Ph-PAS	++P	++P	-		-	-	-	-
3. D-PAS	++++P	++++P	++P		++P	++P	++P	++P
4. AB pH 1	++B	+++B	-		-	-	-	-
5. AB pH 1 - PAS	++++BP	++++BP	++P		++P	++P	++P	++P
6. AB pH 2.5	+++B	++++B	-		-	-	-	-
7. AB pH 2.5 - PAS	++++PB	++++PB	++P		++P	++P	++P	++P
8. AF	+++PL	++++PL	-		-	-	-	-
9. AF - AB pH 2.5	+++BP	++++BP	-		-	-	-	-
10. Azure A pH 1.5	++M	+++M	-		-	-	-	-
11. Azure A pH 3.0	+++M	+++M	+O		+O	+O	+O	+O
12. Azure A pH 5.0	+++M	+++M	+O		+O	+O	+O	+O
13. AB pH 5.6 - .1 M Mg ⁺⁺	++B	++B	-		-	-	-	-
14. AB pH 5.6 - .2 M Mg ⁺⁺	++B	++B	-		-	-	-	-
15. AB pH 5.6 - .4 M Mg ⁺⁺	+B	++B	-		-	-	-	-
16. AB pH 5.6 - .5 M Mg ⁺⁺	+B	+B	-		-	-	-	-
17. MM - AB pH 2.5	++B	+++B	-		-	-	-	-
18. MM - Saponi. - AB pH 2.5	+++B	+++B	-		-	-	-	-

19.	AM - AB pH 2.5	-	-	-	-	-	-
20.	AM - Saponi. - AB pH 2.5	++B	-	++B	-	-	-
21.	AH - AB pH 2.5	-	-	-	-	-	-
22.	Stalidase - AB pH 2.5	++B	-	++B	-	-	-
23.	Hyal. - AB pH 2.5	++B	-	++B	-	-	-
24.	Pepsin - AB pH 2.5	+++B	-	+++B	-	-	-

3) MUSCULARIS :

Results resembled with those obtained in oesophageal muscles, hence it was concluded that only glycogen existed in this layers

4) SEROSA :

Only neutral mucosubstances were identified here.

III) SEXUAL DIMORPHISM :

Histological and histochemical results were identical in both the sexes, suggesting no sexual dimorphism among the mucosubstances in proventriculus.

C) VENTRICULUS (GIZZARD) :

I) HISTOLOGICAL OBSERVATIONS :

It showed usual 4 layers (HE-technique) (Fig.No. +). Mucosa was highly folded. The peculiarity of this region was presence of non-cellular innermost 'keilin' layer consisting of parallel laminae perpendicular to mucosa. Another noticeable peculiarity was that muscularis was considerably well developed (thick) than any other regions of the alimentary tract. The mucosa consisted of only goblet cells. Crypts and glands were well developed. Muscularis mucosa was indistinct.

II) HISTOCHEMICAL OBSERVATIONS :

The results obtained are shown in Table No. 4 and distribution of mucosubstances is illustrated in microphotographs. Some observations are given below in detail.

1) MUCOSA :

1) KOILIN LAYER :

It was non-cellular layer.

11) SURFACE GOBLET CELLS :

The cells reacted intensely with PAS and this activity was resistant to both diastase digestion and Ph-PAS treatment, indicating absence of glycogen as well as neutral mucins (Fig. No. , and). The cells reacted moderately with AB pH 1, the activity was found to be enhanced slightly indicating presence of both sulfo and carboxymucins (Fig.No.), also seen in sequential staining technique (Fig.No.). The presence of sulfomucins was confirmed by intense purple reactivity with AF, noticeable metachromasia even at low pH level, graded decrease in alcianophilia from moderate to poor in CBC technique. Presence of carboxymucins was inferred by their enhanced alcianophilia with AB pH 2.5 than observed at AB pH 1, blue-purple staining with AF AB-pH 2.5, enhanced metachromasia from low towards high pH levels of Azure A, partial restoration of alcianophilia after saponification of previously methylated sections and partial reduction of alcianophilia following AH technique. The carboxymucins were then identified as sialomucins. Thus to summarize surface goblet cells contained sulfomucins (predominant) and sialomucins (poor quantities).

111) CRYPTO-CELLS :

These cells reacted in identical manner as the surface goblet cells, indicating presence of sulfomucins (predominant)

Table No. 4 : Histochemical staining reactivities of mucosubstances in the Ventriculus of A.phoenicurus phoenicurus.

Histochemical Techniques	Koilin layer	M U C O S A					Submucosa	Mascularis	Serosa
		Surface goblet cells	Crypt cells	Glandular Cells					
1. PAS	-	++++P	++++P	++P	++P	++P	++P	++P	
2. Ph-PAS	-	++++P	++++P	-	-	-	-	-	
3. D - PAS	-	++++P	++++P	++P	++P	++P	++P	++P	
4. AB pH 1	-	+++B	+++B	-	-	-	-	-	
5. AB pH 1 - PAS	-	++++PB	++++PB	++P	++P	++P	++P	++P	
6. AB pH 2.5	-	++++B	++++B	-	-	-	-	-	
7. AB pH 2.5 - PAS	-	++++PB	++++PB	++P	++P	++P	++P	++P	
8. AF	-	++++PL	++++PL	-	-	-	-	-	
9. AF - AB pH 2.5	-	++++BP	++++BP	-	-	-	-	-	
10. Azure A pH 1.5	-	+++M	+++M	-	-	-	-	-	
11. Azure A pH 3.0	-	++++M	++++M	+O	+O	+O	+O	+O	
12. Azure A pH 5.0	-	++++M	++++M	++O	++O	++O	++O	++O	
13. AB pH 5.6 - .1 M Mg ⁺⁺	-	+++B	+++B	-	-	-	-	-	
14. AB pH 5.6 - .2 M Mg ⁺⁺	-	++B	++B	-	-	-	-	-	
15. AB pH 5.6 - .4 M Mg ⁺⁺	-	++B	++B	-	-	-	-	-	
16. AB pH 5.6 - .5 M Mg ⁺⁺	-	+B	+B	-	-	-	-	-	
17. MM - AB pH 2.5	-	+++B	+++B	-	-	-	-	-	
18. MM - Saponi. - AB pH 2.5	-	+++B	+++B	-	-	-	-	-	

19.	AM - AB pH 2.5	-	+B	-	-	-
20.	AM - Saponi. - AB pH 2.5	-	++B	-	-	-
21.	AH - AB pH 2.5	-	++B	-	-	-
22.	Sialidase - AB pH 2.5	-	++B	-	-	-
23.	Hyal. - AB pH 2.5	-	+++B	-	-	-
24.	Pepsin - AB pH 2.5	-		-	-	-

and sialomucins (poor quantities) therein.

iv) GLANDULAR CELLS :

The glands were highly defined and reactivities recorded resembled to those exhibited by glandular secretory cells of proventriculus. Hence it was concluded that the ventricular glandular epithelial cells contained only neutral mucins (Fig. No.).

2), 3) and 4) SUBMUCOSA, MUSCULARIS AND SEROSA :

The results obtained here were similar to respective layers in proventriculus. Therefore it was concluded that submucosa and serosa contained only neutral mucins and muscularis contained only glycogen.

III) SEXUAL DIMORPHISM :

The results indicated absence of sexual dimorphism in mucins in ventriculi of male and female waterhens.

D) DUODENUM :

I) HISTOLOGICAL OBSERVATIONS :

The HE stained section revealed presence of usual 4 tunics viz. mucosa, submucosa, muscularis and serosa (Fig.No.), The mucosa was thrown into numerous finger like blunt ended villi projecting into the lumen. The shape of villi, the number of villi and number of goblet cells were the aspects to distinguish duodenum histologically from small intestine (external demarkation lacking). Duodenal mucosa was found to

exhibit a few finger like blunt villi, secondly, goblet cells were few in number as compared to those in small intestine.

The mucosa consisted of single layered dimorphic epithelial cells viz. secretory goblet cells and absorptive columnar cells, both exhibiting basally situated nuclei. Crypts were located inbetween villi, glands were absent. Submucosa was thin and contained connective tissue, it was observed extended into mucosal villi. Muscularis was also thin and consisted of usual inner circular and outer longitudinal muscles serosa was as usual.

II) HISTOCHEMICAL OBSERVATIONS :

The staining reactivities are shown in Table No.5 and distribution of mucosubstances is illustrated in microphotographs.

1) MUCOSA :

1) COLUMNAR EPITHELIUM :

It exhibited poor PAS reactivity which was diastase resistant but labile to Ph-PAS treatment. This indicated absence of glycogen but presence of neutral mucins (poor quantities) (Fig.No.). The cells remained unstained with AB pH 1, AB pH 2.5 and AF, thus indicating absence of acidic mucins in these cells. Presence of only neutral mucins was substantiated from their only pink colouration in sequential staining techniques (viz. AB pH 1 - PAS and AB pH 2.5-PAS), there was no blue tinge at all (Fig.No. and).

111) SURFACE GOBLET CELLS :

These cells in duodenum showed identical staining reactivities with various histochemical techniques, to those shown by the surface goblet cells in proventriculus and glandular epithelial cells in oesophagus. Hence it was concluded that duodenal surface goblet cells contained a mixture of neutral, sulfo (predominant) and sialomucins (poor quantities) (Fig.No. and).

111) CRYPT GOBLET CELLS :

Results obtained were identical to those of surface goblet cells mentioned above. So it was concluded that these cells also contained a mixture of neutral, sulfo and sialomucins. In the distal part of the duodenum, the lamina propria showed a distinct glandular structure, the cells within which showed intense reactivity with AB pH 1 and AB pH 2.5 indicating presence of acidic mucins (Fig.No.).

2), 3) and 4) SUBMUCOSA MUSCULARIS AND SEROSA :

These layers in duodenum reacted in identical manner as the respective layers in oesophagus, proventriculus and ventriculus. So it was concluded that duodenal submucosa and serosa contained neutral mucins and muscularis contained glycogen.

III) SEX DIMORPHISM :

Histological, histochemical results obtained being identical, it was concluded that there existed no sexual

Histochemical Techniques	M U C O S A					Submucosa	Muscularis	Serosa
	Columnar epithelium	Surface goblet cells	Crypt goblet cells					
1. PAS	+P	++++P	++++P			++P	++P	+P
2. Ph-PAS	-	++P	++P			-	-	-
3. D-PAS	+P	++++P	++++P			++P	+P	+P
4. AB pH 1	-	+++B	+++B			-	-	-
5. AB pH 1 - PAS	+P	++++PB	++++PB			++P	++P	+P
6. AB pH 2.5	-	++++B	++++B			-	-	-
7. AB pH 2.5 - PAS	+P	++++PB	++++PB			++P	++P	+P
8. AF	-	+++PL	+++PL			-	-	-
9. AF - AB pH 2.5	-	++++BP	++++BP			-	-	-
10. Azure A pH 1.5	-	+M	+M			-	-	-
11. Azure A pH 3.0	-	++M	++M			-	-	-
12. Azure A pH 5.0	-	+++M	+++M			++O	++O	-
13. AB pH 5.6 - .1 M Mg ⁺⁺	-	+++B	+++B			-	-	-
14. AB pH 5.6 - .2 M Mg ⁺⁺	-	++B	++B			-	-	-
15. AB pH 5.6 - .4 M Mg ⁺⁺	-	++B	++B			-	-	-
16. AB pH 5.6 - .5 M Mg ⁺⁺	-	+B	+B			-	-	-
17. MM - AB pH 2.5	-	+++B	+++B			-	-	-
18. MM - Saponi. - AB pH 2.5	-	+++B	+++B			-	-	-
19. AM - AB pH 2.5	-	-	-			-	-	-
20. AM - Saponi. - AB pH 2.5	-	++B	++B			-	-	-
21. AH - AB pH 2.5	-	+B	+B			-	-	-
22. Sialidase - AB pH 2.5	-	+++B	+++B			-	-	-
23. Hyal. - AB pH 2.5	-	+++B	+++B			-	-	-
24. Pepsin - AB pH 2.5	-	+++B	+++B			-	-	-

Table No. 5 : Histochemical staining reactivities of mucosubstances in the Duodenum of A.phoenicurus phoenicurus.

dimorphism in mucosubstances in duodenums of male and female waterhens.

E) SMALL INTESTINE (ILEUM)

I) HISTOLOGICAL OBSERVATIONS :

In transverse section, ileum appeared almost circular and very thin walled. The sections stained with H-E revealed presence of typical four layers as usual (Fig.No.). Mucosa showed numerous villi projecting into lumen. The villi were broad at proximal ends which tapered abruptly towards distal ends, giving thread like appearance at tips. The villi were more numerous than in duodenum. The cells in mucosa were dimorphic viz. columnar and goblet. The crypts exhibited no goblet cells. Submucosa extended into core of the villi. Muscularis was thin and serosa formed typical outermost tunic

II) HISTOCHEMICAL OBSERVATIONS :

The results are listed in table no. 6 and distribution of mucosubstances is illustrated in microphotographs.

The details of some observations are given below :-

1) MUCOSA :

1) COLUMNAR EPITHELIUM :

In their tinctorial shades, these cells resembled with duodenal columnar epithelial cells. So it was concluded that these cells also contained poor quantities of neutral mucins (Fig.No.).

Table No. 6 : Histochemical staining reactivities of mucosubstances in the small intestine of *A. phoenicurus phoenicurus*.

Histochemical Techniques	M U C O S A					Submucosa	Muscularis	Serosa
	Columnar epithelium	Surface goblet cells	Crypt goblet cells					
1. PAS	++P	++++P	++++P			++P	++P	++P
2. Ph-PAS	+P	+++P	+++P			+P	-	+P
3. D-PAS	++P	++++P	++++P			++P	+P	++P
4. AB pH 1	-	+++B	+++B			-	-	-
5. AB pH 1 - PAS	++P	++++PB	++++PB			++P	++P	++P
6. AB pH 2.5	-	++++B	++++B			-	-	-
7. AB pH 2.5 - PAS	++P	++++PB	++++PB			++P	++P	++P
8. AF	-	+++PL	+++PL			-	-	-
9. AF - AB pH 2.5	-	++++BP	++++BP			-	-	-
10. Azure A pH 1.5	-	+++M	+++M			-	-	-
11. Azure A pH 3.0	-	+++M	+++M			-	-	-
12. Azure A pH 5.0	-	+++M	+++M			+O	+O	+O
13. AB pH 5.6 - .1 M Mg ⁺⁺	-	+++B	+++B			-	-	-
14. AB pH 5.6 - .2 M Mg ⁺⁺	-	+++B	+++B			-	-	-
15. AB pH 5.6 - .4 M Mg ⁺⁺	-	+++B	+++B			-	-	-
16. AB pH 5.6 - .5 M Mg ⁺⁺	-	+++B	+++B			-	-	-
17. MM - AB pH 2.5	-	+++B	+++B			-	-	-
18. MM - Saponi. - AB pH 2.5	-	+++B	+++B			-	-	-
19. AM - AB pH 2.5	-							

20.	AM - Saponi. - AB pH 2.5	-	++B	++B	-	-
21.	AH - AB pH 2.5	-	-	-	-	-
22.	Sialidase - AB pH 2.5	-	+++B	+++B	-	-
23.	Hyal. - AB pH 2.5	-	+++B	+++B	-	-
24.	Pepsin - AB pH 2.5	-	++++B	++++B	-	-

11) GOBLET CELLS :

Both surface and crypt goblet cells were identical in reactivities to those in duodenum. Hence it was concluded that these cells also exhibited a mixture of sulfomucins (predominant), sialomucins (poor quantities) and neutral mucins (Fig. No. and).

2), 3) and 4) SUBMUCOSA, MUSCULARIS, SEROSA :

In all histochemical aspects, these layers in ileum resembled with respective layers in the other parts of the alimentary tract. So it was concluded that submucosa and serosa contained neutral mucins and muscularis contained glycogen.

III) SEXUAL DIMORPHISM :

It was lacking in mucosubstances in small intestine also.

F) LARGE INTESTINE :

I) HISTOLOGICAL OBSERVATIONS :

It is circular in cross section, but more thicker in musculature than small intestine. H-E stained sections revealed 4 tunics as usual from innermost mucosa to outermost serosa (Fig.No.). The mucosal folds were slightly long and narrow and with numerous goblet cells, but number of columnar cells was found to be less. Crypts were well defined but glands were absent. Muscularis mucosa, submucosa, muscularis layers were well defined, especially muscularis was well developed offering thickness to large intestine. It was in turn covered by a typical serosa.

	Histochemical Techniques	M U C O S A					Submucosa	Muscularis	Serosa
		Columnar epithelium	Surface goblet cells	Crypt goblet cells	Crypt goblet cells				
1.	PAS	+P	++++P	++++P	++++P	++P	++P	++P	+P
2.	Ph-PAS	-	++P	++P	++P	-	-	-	-
3.	D-PAS	+P	++++P	++++P	++++P	++P	+P	+P	+P
4.	AB pH 1	-	+++B	+++B	+++B	-	-	-	-
5.	AB pH 1 - PAS	+P	++++PB	++++PB	++++PB	++P	++P	++P	+P
6.	AB pH 2.5	-	++++B	++++B	++++B	-	-	-	-
7.	AB pH 2.5 - PAS	+P	++++PB	++++PB	++++PB	++P	++P	++P	+P
8.	AF	-	++++PPL	++++PPL	++++PPL	-	-	--	-
9.	AF - AB pH 2.5	-	++++BP	++++BP	++++BP	-	-	-	-
10.	Azure A pH 1.5	-	+++M	+++M	+++M	-	-	-	-
11.	Azure A pH 3.0	-	+++M	+++M	+++M	-	-	-	-
12.	Azure A pH 5.0	-	+++M	+++M	+++M	++O	++O	++O	+O
13.	AB pH 5.6 - .1 M Mg ⁺⁺	-	+++B	+++B	+++B	-	-	-	-
14.	AB pH 5.6 - .2 M Mg ⁺⁺	-	+++B	+++B	+++B	-	-	-	-
15.	AB pH 5.6 - .4 M Mg ⁺⁺	-	+++B	+++B	+++B	-	-	-	-
16.	AB pH 5.6 - .5 M Mg ⁺⁺	-	+++B	+++B	+++B	-	-	-	-
17.	MM - AB pH 2.5	-	+++B	+++B	+++B	-	-	-	-
18.	MM - Saponi. - AB pH 2.5	-	+++B	+++B	+++B	-	-	-	-
19.	AM - AB pH 2.5	-	-	-	-	-	-	-	-
20.	AM - Saponi.- AB pH 2.5	-	+++B	+++B	+++B	-	-	-	-
21.	AH - AB pH 2.5	-	+++B	+++B	+++B	-	-	-	-
22.	Sialidase - AB pH 2.5	-	+++B	+++B	+++B	-	-	-	-
23.	Hyal. - AB pH 2.5	-	+++B	+++B	+++B	-	-	-	-
24.	Pepsin - AB pH 2.5	-	+++B	+++B	+++B	-	-	-	-

Table No. 7 : Histochemical staining reactivities of mucosubstances in the large intestine of A.phoenicurus phoenicurus.

II) HISTOCHEMICAL OBSERVATIONS :

They are listed in table no. 7 while the distribution of mucosubstances is illustrated in microphotographs.

Details of some results are given below :-

1) MUCOSA :

1) COLUMNAR EPITHELIUM :

Results were identical to those cells in duodenum and ileum. So this indicated presence of only neutral mucins therein (poor quantities) (Fig.).

11) SURFACE AND CRYPT GOBLET CELLS :

The results of staining exhibited by these cells were identical to those cells in duodenum and ileum. Therefore, it was concluded that these cells also contained Sulfomucins (pre-dominant), Sialomucins (poor quantities) and neutral mucins (Fig.No. , , , and).

2), 3) and 4) SUBMUCOSA, MUSCULARIS AND SEROSA :

Results obtained were identical to the respective layers in duodenum and ileum.

III) SEXUAL DIMORPHISM :

It was not observed.

PLATE No. 1

Captions to Figures

- Fig.1 - T.S. of Oesophagus stained with H-E - 10 x Scanner**
Fig.2 - T.S. of Oesophagus stained with PAS - 10 x Scanner
Fig.3 - T.S. of Oesophagus stained with AB pH 1 - 10 x Scanner
Fig.4 - T.S. of Oesophagus stained with AB pH 2.5-10 x Scanner
Fig.5 - T.S. of Oesophagus stained with AB pH 2.5-PAS-10xScanner
Fig.6 - T.S. of Oesophagus stained with AF - 10 x Scanner.

ABBREVIATIONS

S - Serosa

M - Muscularis

CT - Connective tissue

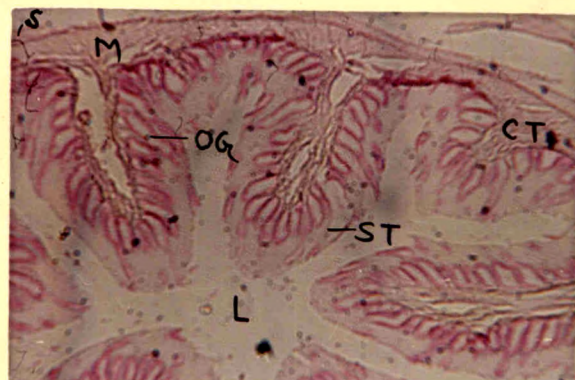
DG - Oesophageal glands

ST - Stratified epithelium.

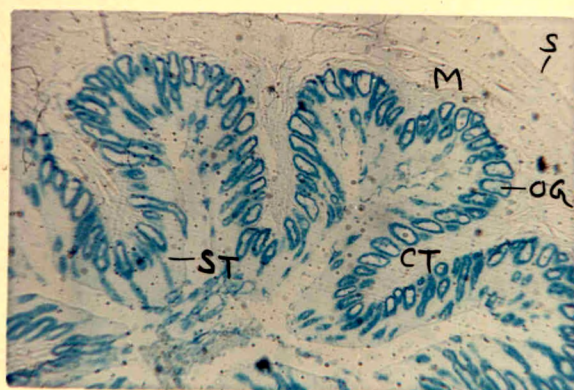
PLATE No. 1



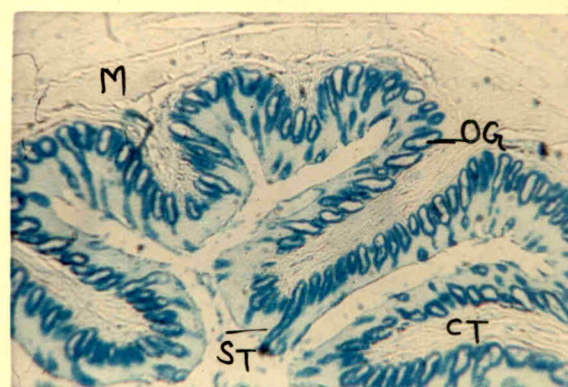
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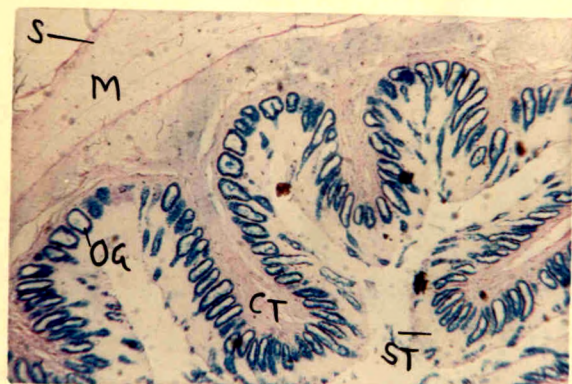
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PLATE No. 2

Captions to Figures

Fig.7 - T.S. of Proventriculus stained with H-E - 8.5 x Scanner

Fig.8 - T.S. of Proventriculus stained with PAS - 8.5 x Scanner

Fig.9 - T.S. of Proventriculus stained with AB pH 1-8.5 x Scanner

Fig.10- T.S. of Proventriculus stained with AB pH 2.5 - 8.5xScanner

Fig.10a-T.S. of Proventriculus stained with AB pH 2.5 - Enlarge

Fig.11 -T.S. of Proventriculus stained with AB pH 2.5 - PAS

ABBREVIATIONS

S - Serosa

CT - Connective tissue

SG - Surface goblet cells

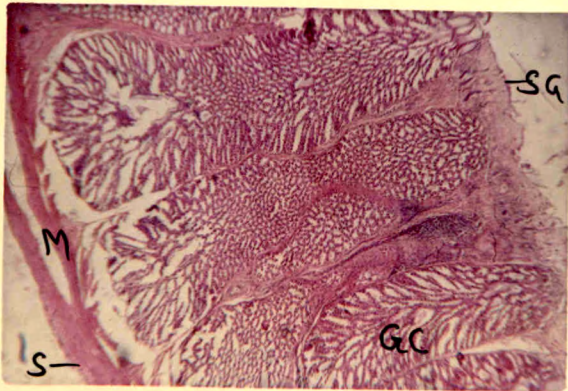
M - Muscularis

GC - gland cells

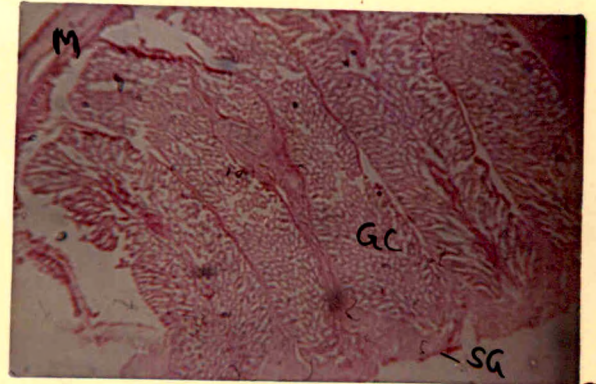
Dc - Duct cells

D - Duct

PLATE No.2



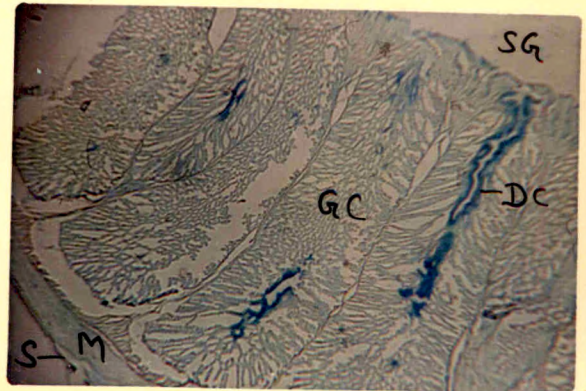
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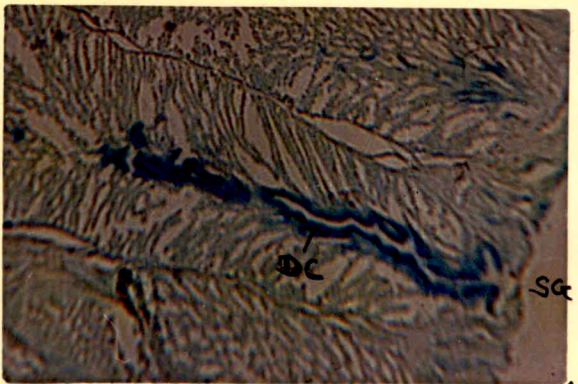
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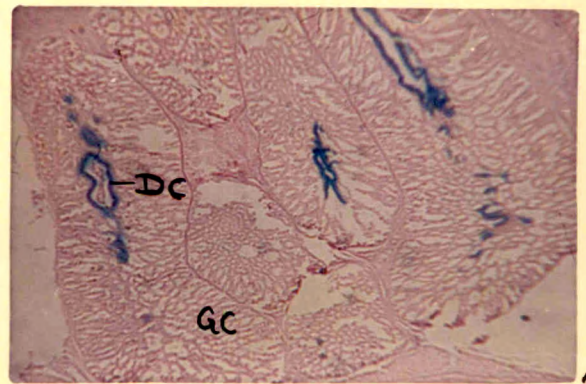
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PLATE No. 3

CAPTIONS TO FIGURES

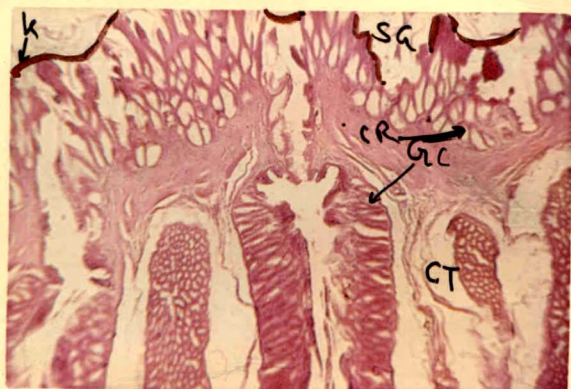
- Fig.12 - T.S. of Ventriculus stained with H-E - 8 x Scanner
(Distal half portion).
- Fig.13 - T.S. of Ventriculus stained with H-E - 8 x Scanner
(Proximal half portion).
- Fig.14 - T.S. of Ventriculus stained with PAS - 8 x Scanner
- Fig.15 - T.S. of Ventriculus stained with Ph-PAS - 8 x Scanner
- Fig.16 - T.S. of Ventriculus stained with D-PAS - 8 x Scanner
- Fig.17 - T.S. of Ventriculus stained with AB pH 1 - 8 x Scanner
- Fig.18 - T.S. of Ventriculus stained with AB pH 2.5 - PAS -
8 x Scanner.

ABBREVIATIONS

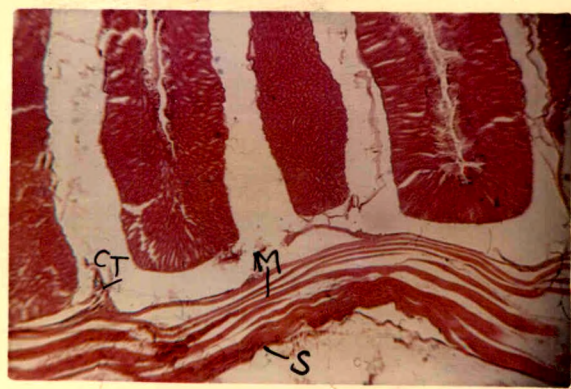
S - Serosa
CT - Connective tissue
SG - Surface goblet cells
CR - Crypt cell

M - Muscularis
K - Koilin layer
GC - gland cell

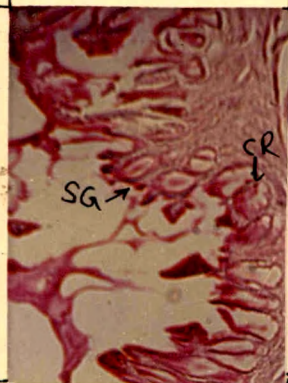
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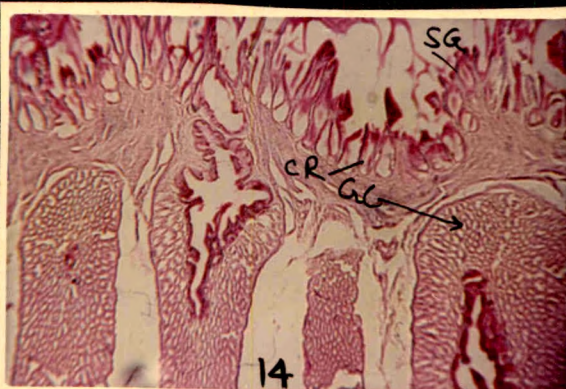
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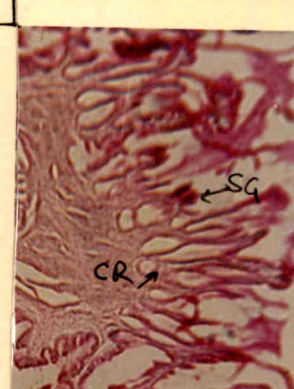
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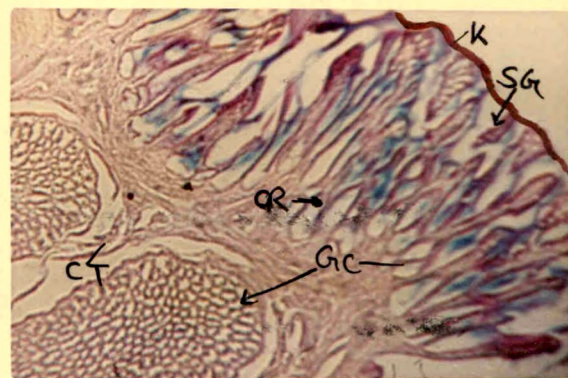
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PLATE No. 4

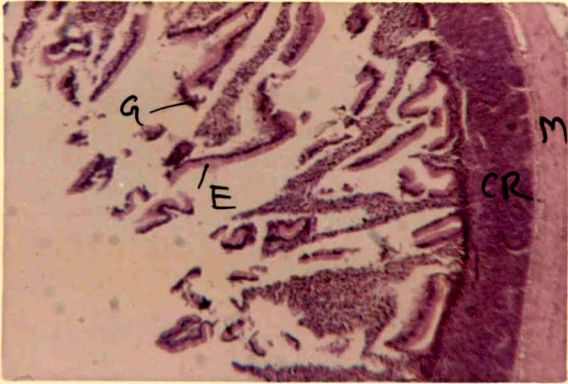
Captions to Figures

- Fig.19 - T.S. of Duodenum stained with H-E - 10 x Scanner
Fig.20 - T.S. of Duodenum stained with PAS - 10 x Scanner
Fig.21 - T.S. of Duodenum stained with AB pH 1 - 10 x Scanner
Fig.22 - T.S. of Duodenum stained with AB pH 2.5 - 10 x Scanner
Fig.23 - T.S. of Duodenum stained with AB pH 1 - PAS -
10 x Scanner
Fig.24 - T.S. of Duodenum stained with AB pH 2.5 - PAS -
10 x Scanner showing gland.

ABBREVIATIONS

S - Serosa	M - Muscularis
CT - Connective tissue	G - Goblet cell
CR - Crypt cell	E - Columnar epithelium.
L - Lumen.	xG - Gland.

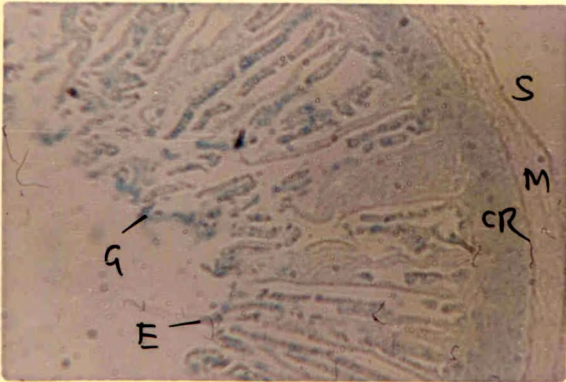
PLATE No. 4



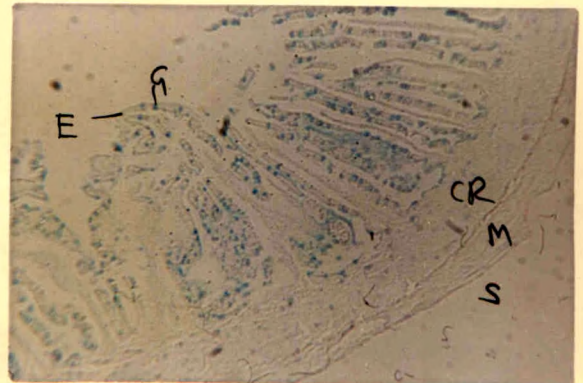
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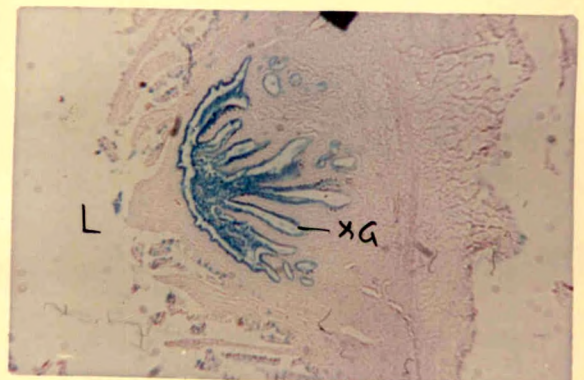
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PLATE No. 5

Captions to figures

Fig.25 - T.S. of Small intestine stained with H-E - 10 x Scanner

Fig.26 - T.S. of Small intestine stained with AB pH 1 - 10xScanner

Fig.27 - T.S. of Small intestine stained with AB pH 2.5 -
10 x Scanner.

Fig.28 - T.S. of Small intestine stained with AB pH 1 - PAS -
10 x Scanner.

Fig.29 - T.S. of Large intestine stained with H-E - 10 x Scanner

Fig.30 - T.S. of Large intestine stained with PAS - 10 x Scanner

ABBREVIATIONS

S - Serosa

CT - Connective tissue

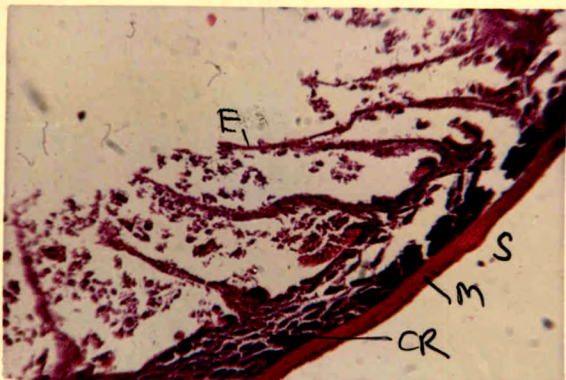
E - Columnar epithelium

M - Muscularis

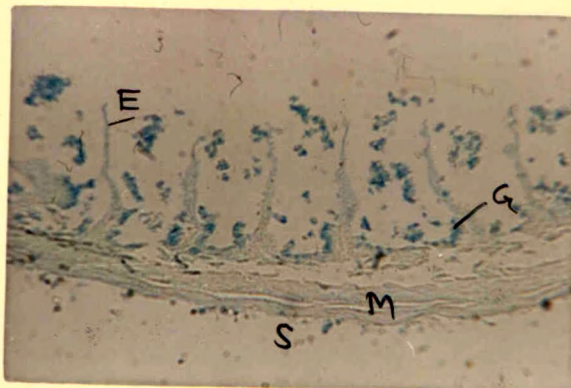
CR - Crypt cell

G - Goblet cell

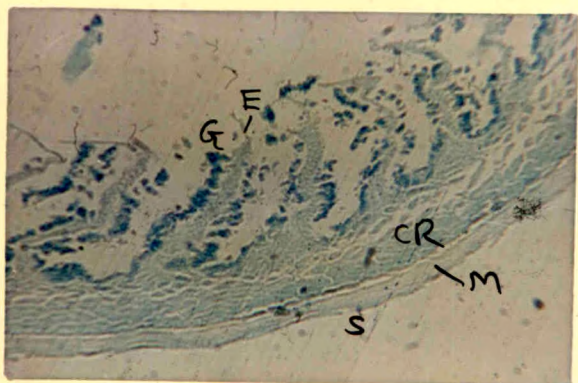
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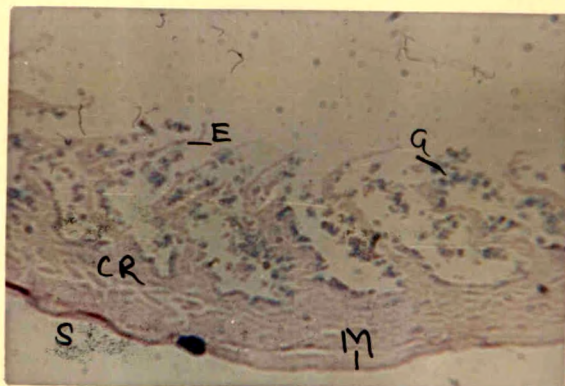
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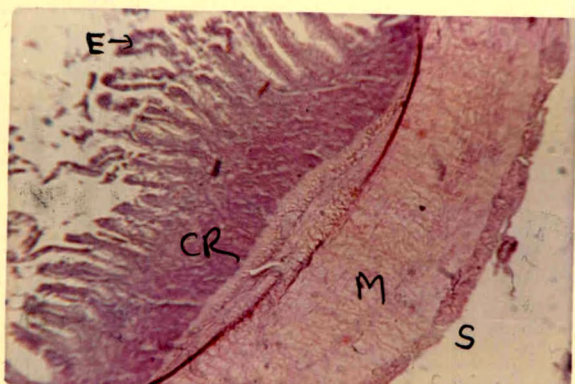
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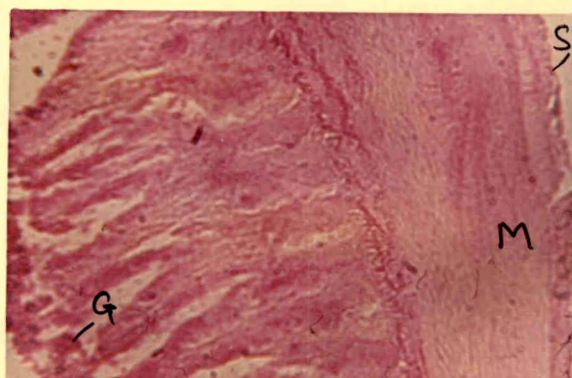
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