introduction

CHAPTER ONE

INTRODUCTION

1. WHAT ARE MUCOSUBSTANCES?
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In the field of histochemistry, there is much with which one can do much. The technology of histochemistry has made great strides, particularly during the last two decades. Histochemistry now consists of a large body of literature and offers investigators a wealth of methods for examining biological research problems. Chemical identification of tissue components is as great as the concern for accurate localisation and distribution of the components. A sound background in chemistry and histology is therefore essential for histochemical studies to be maximally beneficial. Mieschor quoted that 'histology must be carried out on a chemical basis".

A primary difference between the disciplines of histology and histochemistry is that histology is primarily concerned with the study of microscopic morphology while histochemistry is concerned with the chemistry of cells and tissues related to their morphology. Histology is therefore subserved by empirical staining methods, while histochemistry is based on specific chemical reactions. Histochemistry offers the only methods by which chemical components of tissues can be localised regionally within a particular tissue or at a cellular or subcellular level. It is possible with the histochemical methods to study cells and tissues that are too small to be studied by other methods (Vialli, 1966).

A noteworthy thing is that a few histochemical methods are absolutely specific and always reliable. The words of Adams (1965) seem relevant here - "preoccupation with devising histochemical methods that are 'absolutely specific' under all

circumstances is about as realistic as the medieval search for the philosophers' stone".

I) WHAT ARE MUCINS?

Eichwald (1935) studied the mucins chemically. He reported presence of carbohydrate in mucin. He defined the mucin as "a conjugated single compound of a moiety released under certain conditions as a sugar". The classification of polysaccharides has been very confusing and often entirely unsatisfactory. Histochemists sometimes use terms that are different from those used by biochemists and what is worse, the terms are often poorly defined. Furthermore, many polysaccharids have not been isolated, purified, chemically defined. They can be characterised only on the basis of their histochemical reactions. The term mucopolysaccharide has served nobly in the past to describe any chemical compound that consists of a sugar moiety and a protein moiety. According to Hunt (1970), the protein part predominates in glycoprotein and polysaccharide part predominates in mucopolysaccharide. The terms like mucoproteins, mucopolysaccharides, mucosubstances, mucins, mucoids are still used today and are convinient when referring to a broad class of carbohydrate compounds.

The term mucin (slimy, vicid, tenaceous substance) has been in use from 18th Century as evident by Latin and English medical texts. Hammarstein (1895) demonstrated acidic nature of mucin in submaxillary glands. Attempts have been made to develop a system of classification that is more meaningful in

terms of what is known about the chemical nature of compounds. Jeanloz (1960) made the first and most sweeping proposal for such a system of classification. Pearse (1968) presented a classification system that was derived primarily from Jeanloz (1960) but a little bit modified from other authors.

Due to confusion regarding the terms like mucins, mucoids, mucopolysaccharides, mucoproteins, glycoproteins, glucosaminoglucuronoglycans (now replaced by glucosaminoglycans) etc., it is indeed difficult to distinguish them by definitions. A system of histochemical classification of carbohydrates has been proposed by Spicer et al. (1965). This system does justice to our knowledge of the histochemistry of the carbohydrates and also follows the biochemical classification previously outlined as closely as possible. Spicer et al. (1965) suggested a general term 'mucosubstance' for histochemical reference to any carbohydrate rich component. Spicer (1965) while classifying mucosubstances considered 3 aspects viz. 1) Histological site in which they occur, such as epithelial mucosubstances, connective tissue mucosubstances, 2) Chemical nature of mucosubstances such as neutral mucins, acidic mucins (sulfated, non-sulfated), 3) Affinity of the mucosubstance towards histochemical reactions such as .

a) Affinity for basic dyes like Azure A

b) Affinity for AB

c) Persistance of alcianophilia in the graded concentration of $MgCl_2$.

d) Lability towards hyaluronidase.

e) Lability with respect to neuraminidase (sialidase).

In last two decades, the histochemical investigations with reference to mucins have thrown much light on different aspects such as identification, chemical nature, localization, distribution and functional significance of mucins in the animal body.

II) AVIAN ALIMENTARY TRACT :

Whenever and wherever avian fauna is referred, one authority stands before us, Salim Ali. It is said that he has travelled much more than the migratory birds to study the avian fauna. He has described Indian avian fauna. Raghuvira and Dave have described scientific nomenclature of birds of India, Burma and Ceylon. Some others have concentrated their attention on morphology, anatomy and histology of avian fauna. Histological work in connection with the avian digestive tract is reported by many authors and investigators, a few notworthy references come from Feder (1962), Dovidova (1965), Quary (1967), Ghosh and Gyanvati (1967), Herpol (1967), Patt and Patt (1969), Michael (1971), Hodges and Michael (1975), Bayer *et al*. (1975, 1977), Magon and Mohan (1976), Dahm et al. (1980), Kehoe *et al*. (1985), Nitta *et al*. (1985).

More and more research work is bringing out good results to know ins and outs about avian endocrinology, reproduction, respiration, muscles, etc., but the digestive tract however needs more investigations. During last 2 decades, alimentary tracts of a few mammals like rabbit, rat, guinea pig are being a subject for the study of mucins therein, however submammalian

groups especially birds have received less attention, though they deserve more. Lim and Low (1977) reported that the digestive tract of a bird is a peculiar area since mucosal layer shows strikingly variable structure as we go from oesophagus towards rectum. The present investigation "histochemical studies on mucosubstances in the alimentary tract of Amaurornis phoenicurus phoenicurus".(White breasted water-hen) has been undertaken with this point in mind.

III) MUCOSUBSTANCES AND GASTROINTESTINAL TRACT OF VERTEBRATES - A REVIEW OF LITERATURE :

For convinience, comparative review on mucosubstance is taken organwise to illuminate the work done in histochemistry of mucins, in the alimentary tract of vertebrates.

A) OESOPHAGUS -

i) PISCES -

Oesophagus of several fishes has been studied from morphology, anatomy and histological point of views. The credit goes to several workers like Pasha (1964), Bullock (1967), Bucke (1971), Singh (1974), Sinha (1977), Sis et al. (1979) and Chakrabarty et al. (1983), Dangny et al. (1985), Martin et al. (1985).

Pop et al. (1975) considered oesophagus as a neglected part, this might be in accordance to ordinary function of oesophagus as a passage for the food upto stomach, as seen in most vertebrates. Oesophagus of teleostean fishes showed mucous cells which reacted positively with PAS as well as AB (Bullock,

1971; Bucke, 1971) suggesting existance of neutral and acidic mucins therein. Reifel and Travill (1977) studied oesophagus of 10 species of fishes and identified 6 types of mucous cells. They reported 2 kinds of sialomucins (viz. sialidase resistant and sialidase labile), sulfomucins as well as neutral mucins. Chakraborty et al. (1983) reported sulfo and sialomucins in oesophageal mucous cells in Labeo rohita (Fleuroscent microscopic study). Martin and Blaber (1984) reported numerous mucous cells in oesophagus of teleostean fish containing neutral and acidic mucins. They also pointed out that stratified nature of mucosal cells in proximal oesophagus transforms into columnar type distally. Muscularis layer consisted of striated type of muscles. In Pharactolaemus ansorgei (fish), oesophagus (Dangny and Lenglet, 1985), there are folds in mucosa suggesting spiral valve, all covered by villi like structures supported by connective tissue, oblic muscles and numerous mucous cells rich in sulfated mucins and poor in neutral mucins. The mucous cells are more numerous in posterior parts of oesophagus. Jadhav (1985) pointed out that in oesophagus of Clarias magur, mucosa consists of columnar epithelium + two types of goblet cells while in Tilapia mossambica, mucosa comprises only columnar epithelial cells. Another histological difference is that in Clarias, oesophageal glands exist only in distal region while in Tilapia, glands are identified throughout the oesophageal length. Jadhav (1985) identified neutral mucosubstances (poor) in the epithelial cells, sulfomucins in type I goblet cells and neutral + sulfomucins in type II goblet cells. Atypical mucosubstance was

not reported in these two fishes.

ii) AMPHIBIA

Norris (1959) reported PAS positive goblet and glandular cells in the oesophagus of R.pipiens. Loo and Wong (1975) identified sulfomucins and neutral mucins in oesophageal goblet cells in B.melanostictus. Mutkekar (1981) demonstrated 1) neutral mucins in columnar epithelial cells, 2) Neutral + sulfo + sialomucins in goblet cells (type I) and glandular cells (type I), 3) Neutral + Sialomucins in goblet cells (type II) in oesophagus of E.systoma. Mangalware (1981) demonstrated 1) Neutral mucosubstances in columnar epithelium, 2) Sulfomucins (predominant) + trace of sialomucins in the goblet cells and glandular cells in the distal part of oesophagus of B.melanostictus. Suganuma et al. (1981) studied mucins in digestive tract of 5 amphibians viz. tree frog, common frog (R.tigrina), African frog, axolotle larva and Newt. They revealed 1) Neutral mucins in columnar epithelium in Rana, 2) Sialomucins in axolotle, 3) trace of sialomucins in goblet cells in frogs. Patil (1983) reported neutral mucins in ciliated epithelial cells, goblet cells (type II), glandular epithelial cells and neutral + sulfo + sialomucins in the goblet cells (type I) in the oesophagus of R.cyanophlyctis. In R.malbarica, (Jadhav, 1985) ciliated mucosal cells elaborate only neutral mucins while glandular cells of mucosa exhibit a mixture of neutral mucosubstances, sulfomucins and sialomucins. Czopek et al. (1985) pointed out that in Hyla arborea, females show more distinct mucosa than the males in

oesophagus (histological sexual dimorphism).

iii) REPTILIA

Nalavade and Varute (1973) subjected 3 species of Lizards to demonstrate mucins in the oesophagus. They reported that in C.versicolor and M.carinata, mucosa consists of only goblet cells with predominant quantities of neutral, sulfo and sialomucins, while mucosa of H.flaviviridis is dimorphic as it exhibits both goblet and columnar epithelial cells. They pointed out that goblet cells show mixture of neutral + sulfo + sialomucins; while columnar epithelium elucidates neutral mucins + protein masked sialomucins. According to them, oesophagi of above 3 species exhibit 2 types of glands viz. peptic and mucous, showing presence of protein and neutral mucins respectively. Ferri (1977) identified neutral and acidic mucopolysaccharides in the goblet cells of oesophagus in X.merremii (snake). Loo and Swan (1978) worked on E.cunninghami (Australian lizard) to report presence of acidic sulfated mucins in mucosal-epithelium in oesophagus. In oesophagus of M.carinata (Mandlik 1983) 1)Columnar epithelium contains only neutral mucins, 2) goblet cells (type I) - sulfo-mucins, 3) goblet cells (type II) - sulfo + neutral both. This was observed in proximal part of oesophagus. Simultaneously, Mandlik (1983) worked out mucosubstances in distal part of oesophagus to demonstrate only neutral mucins in ciliated epithelial cells in mucosa. El-Taib and Jarrar (1984) reported that in Mauremys caspica, mucosa of oesophagus is lined by ciliated columnar epithelium with numerous goblet cells containing mixture of neutral + acidic moieties of mucosubstances.

They also pointed out absence of mucosal glands, muscularis mucosa and submucosa in oesophagus. Oesophageal mucosal folds in turtle, are few and broad with only goblet cells, while in ground lizard they are more elongated and more in number with two types of goblet cells as well as ciliated epithelial cells, but glands are reported to be absent in both (Jadhav 1985). Further investigation revealed presence of sulfomucins in the goblet cells in turtle (L.punctata lacepede) and type I goblet cells in the ground lizard, sialomucins in type II goblet cells in ground lizard (S.ponticerina).

iv) AVES :

Hanke (1957) described 3 layered tunica muscularis in O.crucis and I.major (in other birds, muscularis is 2 layered). Van Alten and Fennell (1957) reported presence of sulfomucins in oesophageal glands during histogenesis. In majority of birds, oesophageal glands produce acidic mucosubstances mainly carboxylated (Grossi and Millo, 1967; Allenspeak and Berlin, 1971). Warner et al. (1967) found alveolar mucous glands in the lamina propria zone in Japanese quill (Coturnix coturnix japanica). Histochemical studies demonstrated neutral, sulfated as well as non-sulfated mucins (latter probably sialic acid) in the oesophagus of domestic fowl (Rangel et al., 1970). Feder (1972) considered many aspects like feeding habit (type of food), type of mucosubstances and structure of epithelial cells in oesophageal glands in several birds, but he failed to conclude about the exact relationship between the mucin and food consumed. Magon and Mohan (1976) reported on degree of cornification in

oesophagus. They pointed out that cornification is noticable in passer as compared to that in corvus. Malvadkar (1985) worked out mucosubstances in the oesophagus of pond-heron. He demonstrated glycogen in stratified epithelium, neutral + sulfo + carboxymucins in glandular mucosal cells. Jadhav (1985) worked out mucins histochemically in 2 birds viz. Psittacula krameri (frugivorus) and Ceryle radius (carnivorous). He pointed out high degree of cornification in oesophagus of parrot as compared to that observed in kingfisher. Another noteworthy aspect was absence of oesophageal glands in parrot, while in kingfisher, glands were well defined. He identified only glycogen in stratified epithelium in oesophagus of both birds. While glands in kingfisher exhibited neutral + sulfo + carboxymucins. In bobwhite quail, Nitta and Hiroaki (1985) reported 2 kinds of mucosal folds viz. primary thick longitudinal and secondary thin circular. Mucosal folds were covered by squamosal epithelial cells with microfolds and microvilli on free surface.

v) MAMMALIA

Reports are sporadic and are mainly concerned with glycogen contents in oesophagus of man and monkey (Wislochi et al., 1951; Rywlin and Ortega, 1970; Theman et al., 1971; Hopwood et al., 1977a). Carvolho et al. (1968) identified glycogen, neutral mucins, sialic acid in oesophageal glandular cells in armadillo (D.novemcinctus). Rambourg (1969) demonstrated glycoprotein containing sialic acid in rat oesophagus. Lambert (1971), Bescol et al. (1972) demonstrated sulfomucins

in stratified and glandular epithelium in the oesophagus of man. Masuda et al. (1977) showed PAS and AB reactive cells in the oesophagus of man. Hopwood et al. (1977b) reported neutral mucins in brush border region of the epithelial cells in oesophagus of man. Yassien and Tonner (1977) concluded that the oesophageal glands in human are equivalent to minor sublingual salivary glands. Deshmukh (1984) demonstrated heterogenity in distribution of mucins in bats. He showed presence of neutral + sialomucins in keratinised epithelial cells in some bats and neutral + sulfomucins in still some other species of bats. Jadhav (1985) reported that oesophageal mucosa shows higher degree of keratinisation in squirrel (F.pennati) than in monkey (M.carinata). Mucosa in both is devoid of goblet cells as well as glands. In both, keratinised cells exhibit poor quantities of neutral mucins and noticable amount of sulfomucins while stratified squamous epithelial cells show only neutral mucins. (poor).

B) CARDIAC STOMACH

i) PISCES :

Jirage (1970) reported sialomucins, sulfomucins and neutral mucins in gastric epithelial cells of T.mossambica. Bucke (1971) and Kapoor et al. (1975) provided some information in connection with the mucosubstances in the gastric epithelium of teleostean fish (E.lucius). The cells reacted with PAS as well as AB, suggesting existance of neutral and acidic mucins therein. Shafi (1974) studied C.batrachus to demonstrate

presence of acidic sulfated mucopolysaccharides in surface epithelial cells in cardiac stomach. Reifel and Travill (1978) reported heterogenous distribution of neutral, sialo and weakly acidic sulfomucins in the cells located at surface and pit regions of gastric glands in several teleostean fishes. Martin and Blaber (1984) worked out morphology and histology of alimentary tract of several teleostean fishes. They pointed out that gastric glands exist only in cardiac stomach and not in pyloric region. Jadhav (1985) reported presence of only oxyntic type of cells in gastric glands in Clarius and Tilapia. Jadhav (1985) further identified neutral mucins in the epithelial cells in Tilapia, exhibited sulfo + neutral mucins. The goblet cells in the stomach of an insectivorous fish (Clarias) elaborated only neutral mucins whereas these cells in the herbivorous fish (Tilapia) elaborated a mixture of neutral mucosubstances and sulfomucins.

ii) AMPHIBIA

Norris (1959) observed PAS positive gastric surface epithelial cells and alveolar cells in R.pipiens. Loo and Wong (1975) and Mangalwere (1981) reported neutral mucins in columnar epithelial cells and neutral + traces of acidic mucins in mucus neck cells in B.melanostictus. Mogil'naya et al. (1978)studied gastric epitheliocytes and reported on functional aspect of neutral mucins in protection of mucosa. The columnar epithelium of stomach contains neutral mucins as shown by Mutkekar (1981) and Patil (1983) in E.systoma and R.cyanophlyctis respectively.

Suganuma *et al*. (1981) reported traces of sialomucins in c columnar epithelial cells of five different amphibians. Mangalware (1981) reported higher concentration of neutral mucins in surface and crypt-goblet cells in *B.melanostictus*. Mutkekar (1981) and Patil (1983) reported a mixture of neutral + sulfo + sialomucins in goblet cells in *E.systoma* and *R.cyanophlyctis* respectively. The mucus neck cells in gastric glands show only neutral mucins as pointed out by several workers in several animals like frogs (Mutkekar, 1981) and Patil, 1983), toad (Mangalware, 1981), tree frogs, African frog, Newt, Axolotle larva (Suganuma *et al*., 1981). Jadhav (1985) reported absence of mucus glands in proximal region of cardiac stomach in *R.malberica*, gastric glands elsewhere exhibit dimorphic cells *viz*. mucus neck cells and oxyntic cells, showing only neutral mucins.

iii) REPTILIA

Mogil'naya *et al*. (1978) studied gastric epitheliocytes to report presence of neutral carbohydrates and sialosaccharides. Loo and Swan (1978) pointed out neutral mucins in neck cells and a mixture of neutral + acidic mucins in the lining epithelial cells in *E.cunninghami*. In *T.scincodes*, mucus secreting cells contain neutral mucins (Girraud *et al*., 1979). Mandlik (1983) identified only neutral mucins in gastric epithelial cells in *M.carinata*. The mucus neck cells in gastric glands contain only neutral mucins in Australian lizard (Loo and Swan, 1978), *M.carinata* (Mandlik, 1983) and *X.merremii.* (Ferri *et al*., 1975).

El Taib and Jarrar (1984) reported 2 types of cells in stomach of M.caspica, viz. columnar and goblet. They reported that cardiac glands were long, simple, tubular while pyloric glands were short, alveolar. In turtle and ground lizard, gastric mucosal epithelium consists of only goblet cells, while gastric glands exhibit mucus neck cells and oxyntic cells. Among these in mucus neck cells of turtle, atypical mucosubstances were demonstrated, which were AB and CI unreactive but PAS, AF positive and metachromatic. In both, surface goblet cells exhibit neutral mucins, quantity of which is more in turtle (Jadhav 1985).

iv) AVES :

Avian stomach, with no exception, is highly modified organ showing proximal glandular smooth walled proventriculus or cardiac stomach and distal highly muscular tough walled gizzard or ventriculus or pyloric stomach. Patt and Patt (1969) described histology of avian stomach to report duct cells equivalent to mucus neck cells in mammalian stomach. While other glandular cells secrete pepsin and HCl. Histomorphological and proteolytic activity of oxynticopeptic cells of proventricular glands have been worked out in P.krameri, L.schah and A.tirstis by Jain (1976). Luppa (1959) observed presence of hyaluronidase resistant acidic polysaccharide-protein complex in epithelial cells of glandular stomach of embryonic chick. Belanger and Migicovsky (1961) demonstrated in carporation of radiosulfate in the superficial glands and

ducts of proventriculus in chick, indicating presence of sulfomucins in these sites. Mogil'naya and Bogatyr (1983) reported existance of mixture of neutral + sulfo + sialomucins in epithelial cells in avian glandular stomach. Malvadkar (1985) reported presence of neutral + sulfo + sialomucins in surface goblet cells and sulfo mucins (predominant) + poor neutral mucins in glandular duct cells in proventriculus of pond heron. He demonstrated only neutral mucins in glandular secretory cells, but reported no sexual dimorphism in mucins. Jadhav (1985) demonstrated a mixture of neutral + sulfo + sialomucins in surface goblet cells in proventriculi of parrot and kingfisher. He also reported presence of poor quantities of neutral mucins and hyaluronidase resistant sulfomucins in glandular duct cells.

v) MAMMALIA :

Cardiac Stomach of mammal is a much studied organ as far as mucosubstances are considered. Lambert et al. (1968) reported PAS and AB positive mucosubstances in the brush borders of gastric surface epithelial cells in several mammals including man. Glycogen contents in these cells were well demonstrated in dog, cat and man, however these cells lack glycogen in rat, rabbit, mouse, monkey, guinea pig (Fruschelli, 1967). Several other workers worked out mucins in surface epithelium in cardiac stomachs of several mammals - 1) Wattel et al. (1977) demonstrated acidic mucins in rat. 2) Carvalho et al. (1975) showed existance of sulfo-mucins in armadillo (Dasypus). 3) Tyrrko

et al. (1968) demonstrated sulfomucins in dog and man. 4) Spicer and Sun (1966) showed presence of a mixture of sulfo + sialomucins in dog. 5) Gerard et al. (1967) demonstrated glycoprotein and sialidase labile mucins in canine. 6) Ray (1974) showed a mixture of neutral + acidic mucins in pig. 7) Sinitsina (1968) demonstrated neutral + sulfomucins in man. 8) Deshmukh (1984) worked out mucins in cardiac stomach of bat, to show neutral + sulfo + sialomucins therein.

The reports on mucosubstances in mucous neck cells revealed glycogen contents in dog (Tsujimura 1976). Roy (1974) and Carvalho et al. (1975) demonstrated PAS reactivity in gastric glandular cells in dog and armadillo respectively. Contribution of Cuevas and Chavez (1966) is noteworthy. They employed 'Hale succinic unhydride' method to prove an identical distribution of mucosubstances in gastric glands in various mammalian species. Spicer and Sun (1966) reported presence of predominant sulfated mucins in zymogen cells in basal parts of gastric glands in dog. In squirrel (F.pennati) and monkey (M.carinata), the gastric surface goblet cells and mucus neck cells exhibit only sulfomucins (predominant) (Jadhav 1985).

C) PYLORIC STOMACH :

1) PISCES :

The reports on the mucosubstances in pyloric stomach of fishes are scanty, except the work done by Reifel and Travill (1978). They reported heterogenous distribution of both

sialidase labile and resistant types of sialomucins, weakly acidic sulfomucins as well as neutral mucins in pyloric epithelial cells in 8 species of Teleostean fishes. In Clarias (Jadhav, 1985), the pyloric goblet cells exhibit only neutral mucosubstances, while in Tilapia, these cells show neutral mucins (poor) + sulfomucins (predominant). Further study revealed presence of neutral mucosubstances in the columnar epithelial cells in Tilapia, pyloric glands and serosa in both fishes, glycogen in muscularis, neutral mucins and hyaluronic acid in the pyloric submucosa. Martin and Blaber (1984) showed absence of gastric glands in pyloric stomach in Teleostean fishes.

ii) AMPHIBIA :

Norris (1959) demonstrated PAS positive reaction in surface epithelial cells as well as glands in R.pipiens. Suganuma et al. (1981) took a survey of several amphibians to report presence of neutral mucosubstances in surface goblet cells and glandular cells. They pointed out a junctional zone between pyloric stomach and small intestine where epithelial cells show traces of sialomucins. Mutkekar (1981) demonstrated only neutral mucins in goblet cells and glandular cells in frog. Mangalware (1981) gave a supporting evidence in toad. Patil (1983) identified neutral and sialomucins in epithelial cells of R.cynophylyctis. In R.malberica, (Jadhav, 1985), mucosa of pyloric stomach consists of only goblet cells showing neutral mucins (predominant).

iii) REPTILIA :

Ferri et al. (1975) demonstrated neutral polysaccharides in pyloric cells in X.merremii (snake). Mandlik (1983) also reported presence of only neutral mucins in the surface goblet cells of pyloric glands in M.carinata. Gastric mucosal epithelium consists of goblet cells only in ground lizard and columnar cells only in turtle. In both pyloric gastric glands are present. Columnar epithelium contains neutral mucins (poor), but predominant neutral mucins occur in foveolar cells in the pyloric stomach of the turtle. While in ground lizard these cells exhibit poor neutral mucins and predominant sulfomucins respectively. Secondly pyloric glandular cells in turtle elaborate a mixture of neutral + sulfo + sialomucins while those cells in ground lizard show only neutral mucins (Jadhav, 1985).

iv) AVES :

Avian stomach, as mentioned above (cardiac stomach account) is dimorphic structure and plays a dual role. The proximal part (proventriculus) is glandular and meant for storage, digestion of protein and probably fat and HCl synthesis (Patt and Patt 1969). While distal part (gizzard) is meant for food grinding (Magon and Mohan, 1976). Aswamy et al. (1971) described 4 zones in gizzard of P.phillippensis, viz. outermost hardest zone with quinones, second harder zone with tannin, third zone with S-S bond, and fourth zone with collagenous proteins, so rather the 4th zone is softer in nature.

Megil'naya and Rogatyr (1977) demonstrated presence of neutral, sulfo and sialomucins in mucus secretion in gizzard in a few birds. Shah and Panickar (1975) studied quantitative histochemistry to analyse glycogen contents in gizzard in several birds. Malvadkar (1985) demonstrated intense PAS reactivity in surface goblet cells resistant to both Ph-PAS and D-PAS, indicating absence of neutral mucins and glycogen both. The cells exhibit predominant sulfo and poor quantities of carboxymucins. Sulfomucins are hyaluronidase resistant. The mucosubstances in crypt cells were identical as seen in surface goblet cells. However glandular epithelial cells exhibit only neutral mucins. Jadhav (1985) worked out histochemistry of mucosubstances in the alimentary tract of kingfisher and parrot. He reported presence of non-cellular innermost lining 'koilin layer'; and highly muscular nature of gizzard. The mucosa is highly folded with only goblet cells and crypts were well developed in both species, but glands were found only in kingfisher, absent in parrot. He reported absence of neutral mucins, glycogen in surface and crypt goblet cells. These cells exhibit predominant sulfomucins (hyaluronidase resistant) and poor quantities of carboxymucins. Glandular epithelial cells in kingfisher exhibit only neutral mucins.

v) MAMMALIA :

Reports from several workers are - 1) Neutral mucins in surface epithelial cells in cow (Birgele 1969). 2) Sialidase labile and resistant sialomucins in ferret (Poddar and Jacob,

1979). 3) Sulfated and Carboxyl containing mucosubstances in armadillo (Carvalho et al., 1973). 4) Only neutral mucins in surface epithelial cells (type I) and neutral + sialomucins in same cells but type II in bats (Deshmukh 1984). 5) Neutral mucins in glandular epithelial cells in dog (Tsujimura 1976), in ferret (Poddar and Jacob 1979) and in bats (Deshmukh 1984). 6) A mixture of neutral + acidic mucosubstances in glandular cells in cow (Birgele 1969) and in armadillo (Carvalho et al., 1973). 7) Neutral + sialomucins in glandular cells in bats (Deshmukh 1984). In F.pennati and M.carinata, (Jadhav, 1985), surface goblet cells in pyloric stomach elaborate neutral and sulfomucins . Atypical mucosubstance is not reported. Suzuki et al. (1985) demonstrated neutral + sulfomucins in gastric glandular cells in Francois leaf-monkeys (Presbytis francoisi).

D) DUODENUM :

i) PISCES :

In pisces, there is no clearcut demarkation between duodenum and ileum, hence the reports available about the mucins are with reference to small intestine. Bucke (1971) demonstrated PAS and AB reactive cells in mucosal epithelium of intestine in E.lucius. Shafi (1974) reported both Non-sulfated and sulfated acidic mucosubstances in the intestinal goblet cells in C.betrachus. Reifel and Travill (1979) studied 10 species of teleostean fishes. In 8 species, they demonstrated sialidase resistant sialomucins, some weakly acidic sulfomucins in intestinal mucosal cells, in rest 2 species, they showed sialo-

mucins and sialomucins + neutral mucins respectively. In Clarias and Tilapia, duodenal columnar epithelial cells exhibit only neutral mucins while goblet cells elaborate mixture of neutral + sulfo + sialomucins (Jadhav 1985).

ii) AMPHIBIA :

Mutkekar (1981) and Mangalware (1981) pointed out that glands were absent in duodenum of E. systoma and B. melanostictus respectively. Columnar epithelium of mucosa showed PAS reactivity (neutral mucins) as shown by Mutkekar (1981) in frog, Mangalware (1981) and Patil (1983) in toad. The duodenal goblet cells contain sulfomucins in toad (Mangalware 1981), sulfomucins + sialomucins in E. systoma (Mutkekar 1981) and a mixture of neutral + sulfo + sialomucins in the skipper frog (Patil 1983). Jadhav (1985) poihted out presence of neutral mucins in the columnar epithelium and mixture of neutral + sulfo + sialomucins in the goblet cells in the duodenum of R. malberica.

iii) REPTILIA :

Gabe and Saint-Girons (1972) subjected 33 species of saurians to study mucus secreting cells in duodenum. They observed that mucins in goblet cells were stronger in acidity in some lizards and weaker in others. In M. carinata (Mandlik, 1983), duodenal columnar epithelium showed neutral mucins and goblet cells showed mixture of neutral + sulfomucins. The number of goblet cells is more in duodenal mucosa in ground lizard than in turtle (Jadhav 1985). In both, columner epithelium elaborates neutral mucins while goblet cells show sulfo-

mucins (predominant) and sialomucins (poor).

iv) AVES :

Hodges and Michael (1975) studied chief cells in white leghorn cockrel in details. Malvadkar (1985) reported that in pond heron, columnar epithelial cells exhibit poor PAS reactivity which was diastage resistant but PhaPAS labile indicating presence of neutral mucins but absence of glycogen. Surface and crypt goblet cells showed identical results since these cells exhibit predominant sulfomucins and poor quantities of neutral mucins. Jadhav (1985), reported that absorptive columnar cells in kingfisher and parrot exhibit poor quantities of neutral mucins, while goblet cells show a mixture of neutral mucins (poor quantities) + predominant sulfomucins.

v) MAMMALIA :

The available reports reveal much diversity in type of mucosubstances in goblet cells. Silva et al. (1973) reported neutral mucins in goblet cells in duodenum of cat; Carvalho et al. (1972) demonstrated sialomucins in goat's duodenal mucosal cells. Other reports are - 1) Sulfomucins in man (Hoskiss and Zamcheck, 1963); 2) Neutral + Carboxymucins (sialomucins) + sulfated mucosubstances in man. 3) Both sialidase labile and resistant types of sialomucins in ferret, (Peddar and Jacob, 1979). 4) Neutral, sulfo and sialomucins in goblet cells (type I), neutral + sialomucins in goblet cells (type II) and only sialomucins in goblet cells (type III) in Indian bats (Deshmukh, 1984).

The mucosubstances in Brunner's glands have been a subject of studies in several mammals. The credit of available literature goes to Bhide (1978, 1979); Forman et al. (1979); Poddar and Jacob (1979); and Deshmukh (1984). The cells in Brunner's glands exhibit only neutral mucins in human (Sinitsina 1966), in cat (Silva et al., 1973); in Kangaroo, native cat, mouse and bandicoot (Krauce 1973) and in some bats (Forman et al., 1979; Bhide, 1979, Deshmukh, 1984). The cells exhibit acidic mucins in Koala and Wombat (Krauce 1973), sulfomucins in guinea pig (Jennings and Florrey 1956), neutral + carboxy + sulfomucins in goat (Carvalho et al., 1972).

Lesson and Lesson (1967) demonstrated 2 types of cells in Brunner's glands of rabbit, viz. type I cells with only neutral mucins, and type II cells with a mixture of neutral + acidic mucosubstances. Bhide (1979) and Deshmukh (1984) reported the same results in bats. The first successful attempt to demonstrate sexual dimorphism associated with mucosubstances was made by Shackleford and Wilborn (1978) who worked on hamsters. According to them, the Brunner's glands in male contain double quantity of acidic mucosubstances as compared to that in female, however they pointed out that glands in female show intense PAS reactivity as compared to that in male, suggesting presence of predominant neutral mucins in females than in males. In F.pennati and M.carinata (Jadhav 1985), duodenal columnar epithelial cells elaborate only neutral mucosubstances, while goblet cells exhibit only sulfomucins. Further study reveals that in F.pennati, Brunner's glands consist of dimorphic cells

viz. type I cells with only neutral mucosubstances and type II with only sulfomucins. While in M.carinata, Brunner's cells (all alike) elaborate a mixture of neutral, sulfo and sialo-mucins. Takehana et al. (1985) showed presence of neutral + acidic mucins in the duodenal glanduler cells in mink. Further they revealed neutral mucins in the form of α-D-glucose, α-D-mannuse and acidic mucins as sialic acid. Quian et al.(1985) reported absence of Brunner's glands in the duodenum of purpoise (Neophocaena asiaeorientalis).

E) ILEUM (Small intestine) :

i) PISCES :

Very few reports on mucin in ileum of fishes are available. Martin and Blaber (1984) worked out morphology and histology of alimentary tract of several teleostean fishes. They demonstrated secretary goblet cells and columnar absorptive cells in the small intestine. Jadhav (1985) worked out histochemistry of mucosubstances in the small intestine of Clarias and Tilapia, to prove presence of neutral mucins in the absorptive columnar epithelium, sulfomucins in type I goblet cells and sulfo + neutral mucins in type II goblet cells.

ii) AMPHIBIA :

McAvoy and Dixon (1978) reported that the mucosa of ileum consists of dimorphic cells viz. columnar epithelial and goblet cells. Suganuma et al. (1981) demonstrated only neutral mucins in the brush border of columnar epithelial cells. Mutkekar (1981), Mangalware (1981) and Patil (1983) demonstrated PAS

reactivity in columnar epithelial cells in E. systoma, B. melanostictus and R. cyanophlyctis respectively. However, strickingly, they observed heterogenity in goblet cells as far as mucosubstances are considered. They reported that in B. melanostictus (Mangalware, 1981) goblet cells contain only sulfomucins. Suganuma et al. (1981) reported the same in hyla. In E. systoma (Mutkekar, 1981), goblet cells showed mixture of sulfo + sialomucins. While in R. cyanophlyctis (Patil, 1983), the goblet cells exihibit a mixture of neutral + sulfo + sialomucins. In the ileum of R. malbrica (Jadhav, 1985), absorptive columnar epithelial cells show neutral mucins (poor) and goblet cells show sulfo + sialomucins.

iii) REPTILIA :

Anwar and Mohamoud (1975) subjected two Egyptian lizards (M. quinque taeniata and C. ocellatus) to report presence of acidic mucoproteins in goblet cells. In E. cunninghami (Lee and Swan, 1978), goblet cells contain sulfomucins, while those in M. carinata (Mandlik 1978) exhibit both neutral + sulfomucins while traces of neutral mucins were demonstrated in columnar epithelial cells. The columnar epithelial cells in the ileum of turtle and ground lizard (Jadhav 1985) show only neutral mucins, while goblet cells in these reptiles show neutral + sulfomucins, but sulfomucins predominate in quantity in ground lizard while neutral mucins are more in quantity in turtle.

iv) AVES :

Bayer et al. (1975) studied intestinal goblet cell

chick by using scanning and electron microscopy technique. Magon and Mohan (1976) pointed out histological aspects of small intestine of P.domesticus and C.splendens. Malvadkar (1985) demonstrated poor quantities of neutral mucins in columnar epithelium in ileum of pond heron. While goblet cells in pond heron intestine exhibit predominant quantities of sulfomucins and poor quantities of neutral mucins. Jadhav (1985) demonstrated poor quantities of neutral mucins in absorptive columnar cells and serosa in the small intestine of parrot and kingfisher. Goblet cells as usual exhibited sulfomucins (predominant) and traces of sialomucins in both the birds.

v) MAMMALIA :

Chiefly the goblet cells in ileum of several mammals have been subject of studies with regards to mucins therein. It was reported that mucins show specieswise diversity as reported by 1) Only neutral mucins in Sheep and Cattle (Skordinskii et al., 1970). 2) Sulfo + sialidase resistant and labile sialomucins in ferret (Podder and Jacob, 1979). 3) In man, mucins exhibit diversification, since (a) Subbuswamy (1971) reported only neutral mucins in goblet cells, (b)Fillipe and Fenger (1979) demonstrated neutral + sialo mucins, (c) Lev and Spicer (1965) showed presence of neutral + sialic acid containing mucins. 4) Kim (1972) studied small intestine of several vertebrates including, 7 fishes, 5 amphibians, 6 reptiles, 8 birds and 7 mammals. He showed that goblet cells

contain neutral + acidic sulfated mucosubstances which vary according to species and location of goblet cells in the small intestine. In F.pennati and M.carinata, (Jadhav 1985) intestinal columnar epithelium elaborates poor quantities of only neutral mucins. While crypt and surface goblet cells elaborate neutral mucins (poor) and sulfomucins (predominant). Cha and Jung-Ho (1985) pointed out variation in the number of paneth cells along the length of the ileum in mole (Talpa micrura coreana). They reported that the number is highest in middle zone and lowest in proximal zone.

F) LARGE INTESTINE :

i) PISCES :

Large intestine of fishes histologically differs from small intestine in having well developed musculature and more number of goblet cells (Pasha 1964). Reifel and Travill (1979) studied ten species of teleostean fishes and demonstrated sialidase resistant sialomucins, weakly acidic sulfomucins in rectal epithelial cells in 8 species, while in rest two species, they reported neutral and sulfomucins respectively. Jadhav (1985) reported absence of morphological demarcation between small and large intestine of Clarias and Tilapia but histologically, it was pointed out that in Clarias, villi are short and a few while Tilapia exhibits folds in large intestine. Mucosubstances demonstrated are similar to those in small intestine of these two fishes. Martin and Blabber (1984) showed high population of goblet cells in the rectum of teleostean

fishes, the secretion probably makes the defecation easy.

ii) AMPHIBIA :

The columnar epithelial cells of mucosa contain only neutral mucins in traces in E.systoma (Mutkekar 1981), B.melanostictus (Mangalware 1981) and R.cyanophlyctis (Patil, 1983). However goblet cells contain neutral + sulfomucins in frog and toad (Mutkekar 1981 and Mangalware 1981). Patil (1983) distinguished goblet cells into type I cells with only sulfomucins and type II cells with neutral mucin in R.cyanophlyctis. In R.malberica, large intestinal mucosa exhibits broad folds (no villi) with greater population of the goblet cells and a few absorptive columnar cells, which exhibit only neutral mucins, goblet cells show neutral + sulfomucins (Jadhav, 1985).

iii) REPTILIA :

Anwar and Mohmoud (1975) reported acidic mucoproteins in rectal goblet cells of 2 egyptian lizards. In M.carinata (Mandlik, 1983), columnar epithelial cells show traces of neutral mucins, goblet cells (Type I) show traces of sulfomucins and goblet cells (type II) exhibit predominant sulfomucins. In large intestine of turtle and ground lizard (Jadhav, 1985), columnar epithelium contains only poor quantities of neutral mucins. While goblet cells in turtle exhibit neutral + sulfomucins and those in ground lizard exhibit sulfo + sialomucins.

iv) AVES :

Patt and Patt (1969) stated that large intestine differs from small intestine in 3 aspects - 1) Number of villi, 2)height

of the villi and 3) number of goblet cells. In large intestine, number and height of the villi are less but goblet cells are more as compared to those in small intestine. Malvadkar (1985) demonstrated poor quantities of neutral mucins in columnar epithelium and predominant quantities of sulfomucins + traces of neutral mucins in goblet cells in large intestine of pond - heron. Jadhav (1985) stated that mucosal folds are short in hight in large intestine of parrot as compound to those seen in kingfisher's large intestine. So also crypts are well defined in parrot than in kingfisher. He demonstrated poor quantities of neutral mucins in columnar epithelial cells and a mixture of predominant sulfomucins + traces of neutral mucins in goblet and crypt cells in parrot and kingfisher.

v) MAMMALIA :

Most mammals show decrease in the height of villi in large intestinal region. In some, no villi but broad folds are reported. Patt and Patt (1969) demonstrated crypts at the bases of folds and more number of goblet cells as compared to those seen in small intestine. Goblet cells in man exhibit acidic mucins (Subbuswamy, 1971). Goblet cells in rabbit exhibit neutral + acidic mucins (Masuda et al., 1977). Kim (1972) studied several vertebrates from fishes to mammals, to demonstrate existance of neutral + acidic mucins in goblet cells in large intestine. He pointed out diversity in mucins as per the species and location of goblet cells in the large intestine. Jadhav (1985) revealed presence of neutral mucins (poor) in absorptive cells and a mixture of neutral mucins (poor) + pre-

dominant sulfomucins in the goblet cells in the large intestine of two mammals viz. F.penneti and M.carinata.

IV) REASONS THAT LED TO UNDERTAKE THE PRESENT INVESTIGATION :

It is always said that 'the smallest good deed is better than the gradest good intention'. There are several avenues open in the field of histochemistry and biochemistry. Although several methods, techniques are known, much is known about mucosubstances, their location, chemical nature and distribution. Apart from these facts, a small, concise work is undertaken concentrating the studies on mucosubstances in all the organs of digestive tract in one and the same animal viz. white breasted waterhen (A.phoenicurus phoenicurus). Secondly, the bird selected for the present study is omnivorous, the idea behind it was to observe changes, if any, in the mucosubstances according to the feeding habit. If a review is taken in connection with the available literature on mucosubstances, following points could be made out -

I) Mucosubstances have been a subject for histochemical study for last 3 decades, many organs have been studied, however information about mucins in bird's alimentary tract is scanty.

II) Several mammals have been a subject for study of mucosubstances, a few amphibians are also studied in details but except these 2 groups, a little is known about mucins in submammalian groups especially birds.

III) Information about mucosubstances in vertebrate alimentary

canal, reveals that some investigators have selected one organ of a given animal, others have selected altogether a different organ of a different animal. Hence, there is very scanty literature on mucosubstances in all the organs in the alimentary tract of one and the same animal.

IV) Literature available reveals that identification of mucins is confined to PAS, AB reactivities recorded in cells or glands in mucosa, but further identification is not done.

V) Shackleford and Wilborn (1978) pointed out sexual dimorphism in mucins in the Brunner's glands of hamsters. Except this, no information is available on sexual dimorphism among mucins in alimentary tract. So attempt is made to find out sexual dimorphism in mucins, if any, in A.phoenicurus phoenicurus.

VI) Lim and Low (1977) reported that the gastro intestinal tract of a bird illustrates an area of particular interest since the mucosal surfaces are found to be specialised in different parts of the tract.

All these aforementioned aspects led the author to undertake the present investigation on the mucosubstance in the alimentary tract of one of the birds. The aims and objectives of this investigation were to find out histochemical demonstration, characterisation, distribution of mucosubstance in organs right from oesophagus to rectum in A.phoenicurus phoenicurus.

For the present investigation, most recent and well established, histochemical techniques have been employed to achieve a technical and methodological perfection.

V) RESEARCH PLAN :

On the basis of literature and information available in connection with mucins in alimentary tract, it was decided to work out the histology of the organ in brief and histochemical distribution and characterisation of mucosubstance in the alimentary tract of A.phoenicurus phoenicurus in detail.

a) CHOICE OF THE ANIMAL :

Due care was taken while selecting the material for the histochemical studies. So as to see that no work has been carried out subjecting the same animal. Secondly, availability of specimens of both the sexes in adequate number was also considered to make the investigation work continuous upto end. In this connection, A.phoenicurus phoenicurus found to be most suitable material for the research work undertaken.

b) TECHNIQUES TO BE USED :

Experimental research methodology was found to be suitable for the investigation undertaken. Laboratory equipments, stains, chemicals, reagents were listed and their availability was considered while selecting the techniques to be used for present investigation. In accordance to the aims which were determined before investigation started, suitable well tested, well established histochemical techniques were employed (observation

tables illustrate different techniques that were employed so as to find out nature of mucosubstances in different cellular sites in the alimentary tract from oesophagus to large intestine of A.phoenicurus phoenicurus, in both the sexes). Such histochemical techniques are better than biochemical techniques to illustrate tissue and cellular localization of mucosubstances. During the entire laboratory work, precausion was taken for keeping the staining timings constant, since staining intensity keeps great importance for drawing conclusions towards presence, absence and quantity of mucosubstances in the tissue cells. The differences recorded in the intensity of staining were taken as reflections of differences in the concentrations of different types of mucosubstances.

c) CRITICAL EVALUATION OF THE OBSERVATIONS :

The results obtained during experimentation work, were subjected for critical analysis with reference to following aspects -

1) Histology of various organs from oesophagus to large intestine (HE technique).
2) Histochemical characterisation of mucosubstances in different layers from innermost mucosa to outermost serosa in different organs of the digestive tract.
3) The distribution of mucosubstances in different layers.
4) Sexual dimorphism in mucosubstances in A.phoenicurus phoenicurus.
5) Comperision of the results obtained in the present investiga-

tion and the existing information from other birds so as to point out similarities and dissimilarities, if any (comparative chart).

6) To find phylogenetic variation, results obtained in present investigation were compared with those available from literature on mucins in vertebrate alimentary tract, especially in birds.

7) By looking into circumstantial evidences, mucosubstances and their functional significance was studied in various organs of alimentary tract of A.phoenicurus phoenicurus.

d) OUTLINE OF DISSERTATION :

As per the principles of Research Methodology, the present dissertation was divided into four chapters, the first being 'the introduction' with explains some things about the histochemistry field, brief ideas about mucins, a review of literature on morphology, anatomy of avian digestive tract, comparative serial review of literature on mucins in vertebrate alimentary tract, reasons that led to undertake the present short investigation and research plan. Chapter II covers usual aspects like material used and methods employed to make the investigation a success. Chapter III deals with histological and histochemical observations on different organs of alimentary tract of A.phoenicurus phoenicurus. Chapter IV is devoted to the discussion on results obtained in the investigation undertaken and comparision with those obtained in other birds like

A.grayii(Malvadkar, 1985), P.krameri and C.radius (Jadhav, 1985). The discussion is followed by Summary, concluding remarks and complete bibliography of the references cited time to time in various chapters, to make the dissertation perfect giving no scope for erratum.