OBSERVATIONS

OBSERVATIONS

General histological pattern of the digestive tract of Cypraea arebica arebica shows close similarity with that of the chordates. The pattern exhibits mainly four layers; which are mucosa, submucosa, muscular layer and serosa, from inword to outword. Prominance of muscularis mucosa which is a characterstic of chordate histological pattern; is hardly observed in this snail. The histological records are confirmed with Eosin - Haemotoxyline and Mallory's tripple staining techniques.

The histochemical data on some important staining reactions employed in the present investigation of the digestive tract are recorded in the Tables 1 to 7, according to the visually estimated intensity and shades of colour. These shades of colour are grouped into four categories representing intense activity (****), moderate activity (***), weak activity (**) and poor activity (*). Histochemical observations seeking further details are represented along with the interpretations of the histochemical staining reactions.

BUCCAL MASS

MORPHOLOGY

Digestive tract of the Cypraea arebica arebica commence with the mouth opening situated at the base of siphonal canal of the shell. Mouth opens into a voluminous oblong sac of about 1.5 to 2mm in length and

about 1 mm in thickness. It is the toughest muscular organ of the digestive tract. The toughness is due to complex muscular organization mainly on its dorsal aspect. This is probably associated with the mastication of the injested food. On the mantle floor the region of buccal mass shows a small bulged elevation. In a freshly killed snail the mass shows reddish colour. From the posterior corner of the buccal mass there arises a very elongated redula with strong rasping teeth and the redular caecum like watch spring.

HISTOLOGY

Four layered pattern is well exhibited by the buccal mass. In a cross section of the entire buccal mass the muscles shows their accumulation on the dorsal margin (plate No. 1 fig. no. 4). Serosa is the outermost layer comprised of single celled layer of columnar type. Muscles are oriented in much more complex manner to fascinate the smooth working of the redula. Submucosa shows fibrillar pattern subtending the mucosa layer. Mucosa layer is principally made up of two kind of cells - goblet and columnar inervated by small vacuoles. Redula lies in the lumen of the buccal mass, is a ribbon shaped membrane baset on one surface with teeth arranged in definate pattern in identical transverse rows. Both memberane and teeth are of hard consistancy composed of chitin,

CAPTIONS TO PHOTOMICROGRAPHS

PLATE NO - 1

Fig.1 T.S.of buccal mass-ventral region, stained with AB (pH 2.5) - PAS, Note the intense staining in goblet cells (GC), moderate in columnar cells (CC), submucosa (SM) and museles (M) x 264.

Fig.2 T.S.of buccal mass - ventral region, stained with PAS. Note the intense staining in goblet cells (GC), moderate in columnar cells (CC) submucosa (SM) and muscles (M) x 440.

Fig.3 T.S.of buccal mass - lateral region, stained with AB (pH 1.0) - PAS. Note the intense staining in goblet cells (GC), moderate in columnar cells (CC) and submucosa (SM) x 440.

Fig.4 T.S. of buccal mass stained with Mallaorys tripple. Note the gross view with redula (R) and muscles (M) x 40.

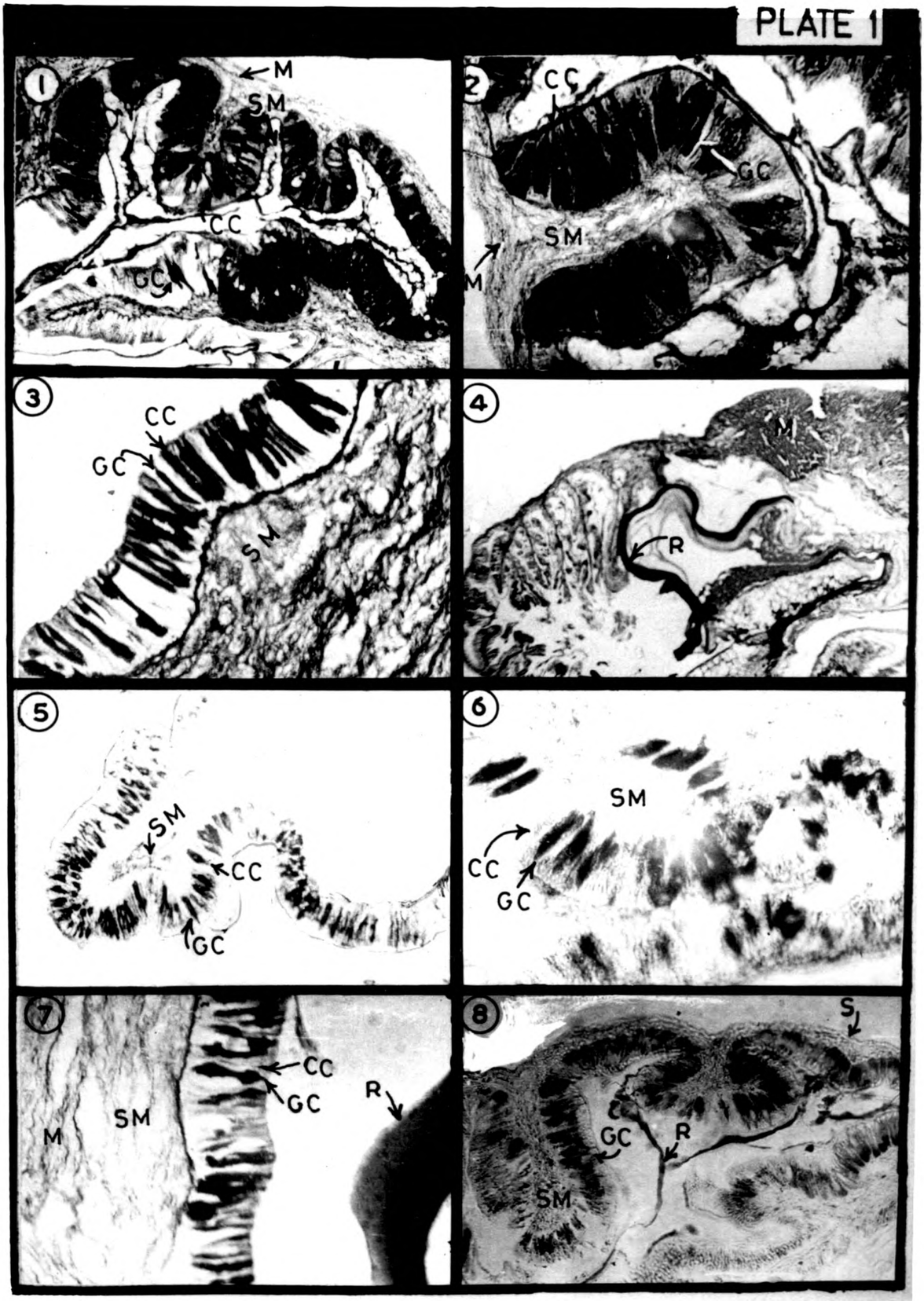
Fig.5 T.S.of buccal mass- ventral region, stained with AF. Note the intense staining in goblet cells (GV), weak in columnar cells (CC) and poor in submucosa (SM) x 264.

Fig.6 T.S.ofbuccal mass - ventral region, stained with acid hydrolysis. Note the moderate staining in goblet cells (GC), and weak in columnar cells (CC) and submucosa (SM) x 440.

Fig.7 T.S. of buccal mass - lateral region, stained with PAS. Note the intense staining in goblet cells (GC) and Redula (R) moderate in columnar cells (CC), and weak in submucosa (M)(SM) and muscles (M) x 440.

Fig.8 T.S. of buccal mass - ventral region, stained with D-PAS. Note the moderate staining in goblet cells (GC), and weak in redula (R), submucosa (SM) and serosa (S) x 264.

PLATE 1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principally. They are well observed in transverse section as coiled part. (Plate 1 fig. No. 4,7 and 8)

HISTOCHEMISTRY

1. ELABORATION OF MUCOSUBSTANCES BY THE REDULA

Intense reactivity with PAS is noticed in the redula which is almost abolished by diastase or saliva digestion and is partially diminished by prior phenyl hadrazine treatment. Redula shows alcianophilia to both pH 2.5 while in sequential standing techniques with AB (pH 1.0, 2.5) - PAS both of the colours are maintained at moderate level at AB (pH 2.5) - PAS and weak at AB (pH 1.0) - PAS. The presence and characterization of acidic mucosubstances is confirmeal by graded Concentration of MgCl2 in Alcian blue dye technique. The alcianophilia is resistant up to 0.2 M Mg Cl2. The alcianophilia is totally absent in acid hydrolysis, mild methylulation and active methylation. Azure A metach hromasia observed only at low PH level. Both sialidase digestion AB (PH 2.5) and hyaluronidase AB (PH 2.5) shows reaction at moderate level.

Thus the chitinous material present in redula contains glycogen, neutral mucins and weakly sulphated acid mucosubstances. But these sulphated acid mucosubstances are AF negetive.

2. ELABORATION OF MUCOSUBSTANCES BY THE MUCOSA.

Mucosa layer of the buccal mass comprises of two kinds of cells - goblet and columnar and exhibits different staining reactions.

The goblet cells are intensely reacting with PAS (Plate No.1 fig. 1, 2 and 7) and at all regions (i.e. ventral, lateral and dorsal). The intensity of reaction shows consistantcy even with the phenylhydrazine treatment at the ventral region but abolishes in lateral and odrsal aspect. The reactivity with diastase digestion is partly diminished at all surfaces. (Plate no.1 fig.8) The cells shows alcianophilia at PH 1.0 and 2.5 at ventral and lateral regions and are negetively alcianophilic at dorsal surface. In the combined sequential staining techniques with AB (PH 2.5) PAS shows intense pink and blue colouration to the ventral and lateral region, while it is weak at the dorsal surface. The reactivity with AB (PH 1.0) - PAS shows intense pink coloration and weak blue colouration at all surfaces (plate no.1 fig. 3) Sulphated mucosubstances are confirmed with intense AF reactivity (plate no.1 fig.5), which is maintained in sequential AF-AB (PH 2.5). The alcianophilia is quite resistant in graded concentration of $MgCl_2$ up to 0.6 M $MgCl_2$. Acid hydrolysis shows partial loss of alcianophilia (Plate no.1 fig. 6) indicates probable presence of sialomucins in goblet cells of ventral region. Partial restoration of alcianophilia

after saponification in this region indicates presence of carboxyle groups, in ventral and lateral regions. No reactions with both methylation and saponification at dorsal margin. Azure A metachromasia is observed only at low PH. Sialidase digestion AB (PH 2.5) and Hyaluronidase AB (PH 2.5) show moderate blue colour.

Columnar cells shows moderate PAS reactivity (plate no.1 fig. 1,2 and 6) which is nearly abolished with distase digestion (plate no.1 and fig. 8) and vanishes completly with prior treatment of phenylhydrazine. Absence of alcianophilia at AB (PH 1.0), at moderate level with AB (PH 2.5), and only pink colouration with sequential staining AB (PH 1.0 & 2.5) PAS are observed. (plate no. 1 fig. 3) Columnar cells are AF positive weakly (plate no.1 fig. no. 5)and the alcianophilia diminishes at graded concentration of MgCl2 after 0.1 M MgCl2. Azure A metachromasia are seen weakly at middle PH level Sialidase digestion AB (PH 2-5) show no reaction.

Thus goblet cells contain glycogen, neutral mucins and weakly as well as strongly sulphated acidic mucins at the ventral and lateral margin and to some extent to the dorsal margin. Columnar cells contains glycogen and nonsulfated mucins.

3. ELABORATION OF MUCOSUBSTANCES BY THE SUBMUCOSA

The fibrilar structure of submucosa is moderatly reactive with PAS (plate no.1 Fig. 1,2 and 7) which is abolished in both diastase digestion and prior phenylhydrazine treatment. The region shows alcianophilia at both AB (pH 1.0 and 2.5) moderately. In the combined sequential staining technique with AB (pH 1) - PAS shows both pink and blue colouration (plate no. 1 fig.3) with moderate intensity, with AB (pH2.5) - PAS only pink colour is maintained. The presence and characterization of acidic mucosubstances was confirmed by graded concentration of $MgCl_2$ in the alcian blue dye technique. The alcianophilia was quite resistant upto 0.2 M MgCl2 and diminishes slowly in 0.4 M and 0.6 M MgCl2. Prior to acid hydrolysis (plate No. 1 fig. 6) methylation shows complete absence of alcianophilia. Azure A metachromasia is observed only at low pH. Both sialidase digestion and Hyaluronidase AB (pH2.5) developes blue colour weakly.

Thus the submucosa contains glycogen, neutral mucing and weakly sulphated acidic mucins.

4. ELABORATION OF MUCOSUBSTANCES BY THE MUSCLAR LAYER

Both circular and longitudinal muscle layers demonstrates weak PAS reactivity (plate No.1 fig. 1,2 and 7) w which is completely abolished by diastase digestion and prior phenylhydrazine treatment. Alcianophilia also lacks

at both AB (pH 1.0 and 2.5) - PAS shows only pink colouration moderately. The presence of characterization of acidic mucosubstances was confirmed by graded concentration of MgCl2 in the Alcian blue dye technique. The alcianophilia was resistant upto 0.2 M $MgCl_2$w which diminishes after acid hydrolysis, mild and active methylation, sapohification indicates complete absence of alcianophilia. Both sialidase and Hyaluronidase AB (pH 2.5) shows no colour.

Thus muscular layer contains mainly glycogen, neutral mucin.

5. ELABORATION OF MUCOSUBSTANCES BY THE SEROSA

Cells of serosa indicate weak PAS reactivity which is completly lost by diastase digestion and prior phenylhydrazine treatment exhibits poor alcianophilic reaction which is maintained in combined sequential staining at AB (pH 2.5) - PAS by showing both pink, blue colourations, but blue colour diminishes completly with AB (pH 1) - PAS and retains pink colour only. All routine techniques hereafter are show no reaction at all. Sialidase and hyaluronidase AB (pH 2.5) shows poor colour.

Thus serosa cells contain in them mainly glucogen and neutral mucins.

PRE OESOPHAGUS

MORPHOLOGY

From the posterior end of the buccal mass the pre-

CAPITONS TO PHOTOMICROGRAPHS

PLATE NO - 2

Fig.1 T.S.of pre-oesophagus - ventral region, stained with PAS. Note the intense staining in goblet cells (GC) weak in columnar cells and poor in submucosa (SM) muscles (M) and serosa (S) x 440.

Fig.2 T.S. of Pre - oesophagus stained with acid hydrolysis. Note the moderate staining goblet cells 9GC), weak in columnar - cells (CC) and weak in sumbucosa (SM) x 440.

Fig.3 T.S. of Pre - oesophagus stained with PAS. Note the intense staining in goblet cells (GC),weak stain in columnar cells (CC), submucosa (SM) muscles (M) and serosa (S) x 264.

Fig.4 T.S. of Pre - oesophagus stained with AB (pH 2.5) - PAS. Note the intense staining in goblet cells (GC), weak in columnar cells (CC) and submucosa (SM) x 440.

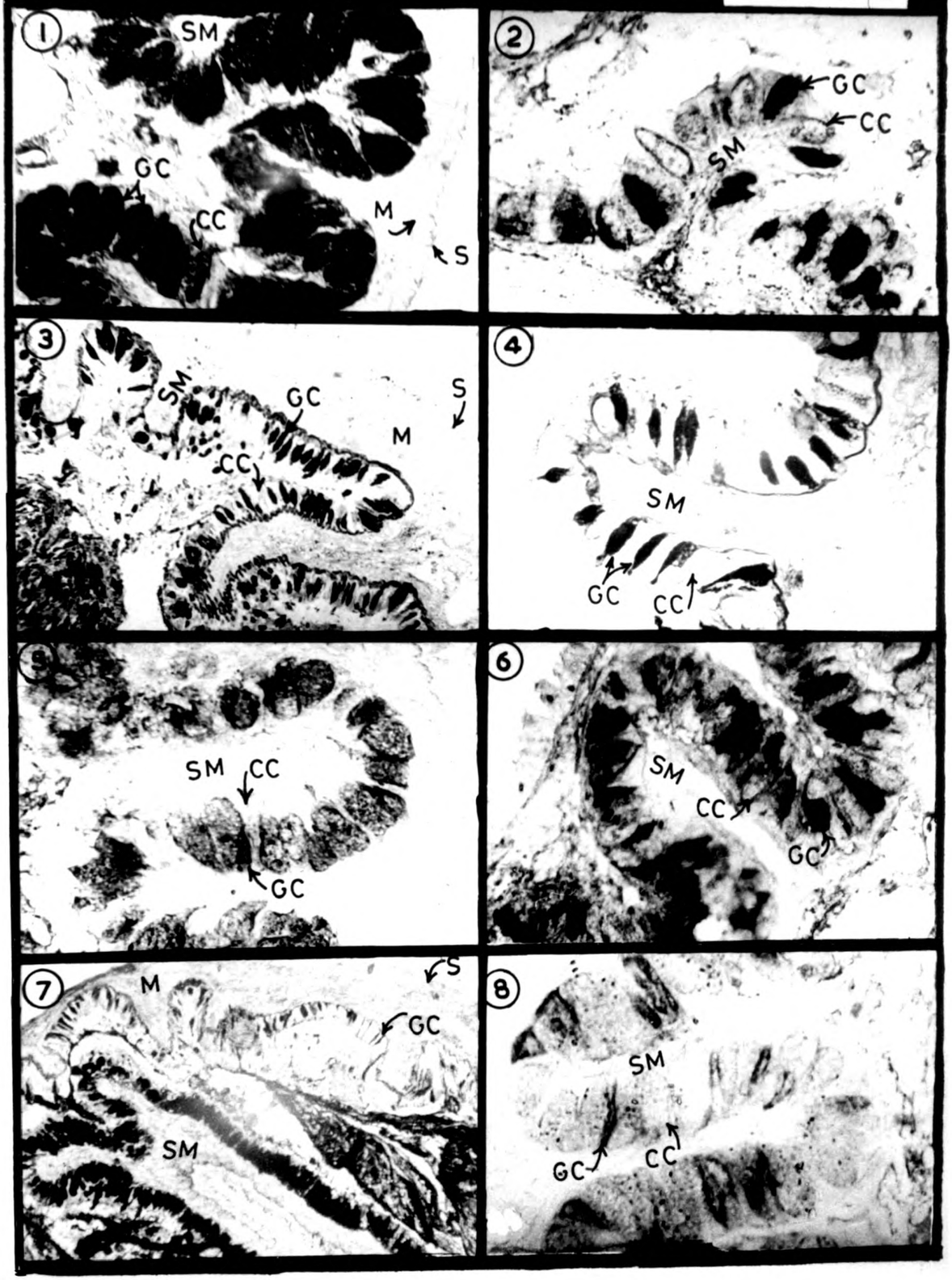
Fig.5 T.S. of Pre - oesophagus stained with AB (pH 5.6) + 0.1 M Mg ++ . Note the intense staining in goblet cells (GC), weak in columnar cells (CC) and submucosa and (SM) x 440.

Fig.6 T.S. of Pre - oesophagus stained with PH - PAS Note the intense staining in goblet cells (GC) poor in columnar cells (CC) and submucosa (SM) x 440.

Fig.7 T.S. of Pre - oesophagus stained with AB (pH 1.0) PAS. Note the moderate staining in goblet cells (GC), weak in submucosa (SM) and poor in muscles (M) and serosa (S) x 132.

Fig.8 T.S. of Pre - oesophagus stained with Mild methylation AB (pH 2.5). Note the weak staining in goblet cells (GC) poor in columnar cells (CC) and no staining in submucosa (SM) x 440.

PLATE 2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SM
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SM
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CC

oesophagus take its origin. This tubular structure then upturn towards the mouth and lies beneath the buccal mass. Pre-oesophegus is short tube of about 2 mm in length and 0.3 to 0.4 mm in diameter with yellowish white colour. The tube on its dorsal surface is masked with diffused glandular tissue, probably representing the buccal glands or salivary glands. Morphology of this, is difficult to describe since it has no definate shape.

HISTOLOGY

Like the buccal mass the pre-oesophegus is also consist of four layers mucosa, submucosa, muscular layer and serosa. Serosa - the outermost layer of pre-oesophagus is formed by single celled layer of columnar type. Muscular layer is arranged in a concentric manner and consist of circular and longitudinal layers, fibrillar arrangement of the submucosa can be detected in cross section while the mucosa layer is made up of two kinds of cells goblet and columnar, interspersed with small vacuoles. Goblet are in large number towards ventral side than the dorsal one.

HISTOCHEMISTRY

1. ELABORATION OF MUCOSUBSTANCES BY MUCOSA

Goblet and columnar are the cell types observed in the mucosa layer and are reacting differently to the

different staining techniques.

Goblet cells are intensly stained with PAS (plate no.2 fig. 1,3) which is constant even with prior treatment of phenylhydrazine (plate no.2 - fig. 6) but after diastase digestion the intensity was lowered to weal level. Alcianophilia is documented by a technique AB (pH 1.0 and 2.5) sequential staining procedures with both AB (pH 1.0 and 2.5) - PAS are intense (plate no.2 fig. 4,7). Sequential staining with AF-AB 2.5 develops only blue colour. Alcianophilic reaction is resistant strongly at 0.1 M MgCl2 but up to 6M MgCl2. Acid hydrolysis maintains the alcianophilia (plate no. 2 fig. 2) but mild methylation (pH 2.5) (Plate no.2 fig. 8) and active methylation reduces partially. Mild methylation with saponification reduces the alcianophilia (plate no.2 fig. 8) weakly and active methylation with saponification reduces it completely. Azure A metachromasia is seen only at low PH. Both sialidase digestion and hyaluronidase AB (pH 2.5)shows moderate reaction.

Columnar cells are weakly reacted with PAS (plate no.2 fig. 1,3) and lost its reactivity with prior phenylhydrazine treatment partially and after distase digestion completely. Alcianophilia with AB (pH 1.0 and 2.5) develops blue colour weakly. Combined sequential staining techniques AB (pH 1.0 and pH 2.5) - PAS (plate

no.2 fig. 4,7) demonstrates both pink and blue colours at weak level. Acidic sulfated mucin are doubted due to the reaction with AF the alcianophilic reaction at graded concentration of $MgCl_2$ is lost substantially (plate no.2 fig. 8) after 1 M Mg^{++} . Acid hydrolysis reduces the alcianophilia (Plate no.2 fig. 2) at pH 2.5. Mild methylation reduced alcianophilia and restored after saponification Azure A technique and enzyme test confirms the presence of sialic acid in them.

Thus goblet cells of mucosa contain in them glycogen, strongly and weakly acidic sulfomucins and neutral mucins Columnar cells contains glycogen, neutral mucins and weakly acidic mucosubstances and sialic acid in them.

2. ELABORATION OF MUCOSUBSTANCES BY SUBMUCOSA

Submucosa showing weak PAS (plate no.2 fig. 1,3) reactivity completely lost by prior phenylhydrazine (plate no.2 fig. 6) and diastase digestion treatment. Sulfated acidic mucosubstances are seen with the technique AB (pH 1.0 and 2.5). In combined AB (pH 1.0 and 2.5) - PAS (plate no. 2 fig. 4,7) sequential staining both pink and blue colours are observed. Carboxylic containing acidic mucin are confirmed by observing their response with AF.

Alcianophilic staining produces involving supression by $MgCl_2$ grades, mild and active methylation and

the saponification. Acid hydrolysis showed and confirmed the presence of strongly sulfated acidic mucosubstances in them (Plate no.2 fig. 2) Azur A technique and enzyme test confirms the presence of sialic acid in them.

Thus glycogen, carboxylic containing sialic acid, strongly sulfated acidic mucosubstances are observed in submucosa.

3. ELABORATION OF MUCOSUBSTANCES BY MUSCULAR LAYER

Both longitudinal and circular muscle layers are prominant in pre-oesophagus. They show weak staining reaction with PAS (plate no.2 fig 1,3) and prior treatment with phenylhydrazine (plate no.2 fig 6). After diastase digestion staining is lost completly. Sulfated acidic mucin are estimated due to positive reaction with AB (pH 2.5) Sequential staining procedures such as AB (pH .1 and 2.5) - PAS showed only pink colour. Muscles are not reacted with AF and combined AF-AB (PH 2.5) techniques. Alcianophilic reaction is resistant at 0.1 M $MgCl_2$, but disappeared with the treatment of 0.2 M, 0.4 M and 0.6 M Mg Cl_2 concentration. Blue colour is evident even after treating sections with acid hydrolysis methylation techniques show ho reaction at all. Azure A and enzyme test confirms the absence of sialic acid.

Thus the muscular layer contains glycogen and weakly sulfated acidic mucins and neutral mucins.

4. ELABORATION OF MUCOSUBSTANCES BY SEROSA

Serosa exhibited moderate PAS reactivity (plate no. 2 fig. 1,3) which is found to be presistant with prior treatment of phenylhydrazine. Abolishment of the reactivity is seen when digested with diastase. Alcianophilia is demonstrated by a technique AB (pH 1.0) but is lost in AB (pH 2.5). Combined sequential staining reactions with AB (pH 1 and 2.5) show poor response. Sulfated acidic mucosubstances were thought due to positive reaction with AF. Both carboxylic containing and sulfated acidic mucosubstances are confirmed by sequential staining reaction with AF-AB (pH 2.5). In graded concentration of MgCl2 alcianophilia is resistant up to 0.1M MgCl2 which decreases with increased concentrations of MgCl2. Acid hydrolysis has maintained the alcianophilla prominantly. Mild methylation treatment persist the alcianophilia but is lost in active methylation strongly. Azure A and enzyme tests confirms the presence of sialic acid.

Thus serosa contains glycogen, sulfated acidic mucosubstances and carboxlic containing acidic mucosubstances.

OESOPHAGEAL BULB

MORPHOLOGY

Preoesophegus at the level of mouth widens into roughly triangular sac which has gray colour. It is about 2 to 3 mm. in length and 1.5mm in thickness at the middle

region. The sac appears to be spongy and hence shows wrinkled nature externally. The sac lies at the ventral aspect of the buccal mass. Thus buccal mass, preoesophugus and oesophageal bulb forms a compact structure may be called buccal complex.

HISTOLOGY

Peculiar four layered pattern is documanted by this organ of digestive tract. Serosa is single celled layer forming the outer boundry of the organ. Muscular layer is seems to be thin as compared with previous two organs. Submucosa has usual fibrillar organization. Mucosa consist of goblet and columnar cells. In cross section the wall if folded., where the lumen is typically almost filled with transverse folds. The folds are clothed with a columnar epithelium interspersed occasionaly with gland cells especially at the tip of the fold. Goblet cells are lacked in this region. One side of the bulb which is containuous with the ventral edge showes folds of smaller height. Here the number of goblet cells is high. Granular cells are the additional cell types observed in this region. Peripherial cells at the external surface of the ventral region is the another histological pecularity of oesophageal bulb.

CAPTIONS TO PHOTOMICROGRAPHS

PLATE NO - 3

Fig.1 T.S. of oesophageal bulb - stained with AB (PH 2.5). Note the folded lumen (FL) muscies layer (M) ands serosa (S) x 79.2

Fig.2 T.S. of oesophageal bulb - ventral region stained with AB (PH 2.5) - PAS. Note the intense staining in goblet cells (GC) and peripheral cells (PC) moderate in columnar cells (CC), submucosa (SM) x 264.

Fig.3 T.S. of oesophageal bulb - ventral region, stained with PAS. Note the intense staining in goblet cells (GC), mast cells (MC) and peripheral cells (PC), moderate staining in granular cell (GR), submucosa (SM) and mucles (M) x 264.

Fog.4 T.S. of oesophageal bulb - ventral region, stained with AF - AB (PH 2.5). Note the intense staining in goblet cells (GV), moderate in granular cells (GR) and weak in submucosa (SM) x 440.

Fig.5 T.S. of oesophageal bulb - ventralr region stained with AF. Note the intense staining in goblet cells (GC), weak in granular cells (GR) and submucosa (SM) and serosa (S)x264.

Fig.6 T.S. of oesophageal bulb - ventral region, stained with AB (PH 2.6) + 0.1 M Mg +++ Note the moderate staining in goblet cells (GC) weak in granular cells (GR) and poor in submucosa (SM) x 440.

Fig.7 T.S. of oesophageal bulb - ventral region, stained with AB (PH 0.1) - PAS. Note the intense staining in goblet cells (GC), and weak in submucosa (SM) x 264.

Fig.8 T.S. of oesophageal bulb - ventral region, stained with PAS. Note the folded lumen (FL) muscles (M) and serosa (S) x 79.2

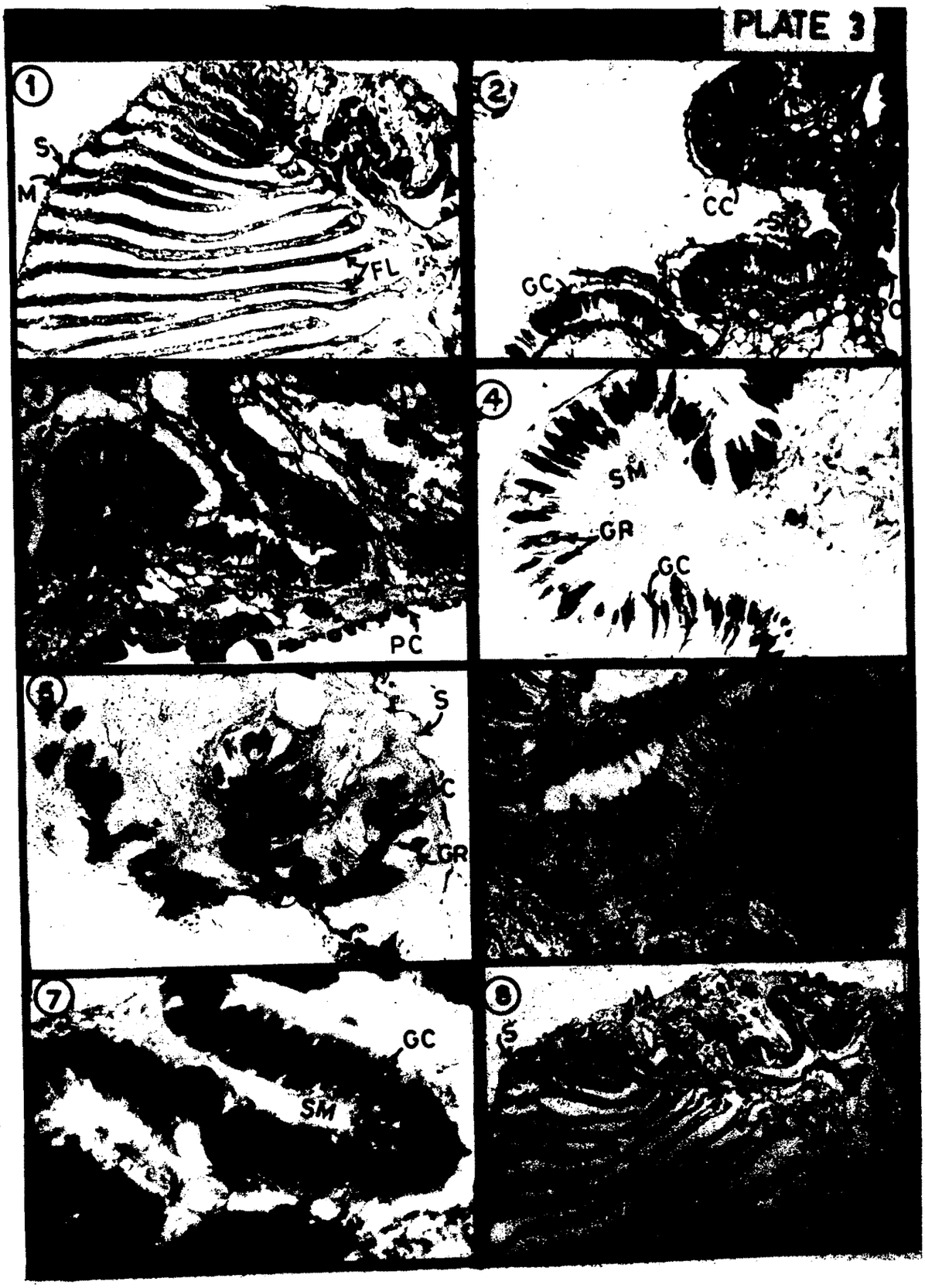
PLATE 3
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HISTOCHEMISTRY

1. ELABORATION OF MUCOSUBTANCES BY THE MUCOSA

Mucosa layer as seen under microscope shows three kinds of the cells goblet, columnar and granular.

Goblet cells are intense PAS reactive (Plate no. 3 fig. no.3 and 8). The intensity is maintained even with the treatment of phenylhydrazine and diminishes slightly with diastase digestion. It confirms the glycogen in the cells. Alcianophilia with AB (pH 1.0) is profuse but is diminished to weak level at AB (pH 2.5). Combined sequential staining teahnique such as AB (pH 1.0 and 2.5) - PAS shows pink and blue colouration with PH 1.0 and only pink with PH 2.5 indicating neutral mucosubtances. (Plate no.3 fig. no.2 and 7). Intense reaction with AF confirms the sulphated mucins (plate no.3 fig. no.5) which is further stressed by developing pink colouration with combined sequential staining technique AF-AB (PH 2.5) (plate no.3 fig.no. 4). The alcianophelia as quite resistant up to 0.1M MgCl2 (Plate no.3 fig. No.6) and decreases with graded concentration up to 0.6M MgCl2. The alcianophilia with acid hydolysis treatment shows no effect. Partial loss of alcianophilia in active methylation confirms the sulfomucins at the site. Azure A shows metachromasia at low PH. Moderate blue colour appears with sialidase digestion and hyaluronidase test at AB (pH 2-5).

Granular cells are moderatly reactive with PAS and the reaction is lost with prior phenylhydrazine treatment and diminishes with diastase digestion. Alcinaophilia with AB (pH 1.0) stains moderatly and no staining is seen with AB (pH 2.5) combined sequential staining techniques AB ($_p$H 1.0 and 2.5) - PAS (plate no.3 fig. no.3 and 7) exhibits both pink and blue colouration. AF reactivity is poor (plate no.3 fig. 5) but with combined sequential staining AF-AB ($_p$H 2.5) it is purple in colour indicating sulphomucins presence. (Plate no.3 fig. 4) Alcianophilia with graded concentration of MgCl2 is resistant up to 0.4M MgCl2 and then decreases considerably. Nearly complete loss of alcianophilia at acid hydrolysis indicates the presence of sulphomucins. Azur A shows metachromasia at low PH. Both sialidase digestion and hyaluronidose techniques at AB ($_p$H 2.5) blue colouration.

No reactivity is observed with the treatment of mild and active methylation and saponification.

Columnar cells shows the reactivity with different staining techniques nearly similar as granular cells.

Thus goblet cells contain in them glycogen, neutral mucins and sulfated mucins, while granular and columnar cells indicate the presence of glycogen, neutral mucins and sulfated acidic mucosubstances.

2. ELABORATION OF MUCOSUBTANCES BY THE SUBMUCOSA

Submucosa is weakly reactive with PAS and this reactivity is lost with prior phenylhydrazine treatment and diastase digestion. Alcianophilia with AB (pH 1.0 and 2.5) is moderate at PH 1.0 and weak at PH 2.5. Combined sequential staining techniques AB (pH 1.0 and 2.5) - PAS develop both pink and blue colours. AF reactivity is poor and only blue colour is maintained in combined sequential staining with AF-AB (pH 2.5) (plate no.3 fig. no.4). Alcianophilia with graded concentration of MgCl2 is resistant poorly. No reactivity is observed with mild andactive methylation and saponification but the acid hydrolysis treatment maintains alcianophilia. Metachromasia is observed with Azur A, while enzyme test confirms the absence of sialic acid.

Presence of glycogen, strong and weak sulfomucin are thus documented in submucosa.

Mast cells are intensly reacted with PAS (plate no.3,8) but lost there reactivity with diastase digestion completly and partially with prior treatment with phenylhydrazine. Poor reaction is observed with the teachniques AB (PH 1.0 and 2.5). Combined sequential staining techniques AB (PH 1.0 and 2.5) - PAS develops both pink and blue colours. Sulfated acidic mucosubstances are confirmed with AF technique which shows purple colour. Alcianophilia with graded concentration of

MgCl2 is poor but moderate intensity persists with acid hydrolysis treatment and with mild methylation as well as saponification. Metachromasia is observed at low PH. While enzyme tests confirms the absence of sialic acid.

Presence of glycogen, sulfated acidic mucosubstances are confirmed in mast cells.

3. ELABORATION OF MUCOSUBSTANCES BY THE MUSCULAR LAYER

Weakly developed muscular layer shows very little reaction with different staining techniques. The layer is dominated only at the ventral region. Weak reactivity is observed with PAS (plate no.3 fig. no.3 & 8) and is lost completly with prior pheylhydrazine treatment diastase digestion. Little reaction is seen with AB (PH 1.0) and poor with AB (pH 2.5) (plate no.3 fig.no.1) In combined sequential staining techniques AB (pH 1.0 & 2.5) $^{-PAS}$ only pink colour is seen. AF reactivity is also negetive. Only slight blue colour appears with a technique AF - AB (pH 2.5). No reaction practically is observed with graded concentration of MgCl2. But acid hydrolysis maintains alcianophilia to weak level. Mild and active methylation and saponification teahniques resulted negetively. At low PH 1.5 Azure A shows metachromasia. Sialidase digestion AB (pH 2.5) and hyaluronidase techniques developes blue colour.

Thus glycogen, sulfomucins and neutral mucosubstances are present in the muscular layer.

4. ELABORATION OF MUCOSUBSTANCES BY THE SEROSA

Serosa of the oesophageal bulb shows peripheral cells which are intensly reactive with PAS (plate no.3 fig.no. 3 and 8). The reactivity is lost completly with prior phenlyhydrazine and distase digestion treatment. Alcianophilia at AB (pH 1.0 and 2.5) is observed at weak level. Combined sequential staining techniques AB (PH 1.0 and 2.5) - PAS shows both blue and pink colours (plate no.3 fig. no.2). The cells are intensly reacted with AF and both blue and purple colours are seen at combined sequential staining AF-AB (pH 2.5). Acid hydrolysis maintains alcianophilia weakly. No reaction is observed with mild and active methylation and saponification. Azure A and enzyme test confirms the absence of sialic acid.

Thus the peripheral cells contain glycogen, and AF positive sulfated acidic mucosubstances.

POST OESOPHAGUS

MORPHOLOGY

Tapered end of the oesophageal bulb comes out from buccal complex as some what thickened posterior oesophagus. It runs below the floor of mantle towords the posteriorly situated midgut gland. The tube is about 3 to 4 mm. in length and of 0.4 to 0.5mm in a diameter; represents white colour.

HISTOLOGY

Cross section of post - oesophagus strictly observes the four layered plan consist of serosa, muscular layer,

submucosa and mucosa. Elaboration of submucosa and muscular layer increases the thickness of the tube. Serosa is comprised of single celled layer of columnar type Peripheral cells are also seen on its surface. Muscular layer is distingwished into (plate no.4 fig.1). Fibrillar pattern of submucosa is evident prominantly. Mucosa layer is formed by two kinds of cells namely goblet and columnar. Inner wall is folded uniformly into 10 to 15 principal folds, which are subfolded two to three times.

HISTOCHEMISTRY

1. ELABORATION OF MUCOSUBSTANCES BY THE MUCOSA.

Goblet and columnar are the kind of the cells that comprises the mucosa.

Goblet are intensly reacted with PAS (plate no.4 - fig. no.1) which is abolished by the prior phenylhydrazine treatment and diminishes in diastase digestion. Moderate staining is observed with AB (pH 1 and 2.5). In combined sequential staining techniques AB (pH 1.0 and 2.5) - PAS the intensity of reaction is maintained at intense level (plate no.4 - fig. no.5). Some goblets are intensely AF positive which is also observed with combined sequential staining of AF-AB (pH 2.5). Alcianophilia with graded concentration of $MgCl_2$ decreases with the increased concentration of $MgCl_2$. Thus indicating the presence of sulfated acidic mucosubstances.

CAPTIONS OF PHOTOMICROGRAPHS

PLATE NO - 4

Fig.1 T.S. of posterior - oesophagus stained with PAS. Note the intense staining in goblet cells (GC) moderate in submucosa (SM), muscles (M) and serosa (S) x 264.

Fig.2 T.S. of stomach stained with PAS. Note the intense staining in goblet cells (GC), moderate in submucosa (SM) and weak in columnar cells (CC) x 83.16.

Fig.3 T.S. of posterior oesophagus stained with acid hydrolysis. Note the moderate staining in goblet cells (GC) and serosa (S) intense in mast cells (MC), weak in submucosa (SM) and poor in columnar cells (CC) x 83.16.

Fig.4 T.S. o f stomach stained with AB (pH 2.5)-PAS Note the intense staining in goblet cells(GC) and submucosa (SM) moderate in columnar cells (CC) and muscles (M) x 83.16.

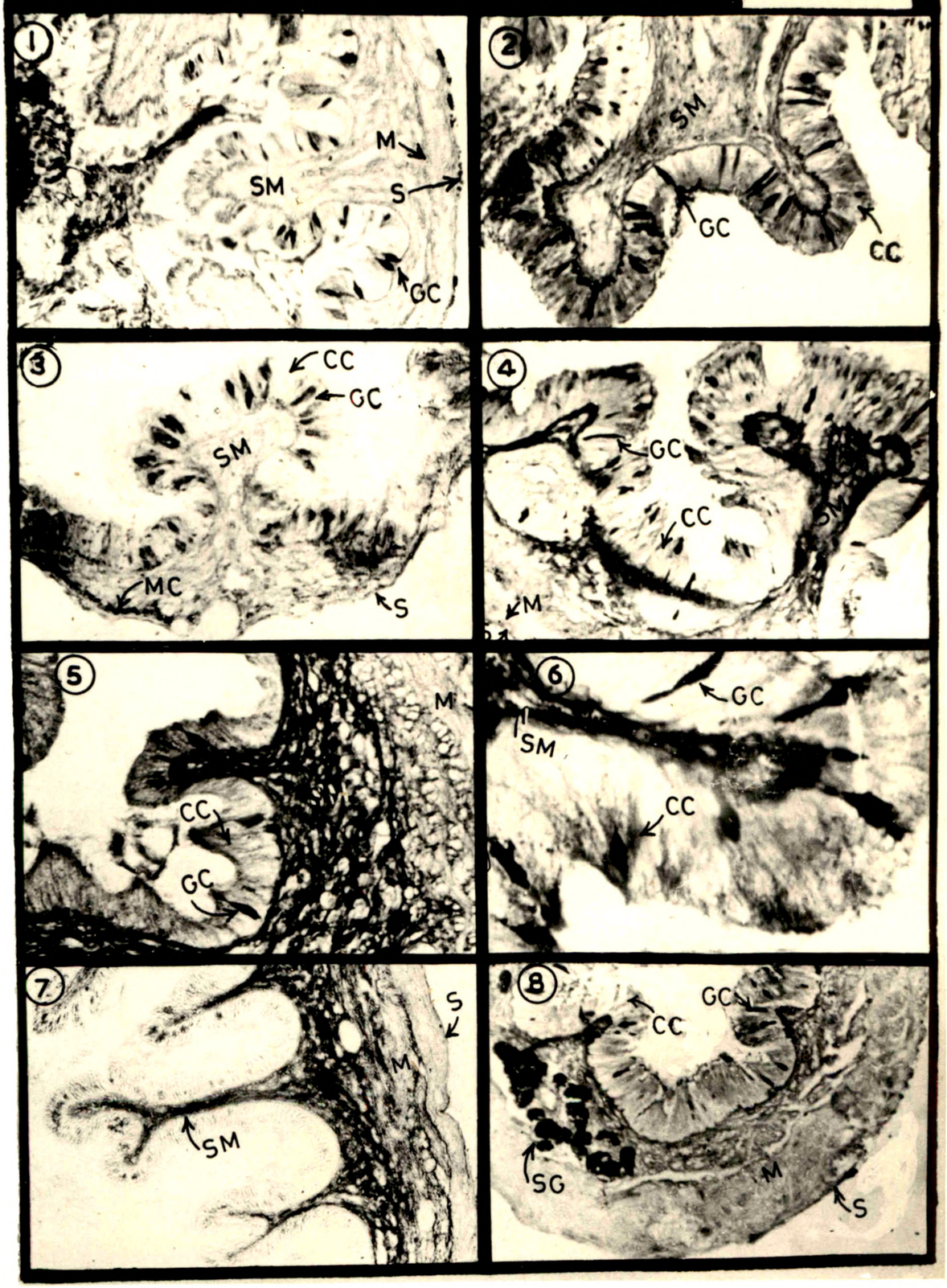
Fig.5 T.S. of porterior oesophagus stained with AB (PH 0.1) PAS. Note the intense staining in goblet cells (GC), submucosa (SM) moderate in columnar cells (CC) and weak in muscles (M) x 277.2

Fig.6 T.S. of stomach stained with AB (PH 1.0)-PAS Note the intense staining in goblet cells (GC) and submucosa (SM) and weak in columnar cells (CC) x 277.2

Fig.7 T.S. of posterior oesophagus stained with AB (PH 0.1). Note the intense staining in submucosa (SM) moderate in serosa (S) and weak in muscles (M) x 264.

Fig.8 T.S.ofstomach stained with PAS. Note the intense staining in goblet cells (GC), stomach gland (SG), weak in columnar cells (CC) and muscles (M) and moderate in serosa (S) x 83.16.

PLATE 4


1
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SM
S
GC
2
SM
GC
CC
3
CC
GC
SM
MC
S
4
GC
CC
SM
M
5
M
CC
GC
6
GC
SM
CC
7
S
M
SM
8
GC
CC
SG
M
S

Blue colour is maintained at moderate level with acid hydrolysis (plate no.4 fig. 3) No reaction is seen with methylation and saponifications. Azure A and enzyme tests concludes the absence of siatic acid.

Columnar cells are moderatly reactive with PAS which diminishes with prior phenylhydrazine treatment and diastase digestion. Moderate reaction is seen with AB (PH 1 and 2.5). Moderate staining was observed with combined sequential staining of AB (pH 1.0 and 2.5) - PAS (plate no.4 fig. no.5). AF reaction with columnar cells is poor. In graded concentration of MgCl2 the cells are resistant upto 0.4M MgCl2. Reaction with acid hydrolysis is very poor (Plate no.4 fig. no.3). Restaining is seen with mild methylation. No result was seen with active methylation and sapnofication. Metachromasia is evident upto PH 3.0. No reaction with silidase digestion AB (PH 2.5) and blue colour is seen with hyaluronidase technique.

Thus goblet cells contain glycogen, netural mucins and strongly sulfated mucosubstances, columnar cells are demonstrating glycogen, sulfated mucins and sialo mucins.

2. ELABORATION OF MUCOSUBSTANCES BY SUBMUCOSA

Moderate reactivity of PAS is observed with submucosa (plate no.4 fig. no.1) which diminishes partly with prior treatment of pheylhydrazine and completly with diastase digestion. Intense reactivity with AB (pH 1.0 and 2.5) is seen indicating sulfated mucosubstances in

them. (plate no.4 fig. no.7). Both pink and blue colours are seen with combined sequential staining with AB (pH 1.0, 2.5) - PAS (plate no.4 fig. no.5). Here the intensity is more at P pH 2.5 than pH 1.0 indicating strongly acidic sulfomucins. Alcianophilia as quite resistant with graded concentration of $MgCl_2$ upto 4M $MgCl_2$ Blue colour is persistant to a weak level with the treatment of acid hydrolysis (plate no.4 fig. no.3). Mild methylation decreases the reactivity to a poor level and is absent with active methylation and saponification. Azure a and enzyme tests confirms the absence of sialic aicid.

Presence of glycogen, strongly acidic sulfomucins and neutral mucins are observed in submucosa.

Mast cells are intensly geacted with PAS but lost there reactivity with distase digestion and phenyhydrazine treatment completly. Moderate reaction is observed with the technique AB (pH 1 and 2.5). Combined sequential staining technique AB (pH 1 and 2.5)- PAS develops both pink and blue colour at pH 1.0 and only pink at pH 2.5. Alcianophilia with graded concentration of $MgCl_2$ is weak but intense intensity persist with acid hydrolysis treatment. No reaction is seen with mild and active methylation. No reaction is seen with milk and active methylation and saponification. Absence of the sialic acid is confirmed by Azure A and enzyme tests.

Presence of glycogen and sulfated acidic muscosubs-

tances are confirmed in most cells.

3. ELABORATION OF MUCOSUBSTANCES BY MUSCULAR LAYER

Both circular and longitudinal muscle layers are prominant in post oesophagus. They are weakly reacted with PAS (plate no.4 fig. no.1) and show no reaction with treatment of phenyhydrazine and diastase digestion. Both AB (pH 1 and 2.5) show weak reaction. In combined sequential staining teachnique AB (pH 1 and 2.5) - PAS both pink and blue are seen (plate no.4 fig. no.5) AF reaction is negetive. While in combined sequential staining of AF - AB (pH 2.5). Only blue colour is maintained. Graded concentration of $MgCl_2$ show alcianophilia upto 0.5M $MgCl_2$. Weak blue colour is noticed with the treatment of acid hydrolysis (plate no.4 and fig. no.3). No reaction is obsesned with mild and active methylation and saponification.

Thus muscular layer contain in it glycogen and strongly sulfated mucins. Metachromasia is seen with Azur A and blue colour is not abolishes with sialidase digestion and hyaluronidase techniques.

4. ELABORATION OF MUCOSUBSTANCES BY SEROSA

The reactivity of serosa with different staining techniques shows close similarity with that of muscles and hence glycogen and sulfated acidic mucins are confirmed in it.

STOMACH

MORPHALOGY

Post eosophagus after presuing long streight course posteriorly enters the stomach which lies embeded in the visceral mass. This knob like sac is tubular continuation not definately detimited externally from the post cesophagus. It is about 0.4 to 0.5 mm in diameter and about 1 mm in length.

HISTOLOGY

Stomach represents all four layers of general pattern, i.e. serosa, muscular layer, submucosa and mucosa. Interior of the organ is drawn into folds which are less conspicious than post-oesophagus and are of smaller height. Single celled layer serosa, well occupied musclar layer, fibrillar submucosa with stomach glands and goblet and columnar celled mucosa are the histological features. They are confirmed by normal eosin haemotoxylen and Mallaroy's tripple techniques.

HISTOCHEMISTRY

1. ELABORATION OF MUCOSUBSTANCES BY MUCOSA

Both goblets and columnar cells are comprising the mucosa layer but the number of goblets are few as compared to preerious organs.

Goblets get intense stain with PAS (plate no.4 fig. 2 and 8) but are not stained with diastase digestion and prior phenylhydrazine treatment. AB (pH 1.0 and 2.5)

demonstrates weak reaction while combined sequential staining with AB (pH 1.0 and 2.5) - PAS show pink colour only (Plate no.4 fig. 4 and 6) with graded concentration of $MgCl_2$ alcianophilia as seen only at 0.1 M $MgCl_2$ which is lost in further gradation. No staining was observed in mild and active methylation and saponification. Hence glycogen and sulfated acidic mucosubstances are observed in goblet cells. Both Azure A and enzyme test techniques shows positive results.

Columnar cells show weak reactivity with PAS (plate no.4 fig. 1 and 8) which is abolished completly by prior phenylhydrazine and diastase digestion technique. Weak colouration has been seen with AB (pH 1 and 2.5). Only pink colour developes when treated with combined sequential staining of AB (pH 1.0 and 2.5) - PAS (Plate no.4 fig. 4 and 6). Graded concentration of $MgCl_2$ is quite resistant upto .6 M $MgCl_2$ indicating strongly sulfated mucosubstances. No reaction was indicated with rest of staining techniques. Positive results with Azure A and enzyme test techniques are demonstrated.

Thus columnar cells contain glycogen neutral mucins and strongly sulfated acidic mucosubstances.

2. <u>ELABORATION OF MUCOSUBSTANCES BY SUBMUCOSA</u>

Submucosa is characterized by presence of mast cells and stomach gland (Plate no.4 fig. 8).

Intense reactivity with PAS is observed by both stomach glands and mast cells (Plate No. 4 fig. 8) which has been vanished completly with the prior phenylhydrazine treatment and distase digestion. AB (pH 1 and 2.5) shows weak reactivity. In the combined sequential staining of AB (pH 1.0 and 2.5) - PAS (plate no.4 fig. 4 and 6) mast cells show both pink and blue colour and stomach glands only pink colour. Poor AF and AF-AB (pH 2.5) reactivity is seenin mast cells. Both mast cells and general submucosa are quite resistant to graded concentration of $MgCl_2$ indicating presence of sulfated acidic mucosubstances. No reactivity is seen for graded concentrations of $MgCl_2$. Acid hydrolysis develops blue colour weakly for mast cells and submucosa in general but absent in stomach gland. Mild methylation supresses alcianophilia and restroration occurs at saponification indicating scialic acid in them. Active methylation and saponification show no result. Azure A shows metachromasia at low PH enzyme tests confirms with positive staining absence of enzymes in mast cells but negetive stain with stomach glands.

Thus mast cells contain in the glycogen, strongly sulfated acidic mucosubstances. Stomach gland exhibits glycogen, weak sulfated acidic mucosubstances and sialic aicd. Submucosa in general shows glycogen and strongly sulfated mucosubstances.

3. ELABORATION OF MUCOSUBSTANCES BY MUSCULAR LAYER

Prominant circular and longitudinal muscle layers are weakly responding to PAS. (Plate no.4 fig. 8). Abolishment

of activity of PAS is seen with prior phenylhydrazine and diastase digestion techniques. AB (pH 1 and 2.5) shows poor reactivity. Both pink and blue colours are developed when treated for combined sequential staining of AB (pH 1 and 2.5) - PAS (Plate no.4 fig. 4). AF technique gives negetive result. Poor resistances with graded concentration of $MgCl_2$ is the indication of weak acidic mucosubstances. Acid hydralysis does not maintain alcianophilia. Restoration of alcianophilia after saponification indicates proboibility of siatis mucins.

Thus musclear layer contains in it glycogen weakly acidic sulfomucin and sailomucins. Azure A maintains chromasia and presence of sialic acid is confirmed by enzyme tests such as sidlidase digestion and hyaluronidase technique.

4. ELABORATION OF MUCOSUBSTANCES BY SEROSA

Serosa layer with the differential staining techniques shows close similarly with that of muscle but of hister concentrations, hence comprised of glycogen weakly acidic sulfomucins and sialomucins.

INTESTINE

MORPHOLOGY

The intestine proceeds anteriorly from stomach where it is not externally delimited. It forms a single loop in the visceral mass where it is intermingled with midgut

gland, Intestine walls are thin and has been widen considerablly throughout its course in the visceral mass. Thinness of the wall gives intestinate a transperancy.

HISTOLOGY

Mucosa, submucosa, muscular layer and serosa are forming histological picture of the intestine. (plate no.5 fig. 2). Single celled layer of columnar type forms serosa. Muscular layer is very much feebly developed and represents thin layer. Submucosa along with fibrillar material contains mast cells; while goblet, columnar and granular cells are chief constitutents of mucosa. Inside wall show much less folding as compared to stomach and other previous structures of the tract.

HISTOCHEMISTRY

1. ELABORATION OF MUCOSUBSTANCES BY MUCOSA.

Mucosa of intestine is formed by three kinds of cells goblet, columnar and granular. They show differential staining reactions with the different techniques.

Goblets are moderately stained with PAS (plate no. 5 fig. 5) while the prior phenylhydrazine and diastase digestion treatment abolishes the staining partially. AB (pH 1.0) reactivity with goblets are moderate but with AB (pH 2.5) it is poor. With the combined sequential staining AB (pH 1.0 and 2.5)- PAS the cells are getting both blue as well as pink colour moderatly (plate no.5 fig.5). The cells are also AF positive exhibiting weak stain /

CAPTIONS TO PHOTOMICROGRAPHS

PLATE NO - 5

Fig.1 T.S. of rectum stained with PAS. Note the intense stained mucosa (MU), moderate in submucosa (SM), weak in muscles (M) and moderate in serosa (S) x 79.2.

Fig.2 T.S. of intestine stained with Mallorys tripple. Note the goblet cells (GC), mucosa (MU) submucosa (SM) and serosa (S) x 83.16.

Fig.3 T.S. of rectum stained with AB (pH 0.1) PAS. Note the intense stained goblet cells (GC), mast cells (MC) moderate in submucosa (SM), and serosa (S) and weak in muscles (M) x 440.

Fig.4 T.S. intestine stained with AB (pH 2.5)- PAS. Note the intense staining in mast cells (MC) moderate in serosa (S) poor in muscles (M), mucosa (MU) and submucosa (SM) x 594.

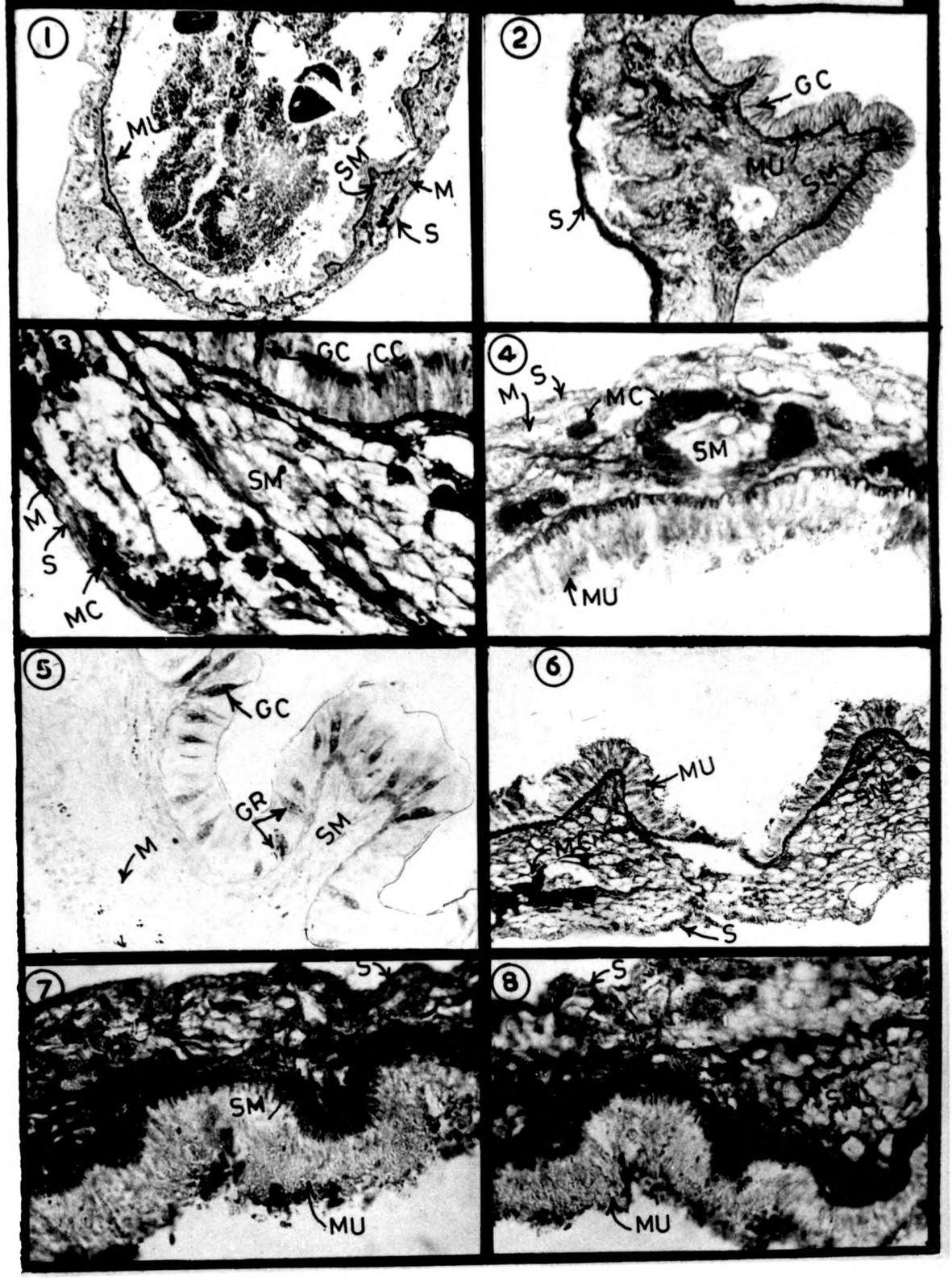
Fig.5 T.S. of intestine stained with PAS. Note the moderate staining in goblet cells (GC), weak in granular cells (GR) poor in submucosa (SM) and muscles (M) x 440.

Fig.6 T.S. of rectum stained with PAS. Note the intense staining in mast cells (MC), mucosa (MU), moderate in submucosa (SM) and serosa (S) x 264.

Fig.7 T.S. of rectum stained with acid hydrolysis. Note the intense staining in submucosa (SM), moderate in serosa and mucosa (MU) x 440.

Fig.8 T.S. of rectum stained with AF. Note the intense staining in submucosa (SM), moderate in mucosa (MU) and weak in serosa (S) x 440.

PLATE 5


1
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2
GC
MU
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3
GC
CC
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4
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S
MC
SM
MU
5
GC
GR
SM
M
6
MU
MC
S
7
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SM
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8
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S
MU

and developes both purple and blue colours with combined sequential staining. With graded concentration of $MgCl_2$ alcianophilia is quite resistant up to 0.6M $MgCl_2$ - Acid hydrolysis fails to abolish alcianophilia at PH 2.5 indicating presence of weak sulfomucins. Mild methylation shows to effect to alcinophilia but active methylation shows complete loss of alcianophilia. Metachromasia is observed with Azure A at low PH. Exzymes tests are positive.

Thus the goblets are containing glycogen, neutral mucins and weak sulfated acid mucosubstances.

Columnar cells are weakly stainable with PAS, which exhibit abolishment of staining to the poor level by diastase digestion and prior phenylhydrazine treatment. Alcianophilia to both AB (pH 1.0 and 2.5) show no reaction and is combined sequential staining only pink colour appears. Poor AF reactivity and complete absence of staining at combined sequential staining technique AF-AB ($_p$H 2.5) is recorded. With the graded concentration of $MgCl_2$ alcianofilia is resistant upto 0.6M $MgCl_2$ - Acid hydrolysis fails to abolish the alcianophilia completly. Blue colour is not at all observed at mild and active methylation and saponification, enzyme test confirms. the absence of enzymes in columnar cells.

Presence of glycogen and neutral mucins are well documented in columnar cells.

Granular cells are weakly reactive with PAS,(plate no.5 fig. 4) but the reactivity is lost completly with phenylhydrazine treatment and with diastase digestion, Alcianophilia to both AB (pH 1.0 and 2.5) exhibits weak reaction, while in combined sequential staining AB (pH 1.0 and 2.5) - PAS only pink colour make its appearance. AF reaction is poor and both purple and blue colours are seen in AF - AB (pH 2.5) technique. The alcianophilic reaction is weak by the addition 0.4M, 0.2M $MgCl_2$. Mild Acid hydrolysis maintains blue colour at weak level. Mild methylation demonstrates poor response and the response is lost completly in active methylation and saponification. Metachromasia is represented at Azure A. Enzyme tests show positive results.

Thus presence of glycogen neutral mucins and weakly sulfated mucins are confirmed in granular cells.

2. ELBORATION OF MUCOSUBSTANCES BY SUBMUCOSA

This layer is consist of mast cells. (Plate no.5 fig. 4). Submucosa in general is porrly reacted with PAS and no reaction is observed with prior phenylhydrazine and distase digestion treatment. Alcianophilia to AB (pH 1.0) shows weak and to AB (pH 2.5) shows poor results. In combined sequential staining technique AB (pH 1.0) - PAS only blue colour and with AB (pH 2.5) - PAS both blue and pink colour are estimated. AF reaction is poor and purple colour is lost in combined sequential staining AF - AB (pH 2.5). In graded concentration of $MgCl_2$ alci-

anophilia is resistant upto 0.6M $MgCl_2$- Treatment with mild methylation and saponification developes blue colour poorly. Active methylation shows no colour at all. At low PH of Azure A chromasia is noted. Enzyme tests gives positive results.

From the above reactions glycogen, strong and weak sulfomucins are demonstretable in submucosa.

Mast cells show intense reactivity with PAS (plate no.5 fig. 4) which is reduced at diastase digestion partially and lost completly at prior phenylhydrazine treatment. Alcianophilia to AB (PH 1.0) is at weak level and poor reaction is observed at AB (pH 2.5). On combined sequential staining technique AB (pH 1.0 and2.5)- PAS pink colour is presistant. (Plate no.5 fig. 4). AF reactivity is also present at weak level. Both purple and blue colours are seen in AF-AB (pH 2.5) technique. Alcianophilia is resistant upto 0.6M MgCl2 in graded concentration of MgCl2. Abolishment of alcinophilia fails with acid hydrolysis treatment. Metachromasia is seen with Azure A at low PH. Enzyme tests give positive results.

Mast cells thus contain in then glycogen and acidic sulfated mucosubstances.

3. ELABORATION OF MUCOSUBSTANCES BY MUSCLE LAYER

Muscle layer is poorly reacted with PAS (plate no. 5 fig. 5) and no reaction is seen with phenylhydrazine

and diastase digestion technique. Alcianophilia at AB (PH 1.0) is at poor level and reaction was seen at AB (pH 2.5). In combined sequential staining AB (pH 1.0)- both pink and blue colours are seen but only pink colour is observable at AB (pH 2.5) - PAS (plate no.5 fig.4). In graded concentration of $MgCl_2$ alcianophilia is maintain upto high grades of $MgCl_2$. Acid hydrolysis technique results at poor level. No reactions are observed in rest of techniques i.e. methylation milH and active methylation and saponification etc. Azure A and enzyme tests confirms the absence of silic acid.

Thus muscles are containing in them glycogen neutral mucina and weak acidic sulfated mucins.

4. ELABORATION OF MUCOSUBSTANCES BY SEROSA

PAS reactivity with serosa in weak and it is lost in phenylhydrazine and distase digestion treatment. Alcianophilia to AB (pH 1.0) is weak and poor to AB (pH 2.5). In combined sequential staining techniques AB (pH 1.0 and 2.5) - PAS both pink and blue colours are seen. (Plate no.5 fig. 4). Purple colour developes poorly with AF and both purple and blue colours are demonstrated in AF - AB (pH 2.5) technique. In graded concentration of $MgCl_2$ alcianophilia is qmite resistant upto 0.6 M $MgCl_2$. Blue colour fails to disappear to acid hydrolysis which confirms the acidic sulfomucins. No reactions were seen with mild and active methylation and saponification.

Azure A demonstrates metachromasia and the enzymes tests are positive.

Thus serosa contains glycogen, sulfated acidic mucosubstances.

RECTUM

MORPHOLOGY

Re-ctum is streight thbe emerging from visceral mass as a yelloish tubular part. It travelles on the floor of mantle along the margin of ctenidum and opens as anus into mantle cavity. The length is about 3 to 4 mm. It is opaque tube as compared to intestine.

HISTOLOGY

Histologycally the wall ofrectum is very thin. Serosa is single celled layer of columnar type. Muscle layer is thin but submucosais quite prominant. It contains pigmented material possiblly the rectal gland. Distinction of goblet cells is very poor, and are in few number. The folded nature of inner wall is practically abolished in the rectum.

HISTOCHEMISTRY

1. ELABORATION OF MUCOSUBSTANCES BY MUSOSA

Goblets and columnar cells are the cellular componants of the mucosa.

Goblets are intensly stained with PAS, this staining is diminished by phenylhydrazine treatment and lost comp-

pletely by diastase digestion. Alcianophilia are poor with both AB (pH 1.0 and 2.5). Only pink colour is observable intensly with combined sequential staining to AB (pH 1.0)-PAS (plate no.5 fig 3). While moderate colour is observable to AB (pH 2.5) - PAS. AF staining is poor and pink colour developes to AF-AB (pH 2.5) technique. Alcianophilia is reduced with graded concentration of $MgCl_2$. Poor staining reaction is seen with acid hydrolysis. No reaction seen with Azure A and enzyme tests are positive. Mild emhtylation, active methylation saponification etc.

Thus glycogen, neutral mucins and weakly sulfated acidic mucosubstances are present in goblet cells.

Columnar cells are moderatly stainable with PAS, the reactivity is partiallym maintained in phenylhydrazine and distase digestion treatment. No staining reaction has been seen with AB (pH 2.5) but to poor level with AB (pH 1.0). Combined sequential staining technique show both pink and blue colours to AB(pH 1.0) PAS (plate no.5 fig.3) and with AB (pH 2.5)-PAS,which confirm the neutral mucins. Both AF and AF-AB (pH 2.5) reactivity is negetive. Alcianophilia are resistant with graded concentration of $MgCl_2$ upto 0.4M $MgCl_2$. Alcianophilia with acid hydralysis seen only at basal region of cells. No reaction are seen with milk and active methylation. Azure A and enzyme tests are positive.

Thus glycogen, neutral mucins and weakly sulfated mucins are confirmed in columnar cells.

Brush border of mucosa is typically stained with PAS intensly and reduced by prior phenylhydrazine treatment, at the level of poor and moderate with diastase digestion. Alcianophilia persist intensely at AB (pH 1.0) In combined sequential staining such as AB (pH 1.0) - PAS shows both pink and blue colours. The border is AF positive (plate no.5 fig. 8) and at AF-AB (pH 2.5) only purple colour is seen. In graded concentration of $MgCl_2$ alcianophilia is resistant upto 0.6 M $MgCl_2$ while it fails to abolish with acid hydrolysis (plate no.5 fig. 7). Mild methylation exhibits alcianophilia at poor level. No reaction is observed with saponification and active methylation. Metachromasia is observed at low pH and enzyme tests show positive results.

Thus brush border exhibits neutral mucins and strongly sulfated acidic mucosubstances.

2. ELABORATION OF MUCOSUBSTANCES BY SUBMUCOSA

Submucosa is moderately stainable with PAS, (plate no.5 fig. 1 and 6) and the stain is lost completly with prior treatment of phenylhydrazine and diastase digestion. Alcianophilia to AB (pH 1.0) is weak and to AB (pH 2.5) is poor. Both blue and pink colours are developed with combined seqmential staining technique AB (pH 1.0) PAS (plate no.5 fig. 3) and only pink colour is seen

with AB (pH 2.5) - PAS. AF reactivity is intense (plate no.5 fig. 8) but combined sequential staining such as AF - AB (pH 2.5) the reactivity is lost. Alcianophilia in graded concentration of $MgCl_2$ is resistant upto 0.4M $MgCl_2$. Alcianophilia is maintained persistantly with acid hydrolysis treatment (plate no.5 fig. 7). A poor reactivity is observed in mild methylation and saphonification. Azure A and enzyme tests gives positive results.

Thus submucosa contain in them glycogen and strongly sulfated mucins.

Mast cells in submucosa are moderatly stainable with PAS (Plate no.5 fig. 1 and 6) which is reduced with prior phenylhydrazine treatment and lostcompletly with diastase digestion. Both AB (pH 1.0 and 2.5) show weak reactivity. Alcianophilia in AB (pH 1.0)- PAS [redacted] (plate no.5 fig. 3) shows pink colour only, while no colour is observable with AB (pH 2.5) - PAS. AF technique shows intense reaction with mast cells and in sequential staining technique AF - AB (pH 2.5) only purple colour is maintained. Alcianophilia in graded concentrations of $MgCl_2$ is resistant upto 0.4 $MgCl_2$. Blue colour is not abolished in acid hydrolysis treatment. Azure A and enzyme tests are positive.

Thus mast cells contains in them glycogen, neutral mucins and strongly sulfated mucosubstances.

3. ELABORATION OFM MUCOSUBSTANCES BY MUSCLULAR LAYER

Muscle fibers of this layer show weak PAS (plate no.5 fig. 1) reactivity which was completely abolished by prior phenylhydrazine and distase digestion treatment indicates the presence of glycogen. Poor alcianophilia is observed with AB (pH 1.0). In combined sequential staining technique like AB (pH 1.0) - PAS shows pink and blue colouration (Plate no.5 fig. 5). The layer is AF negetive. In graded concentration of $MgCl_2$ alcianophilia is resistent upto 0.4 M $MgCl_2$ and acid hydrolysis fails to abolish it. (Plate no.5 fig. 7). Mild and active methylation shows no stain at all. Metachromasia is persistant at low level of PH. Enzyme tests show weak blue colouration.

This indicates presence of glycogen and sulfated acidic mucins.

4. ELABORATION OF MUCOSUBSTANCES BY SEROSA

The different staining reactions exhibited by serosa are showing close similarity with that of submucosa in general . (Plate no.5 fig. no.1,3,6 and 7).

Thus the overall inference from the staining reactions is identical with that of submucosa, which shows the presence of glycogen, and strongly sulphated acidic mucosubstances.