CHAPTER - THREE

OBSERVATIONS

Histological and Histochemical Observations on mucosubstances in various organs in the alimentary canal of *P. posthuma* and *H. granulosa*.

A critical evaluation of the histological, histochemical and biochemical studies published in the last two or three decades on the alimentary tract, significantly shows that metabolites such as mucosubstances, lipids, proteins and enzymes have been mainly studied in the vertebrates. Very little attention has been paid to the alimentary canal of the invertebrates. This is particularly true for the mucosubstances in the alimentary canal of the annelids. Although earthworm, P. posthuma has been known for a long time their anatomy has been closely studied by Bahl (1926) and there are still considerable gaps in the knowledge of digestive process and histochemistry of various organs in the alimentary canal. Most of the work was orientated for the study of enzymes that take part in the digestion of food. The Indian cattle leech, H. granulosa was studied by Bhalia, (1941) for its morphology and anatomy and initial observations on histology of various organ-systems. Thereafter, no work has been carried out on these two annelids either for histology or for histochemistry. The present report concerns with the mucosubstances in the various organs in the alimentary canal of P. posthuma and H. granulosa. The results obtained in the present investigation on histology and histochemistry of mucosubstances in the alimentary canal of P. posthuma and H. granulosa are presented hereafter.

The histochemical results are recorded in tabulated form and histochemical distribution of mucosubstances in various organs are illustrated in numerous photographs.

OBSERVATIONS :

I. P. posthuma :

A) Alimentary Canal :

The alimentary canal of P. posthuma appeared as a straight tube running along the entire length of the body. The mouth and anus constituted the anterior and posterior openings respectively. It was regionated, morphologically into buccal chamber, pharynx, oesophagus, gizzard, stomach and intestine. The mouth was situated ventral to the prostomium, lead into a short and narrow buccal chamber. The buccal chamber extended upto the middle of the third segment. The buccal chamber was followed by a spacious pear-shaped muscular pharynx that extend upto fourth segment. Behind the pharynx was the oesophagus that ran upto the seventh segment as a thin walled, short narrow tube. In the eighth segment oesophagus modified into a prominent, oval, hard and thick walled muscular organ, the gizzard. The gizzard was followed by a short narrow tube, the stomach, which extended from segments ninth to fourteenth. The region next to the stomach was a long, wide, thin walled tube that extended from

fourteenth segment to the last. The intestine was divided into three parts. viz. pre-typhlosolar region, typhlosolar region and the post-typhlosolar region or rectum. The pretyphlosolar region ran from fifteenth to twenty sixth segments and in twenty-sixth segment gave off two short conical outgrowth, the intestinal caecae. which were extended forward over three or four segments. The typhlosolar intestine was the longest part of the intestine and extended from twenty - sixth segment backward, but stopping short of the last twenty-three to twenty-six segments. The intestine in the last twenty-three segments was the post-typhlosolar region or the rectum that communicated outside through the terminal anus.

B. Histological and Histochemical Observations :

The histology of the various organs in the alimentary canal of P. posthuma is described by making observations on H - E stained preparations. The mucosubstances in the various organs in the alimentary canal were investigated by employing a series of histochemical techniques. The localization and variations in the mucosubstances are recorded in the tabulated form according to the visually estimated intensity of staining and shade. A very intense or strong reaction is designated as ++++, an intense staining reaction as +++, moderately positive reaction as ++, poor or weak reaction as +, trace reaction as ±

and negative reaction as - . The results obtained with various histochemical techniques for mucosubstances are recorded in Table No. 1.

The histochemical localization in mucosubstances in various regions of the alimentary canal are shown in photomicrographs (Figs. 1 to 18). The wall of the alimentary canal consisted, histologically, of four layers : the outer layer of peritonial epithelium, the layer of longitudinal muscles, the layer of circular muscles and the internal epithelial lining of the gut.

1. Buccal Chamber :

Histologically, the wall of the buccal chamber was thin, but was surrounded by a mass of muscular strands which filled up the greater part of the coelomic cavity of this region. the muscles were non-straited. The inner buccal epithelium was formed of tall cylindrical cells covered with a thin cuticle. The epithelial cells were without cilia. The internal lining of the epithelium was thrown into longitudinal folds. Mucous gland cells were observed in the epithelium.

Histochemical Observation :

The histochemical reactivities of various

mucosubstances observed in the buccal chamber are recorded in Table No. 1 according to the visually estimated intensity of staining and shade. The histochemical distribution of mucosubstances in buccal cavity is shown in photomicrographs (Figs. 1 and 2).

The peritoneal epithelium exhibited weak staining with PAS (Fig. 1) which was diastase resistant but could completely be blocked by phenyl hydrazine pretreatment. This layer exhibited only pink to magenta colouration 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 orthrochromatic staining was evident mainly at higher pH levels. These results indicated the presence of only neutral mucosubstances in peritoneal epithelium.

Both circular and longitudinal muscle layers (Fig. 1) exhibited weak PAS staining which could be blocked by phenylhydrazine pretreatment and abolished by diastase digestion indicating 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 orthrochromatic 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.

The epithelial cells in the inner epithelium of the buccal chamber showed poor PAS staining (Fig. 1) which was diastase resistant but could be completely blocked by phenylhydrazine pretreatment. These initial staining reactivities indicated absence of glycogen but presence of neutral mucosubstances in them. These cells remained unstained with AB pH 1.0, AB pH 2.5, C.I. and AF and exhibited only blue orthrochromatic staining at higher pH levels. These results remained unaltered even after pepsin digestion and indicate absence of acidic mucosubstances. The sequential staining procedures such as AB pH 1.0 - PAS, AB pH 2.5 - PAS, and C.I. - PAS showed no tinge of blue colouration and thus, substantiate the presence of neutral mucosubstances in these cells.

Mucous gland cells : Scattered in the inner epithelium were the mucous gland cells, whcih were abundent on the floor of the prostomium. These cells exhibited intense PAS reactivity, (Fig. 1), which was unaffected by diastase digestion but could partly be blocked by prior phenyl hydrazine treatment. After phenylhydrazine treatment only poor PAS reactivity was evident which indicated the presence of neutral

CAPTIONS TO FIGURES

Fig. 1 Section passing through the Buccal Chamber of P. posthuma stained with PAS to show internal epithelium (IE), circular muscle (CM), longitudinal muscle (LM), peritoneal epithelium (P), and mucous gland cell (GC) x 400.

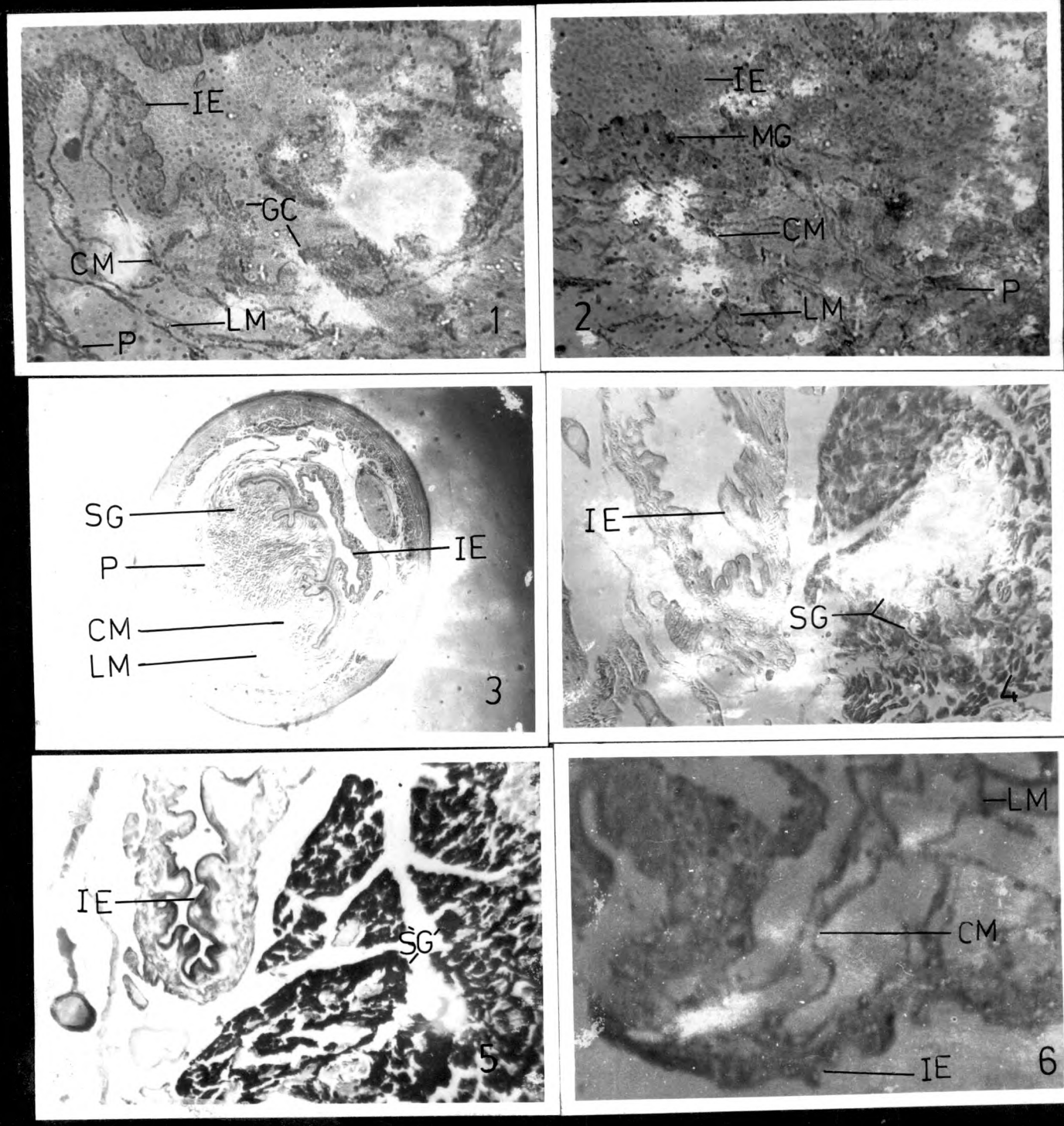
Fig. 2 Section passing through the Buccal Chamber of P. posthuma stained with AB 1.0 - PAS to show only PAS activity in internal epithelium (IE), circular muscle (CM), longitudinal muscle (LM), peritoneal epithelium (P) and Both PAS and Alcian blue react in mucous gland cells (MG) X 400.

Fig. 3 Section passing through the Pharynx of P. posthuma stained with HE to show internal epithelium (IE), circular muscle (CM), longitudinal muscle (LM), peritoneal epithelium (P), and pharyngeal gland (SG) X 300.

Fig. 4 Section passing through the Pharynx of P. posthuma stained with PAS to show intense staining in internal epithelium (IE), and pharyngeal glands (SG) X 400.

Fig. 5 Section passing through the Pharynx of P. posthuma stained with AB 2.5 - PAS to show intense staining activity in internal epithelium (IE), and pharyngeal glands (SG) X 400.

Fig. 6 Section passing through the Oesophagus of P. posthuma stained with AB 2.5 - PAS to show AB activity more in internal epithelium (IE), and less in circular muscle (CM) and longitudinal muscle (LM) X 800.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6

mucosubstances but absence of glycogen.

The mucous gland cells were poorly stained with AB at pH 1.0 and AB pH 2.5 (Fig. 2). These results indicated the presence of sulfomucins in 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, weak metachromasia with azure A at pH 1.5, persistat alcianophilia in CEC technique in presence of 0.2 M Mg^{++} and abolishing of alcianophilia by active methylation which could not be restored after subsequent saponification. The sulfomucins were hyaluronidase resistant. These results thus, revealed presence of neutral mucosubstances (predominant) and sulfomucins (traces) in the mucous gland cells in the inner epithelium. The cuticle showed no staining reactivities with any of the histochemical techniques.

2. Pharynx :

The pharynx was thick and muscular part of the alimentary canal. In cross-section the pharynx was dorsoventrally compressed oval in outline (Fig. 3). The cells of the peritoneum forming outermost covering of the gut were tall and narrow with distinct nuclei. The muscular layer was well developed to form the greater part of the pharyngeal bulb. The

thick mass of the muscular layer was vascular. The lateral walls of the pharynx were pushed inside forming a narrow horizontal shelf on each side. The two shelves were united anteriorly and posteriorly dividing the pharyngeal cavity into a dorsal salivary chamber and ventral conducting chamber. The musuclature lying dorsal to the salivary chamber contained aggregates of the pharyngeal gland cells (Fig. 3). The gland cells were large in size but vary in shape. The gland cells possessed the salivary channels which traversed the musculo-vascular tissue and on reaching the pharyngeal epithelium divided into many fine ductules which penetrated the epithelial cells and terminated in discharge pockets near their free surface. These pockets opened into the salivary chamber. The pharyngeal epithelium consisted of columnar cells which were ciliated on the roof of the pharynx but not on the floor.

HISTOCHEMICAL OBSERVATIONS :

The outer peritoneal epithelial layer (Fig. 4) contained only diastase resistant PAS reactive neutral mucosubstances. The tinctorial affinities of the peritoneal epithelium were identical to those exhibited by the buccal chamber. The longitudinal and circular muscles in the wall of the pharynx contained glycogen but not acidic or other neutral mucosubstances. Their histochemical reactivities were similar to

those exhibited by the buccal chamber musculature.

The inner epithelial layer cells (Fig. 4 and 5) contained only diastase resistant PAS reactive neutral mucosubstances and absence of glycogen in them. These cells remained unstained with AB pH 1.0, AB pH 2.5, C.I. and AF. The pepsin digestion had no effect on these staining reactivities. The histochemical reactivities were similar to those exhibited by the epithelium in buccal chamber. Few mucous gland cells occured in the pharyngeal epithelium. The histochemical reactivities of these gland cells showed similar results as in the gland cells in the buccal chamber and revealed the presence of neutral mucosubstances, and sulfomucins in the mucous gland cells in the inner epithelium of pharynx.

The pharyngeal glands formed a rich glandular structure of varying shape in the musculature above the salivary chamber in pharynx. These pharyngeal gland cells reacted very intensely towards PAS (fig. 4). 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. The moderate alcianophilia at AB pH 1.0 indicated presence of sulfomucins which were predominant. The increased alcianophilia at pH 2.5 (Fig. 5) indicated

presnece of carboxymucins in these cells.

The conclusion that the pharyngeal gland cells contained 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 alcianophilia in CEC technique in presence of 0.5M Mg^{++} and blockade of alcianophilia by active methylation which could not be restored completely. The sulfomucins were hyaluronidase resistant.

The presence of carboxymucins in the pharyngeal gland cells was indicated by increased alcianophilia at AB pH 2.5 (Fig. 5), than at pH 1.0. This conclusion was substantiated by their intense C.I. reactivity, purple - blue staining with AB pH 2.5 - PAS, and C.I. - PAS sequence, blue purple staining in AF - AB pH 2.5 sequence, enhanced metachromasia with azure A at higher pH (3.0 and above) and only partial restoration of their alcianophilia following saponification of actively methylated sections. The carboxymucins were further identified as sialomucins since their alcianophilia was partly reactive to acid hydrolysis and neuroaminidase. Thus, the aforementioned results indicated the presence of neutral mucosubstances in less amount, sialomucins also in less amount and predominance of sulfomucins in the

pharyngeal gland cells.

3. Oesophagus :

Behind the fourth segment the pharynx was followed by the oesophagus which extended upto the eighth segment. It was a thin walled tube. The peritoneal epithelial layer consisted of tall and narrow cells. The layers of longitudinal and circular muscles were well developed. The longitudinal muscle layer was thicker than the circular infront of the gizzard. The internal epithelial layer was transversely folded and consisted of tall columnar cells.

HISTOCHEMICAL OBSERVATIONS :

The staining reactivities of the peritoneum of the small oesophagus were identical to those described for earlier organs. Hence it was concluded that the peritoneum in oesophagus also contained only diastase resistant PAS reactive neutral mucosubstances.

As in pharynx and buccal chamber the longitudinal and circular muscle layers in oesophagus (Fig. 6) also contained glycogen. The muscle layers exhibited histochemical reactivities which resembled to those described for buccal chamber and

CAPTIONS TO FIGURES

Fig. 7 Section passing through the Gizzard of P. posthuma stained with HE to show internal epithelium (IE), cuticle (C), circular muscles (CM) and peritoneal epithelium (P) X 400.

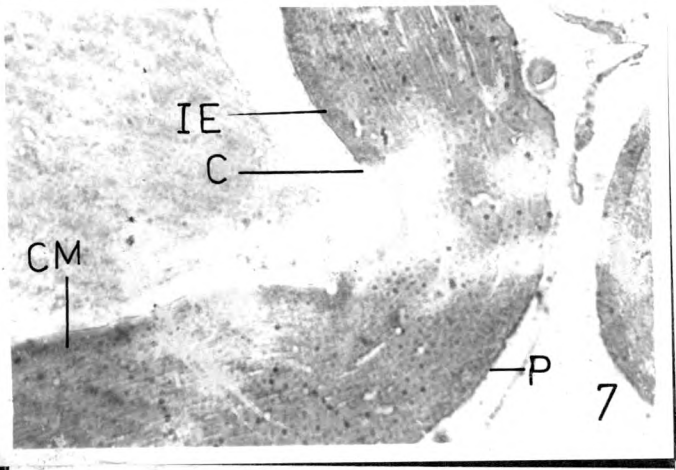
Fig. 8 Section passing through the Gizzard of P. posthuma stained with AB 2.5 - PAS to show intense activity of only PAS In internal epithelium (IE), and circular muscles (CM) X 800.

Fig. 9 Section passing through the Stomach of P. posthuma stained with PAS to show intense staining in mucous gland cells (MG), internal epithelium (IE), and circular muscles (CM). X 600.

Fig. 10 Section passing through the Stomach of P. posthuma stained with AB 2.5 - PAS to show AB staining in gland cells (G), and PAS staining in internal epithelium (IE) and circular muscles (CM). X 800.

Fig. 11 Section passing through the Pretyphlosole Intestine of P. posthuma stained with AB 2.5 - PAS to show intense AB and PAS activity in gland cell (GC), chloragogen cell (CC), and internal epithelium (IE) and only PAS activity in circular muscle (CM) and longitudinal muscle (LM). X 800.

Fig. 12 Section passing through the Typhlosole Intestine of P. posthuma stained with HE to show typhlosole (T), internal epithelium (IE), circular muscles (CM), longitudinal muscles (LM) and peritoneal epithelium (P) X 600.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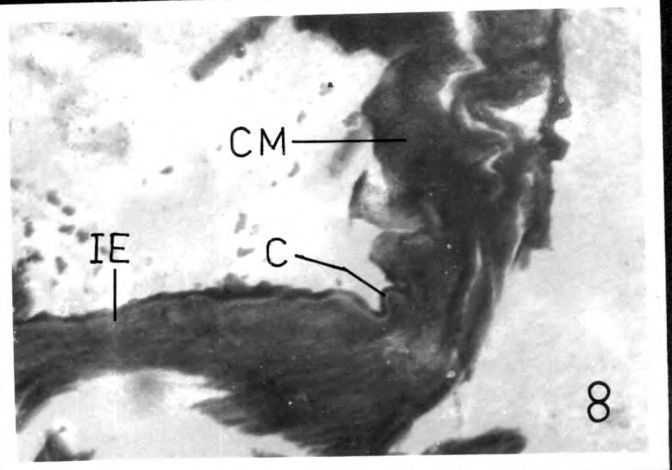
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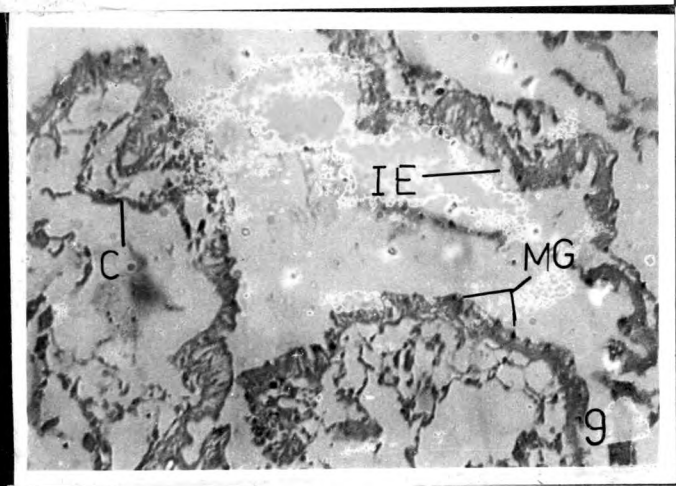
IE
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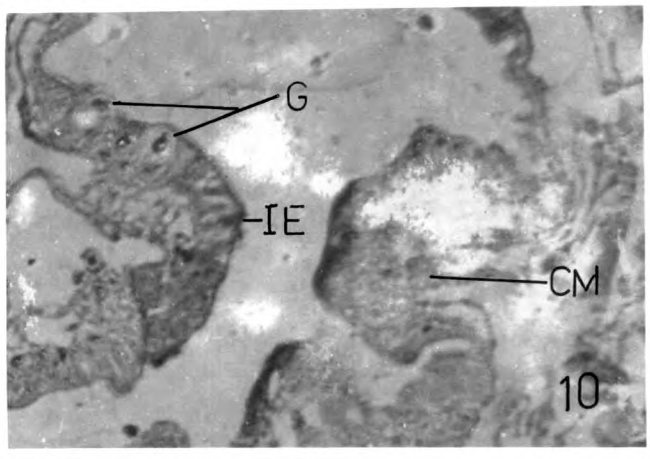
MG
9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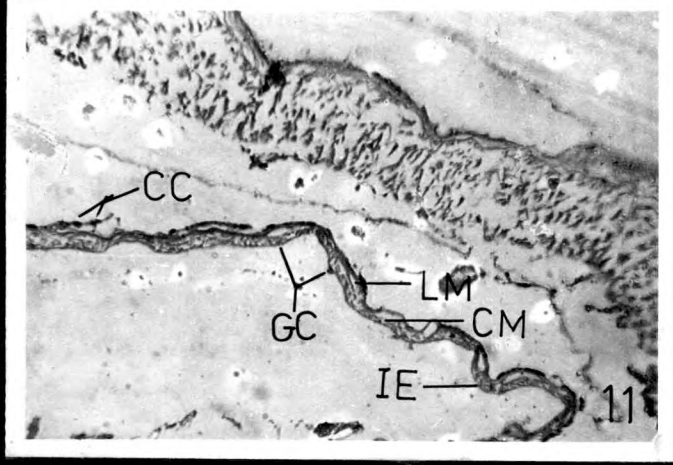
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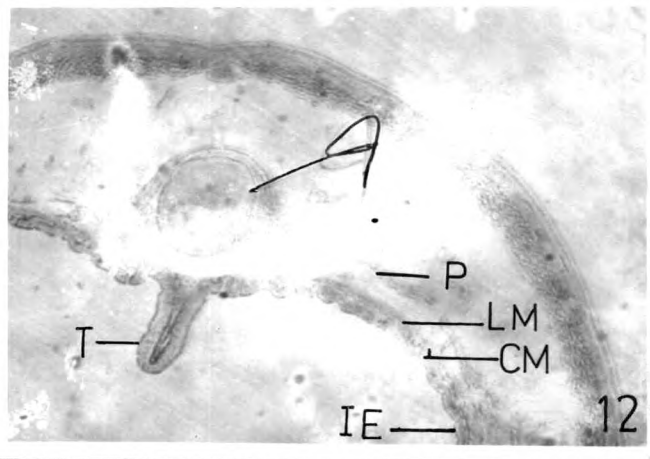
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pharynx.

The inner epithelial cells in the oesophagus (Fig. 6) contained only diastase resistant PAS reactive neutral mucosubstances. The mucous gland cells were few in number and contained neutral mucosubstances and sulfomucin which were slightly in less amount. These observations were based on the staining reactivities of the innerepithelium and mucous gland cells in the inner epithelium of oesophagus, which were identical to those described for inner epithelium and mucous gland cells in buccal chamber and pharynx.

4) Gizzard :

The oesophagus in eighth segment was modified into a prominent, oval, hard and thick-walled muscular organ the gizzard. Histologically, the outer peritoneal epithelium was formed of tall and narrow cells. The entire thickness of the wall of the gizzard was made up of circular muscle fibres only (Fig. 7)and there was total lack of the longitudinal muscles. The internal epithelial lining of the gizzard consisted of cuboidal epithelial cells lined with a thick internal cuticle.

HISTOCHEMICAL OBSERVATIONS :

The staining reactivities of the Peritoneum of

gizzard were slightly identical to those described for peritoneal epithelium in earlier organs described so far. Hence, it was concluded that the peritoneum in gizzard also contained only diastase resistant PAS reactive neutral mucosubstances.

The circular muscles in the wall of gizzard (Fig. 8) contained slightly enhanced quantities of glycogen but not acidic and other neutral mucosubstances. There were no longitudinal muscles in the gizzard. The histochemical reactivities were similar to those described for muscular layers in buccal chamber, pharynx and oesophagus.

The inner epithelial layer of the gizzard consisted of cuboidal epithelial cells lined with a thick layer of cuticle. These epithelial cells exhibited intense (Fig. 8) PAS reactivity which could completely be blocked by phenylhydrazine pretreatment but was diastase resistant indicating absence of glycogen but presence of neutral mucosubstances in them. This conclusion was further supported by their only pink to magenta colouration with sequential staining techniques such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. - PAS, and only blue orthrochromatic staining with azure at higher pH levels. The absence of acidic mucosubstances was inferred from their negative reactivity, with AB pH 1.0, AB pH 2.5, C.I. and AF. Pepsin digestion had no effect on these staining reactivities.

The cuticle remained unaffected with all histochemical reactivities.

5. Stomach :

Histologically, the stomach resembled oesophagus in its outer peritoneal epithelial layer and muscular layer except that in peritoneal layer few chloragogen cells were observed. The folding of the stomach epithelium attained a considerable degree of complexcity and thrown into the transverse folds. The epithelial cells were ciliated and glandular in nature. The wall of the stomach was highly vascular and glandular.

HISTOCHEMICAL OBSERVATIONS :

The histochemical reactivities of the peritoneal layer of the stomach were practically similar (fig. 10) to those of buccal chamber, pharynx, oesophagus and gizzard. Hence it was concluded that the serosa contained diastase resistant PAS reactive neutral mucosubstances. Chloragogen cells which were scattered in the peritoneal layer also indicated presence of glycogen as the PAS staining reactivity could be blocked by phenyl-hydrazined pretreatment and abolished by diastase digestion.

The longitudinal and circular muscles in the wall of stomach (Fig. 9) contained glycogen. Their tinctoreal affinities were identical to those exhibited by the muscle layers in buccal chamber, pharynx, oesophagus and gizzard.

The inner epithelial layer cells (Fig. 9) contained only diastase resistant PAS reactive neutral mucosubstances and absence of glycogen in them. These cells remained unstained with AB pH 1.0, AB pH 2.5, C.I., and AF The pepsin digestion had no effect on these staining reactivities. The histochemical reactivities were similar to those exhibited by the inner epithelium in buccal chamber, pharynx, oesophagus and gizzard.

The staining reactivities of the epithelial cells in the inner epithelium were identical to those described for buccal chamber and oesophagus. Hence it was concluded that the epithelial cells of the inner epithelial layer contained only neutral mucosubstances and there was no glycogen in them. The gland cells (Fig. 10) also exhibited the staining reactivities which were similar to those described for buccal chamber, pharynx. and oesophagus. These gland cells contained sulfomucins in traces and neutral mucosubstances in predominant amounts. These conclusions are based upon the histochemical reactivities of the mucous gland cells in the inner epithelial layer of the stomach.

CAPTIONS TO FIGURES

Fig. 13 Section passing through the Typhlosole Intestine of P. posthuma stained with PAS intense activity in internal epithelium (IE), gland cells (GC), chloragogen cell (CC). X 1200.

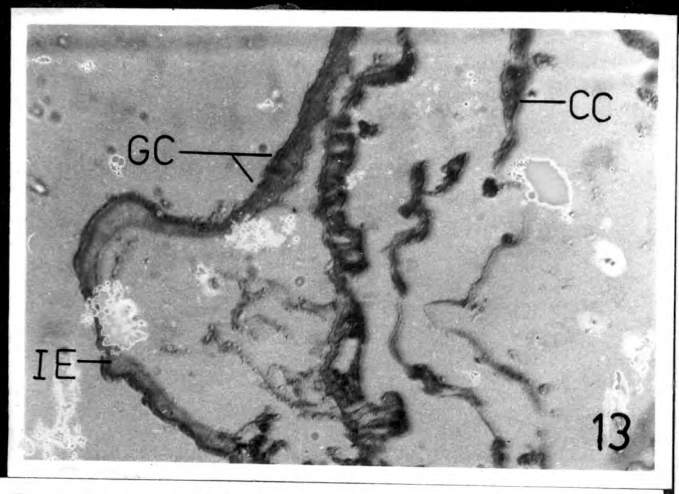
Fig. 14 Section passing through the Typhlosole Intestine of P. posthuma stained with AB 1 - PAS to show different types of gland cells (G_1 and G_2) and intense AB staining in internal epithelium (IE) X 1200.

Fig. 15 Section passing through the Typhlosole Intestine of P. posthuma stained with AB 2.5 to show intense staining in internal epithelium (IE), gland cells (GC) and chloragogen cells (CC) X 1200.

Fig. 16 Section passing through Typhlosole Intestine of P. posthuma stained with AB 2.5 - PAS to show AB and PAS staining in gland cell (GC), internal epithelium (IE) and peritoneal epithelium (P) X 800.

Fig. 17 Section passing through the Posttyphlosole (rectum) of P. posthuma stained with PAS to show PAS activity in gland cell (GC), chloragogen cells (CC), circular muscle (CM) and longitudinal muscle (LM) X 800.

Fig. 18 Section passing through the Posttyphlosole (rectum) of P. posthuma stained with AB 2.5 to show intense staining in gland cell (GC), internal epithelium (IE) and less activity in circular muscles (CM), longitudinal muscles (LM) and peritoneal epithelium (P) X 800.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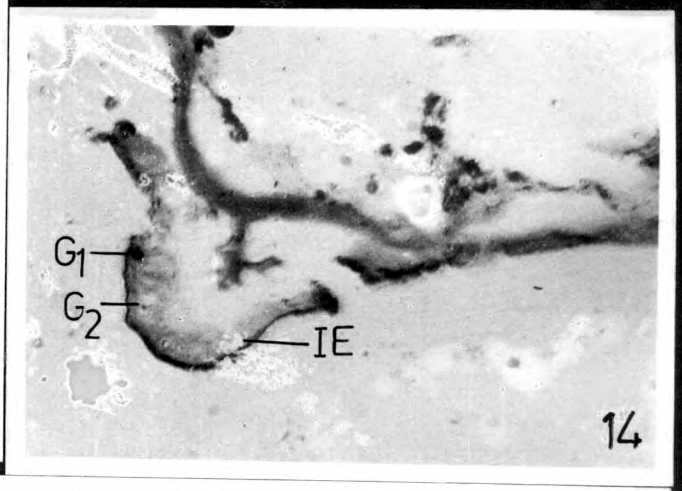
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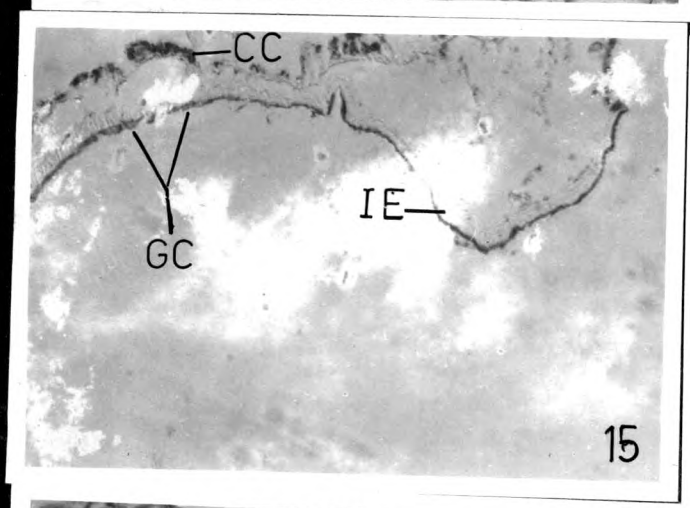
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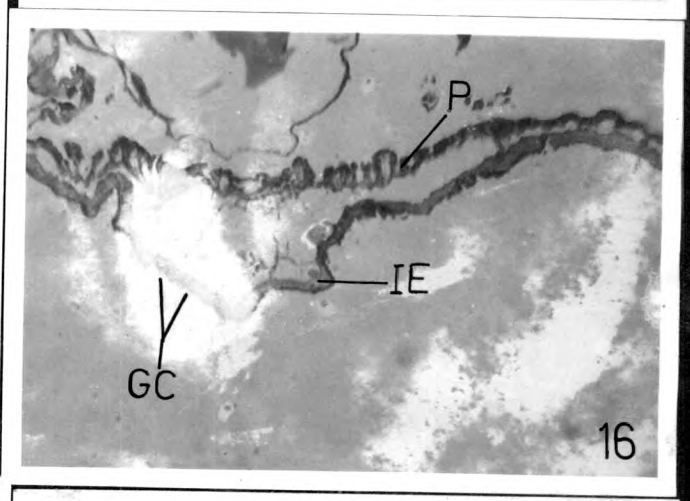
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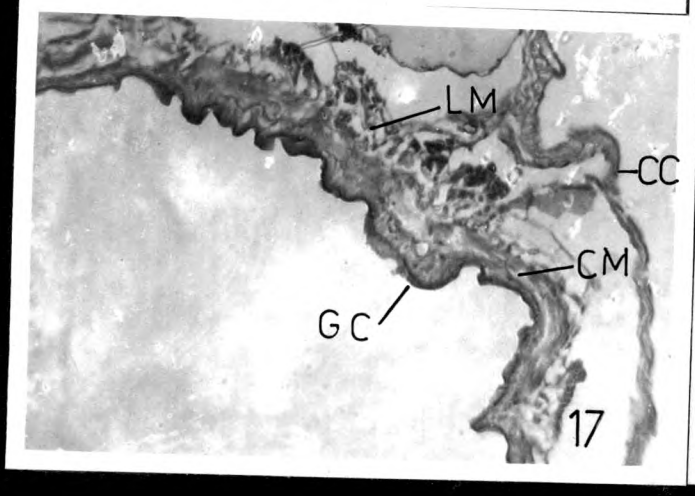
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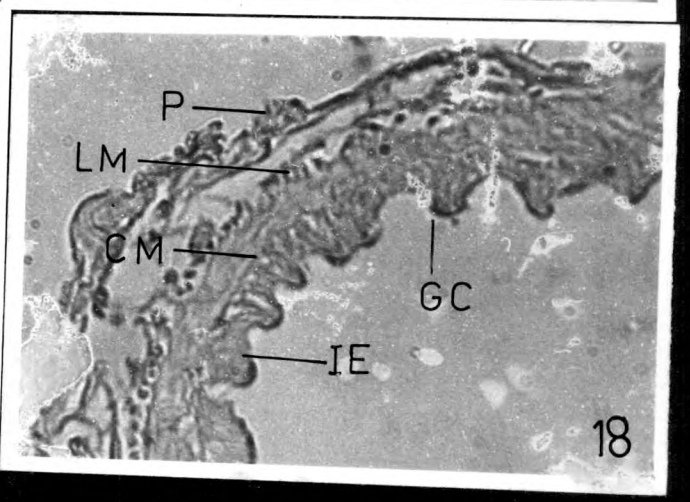
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6. Intestine :

The intestine was a long, wide and thin walled tube extending from fifteenth segment to the last. It had beaded appearance due to constrictions corresponding to septa but buldging in each segment. The intestine was divided into pre-typhlosolar, (Fig. No. 11) typhlosolar, (Fig. 12) and post-typhlosolar (or rectum) (Fig. No. 17) regions. The histological structure in all the three regions was identical. The outer peritoneal epithelium consisted of tall epithelial cells. Some of these cells contained chloragosomes, a yellow refractile granules. These cells, the chloragogen cells (Fig. 12) were scattered throughout in the intestinal peritoneal epithelium. In intestine, the longitudinal and circular muscle layers were feebly developed. The internal lining of the epithelium was ciliated, folded, vascular and glandular. The ciliated cells were elongated and narrow and showed characteristic intracellular fibrils. Four or five of the ciliated cells surrounded a glandular cell. The cytoplasm of the glandular cells exhibited vacuolated appearance. The glandular cells were abundent in the typhlosolar region and their number reduced considerably in the post-typhlosolar region or in rectum. The intestinal caecae which occured in the pre-typhlosolar region of the intestine showed similar histological structure as described for the intestine in general.

HISTOCHEMICAL OBSERVATIONS :

The peritoneal epithelium of intestine, in all the three regions and intestinal cæecae exhibited practically similar staining reactivities to the peritoneal epithelium of buccal chamber, pharynx, oesophagus, stomach and gizzard. Hence it was concluded that the peritonium contained diastase resistant PAS reactive neutral mucosubstance. The chloragogen cells exhibited intense PAS staining (Figs. 11, 13, and 17). Which could be blocked by phenylhydrazine pretreatment and abolished by diastase digestion indicating presence of glycogen in them.

As in buccal chamber, pharynx, oesophagus, gizzard and stomach, the longitudinal and circular muscle layers in pre-typhlosolar, typhlosolar and post-typhlosolar intestine also contained glycogen. The muscle layer exhibited (Fig. 11, 13 and 17) histochemical reactivities which resembled to those described for all earlier organs.

The inner epithelial layer of the pre-typhlosolar, typhlosolar and post-typhlosolar (rectum) regions of the intestine showed distribution of glandular cells amongst the ciliated cells. The epithelial cells were tall, columnar in nature. The number of glandular cells appeared to be increased in the typhlosolar (Fig. 15, 16) region whereas the other two regions contained little less number (Fig. 11 and 18) of these gland cells. The

histochemical reactivities in epithelial cells and glandular cells in all the three regions revelaed similar results.

The epithelial cells showed poor PAS staining (Fig. 13) which was diastase resistant but could be blocked by prior phenylhydrazine treatment. These initial staining reactivities indicated absence of glycogen but presence of neutral mucosubstances in them. 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, AB at pH 2.5), C.I., AF and only blue orthrochromasia with azure A at higher pH levels. These results indicated the presence of neutral mucosubstances in the epithelial cells.

Gland cells : Although the glandular 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. 14). The gland cells which exhibited alcianophilia at pH 1.0 are referred to as G_1 gland cells and those which remained unstained are referred to as G_2 gland cells. With AB pH 1.0 - PAS staining procedure G_1 cells appeared purple blue and G_2 cells pink or magenta.

G_1 Gland Cells :

These cells exhibited an intense PAS reaction (Figs. 11, 13 and 17). Their PAS reactivity was diastase resistant but could be partly blocked by phenylhydrazine pretreatment indicating the absence of glycogen but presence of neutral mucosubstances. More over, these cells exhibited moderate alcianophilia at pH 1.0 (Fig. 14) and an intense alcianophilia at pH 2.5 (Fig. 15 and 16) which indicated the presence of both sulfomucins (predominant) and carboxymucins in less quantities). The presence of sulfomucins in them was 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, 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.

The presence of carboxymucins in G_1 gland cells was supported by their enhanced alcianophilia at pH 2.5 (Fig. 15) 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 sections. The carboxymucins were identical as

sialomucins since their alcianophilia was partly sensitive to acid hydrolysis and neurominidase digestion. Hyaluronidase digestion and pepsin digestion has no effect on these tinctorial affinities. Thus the G_1 gland cells contained neutral mucosubstances, sulfomucins and sialomucins.

G_2 Gland Cells :

These cells also exhibited an intense PAS reactivity (Fig. 11, 13 and 17) which could partly be blocked by prior phenylhydrazine treatment 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 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. 15) indicated the presence of carboxymucins in them. The presence of carboxymucins in these cells was also inferred from their intense blue staining reactivity with C.I., purple-blue colouration with AB pH 2.5-PAS, and C.I. - PAS sequence, only blue staining in AF - AB pH 2.5 sequence, intense metachromasia with azure A at pH 3.00 and above, suppression of their

alcianophilia in CEC technique by the addition of 0.1M 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 hydrclysis and neuraminidase digestion complete abolished their alcianophilia. Hyaluronidase and pepsin digestion had no effect on the aforementioned histochemical results. Thus the G_2 gland cells elaborated neutral mucosubstances and sialomucins.

The histochemical reactivities of the different regions and their histological layer showed no variations in the earthworm normally fed and fed with blotting papers, selected for the present investigation.

II. H. granulosa :

A. Alimentary Canal :

The alimentary canal of H. granulosa was a straight tube running the mouth to the anus. It consisted the pre-oral chamber, the buccal cavity, the pharynx, the oesophagus, the crop, the stomach, the intestine and the rectum.

The pre-oral chamber was a cup-shaped depresion on

the ventral aspect of the anterior sucker. The prostomium and the first four segments of the body formed the roof of the pre-oral chamber, while the circular rim of the sucker formed its outer boundary. At the base of the pre-oral chamber was the tri-radiate mouth guarded by the velum which formed an almost complete partition between the pre-oral chamber in front and the buccal cavity behind.

The mouth was followed by a very short chamber, the buccal cavity, lying just behind the velum. The mucous membrane of the buccal cavity presented three deep crypts, in each of which was embedded a Crescentic jaw. Each jaw was a laterally compressed muscular cushion with a semi-circular profile. Of the three jaws one jaw was medio-dorsal, while the outer two were ventro-lateral in position. The cuticular covering of each jaw was thickened along its summit to form a dentigerous ridge bearing a row of minute monostichodont teeth (denticles).

Evenly scattered over both the flanks of a jaw were small button shaped salivary papillae, each of which bears a number of openings of the salivary glands.

The buccal cavity continued through a very narrow aperture, into the pharynx, which was an oval sac extending

from the fifth to the eight segment. It was characterised by a great development of muscles in its wall. The lumen of the pharynx varies in outline in different regions. In a transverse section of the anterior end, in the region of the nerve collar the pharyngeal lumen had the form of a triangle. The muscles of the pharynx were cut in the form of three sectors, a medio-dorsal and two ventro - laterals. A section of the middle region of the pharynx showed a much wider lumen produced into six angular diverticula. The wall presented six folds, which may give rise to secondary folds thus increasing the number of folds from six to twelve.

The salivary glands were large masses of pyriform unicellular glands covering and surrounding the wall of the pharynx and even extending behind it. They filled the entire space between the pharynx and the body wall.

The oesophagus has a short narrow region of the gut through which the pharynx leads into the crop. It was separated from the pharynx by bands of muscles which stretch across the oesophagus from one side of the body wall to the other. The lumen of the oesophagus was very narrow. The crop formed the largest region of the alimentary canal, occupying about two-third of the visceral space and extending from somite 9^{th} to 18^{th}. It consisted of a metameric series of ten thin walled chambers which passed into one another through

a series of more or less circular openings, each surrounded by a sphincter. Each chamber of the crop was divided by a shallow constriction into a small anterior and a broad posterior part which produced into a pair of lateral outgrowths, the caecae. Of these caecae or diverticulae, the anterior two or three pairs were usually small and irregular, but the succeeding ones go on gradually increasing in size. All the caecae pointed backward and some of the larger ones may extend far back into succeeding somites. The posterior part of each chamber was in the form of a broad inverted funnel. The tenth or the last chamber of the crop in somite 18^{th} was the largest, its caecae were prolonged backward to segment 22^{nd} or even further backward, forming two greatly elongated blind sacs, one on each side of the intestine, which was present dorsally above and between them. Each of these long caecae gave off, on its outer side, several small secondary caecae, which become apparent when the crop was distended. Posteriorly the crop becomes narrow and formed a long funnel-shaped tube in segment 18^{th} which entered the stomach.

The stomach was a small chamber in segment 19^{th}, as its anterior end was dilated, it presented a heart-shaped appearance, its walls were produced internally into transverse folds which anastomosed with one another. The stomach continued behind into the intestine which was not clearly

differentiated externally from the stomach. It was a straight narrow tube extending from the 20^{th} to the 22^{nd} somites, its inner lining formed numerous longitudinal and transverse folds resembling spiral villi. The wall of the intestine was comparatively thinner than that of the stomach and was supplied with numerous haemocoelomic capillaries. At its posterior end the intestine became narrow and passed into the rectum which was a simple thin-walled tube, extending from the 22^{nd} to the 26^{th} segment and opening to the exterior through the anus located dorsally over the posterior sucker.

B. Histological and Histochemical Observations :

The histology of the various organs in the alimentary canal of H. granulosa was described by making observations on H - E stained preparations. The mucosubstances in the various organs in the alimentary canal were investigated by employing a series of histochemical techniques. The results obtained with various histochemical techniques for mucosubstances are recorded in Table No. 2. The histochemical localization in mucosubstances in various region of the alimentary canal are shown in photomicrographs (Figs. 19 to 30).

Histologically, the alimentary canal consisted of inner layer of columnar epithelial cells separated by a basement

membrane from an outer layer of connective tissue. The connective tissue contained muscle fibres and haemocoelomic capillaries. The columnar epithelium was lined by cuticle in fore and hind guts and contained scattered goblet cells in the crop.

1. **Pre-oral Chamber and Buccal Cavity** :

Histologically, the cuticle and the epidermis of the body wall were continued into the pre-oral chamber, the cuticular lining of which was very thin, while the epithelial cells were narrow, elongated and more closely packed together than those of the body wall. The tubular glands in pre-oral chamber and mucous gland in buccal cavity were present in fairly large numbers. The mucous membrane of the buccal cavity presented three deep crypts, in each of which was embedded a cresentic jaw. The epithelial cells in buccal cavity formed a thin layer of flattened and drawn out epithelial cells which were covered by thin cuticle. Fine strands of richly nucleated connective tissue fibres occured between the epithelium and the circular layer of muscles.

HISTOCHEMICAL OBSERVATIONS :

The muscles in pre-oral chamber and buccal cavity (Fig. 19 and 20) exhibited weak PAS staining which could be

blocked by phenylhydrazine pretreatment and abolished by diastase digestion indicating presence of glycogen in them. The muscles remained unstained with AB pH 1.0, AB pH 2.5, and AF and exhibited only blue orthrochromatic staining with Azure A thus, showing 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.

The connective tissue showed poor to weak PAS reactivity (Figs. 19 and 20). 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 absence of sulfomucins in them. Their alcianophilia at pH 2.5 and C.I. respectively revealed the presence of carboxymucins in connective tissue. This conclusion was further substanciated from their purple-blue staining with AB pH 2.5 - FAS 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 their reversible blockade of their alcianophilia of in methylation - sapcnification 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, neurominidase and pepsin digestion had no effect on these staining reactivities. These results indicated presence of neutral mucosubstances and hyaluronic acid in the connective tissue of pre-oral chamber and buccal cavity.

The epithelial cells of the pre-oral chamber and buccal cavity exhibited poor PAS reactivity (Figs. 19 and 20) which was diastase resistant but could be completely blocked by prior phenylhydrazine treatment. These primary staining reactivities indicated absence of glycogen but presence of neutral mucosubstances. These cells remained unstained with AB pH 1.0, AB pH 2.5, C.I. and AF and exhibited only blue orthrochromatic staining at higher pH levels. These histochemical results remained unchanged even after pepsin digestion. These histochemical results indicated the absence of acidic mucosubstances. The presence of neutral mucosubstances was 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 colouration. The epithelium of the crypts that surrounded the jaws (Fig. 20) and the salivary papillae on the jaws showed similar histochemical reactivities indicating presence of neutral mucosubstances in them. The cuticle remained unaffected in all these staining reactivities.

CAPTIONS TO FIGURES

Fig. 19 Section passing through the Buccal Mass (Jaw) of H. granulosa stained with PAS to show intense activity in jaw (J), epithelium (E), salivary papilae (SP) X 1200.

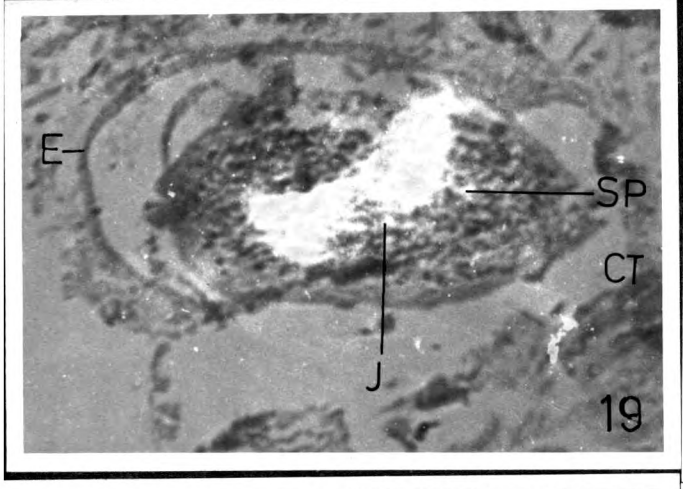
Fig. 20 Section passing through the Buccal Mass of H. granulosa stained with PAS to show intense activity in epithelium (E) and muscles (M) X 800.

Fig. 21 Section passing through the Pharynx and salivary gland of H. granulosa stained with HE to show salivary gland (SG), epithelium (E), muscles (M), connective tissue (CT) X 800.

Fig. 22 Section passing through the Pharynx of the H. granulosa stained with PAS to show staining reaction in epithelium (E), muscles (M) and connective tissue (CT) X 800.

Fig. 23 Section passing through the Oesophagus of H. granulosa stained with AB 2.5 - PAS to show AB activity in epithelium (E), muscles (M), and connective tissue (CT) X 1000.

Fig. 24 Section passing through the Crop of H. granulosa stained with HE to show central chamber (CC), lateral chamber (LC), epithelium (E), muscles (M) and connective tissue (CT) X 800.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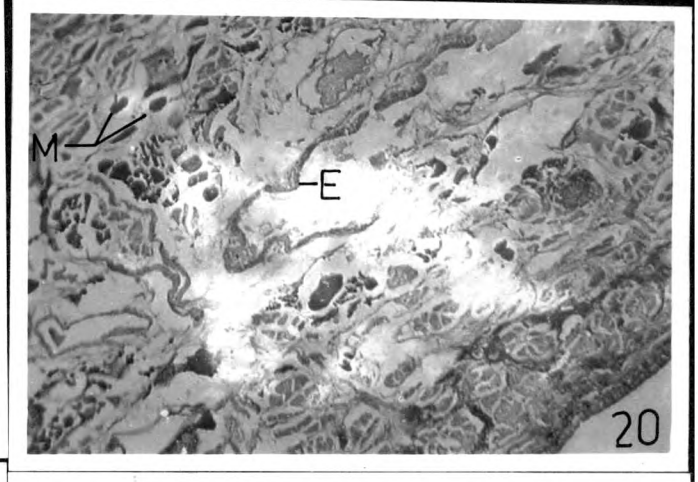
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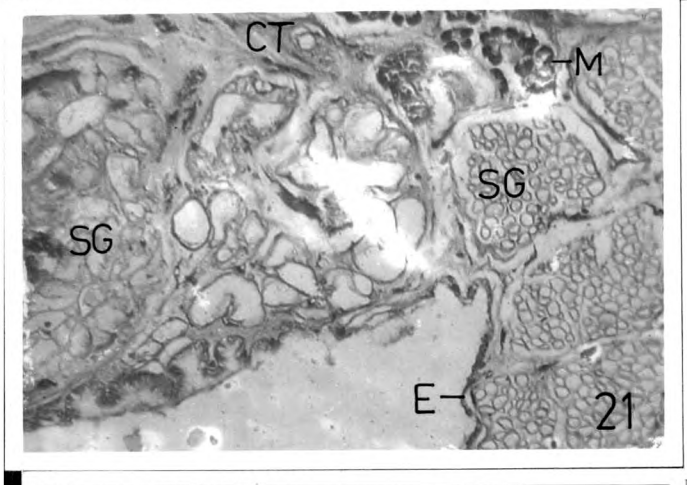
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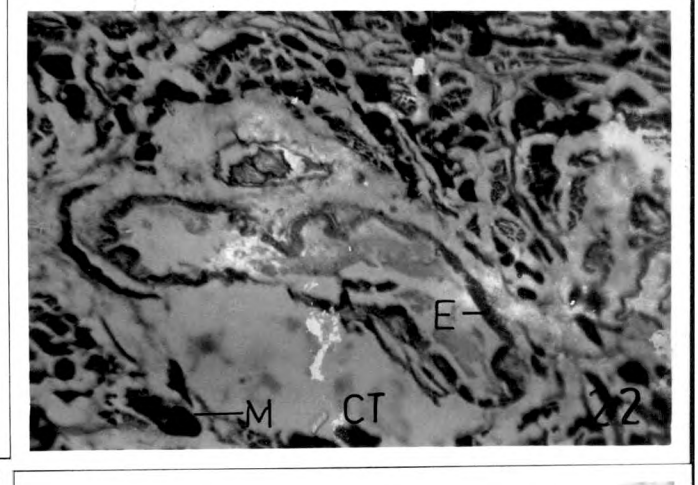
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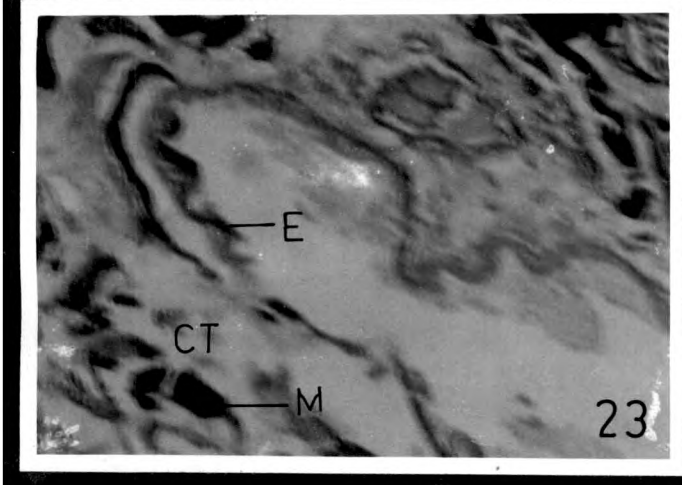
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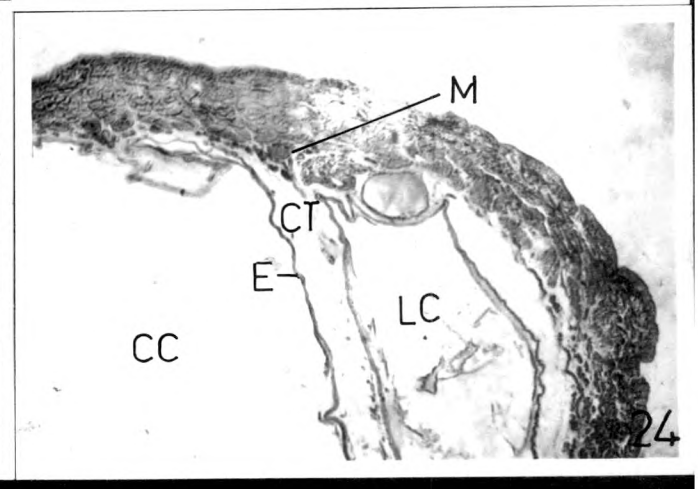
M
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24

The tubular gland cells in the pre-oral chamber and mucous gland cells in the buccal cavity (Fig. 20) exhibited intense PAS staining 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 absence of glycogen and presence of neutral mucosubstances. Further, these cells were poorly stained with AB at pH 1.0 and their staining intensity was not enhanced at pH 2.5. These results indicated the presence of sulfomucins in traces but absence of carboxymucins. The presence of sulfomucins in these cells was substantiated from their weak purple staining with AF alone or AB pH 2.5 step afterwards, weak metachromasia with azure A at pH 1.5, persistant alcianophilia in CEC technique in presence of 0.2M Mg^{++} and loss of alcianophilia by active methylation which could not be restored after subsequent saponification. The sulfomucins were hyaluronidase resistant. These results thus, revealed presence of neutral mucosubstance (Predominant) and sulfomucins (traces) in these cells.

2. Pharynx :

The cuticular lining and epithelium of the pharynx (Fig. 21) were similar to the buccal cavity, but the muscular layer was highly developed and consisting of three sets of

muscles - i) the longitudinal layer, situated just outside pharyngeal epithelium, and consisting of bundles of small fibres separated from one another by radial muscles passing in between them; (ii) the circular layer, which lies outside the longitudinal and consists of a series of loop - like rings surrounding the pharynx and lying one behind the other, the radial muscles making their way between successive rings of circular muscles, and (iii) the radial muscles, which do not form a regular layer but run in bundles in such a way that one end of each muscle was inserted on the pharyngeal epithelium, while the other runs right through the wall of the pharynx and was inserted on the body wall. The number of bundles of the radial muscles was very large.

HISTOCHEMICAL OBSERVATIONS :

The longitudinal, circular and radial muscles in the pharynx exhibited intense PAS staining (Fig. 22) which could completely be blocked by phenylhydrazine pretreatment and abolishment by diastase digestion indicating presence of glycogen in them. No staining reactivities were identified with AB pH 1.0, AB pH 2.5, C.I. and AF and exhibited only weak blue orthrochromatic staining with azure A. Thus, indicating the absence of acidic mucosubstances. The muscle appeared weak pink in sequential staining techniques such as AB 1.0 - PAS, AB

pH 2.5 - PAS and C.I. - PAS.

The connective tissue in the pharynx (Fig. 22) 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 pre-oral chamber and the buccal cavity.

The epithelial cells of the pharynx (Fig. 22) showed identical staining affinities to those exhibited by the epithelium of the pre-oral chamber and buccal cavity. Thus it was concluded that the epithelial cells of the pharynx contained diastase resistant and PAS reactive neutral mucosubstances.

The salivary gland cells (Fig. 22) exhibited intense PAS reaction. Their PAS reactivity was diastase resistant but could partly be blocked by prior phenylhydrazine treatment indicating absence of glycogen but presence of neutral mucosubstances. These cells exhibited intense alcianophilia at pH 1.0 and moderate alcianophilia at pH 2.5 which indicated the presence of sulfomucins (in less quantities) and carboxymucins (predominent). The presence of sulfomucins in salivary glands was further, inferred from their moderate reactivity with AF alone and blue purple staining with AF - AB pH 2.5 sequence,

moderate metachromasia with azure A at pH 1.5, persistant alcianophilia in CEC technique upto 0.5 M Mg^{++} and complete loss of alcianophilia by active methylation and failure to restore it completely after saponification. The sulfomucins were hyaluronidase resistant.

The presence of carboxymucins in the salivary glands was evident from their moderate alcianophilia at pH 2.5 and weak C.I. reactivity, metachromasia with azure A at pH 3.0 and above and partial restoration of their alcianophilia after saponification of previously methylated sections. The carboxymucins were identified as sialomucins as their alcianophilia was partly sensitive to acid hydrolysis and neuraminidase digestion. Hyaluronidase and pepsin digestion had no effect on these staining reactivities. The salivary gland cells contained neutral mucosubstances (in less amount), sulfomucins (predominant) and sialomucins (in less amount).

3. Oesophagus :

The oesophagus was a short narrow region of the gut through which the pharynx leads into the crop. Histologically, the epithelial lining of the oesophagus was highly folded and consists of very narrow elongated cells, while the basement membrane was thin. A loose connective tissue and a few

circular muscle-fibres were present under the oesophageal epithelium.

HISTOCHEMICAL OBSERVATIONS :

The circular muscles in the wall of oesophagus (Fig. 23) contained glycogen but not acidic or other neutral mucosubstances. Their histochemical reactivities were similar to those exhibited by the circular muscles in pre-oral chamber and buccal cavity.

The connective tissue of the oesophagus (Fig. 23) contained neutral mucosubstances and hyaluronic acid. This conclusion was based on staining reactivities of the connective tissue which were identical to those described for the connective tissue of buccal chamber and pharynx.

The epithelial cells of the oesophagus contained diastase resistant and PAS reactive neutral mucosubstances but not the glycogen. This was evident from the staining reactivities of the oesophageal epithelium which were similar to those described for the epithelium of the buccal cavity and pharynx.

4. Crop :

The crop forms by the largest region of the alimentary canal. Histologically, the wall of the crop (Fig. 24) had a thin epithelial lining of prismatic cells which show closely-set rodlets along their free border. The cytoplasm was alveolar. A small oval nucleus lies in the basal part of each cell. A few goblet cells were seen at several places amongst the ordinary epithelial cells. The basement membrane was thin and the connective tissue layer enveloping the crop contains numerous muscle - fibres which form a contractile net. Numerous haemocoelomic capillaries were present in the connective tissue.

HISTOCHEMICAL OBSERVATIONS :

The muscles in the wall of the crop (Fig. 25) exhibited intense PAS reaction which could be completely blocked by prior phenylhydrazine treatment and abolished by diastase digestion indicating absence of glycogen but presence of other neutral mucosubstances. This conclusion was substantiated by the staining reactivities of the muscles in the wall of crop which were practically similar to those described for the muscles in the pharynx.

The connective tissue, like the earlier organs described

so far, of the crop contained neutral mucosubstances and hyaluronic acid. The connective tissue of the crop showed practically identical staining affinities to the connective tissue of buccal chamber and pharynx.

The inner epithelium of the crop also contained neutral mucosubstances. The epithelial cells exhibited histochemical reactivities which resembled to those described for epithelial layer of pharynx and oesophagus.

The goblet cells, in the crop reacted intensely towards PAS (fig. 25). 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. Moderate alcianophilia at pH 1.0 indicated the presence of sulfomucins which were predominent. The enhanced alcianophilia at pH 2.5 indicated the presence of carboxymucins too. 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 pH 1.5, persistant alcianophilia 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.

CAPTIONS TO FIGURES

Fig. 25 Section passing through the Crop of H. granulosa stained with PAS to show staining activity in goblet cells (G), epithelium (E) and connective tissue (CT) X 800.

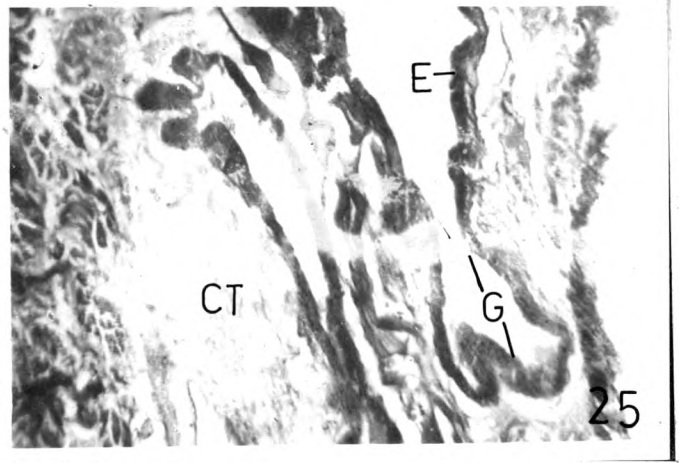
Fig. 26 Section passing through the Stomach of H. granulosa stained with HE to show epithelium (E), muscles (M) and connective tissue (CT) X 1200.

Fig. 27 Section passing through the Stomach of H. granulosa stained with AB 1 - PAS to show AB and PAS activity in epithelium (E) and connective tissue (CT) X 2000.

Fig. 28 Section passing through the Intestine of H. granulosa stained with HE to show epithelium (E) and connective tissue (CT) X 2400.

Fig. 29 Section passing through the Intestine of H. granulosa stained with AB 2.5 - PAS to show lateral caecae (LC) and AB and PAS activity in epithelium (E) muscles (M) and connective tissue (CT) X 1200.

Fig. 30 Section passing through the Rectum of H. granulosa stained with PAS to show lateral caecae (LC) and rectum (R) and PAS activity in epithelium (E), muscles (M), connective tissue (CT) X 800.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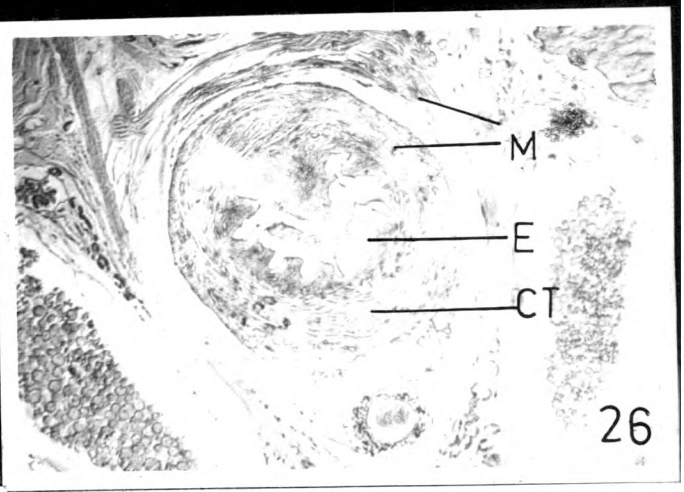
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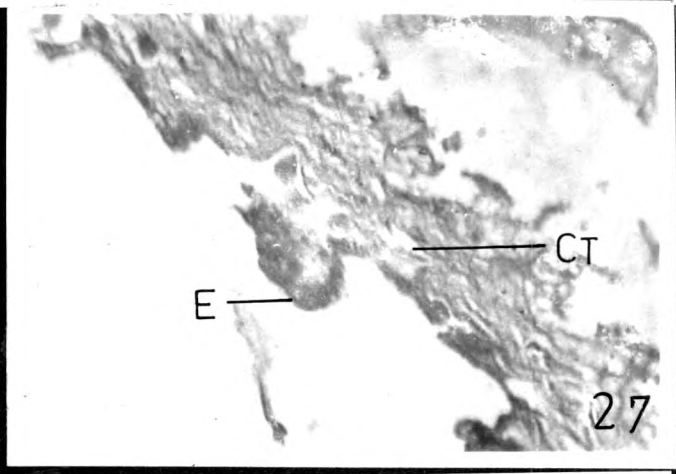
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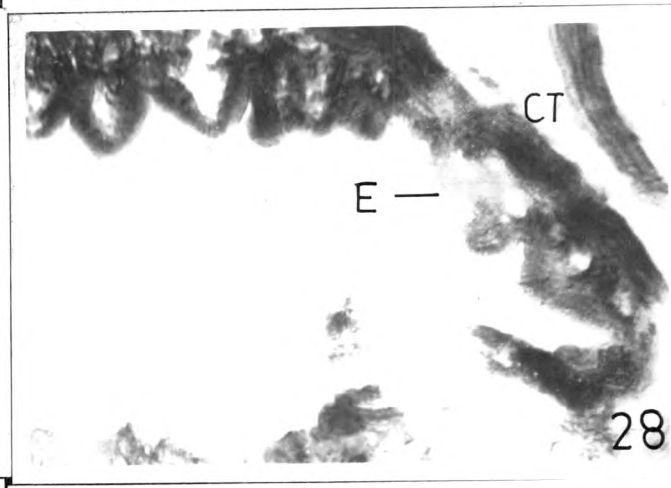
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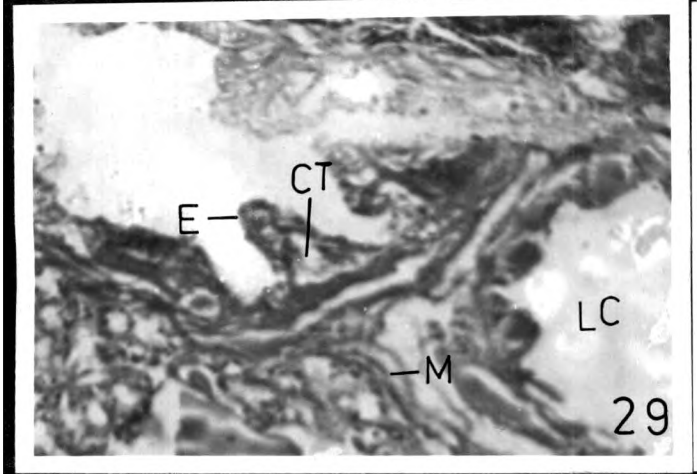
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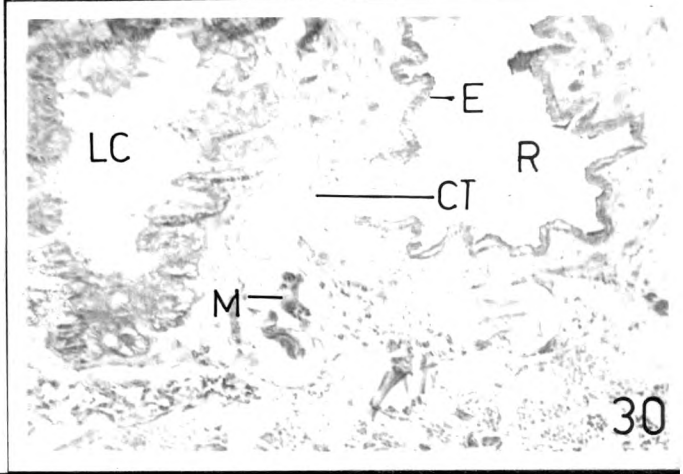
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The enhanced alcianophilia at pH 2.5 than at pH 1.0 indicated the presence of carboxymucins in the goblet cells. This view was substantiated by their intense C.I. reactivity, purple - blue staining with AB pH 2.5 - PAS and C.I. - PAS sequence, blue - purple staining in AF - AB pH 2.5 sequence, enhanced metachromasia with azure A at pH 3.0 and above and only partial restoration of their alcianophilia following saponification of actively methylated 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).

5. Stomach :

The stomach was a small chamber in segment 19^{th}. It presents a heart shaped appearance, its wall were produced internally into transverse folds.

The lining of the stomach was thrown into folds, each consisting of a core of connective tissue covered over by a single - layered prismatic epithelium. The epithelial cells were taller and narrower than those of the crop, but present a layer of rodlets along their free border, like the cells of the crop. A

small oval nucleus lies in the middle of each cell. The thin basement membrane was surrounded by fairly thick layers of connective tissue and circular muscle - fibre, penetrated by a large number of haemocoelomic capillaries.

HISTOCHEMICAL OBSERVATIONS :

The muscles in the wall of the stomach (Fig. 27) contained glycogen. Their tinctorial affinities were identical to those exhibited by the muscles in the pharynx and crop.

The connective tissue in the large intestine (Fig. 27) contained neutral mucosubstances and hyaluronic acid. This conclusion was based on staining reactivities of the connective tissue which were practically identical to those described for the connective tissue in pre-oral chamber, buccal cavity, pharynx, oesophagus and crop.

The epithelial cells in the inner layer of the stomach showed presence of diastase resistant PAS reactive neutral mucosubstances but absence of glycogen and acidic mucosubstances in them. Their histochemical reactivities were similar to those exhibited by the epithelial cells of buccal cavity, pharynx, oesophagus and crop.

6. Intestine and Rectum :

It was straight narrow tube, extending from the 20th to 22nd somites, its inner lining forms numerous longitudinal and transverse folds resembling spiral villi. The wall of the intestine was comparatively thinners than that of the stomach and was supplied with numerous haemocoelomic capillaries.

Histologically, even though the wall of the intestine was thinner but its inner folds were more numerous than those of the stomach (Fig. 28). The folds or villi were narrow and elongated and their distal ends hanged freely into the lumen. The basement membrane was very thin and was surrounded by loosely arranged connective tissue fibres within which was a meshwork of haemocoelomic capillaries.

The rectum was a simple thin walled tube without any inner folds. Opening to the exterior through the anus.

The rectum was lined by an epithelium of prismatic cells covered by a thin layer of cuticle. The cells were short and narrow and had rodlets on their borders. The connective tissue around the epithelium contained circular and longitudinal muscles and haemocoelomic capillaries.

HISTOCHEMICAL OBSERVATIONS :

The muscles in the wall of the intestine (Fig. 29) and rectum (Fig. 30) also contained glycogen. This conclusion was based on their staining affinities towards different dyes which were practically identical to those exhibited by the muscles in pharynx, crop and stomach.

The connective tissue in the intestine (Fig. 29) and the rectum (Fig. 30) 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 the connective tissue in buccal cavity, pharynx, oesophagus, crop and stomach.

The histochemical reactivities of the epithelial layer of the intestine (Fig. 29) and the rectum (Fig. 30) were practically similar to the epithelial layer of buccal cavity, pharynx, oesophagus, crop and stomach. Hence it was concluded that the epithelial layer contained diastase resistant PAS reactive neutral mucosubsences but absence of glycogen and absence of acidic mucosubstances in them.