Introduction

CHAPTER ONE

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1. What are Mucosubstances?

The term 'mucus' denoting a slimy vicid and tenaceous substance of the animal body was freely used in Latin and English medical text books of 18th century. It was Eichwald (1935) who provided the first chemical evidence for the presence of carbohydrates in mucins. He defined mucin as "a conjugated single compound of a moeity released under certain conditions as a sugar."

Polysaccharides may be regarded as condensation polymers of monosaccharides resulting from the formation of glycosidic linkage by elimination of water. With many polysaccharides, systematic nomenculature and classification presents considerable problems and it has, therefore, been usual to group the polysaccharides in broad divisions according to their source and biological function. In the animal kingdom, a major class obtained in this way comprises the amino sugar containing polysaccharides, for which the name 'mucopolysaccharide' has been adopted.

The carbohydrates form a very large group of compounds of enormous theorotical and practical importance. Research on these compounds has undergone a significant advance because of new tools, techniques and stains, in last two decades. The term mucopoly-saccharides, introduced by Meyer (1938), is now well established in literature of vertebrate biochemistry. Due to the confusion regarding the terms such as mucins, mucoids, mucopolysaccharides, mucoproteins, glycoproteins, glycosaminoglycans etc. it is indeed

difficult to distinguish them by definitions. Spicer et al. (1965) classified carbohydrate-rich tissue components and suggested "mucosubstance", a general term for histochemical reference to any carbohydrate-rich component.

The histochemical classification of mucosubstances is suggested by Spicer et al. (1965) in which the mucosubstances are named by (i) staining site in which they are found, and (ii) subgrouping them by their chemical moieties, as neutral mucosubstances, sulfomucins, sialomucins etc. It is suggested that further subdivisions of these subtyped mucosubstances can be achieved by means of following histochemical reactions :

i) Affinity for basic dyes such as Azure A.
ii) Affinity for Alcian blue.
iii) Affinity for Alcian blue in presence of graded concentration of $MgCl_2$.
iv) Lability with respect to testicular hyaluronidase.
v) Lability with respect to vibrio cholerae neuraminidase.

The last two to three decades have witnessed an appreciable upsurge in our understanding of mucosubstances, their chemical nature, identification of individual mucosubstance and functional significance of mucosubstances in relation to life in general.

2. Review of Literature on Avian Digestive Tract

Salim Ali (1964), a notable orninthologist, has described avian fauna of this country. The morphology, anatomy and histology of the avian digestive system have been worked by some

scientists. The important references on avian digestive morphology and anatomy are those of Rosenberg (1941), Leopold (1953), Farner (1960), Marshall (1960), Bradley and Grahame (1960), Colhaum (1961), Naik and Dominic (1962), Toner (1964), Sturkie (1965), Pastea et al. (1967), Cymborowski (1968), Rawal (1968, 1971), Hegde (1970), Hegde and Hooli (1970), Hill (1970), Singh (1973), Mc nab (1973), Vornov (1973, 1974), Papadopol (1974a, 1974b), Borkenhagen (1976), Magon and Mohan (1976), Amonova (1977), Crompton et al. (1979) and Oelhafen-Gandolla and Ziswiller (1981). Recent literature on histology of alimentary tract of birds is reported by Feder (1962), Davidova (1965), Quay (1967), Ghosh and Gynvati (1967), Herpol (1967), Patt and Patt (1969), Michael (1971), Hodges and Michael (1975), Bayer et al. (1975, 1977), Magon and Mohan (1976) and Dahm et al. (1980).

Sturkie (1965) pointed out that "Since last three decades there has been considerable increase in research activity in avian endocrinology, reproduction and modest increase in other areas. Much work, however, remains to be done on such systems as respiration, muscle, nerve and digestion".

Although mucosubstances have been studied from vertebrate alimentary canal, the laboratory animals such as rabbit, rat, pig etc. are the focal points for such studies. In this regards the various organs of gastrointestinal tract of submammalian vertebrates have not received much attention as they deserve. Recently Lim and Low (1977) reported that "The gastrointestinal

tract of avian forms is an area of particular interest since the mucosal surfaces are known to be highly specialized in different parts of tract." This stimulated to undertake the present investigation on mucosubstances in gastrointestinal tract of pond heron, Ardeola gryii.

Some idea of the work done on alimentary tract of vertebrates will be obtained from following brief review.

3. Review of literature on mucosubstances in gastrointestinal tract of vertebrates

A) Esophagus

Pope et al. (1975) commented that the esophagus is much neglected part of gastrointestinal tract. But there are many reports on the anatomy, morphology and in some cases on histology of esophagus in several fishes (Pasha, 1964; Bullock, 1967; Bucke, 1971; Singh, 1974; Sinha, 1977; Sis et al., 1979; Chakrabarty et al., 1983). Among the fishes, teleostean esophageal mucous cells have been reported to react with PAS and AB (Bullock, 1967; Bucke, 1971). Recently, in a detailed investigation on the esophagi of ten species of fishes, Reifel and Travill (1977) have identified six types of mucous cells, but in a given fish only two types of mucous cells were observed. They reported a heterogenous distribution of sialidase resistant sialomucins, sialidase labile sialomucins, sulfomucins and neutral mucins in mucous cells Chakrabarty et al. (1983) studied with fleurescence microscopy the localization of sulfo-and sialomucins in esophageal mucous cells of L. rohita.

Norris (1959) reported on PAS reactive goblet cells and glands in the esophagus of Rana papiens. Loo and Wong (1975) identified mainly sulfomucins, small amount of sulfated mucins and some neutral mucins in esophageal goblet cells of the toad, Bufo melanostictus. Mutkekar (1981) investigated histochemically the presence of neutral mucosubstances in the columner epithelial cells; a mixture of neutral -, sulfo- and sialomucins in type-I goblet cells and type-I cells in glands and neutral - and sialomucins in type-II goblet cells and type-II cells in glands in esophagus of Euperodon systoma. Patil (1983) analysed neutral mucosubstances in the ciliated epithelial cells, type-II goblet cells and mucous cells in the glands and the presence of neutral-, sulfo- and sialomucins in the type-I goblet cells in the esophagus of R. cyanophlyctis. Mangalware (1981) reported on the presence of only neutral mucosubstances in the columner epithelial cells and predominant sulfomucins with traces of sialomucins in the goblet cells and glands in the distal portion of esophagus of B. melanostictus. Suganuma et al. (1981) studied mucosubstances in alimentary tract of several vertebrates including five amphibians viz. tree frog, common frog, African frog, axolotle and newt. Their histochemical studies revealed the presence of neutral mucosubstances in columner epithelial cells in frogs, sialomucins in the corresponding cells in axolotle and small amount of sialomucins in goblet cells in the frogs.

Varute & Nalavade (1973) demonstrated mucosubstances in esophagi

of three species of lizards. The epithelium in esophagi of C. versicolar and M. carinata consisted of only goblet cells which elaborated neutral mucosubstances, sulfomucins and sialomucins. The mucosa in H. flaviviridis exhibited the presence of goblet cells and columner epithelial cells, the former cells elaborated neutral -, sulfo- and sialomucins and the latter type only neutral mucosubstances and protein masked sialomucins. The esophageal glands in all the three species of lizards were peptic glands (protein elaborating) and mucous glands (elaborating only neutral mucosubstances). Loo and Swan (1978) demonstrated acidic sulfated mucins in esophageal epithelium of the Australian lizard, E. cunninghami. Ferri et al. (1977) identified sialic, neutral and acidic polysaccharides in the goblet cells of the esophagus of the snake, X. merremii. Mandlik (1983) showed the presence of neutral mucosubstances in ciliated epithelial cells, sulfomucins in type-I goblet cells and sulfomucins and neutral mucosubstances in the type-II goblet cells in the proximal part and only neutral mucosubstances in the cells and mucous cells in the glands of distal esophagus in Indian skink, M. carinata.

The studies on microanatomy of the upper-digestive tract of Japanese quill found alveolar mucous glands in the tunica propria of esophagus (Warner et al., 1967). Feder (1972) observed the structure of epithelium and glands in the esophagus of various birds differing in their feeding habits. But he failed to find the relationship between the type of mucosubstances and the type of the food. Magon and Mohan (1976) found greatest cornification

in the esophagus of Passer than in the Corvus. Hanke (1957) described three muscular layers in the esophagi of O. crucis and I. major. The other birds contain only two muscular layers. In majority of birds, esophageal glands produce acidic muco-substances which are mainly corboxylated (Grossi and Millo, 1967; Allenspeck and Berlin, 1971). On the other hand, Van Alten and Fennell (1957) reported on the presence of sulfomucins in esophageal glands of birds during histogenesis. Histochemical studies demonstrated the presence of neutral polysaccharides, sulfated and non-sulfated acid polysaccharides (the latter probably sialic acid) in the esophageal glands of the fowl, G. domesticus (Rangel et al., 1970).

There are sporadic reports in the literature mostly concerned with glycogen in normal esophagus of man and monkey (Wislocki et al., 1951; Rywlin and Ortega, 1970; Theman et al. 1971; Hopwood et al., 1977a). Rambourg (1969) reported the presence of a mucosubstance, probably a glycoprotein containing sialic acid, between the esophageal cells of rat. Hopwood et al. (1977b) reported on the neutral mucosubstances in normal human esophageal epithelium. These mucosubstances were observed in the cell coat and membrane coating the granules. The presence of neutral mucosubstances and sialomucins in esophageal keratinised epithelium in some bats but neutral mucosubstances and sulfomucins in other few bats was shown by Deshmukh (1984).

Carvalho et al. (1968) identified glycogen, sialic acid and neutral polysaccharides in the mucins of esophageal glands of armadillo, D. novemcinctus. Masuda et al. (1977) showed the

presence of PAS and AB positive cells in the esophagus of many. The presence of sulfomucins has been reported in the middle layer of the esophageal epithelium (Bescol - Liversac and Guillam, 1972) and esophageal glands (Lambert, 1971). Al Yassian and Tonner (1977) considered that the glands in the human esophagus can be compared with mucous secreting minor salivary glands.

B) Cardiac stomach

Mucosubstances in gastric epithelial cells have been reported in many teleosts (Kapoor et al., 1975). The epithelial cells in the stomach of E. lucius have been shown to react with PAS and AB (Bucke, 1971). Jirage (1970) described sialomucins and sulfomucins in the striated border of gastric epithelial cells and basophilia with AB and presence of neutral mucins in gastric glands of T. mossambica. Shafi (1974) demonstrated acidic sulfated mucopolysaccharides in the gastric surface epithelial cells of C. batrachus. A heterogenous distribution of neutral - , sialo - and weakly acidic sulfomucins was found in the surface epithelial cells and cells lining pit regions in the stomach of eight different species of teleosts (Reifel and Travill, 1978).

Norris (1959) reported PAS reactive surface epithelial and foveolar cells in the stomach of R. pipiens. The presence of neutral mucins in columner cells and neutral mucins and traces of acidic mucins in mucous neck cells in stomach was observed in B. melanostictus (Loo and Wong, 1975; Mangalware, 1981). The columner epithelial cells showed neutral mucosubstances in

E. systoma (Mutkekar, 1981) and R. cyanophlyctis (Patil, 1983). On the other hand Suganuma et al. (1981) demonstrated traces of sialomucins in these cells of five different amphibians. Goblet cells were identified in the gastric surface epithelium which contained higher concentration of neutral mucosubstances in B. melanostictus (Mangalware, 1981) and a mixture of neutral, sulfo - and sialomucins in E. systoma (Mutkekar, 1981) and R. cyanophlyctis (Patil, 1983). Mogil'naya et al. (1978) studied gastric pavement epitheliocytes in amphibians and reported that protection of stomach is connected with neutral carbohydrate components. Norris (1959) reported on PAS reactive mucous neck cells in the gastric glands of R. pipiens. The mucous neck cells have elaborated only neutral mucosubstances in frogs (Mutkekar, 1981; Patil, 1983), toad (Loo and Wong, 1975; Mangalware, 1981) and five different amphibians viz. frogs, newt and axolotle (Suganuma et al., 1981).

Mogil'naya et al. (1978) studied gastric pavement epitheliocytes in reptiles and reported that neutral carbohydrates and sialosaccharides performed the barrier function. Loo and Swan (1978) demonstrated the presence of neutral mucins in neck cells and both neutral and acidic mucins in the lining epithelial cells in stomach of E. cunninghami. Giraud et al. (1979) found PAS reactive mucous cells in the stomach of lizard, T. scincodes. Mandlik (1983) identified only neutral mucosubstances in gastric epithelial cells in M. carinata. The mucous neck cells in the gastric glands contained only neutral mucosubstances in the

Australian lizard (Loo and Swan, 1978), Indian Skink (Mandlik, 1983) and a snake, X. merrimii (Ferri et al., 1975).

The proximal stomach in birds is known as proventriculus which is glandular and the distal stomach is known as gizzard or ventriculus which is muscular. Patt and Patt (1969) described histology of avian stomach and reported that duct cells resemble mucous neck cells and the other cells in glands secrete pepsin and produce HCl. Two distinct chambers - anterior proventriculus and posterior gizzard - were identified in P. domesticus and Corvus splendens (Magon and Mohan, 1976). Histomorphological and proteolytic action studies of HCl and pepsin producing oxyntico-peptic cells were studied in the proventricular glands of Psittacula krameri, Lanius schah and Acridotheres tristis (Jain, 1976). Mogil'naya et al. (1978) reported the presence of neutral carbohydrates, sialosaccharides and sulfosaccharides in gastric epitheliocytes of birds. Luppa (1959) observed the presence of hyaluronidase resistant acid polysaccharide protein complex in lining epithelium of glandular stomach of embryonic chick. Recently, Mogil'naya and Bogatyr (1983) reported on the presence of neutral glycoproteins, sialo- and sulfoglycoproteins in epithelial lining of avian glandular stomach.

The mammalian stomach is a much studied organ as far as mucosubstances are concerned. To some extent the mucosubstances have been identified in the stomach of several mammals. Lambert et al. (1968) reported PAS and AB reactive mucosubstances in the surface coating of gastric mucous cells in several animals including man. The gastric epithelial mucosa cells have been reported

to contain glycogen in dog, man, cat but absence of glycogen in rat, rabbit, mouse, monkey, guinea pig (Fruschelli, 1967), acidic carbohydrates in rat (Wattel et al., 1977), sulfomucins in armadillo (Carvalho et al., 1975), human and dog (Tyrrko et al., 1968), sulfo- and sialomucins in dog (Spicer and Sun, 1966), glycoprotein and sialidase susuptible mucin in canine (Gerard et al., 1967), neutral and acidic mucosubstances in pig (Roy, 1974), and neutral and sulfomucins in man (Sinitsina, 1968). Deshmukh (1984) demonstrated a heterogenous distribution of neutral -, sulfo- and sialomucins in these cells of bats.

The mucous neck cells have been reported to contain glycogen in dog (Tsujimura, 1976). These cells exhibit PAS reactivity in gastric glands in dog (Roy, 1974) and armadilla (Carvalho et al., 1975). Cuevas-Chavez (1966), by employing a new method as Hale succinic unhydride, found identical distribution of mucosubstances in gastric glands of diverse animal species. Spicer and Sun (1966) observed much sulfated mucosubstances in zymogen cells in the deep glands of cardiac stomach of dog.

C) Pyloric stomach

Reifel and Travill (1978) studied pyloric epithelial cells in eight species of teleosts and reported a heterogenous distribution of sialidase sialomucins, sialidase labile sialomucins, weakly acidic sulfomucins and neutral mucusoubstances.

Norris (1959) demonstrated PAS reactive cells in the pyloric epithelium and glands in R. pipiens. Suganuma et al. (1981) reported the presence of neutral mucosubstances in surface goblet

cells and pyloric glands in the amphibians studied. They also reported traces of sialomucins in the epithelial cells in pyloric stomach of amphibians, near the junction of stomach and intestine. On the other hand, Mutkekar (1981) in frog and Mangalware (1981) in toad demonstrated only neutral mucosubstances in the goblet cells and pyloric glands. Patil (1983) identified neutral mucosubstances and sialomucins in the foveolar epithelial cells in pyloric stomach of R. cynophlyctis.

Neutral polysaccharides in the pyloric cells were demonstrated in the snake, X. merremii (Ferri et al., 1975). Mandlik (1983) also analyzed presence of only neutral mucosubstances in the surface goblet cells and pyloric glands in M. carinata.

The bird stomach is a dual structure. The anterior proventriculus is glandular and functions in food storage, protein and fat digestion and HCl secretion (Patt and Patt, 1969). The posterior gizzard or ventriculus is involved in food grinding. Magon and Mohan (1976) also described posterior gizzard in Passer and Corus. Aswamy et al. (1976) described four zones in gizzard lining of the piscivore bird, Pelacanus phillippensis. The outermost zone was hardest due to quinones, second was hard due to tanning, third had S - S bond and fourth contained collagenous protein. Mogil'naya and Bogatyr (1977) have demonstrated the presence of neutral carbohydrates, sulfosaccharides and sialosaccharides in mucus secretion of avian gizzard. Shah and Panicker (1975) reported on quantitative glycogen contents of gizzards in several birds.

Among the mammals, the surface epithelial cells in the pyloric stomach contain neutral mucosubstances in cow (Birgele, 1969), sialidase labile and resistant sialomucins in ferret (Poddar and Jacob, 1979) and sulfate and carboxyl containing mucopolysaccharides in armadillo (Carvalho et al., 1973). Deshmukh (1984) analyzed type-I cells in surface epithelium of pyloric stomach containing only neutral mucosubstances but the type-II cells contained neutral substances and sialomucins in the bat, Pipistrellus ceylonicus chrysothrix. The elaboration of mucosubstances in the pyloric glands of various mammals differ slightly. Mucosubstances have been identified in the pyloric glands as neutral mucosubstances in dog (Tsujimura, 1976), ferret (Poddar and Jacob, 1979) and bats (Deshmukh, 1984), and neutral and acidic mucopolysaccharides in cow (Birgale, 1969) and armadillo (Carvalho et al., 1973). The presence of neutral mucosubstances and sialomucins was shown in the pyloric glands of bats, T.kacchensis and P.ceylonicus chrysothrix (Deshmukh, 1984).

D) Duodenum

Mutkekar (1981) and Mangalware (1981) reported on the absence Brunner's glands in duodenum of E.systoma and B.melanostictus respectively. In the columer epithelial cells the neutral mucosubstances were observed in frogs (Mutkekar, 1981; Patil, 1983) and toad (Mangalware, 1981). The duodenal goblet cells contained sulfomucins in toad (Mangalware, 1981), sulfomucins and sialomucins in balloon frog (Mutkekar, 1981) and a

mixture of neutral -, Sulfo- and sialomucins in the skipper frog (Patil, 1983).

Gabe and Saint-Girans (1972) studied mucous secreting cells in the duodenum of 33 species of saurians and observed that mucins are stronger in acidity in goblet cells of some lizards and weaker in the others. Presence of neutral muco-substances was demonstrated in the duodenal columner epithelial cells and neutral mucosubstances and sulfomucins in the duodenal goblet cells of M. carinata (Mandlik, 1983).

The fine structure of chief cells was described in the white leghorn cockrel (Hodges and Michael, 1975).

The available literature shows a species diversity in the type of mucosubstances in the duodenal goblet cells. The duodenal goblet cells have been observed to contain neutral mucosubstances in cat (Silva et al., 1973), sialomucins in goat (Calvalho et al. 1972), sulfomucins in man (Hoskiss and Zamcheck, 1963) and neutral mucosubstances, carboxymucins, sialomucins and sulfated mucopoly-saccharides in man (Sinitsina, 1966). Poddar and Jacob (1979) reported sialidase resistant and -labile sialomucins in the duodenal cells of ferret. Deshmukh (1984) investigated the presence of neutral -, sulfo - and sialomucins in type-I goblet cells and neutral - and sulfomucins in type-II goblet cells and neutral- and sialomucins in type-III goblet cells in bats. The mucosubstances in Brunner's glands have been studied in several mammals. Available literature on this has been reviewed by Bhide (1978, 1979), Forman et al. (1979), Poddar and Jacob (1979

and Deshmukh (1984). The cells of Brunner's glands elaborate only neutral mucosubstances in human (Sinitsina, 1966), cat (Silva et al., 1973), kangaroo, native cat, marsupial mouse and bandicoot (Krause, 1973) and some bats (Forman et al., 1979; Bhide, 1979; Deshmukh, 1984), acidic mucosubstances in koala and wombat (Krauce, 1973), sulfomucins in guinea pig (Jennings and Florrey, 1956) and neutral mucosubstances, carboxymucins and sulfomucins in the goat (Carvalho et al., 1972). Two types of cells - some elaborating only neutral mucosubstances and others elaborating neutral and acidic mucosubstances - have been reported in Brunner's glands of rabbit (Leeson and Leeson, 1967; More and Bayle, 1972) and some bats (Bhide, 1979; Deshmukh, 1984). An interesting observation was investigated by Shackleford and Wilborn (1978), showing sex dimorphism in the Brunner's glands of hamsters. These glands in male contained double quantity of acidic mucosubstances than in the female and the glands in female elaborated stronger PAS reactivity than in male.

E) Small intestine (Ileum)

PAS and AB reactive cells were observed in the intestinal epithelium of the fish, E. lucins (Bucke, 1971). Shafi (1974) demonstrated acid mucosubstances and sulfated acid mucosubstances in the intestinal goblet cells of C. betrachus. Reifel and Travill (1979) studied mucous cells in intestine of ten species of teleosts and their results revealed the presence of sialidase resistant sialomucins and some weakly acidic sulfomucins in the mucous cells of eight species, only sialomucins in one fish and

sialomucins and neutral mucosubstances in other fish.

Mc Avoy and Dixon (1978) showed the presence of columner cells and goblet cells in the small intestine of toad, X. laevius. The brush border of columner cells reacted with only BAS in R. nigromaculata (Suganuma et al., 1981). Neutral mucosubstances have been identified in the columner cells in E. systoma (Mutkekar, 1981), B. melanostictus (Mangalware, 1981) and R. cyanophlyctis (Patil, 1983). The intestinal goblet cells exhibited some species variations. The goblet cells contained sulfomucins in B. melanostictus (Mangalware, 1981), and Hyla arborea japonica (Suganuma et al., 1981), sulfomucins and sialomucins in E. systoma (Mutkekar, 1981) and a mixture of neutral -, sulfo - and sialomucins in R. cyanophlyctis (Patil, 1983).

Anwar and Mohmoud (1975) reported the presence of goblet cells in the ileum of two Egyption lizards, M. quinque taeniata and C. ocellatus which elaborated acidic mucoproteins. On the other hand, these cells contain sulfomucins in the Australian lizard, E. cunninghami (Loo and Swan, 1978) and neutral mucosubstances and sulfomucins in Indian skink, M. carinata (Mandlik, 1983). Mandlik (1983) demonstrated traces of neutral mucosubstances in columner epithelial cells in small intestine of M. carinata.

Scanning and transmission electron microscopes revealed ultrastructure of intestinal goblet cells of chicks (Bayer et al., 1975). Magon and Mohan (1976) also observed similar histology in small intestine of Passer domesticus and Corvus splendens.

The mucosubstances have been studied mainly from the goblet

cells of small intestine of various mammals. These mucins showed species diversity. These cells contain only neutral mucosubstances in sheep, cattle (Skordinskii et al., 1970) and sulfomucins, sialidase resistant sialomucins and sialidase labile sialomucins in ferret (Poddar and Jacob, 1979). Many workers have reported different results for these cells in the same animal. For example, human small intestinal goblet cells have been reported to contain neutral mucosubstances (Subbuswamy, 1971), neutral mucosubstances and sialomucins (Fillipe and Fenger, 1979) and PAS reactive, sialic acid containing and sulfated mucins (Lev and Spicer, 1965). Kim (1972) studied small intestine and large intestine of 7-mammals, 8-birds, 6-reptiles, 5-amphibians and 7-fishes. The mucins in their goblet cells contained neutral and acidic sulfated mucosubstances and they showed variations according to species and inhabited regions.

F) Large intestine

Rectal epithelium in fish, M. cyprinoides contain large number of goblet cells and thick musculature than small intestine (Pasha, 1964). Reifel and Travill (1979) studied the mucosubstances in mucous cells in rectal intestine of ten teleosts. They observed the presence of sialidase resistant sialomucins and weakly acidic sulfomucins in these cells of eight fishes, only neutral mucosubstances in one fish and only sulfomucins in one fish.

The columner epithelium in the large intestine contain only

traces of neutral mucosubstances in E. systoma (Mutkekar, 1981), R. cyanophlyctis (Patil, 1983) and B. melanostictus (Mangalware, 1981). The goblet cells contain neutral - and sulfomucins in E. systoma (Mutkekar, 1981) and B. melanostictus (Mangalware, 1981). On the other hand, Patil (1983) distinguished type-I goblet cells containing only sulfomucins and type-II goblet cells with neutral mucosubstances in large intestine of R. cyanophlyctis.

Anwar and Mohmoud (1975) reported on the presence of acidic mucoproteins in rectal goblet cells of two Egyptian lizards. The trace quantities of neutral mucosubstances in columner cells and sulfomucins (less quantities) in type-I goblet cells and sulfomucins (predominent) in type-II goblet cells were reported in large intestine of M. carinata (Mandlik, 1983).

In birds, the large intestine differs from the small intestine only in the decrease in number and height of villi and the increase in goblet cell concentration (Patt and Patt, 1969).

In the large intestine of mammals also there is decrease in number or absence of villi, instead broad folds are present. The number of goblet cells increase and well developed crypts are present at bases between the folds (Patt and Patt, 1969). Subbuswamy (1971) reported on presence of acidic mucosubstances in human large intestinal goblet cells. Masuda et al. (1977) recorded PAS and AB reactivities in goblet cells of large intestine of rabbit. Kim (1972) identified neutral and acidic mucosubstances in goblet cells of large intestine of 7-mammals, 8-birds, 6-reptiles, 5-amphibians and 7-fishes. Some variations

were also noted according to species and the regions where goblet cells are present.

4. Reasons that led the undertaking of the present investigation

A critical evaluation of the available literature on animal mucosubstances in general and alimentary tract mucosubstances in particular reveals that there are several avenues open for young scientists working on histochemistry and biochemistry of mucosubstances in the vertebrate alimentary tract. The literature on animal mucosubstances significantly shows that :

i) In general, our status of knowledge on mucosubstances in the vertebrate alimentary tract is poor as compared to other tissues and organ-systems.

ii) Moreover, the work so far done on alimentary tract shows that mammals are the focal point for such investigations. A little is known about these mucosubstances in the alimentary tract of submammalian vertebrates.

iii) Whatever work has been reported on the mucosubstances in vertebrate alimentary tract, clearly shows that some investigations have included one organ of a given animal, others have selected altogether a different organ of a different animal. Hence, there is very scanty literature on detailed investigation on mucosubstances in all the organs of the alimentary tract of one and the same animal.

iv) In some cases only PAS reactivity or AB reactivity of a given cell type in mucosa or the gland has been reported

but the mucosubstances have not been further identified.

v) No information is available on sexual dimorphism of alimentary tract mucosubstances in vertebrates except a few mammalian duodenal mucosubstances.

vi) Recently, Lim and Low (1977) reported that "The gastro-intestinal tract of avian forms is an area of particular interest since the mucosal surfaces are known to be highly specialized in different parts of tract."

The above critical evaluation of the existing literature on vertebrate alimentary tract mucosubstances makes it very clear that a detailed investigation of mucosubstances in sub-mammalian vertebrate alimentary tract is essential to clarify their nature and role in physiology of the tract and also to auqment the understanding of animal mucosubstances in general.

All these aforementioned points prompted to undertake the present investigation on the mucosubstances in alimentary tract of one of the birds. The work included in the present dissertation concerns with the mucosubstances, their histochemical characterization and distribution in various organs of the alimentary tract of the pond heron, A. grayii. For this research most recently developed and recommended histochemical techniques have been employed to achieve a technical and methodological perfection.

5. Plan of the present investigation

Keeping in view the above mentioned reasons and the amount of work done on the alimentary tract mucosubstances in birds, it

was decided to work out the histology of various organs and distribution and characterization of mucosubstances in the alimentary tract of A.grayii.

a] Choice of the animal

While selecting the animal for present studies due care was taken to select such a bird wherein no work has been carried out on histology and mucopolysaccharide histochemistry in any organ of the alimentary tract. Secondly, the animals of both the sexes should be available in required number throught the course of the investigation. Therefore, the pond heron/paddy bird, A.grayii, was found most suitable for the present investigation.

b] Choice of the techniques

For the present studies the Experimental Research methodology was employed by the investigator. According to the availability of the chemicals, stains and reagents in this laboratory, several histochemical methods were thought to be employed. Since the present investigation aims at a detailed study of nature of mucosubstances in different cellular sites in alimentary tract from esophagus to large intestine of A.grayii, in both the sexes, and to characterise them histochemically recent and well established histochemical techniques were employed. The different techniques involving PAS reactivity, modifications in PAS reactivity following diastase digestion and phenylhydrazine pretreatment, alcinophilia at different pH levels, aldehyde fuchsin staining, sequential staining such as AB pH 1.0 - PAS, AB pH 2.5 - PAS, AF-AB pH 2.5, metachromasia at various pH levels, critical electrolyte concentra-

tion, methylation, saponification, acid hydrolysis, enzyme digestions such as hyaluronidase digestion, neuraminidase digestion and pepsin digestion have been employed. Such histochemical techniques are better than biochemical techniques to illustrate tissue and cellular localization of mucosubstances. In the present investigation, the staining timings were kept constant throughout the work, and differences, if any, in the intensity of staining were taken as reflections of differences in the concentrations of the different types of mucosubstances.

C] Critical evaluation of the observations

It was decided to analyze critically the results obtained in the present investigation on alimentary tract of A.grayii in relation to :

i) Histology of various organs such as esophagus, stomach (proventriculus and gizzard), duodenum, small intestine (ileum) and large intestine.

ii) Histochemical characterization of mucosubstances in different layers from mucosa to serosa in different organs of the tract.

iii) The distribution of mucosubstances in different layers from mucosa to serosa in different organs of the tract.

iv) Sex dimorphism, if any, in the various organs in male and female pond herons.

v) Comparision of the results obtained in the present investigation and the existing literature on other birds so as to find out similarities and dissimilarities, if any.

vi) Comparision of the results obtained in the present investi-

gation and existing literature on vertebrate alimentary tract to find out the phylogenetic variations.

vii) Functional significance of mucosubstances in the various organs of alimentary tract based on circumstantial evidences.

d] Presentation of the dissertation

It was decided to divide the present dissertation into four chapters, the first chapter being on the introduction which gives a brief idea of the meaning of mucosubstances, a review of recent literature on morphology, anatomy and histology of avian alimentary tract, the existing literature on mucosubstances in the vertebrate alimentary tract, the reasons that led to undertake the present research work and the plan of present investigation. The second chapter deals with the material and methods employed in the present investigation. Detailed histological and histochemical observations on esophagus to large intestine of A. grayii are listed in third chapter. The fourth chapter is devoted to the discussion on results obtained in the present investigation and the existing literature. The last chapter will be followed by summary and concluding remarks. A complete bibliography of the literature cited in the various chapters of the present dissertation will be given at the end of the dissertation.