

CHAPTER - FOUR

D I S C U S S I O N

DISCUSSION ON HISTOLOGY AND  
HISTOCHEMISTRY OF MUCOSUBS-  
TANCES IN ESOPHAGUS, CARDIAC  
STOMACH, PYLORIC STOMACH,  
DUODENUM, SMALL INTESTINE,  
AND LARGE INTESTINE OF THE  
FROG, E. systoma

## D I S C U S S I O N

The present investigation on mucosubstances in the mucosa, glandular structures, submucosa, muscularis and serosa of the alimentary tract of the frog, E. systoma was undertaken with a view to augment the animal mucosubstances in general and alimentary tract mucosubstances in particular. The present chapter deals with the discussion on the results obtained in the present investigation and the existing literature on mucosubstances in the vertebrate alimentary tract. The present thesis deals with the mucosubstances present in various layers from mucosa to serosa in esophagus, cardiac stomach, pyloric stomach, duodenum, small intestine and large intestine both in male and female frogs, E. systoma. As it is brought to the notice the paucity of literature on amphibian and particularly the anuran alimentary tract, it was hoped that such a study will provide the knowledge of alimentary tract of the particular frog investigated, species differences if any, in the alimentary tract mucosubstances and similarities and/or dissimilarities in alimentary tract mucosubstances when compared to the mammalian and submammalian vertebrate alimentary tract mucosubstances. The purpose of the present discussion is to view critically the results obtained in the present investigation and the information available through the existing literature.

1) Esophagus

The esophagus is a muscular, extensible tube with thin wall, the function of which is to conduct food materials from pharynx to the stomach. On occasions it acts to prevent the entry of water or air from the mouth or regurgitation of gastric

contents (Ingelfinger, 1958). The mucosa of esophagus in E.systema consisted of columnar epithelial cells and goblet cells. Similar histology is also described for amphibian esophagus by Noble (1954) and Reeder (1964). The presence of esophageal glands has been documented for many amphibian genera (Swiecicki, 1876; Langley, 1881; Kingsbury, 1894; Bensley, 1900; Biedermann, 1911; Herter, 1941; Eksaeva, 1958; Reeder, 1964). These glands have been shown to be protein secreting or peptic glands. Some of them have also been reported to secrete mucus (Reeder, 1964). These glands were believed by Kingsbury (1894) to be homologous with gastric glands and concentrated in the posterior portion of the esophagus. In the present investigation also glands were observed in the posterior portion of the esophagus containing two types of secretory cells. On the other hand absence of peptic glands in the esophagus has been reported in urodeles, Siredon and Salamandra and in anurans, Cystignathus, Bombinator and Pipa (Pernkopf and Lehner, 1937). The presence of columnar cells and goblet cells in the mucosal epithelium is also reported in frog (Norris, 1959) and toad (Loo and Wong, 1975). In the present investigation also columnar epithelium and two types of goblet cells were identified in the mucosal epithelium of esophagus in E.systema. The submucosa, muscularis and serosa were as in the other amphibians reported by Patt and Patt (1969).

Histological as well as histochemical results revealed the absence of sexual dimorphism in the esophagus of this frog. The existing literature also shows that there is no sex

dimorphism at histological level or histochemical level in esophagus of the other vertebrates. The sex dimorphism observed in the duodenum of the mammals will be discussed later.

The mucosubstances observed in the columnar epithelial cells, goblet cells, esophageal glands, connective tissue in the submucosa, muscularis and serosa of the esophagus of the frog investigated show mostly the same tinctorial affinities to those exhibited by identical mucosubstances in other organ-systems of the vertebrates in general. In their PAS reactivities, modifications in the PAS reactivities by phenylhydrazine and diastase, alcianophilia, C.I. reactivity, metachromasia, sequential staining techniques, modifications in alcianophilia by methylation, saponification, acid hydrolysis and enzyme digestion tests they resembled to similar mucosubstances in other organs. No special type of mucosubstances such as sulfated sialomucins, KOH-labile mucosubstances, high pH alcianophilic mucosubstances, azurophilic but non-alcianophilic and alcianophilic but non-azurophilic mucosubstances were found in the various esophageal sites of this frog.

Another interesting point of discussion concerns with the distribution of the mucosubstances in the various tissues and cell types in the esophagus of the frog investigated. The histochemical results revealed the presence of neutral mucosubstances in the columnar epithelial cells, neutral mucosubstances, sulfomucins and sialomucins in type-I goblet cells and type-I cells in glands, neutral mucosubstances and sialomucins in type-II goblet cells and type-II cells in the glands, neutral mucosubstances and hyaluronic acid in the submucosal connective tissue,

glycogen in muscle layers and neutral mucosubstances in the serosa.

In similar studies Norris (1959) reported on PAS positive goblet cells and glands in the esophagus of R. pipiens. Loo and Wong (1975) identified mainly sialomucins, small amount of sulfated mucins and neutral mucins in esophageal goblet cells and granules in the columnar epithelial cells in esophagus of the toad. The present investigation revealed two types of cells in the esophageal epithelium and glands.

The presence of hyaluronic acid in the connective tissue is now well established (Meyer, 1947; Wislocki et al., 1947; Duran - Renals, 1958; Jackson, 1964; Mathews, 1967; Nalavade, 1975; Gaikwad, 1981). The presence of neutral mucosubstances has been shown in the connective tissue of the several vertebrate tongues (Nalavade, 1975; Gaikwad, 1981). In the present investigation also hyaluronic acid and neutral mucosubstances have been identified in the connective tissue of the esophagus of the frog.

The presence of glycogen in the muscles is also well established (Nalavade, 1975; Nalavade and Varute, 1973 b; Gaikwad, 1981). The present histochemical results also revealed the presence of glycogen in esophagus muscularis of the frog. The importance of glycogen as an energy source for muscle contraction has been known for many years (Parnas and Wagner, 1914; Meier and Meyerhof, 1924). Numerous reports are found in the literature concerning concentrations of muscle glycogen as influenced by various nutritional, hormonal and other physiological factors (Stetten and Stetten, 1960).

The serosa of the esophagus of frog investigated contained only diastase resistant PAS reactive neutral mucosubstances.

The next interesting aspect is to view comparatively the mucosubstances in the esophagus of the frog under present investigation and the mucosubstances reported in the esophagus of the other vertebrates to find out similarities or differences if any, and to draw a common vertebrate plan of distribution of mucosubstances in the vertebrate esophagus in general.

Pope et al. (1975) reported that the esophagus is a much neglected part of the gastrointestinal tract. Bullock (1967) and Bucke (1971) demonstrated PAS and AB reactive mucous cells in the esophagus of the fishes. Recently Reifel and Travill (1977) described six types of mucous cells in the esophagi of ten teleost fishes, of which only two of them were found in each fish. They reported the presence of sialidase resistant sialomucins and some sulfomucins in type-A cells and neutral mucosubstances with some sialomucins in type-B cells in esophagus of A. rupestris, L. macrochirus, M. salmoides, and P. nigromaculatus. Type-A cells contained sialomucins and type-B cells a mixture of sulfomucins and sialomucins in esophagi of N. crysoleucas and P. promelas. They identified sulfomucins and some sialomucins in type-C cells and only neutral mucosubstances in type-D cells in esophagi of E. americanus and E. lucius. Similar mucosubstances were identified in two types of mucous cells in I. nebulosus but they named the cells as E and F respectively. The type-A cells contained sialidase resistant sialomucins and some sulfomucins and only sialomucins in type-B cells in esophagus of P. flavescens. Chan (1941) suggested that such a presence of two or more carbohydrates

in an epithelium could indicate two levels of maturation in the formation of mucous secretion.

Among the other vertebrates Varute and Nalavade (1973) demonstrated neutral mucosubstances, sulfomucins and sialomucins in mucosal goblet cells in esophagus of garden lizard, wall lizard and skink and neutral mucosubstances and protein masked sialomucins in columnar epithelial cells in esophagus of wall lizard. All these three lizard species consisted of two types of esophageal glands, peptic glands elaborated only proteins (probably enzymes) and mucous glands which elaborated only neutral mucosubstances.

In majority of the birds, esophageal glands produce mainly carboxylated mucosubstances (Grossi and Millo, 1967; Allenspach and Berlin, 1971). On the other hand Van Alten and Kennell (1957) found sulfomucins in esophageal glands of the birds during histogenesis.

There are few reports on the presence of glycogen in esophagus of the man and monkey (Wislocki et al., 1951; Rywlin and Ortega, 1970; Themann et al., 1971; Hopwood et al., 1977 a). A glycoprotein containing sialic acid has been reported between esophageal cells in rat (Rambourg, 1969). Hopwood et al. (1977 b) demonstrated mucosubstances predominantly neutral one in the cell coat and membrane coating vesicles in normal human esophagus. The presence of glycogen, neutral polysaccharides and sialic acid has been reported in the esophageal glands of armadillo (Carvalho et al., 1968). Masuda et al. (1977) showed the presence of PAS and AB reactive cells in the esophagus of man and rabbit.

The presence of sulfomucins has been reported in the middle layer of esophageal epithelium (Bescol-Liversac and Guillam, 1972) and esophageal glands (Lambert et al., 1971). Al Yassin and Toner (1977) reported that the glands in human esophagus are comparable with the minor salivary glands. Sekino and Murata (1978) studied human esophageal acid glycosaminoglycans by electrophoresis and enzyme assays. They identified hyaluronic acid, dermatan sulfate, heparin sulfate, chondroitin -4- sulfate and chondroitin -6- sulfate, all of these increased with advancing age.

The present investigation indicates that in this frog at least epithelial and glandular mucosubstances are secreted to form the esophageal mucus consisting neutral mucosubstances, sulfomucins and sialomucins. These mucins may protect the esophageal mucosa and may act as lubricants. Certain circumstantial evidences also support this conclusion. Hopwood et al. (1977 a) after studying the glycogen in the human esophagus reported that the marked amount of this non-sulfated mucosubstance between most of the esophageal cells suggests that this, and possibly phospholipids may play a role in protecting the epithelium against luminal contents. Varúte and Nalavade (1973) suggested that esophageal mucosubstances may be acting as lubricants during transport of the prey through the esophagus. In this regard the sulfomucins are known to function as biological lubricants as reported by Goudsmit (1972) and Lison (1960). Such mucosubstances may also aid in the protection of the lower part of the esophagus being digested by the gastric juice as suggested by Guyton (1964). Possibly the mucosubstances observed in the present investigation may be playing the role in protection of the esophageal mucosa



and as lubricants. This is only a suggestion and it should be confirmed further by experimental studies.

## 2) Cardiac stomach

It is suggested by Barrington (1942) that the original function of the stomach was that of storage, thus permitting active feeding during brief periods of activity or optimal thermal condition, and subsequent more leisurely but continuous processing of the stored food. Since such aggregations of dead food would tend to undergo bacterial disintegration, with production of toxic by-products, hydrochloric acid secretion may have evolved early as an inhibitor of such bacterial activity (Jordon, 1929). In addition, the presence of stomach would hasten the death of the prey taken alive (Dorris, 1935) would decalcify ingested skeletal materials.

In the present investigation the stomach was distinguished into cardiac stomach and pyloric stomach. The cardiac stomach consisted of a strongly glandular mucosa, a thin submucosa, a well organized muscularis and a typical serosa. The lining of the cardiac stomach consisted of goblet cells and columnar epithelial cells. The chief glands or gastric glands contained only one type of cells in the deeper region (the so called oxyntic cells) and mucous neck cells near gastric pit. In the proximal part (near esophagus) few mucous glands were also seen.

In amphibian stomach, the presence of goblet cells has been reported earlier (Tschassownikov, 1927; Norris, 1959; Loo and Wong, 1975; Leeson, 1975). The presence of columnar cells in the amphibian stomach is reported by Loo and Wong (1975). The chief glands are present in the corpus which comprise 70% to 80% of the

stomach in Bufo and Necturus (Kingsbury, 1894; Barrington, 1942); those near the esophagus are described as transitional between the esophageal glands and mucoid glands of the pyloric stomach. The characteristic branched tubular glands consist of finely granular basal zymogen cells, followed by approaching the lumen by large mucous neck cells (Lim, 1922; Barrington, 1946). The basal finely granular zymogen cells in Rana appear to be responsible for secretion of both small amounts of pepsinogen and hydrochloric acid. Langley (1881) suggested that the mucous neck cell is a homologue of the pyloric mucogenic cell. Sedar (1961) has described the ultrastructure of the oxyntic cells in gastric glands of the frog, R. catesbiana. The submucosa, muscularis and serosa are described in amphibian stomach by Grützner (1901), Kuntz (1924) and Patt and Patt (1969).

As in the esophagus the histological and histochemical studies on cardiac stomach of the frog investigated; indicated the absence of sexual dimorphism. In the existing literature also there are no reports on sex dimorphism in amphibian stomach.

The mucosubstances in different cellular sites from mucosa to the serosa exhibited staining reactivities identical to neutral mucosubstances, glycogen, sialomucins, sulfomucins and hyaluronic acid containing other tissues or organ-systems. In the cardiac stomach of E. systoma also there is no indication of the presence of any atypical mucosubstances.

With regard to the distribution of mucosubstances the present investigation revealed the presence of neutral mucosubstances in columnar epithelial cells, mucous neck cells and serosa of the cardiac stomach. The muscularis contain only glycogen. The goblet



cells in the surface epithelium of the mucosa contain a mixture of neutral mucosubstances, sulfomucins and sialomucins, whereas the mucous glands contain predominantly neutral mucosubstances and traces of sulfomucins. The cells in the gastric glands (oxyntic cells) contain no mucosubstances. The presence of neutral mucosubstances and hyaluronic acid was identified in the connective tissue.

In similar histochemical studies Norris (1959) demonstrated PAS reactivity in the surface epithelium, foveolar epithelium and mucous neck cells in stomach of R. pipiens. Loo and Wong (1975) reported on the presence of neutral mucosubstances in the columnar cells and neutral mucosubstances and traces of acidic mucosubstances in the mucous neck cells in the stomach of B. melanostictus. Forte and Forte (1970) studied carbohydrates in acid secreting cells of the frog stomach. They reported that all the cell fractions were extremely low in acidic sugars, uronic acids, sialic acids but neutral sugars and hexosamine were predominant. Beesley and Forte (1973) also obtained exceptionally high quantity of carbohydrates (260  $\mu$ g/mg protein) from membranes, and about 73 % of the sugars were found to be associated with proteins.

The presence of neutral mucosubstances and hyaluronic acid in the connective tissue, glycogen in muscles and neutral mucosubstances in the serosa of the other organ-systems and tissues has already been discussed in relation with these tissues in the esophagus.

Another interesting point of discussion is to compare the mucosubstances in the stomach of the E. systema and the stomach of other vertebrates to find out the similarities or dissimilarities

if any, and to draw the vertebrate plan of distribution of gastric mucosubstances.

Among the fishes, Bucke (1971) demonstrated PAS and AB reactive cells in the gastric mucosa. Kapoor et al. (1975) also described mucosubstance containing gastric epithelial cells in many teleosts. Recently Reifel and Travill (1978) studied mucosubstances in the stomach (corpus) of eight teleost fishes. They identified sialomucins in the surface epithelial cells in E.americanus and E.lucius but only neutral mucosubstances in A.rupestris, L.macrochirus, M.salmoides, P.nigromaculatus, E.nebulosus and P.flavescens. They further reported the presence of weakly acidic sulfomucins in A.rupestris, L.macrochirus, P.nigromaculatus; sialomucins in E.americanus and E.lucius and neutral mucosubstances in M.salmoides, E.nebulosus and P.flavescens in the gastric pit cells of the corpus.

Mogil'naya et al. (1978) demonstrated neutral carbohydrates and sialosaccharides in the gastric pavement epitheliocytes of the reptiles. Loo and Swan (1978) showed the presence of neutral mucins in the neck cells and both neutral and acidic mucins in the surface epithelial cells in stomach of the Australian lizard, E.cunninghami. Mogil'naya et al. (1978) also demonstrated the presence of neutral carbohydrates and sialosaccharide in gastric epitheliocytes of the birds.

To some extent detail studies have been carried out on mucosubstances of the mammalian stomach. Krause and Leeson (1974) described aglandular gastric mucosa in duckbill platypus and echidna, wherein the lining cells were stratified squamous type. Fruschelli (1967) demonstrated glycogen in gastric mucosal epithe-

lium in cat, dog and man but its absence in rat, rabbit, mouse, guinea pig and monkey. Recently Tsujimura (1976) also reported the presence of glycogen in surface epithelium of canine cardiac and fundic stomach.

Lambert et al. (1968) showed PAS and AB reactive mucosubstances in surface coat of gastric mucous cells of various animals including man. Sheahan and Jervis (1976) studied eleven species of mammals and reported that neutral mucosubstances are most evident in the stomach. Spicer et al. (1978) demonstrated neutral mucosubstances in the superficial epithelium and cells in foveolae of rat stomach. On the other hand Wattel et al. (1977) reported on acid carbohydrates in surface mucous cells in rat stomach. Spicer et al. (1967 a) considered the presence of non-sulfated acidic mucosubstances in mucosal area close to the lumen in dog stomach as the cells in this area failed to incorporate  $S^{35}O_4$ . Gerard et al. (1967) reported that surface epithelium in canine stomach produced glycoproteins.

Sinitsina (1968) demonstrated neutral and acidic mucins in the superficial epithelium in human stomach. Similar studies by Roy (1974) indicated the presence of neutral and acidic mucosubstances in the surface epithelial cells of pig stomach. Carvalho et al. (1974) identified proteins rich in  $\alpha$ -amino radicals, tryptophan and arginine and sialomucins in the gastric lining and pit epithelium of armadillo. Goldman and Ming (1968) in their studies on gastric mucosa identified the presence of sulfated and non-sulfated acid mucosubstances. Spicer and Sun (1966) reported the presence of carboxymucins (sialic acid rich) and sulfomucins in the gastric epithelium of dog. Sulfomucins have also been

reported in human and canine gastric epithelium by Tyrkko et al. (1968). Woussen - Colle et al. (1975) analyzed glycoproteins present in mucosa and secretion from fundus and antrum of the dog. They resembled to the blood group substances but differed in their fucose, sialic acid and sulfate content.

The mucous neck cells have been reported to exhibit PAS positive reaction in the stomach of armadillo (Carvalho et al., 1975) and pig (Roy, 1974). Tsujimura (1976) demonstrated glycogen in mucous neck cells in canine gastric glands. Other investigators have identified only neutral mucosubstances in the mucous neck cells in stomach of rat (Wattel et al., 1977; Spicer et al., 1978) and dog (Spicer and Sun, 1966; Spicer et al., 1967 a; Gerard et al., 1967). In the present investigation also neutral mucosubstances were identified in the mucous neck cells in frog stomach.

Tsujimura (1976) found absence of glycogen in chief and parietal cells of gastric glands in canine stomach. On the other hand Gerard et al. (1967) reported the presence of sulfomucins in chief cells, in canine stomach. The parietal cells have been reported to contain neutral mucosubstances in dog stomach (Gerard et al., 1967; Spicer et al., 1967 a). Spicer and Sun (1966) and Spicer et al. (1967 a) demonstrated sulfated mucosubstances in the zymogenic cells of gastric glands in dog. In the present investigation the single type of peptic cells (oxyntic cells) in the gastric glands of the frog contained no mucosubstances.

Biochemical assays (Werner, 1953; Schragar, 1964; Schragar and Oates, 1968; Gerard et al., 1967; Martin et al., 1968), immunological methods (Hakkinen et al., 1968) and autoradiographic studies (Dziewiatkowski, 1956; Jennings and Florey, 1956; Cornet et al.,

1964 a,b; Shimamoto et al., 1973) have also indicated the presence of sulfoglycoproteins in gastric juice and mucosa of several mammals. In the present investigation, the isolated gastric mucus has not been studied but the mucosubstances have been reported in the gastric secretion of several animals, the literature on which is reviewed in the introductory chapter (Chapter- one).

The aforementioned discussion points out that there are wide variations in the type of mucosubstances in different types of cells and species diversity also. Sheahan and Jervis(1976) also mentioned that "No information exists which explains why gastrointestinal mucosubstances are of various types, why the various types exist in specific topographic relationship to one another in specific site or why there is such a wide variation in the types seen in the same region in a variety of animal species."

Monceaux (1935) reported that mucin absorbs all acidity beyond the physiological pH. Kent (1971) opined that secreted mucus carries out an obvious lubricant function and may possibly play a role in the prevention of dehydration. There are several reports on importance of mucosubstances (particularly sulfomucins) in protection of the gastric mucosa against peptic activity or peptic digestion(Komarov, 1936; Levey and Sheinfeld,1954; Cook and Drill, 1967; Texter et al.,1967; Lambert et al.,1968; Martin et al.,1968; Mikuni and Hotta, 1979). The mucosubstances secreted in frog stomach may be also protecting the mucosa against peptic activity and HCl. This is only a suggestion and it should be provided by experimental studies.

### 3) Pyloric stomach

The pyloric stomach in the frog investigated was short and

narrow as compared to the cardiac stomach. The pyloric stomach of E.systema consisted of a strongly glandular mucosa, a thin sub-mucosa, a well organized muscularis and a typical serosa. The single layered epithelium consisted of mainly goblet cells. Norris(1959) also reported the presence of columnar mucous cells in the surface epithelium of the pyloric stomach of R.pipiens. The cells in the pyloric glands in E.systema resembled to those of the surface epithelial (goblet) cells except these cells were larger than the goblet cells in mucosal lining. Norris(1959) described that pyloric glands in the stomach of R.pipiens are lined exclusively by large rounded mucous cells as in esophageal or fundic glands. Reeder (1964) also described that characteristic pyloric glands in Rana extend 3 to 4 mm anterior from the duodenum and are entirely mucogenic.

As in esophagus and cardiac stomach, the histological and histochemical studies on pyloric stomach of E.systema revealed absence of sexual dimorphism.

The histochemical reactivities of the mucosubstances from mucosa to the serosa in pyloric stomach resembled to those exhibited by glycogen, neutral mucosubstances and hyaluronic acid, containing other organ-systems and tissues. The histochemical results indicated the absence of atypical mucosubstances in the pyloric stomach of the frog investigated.

Regarding the distribution of mucosubstances the present histochemical results revealed only neutral mucosubstances in the entire mucosal goblet cells, cells in the pyloric glands and serosa. The connective tissue in the submucosa contained neutral mucosubstances and hyaluronic acid, whereas the muscularis contained



glycogen. Similar histochemical studies by Norris (1959) also indicated only PAS reactive mucous cells and glands in the pyloric stomach of R. pipiens.

The next point of discussion is the comparison between the results obtained in the present investigation and those reported on pyloric stomach mucosubstances in other vertebrates. Reifel and Travill (1978) studied mucosubstances in pyloric epithelial cells in stomachs of eight species of teleosts. They identified in pyloric epithelial cells only neutral mucosubstances in M. salmoides, I. nebulosus and P. flavescens, sialomucins in E. lucius, sialidase resistant sialomucins in P. nigromaculatus and sialidase resistant sialomucins with small amount of weakly acidic sulfomucins in A. rupestris, L. macrochirus and E. americanus. Mogilnaya and Bogatyr (1977) reported the presence of neutral, sulfomucins and sialomucopolysaccharides in the mucus secretion of the avian gizzard.

Among the mammals, Carvalho et al. (1973) demonstrated sulfated and carboxyl containing mucopolysaccharides in the surface epithelium of pyloric stomach in armadillo, whereas Poddar and Jacob (1979) showed sialidase resistant and sialidase labile sialomucins in the superficial cells in pyloric stomach of ferret. On the other hand Birgele (1969) identified only neutral mucosubstances in pyloric stomach epithelium in abomasum of cow. The present investigation showed only neutral mucosubstances in epithelial goblet cells of the frog.

Poddar and Jacob (1979) identified only neutral mucosubstances in pyloric glands of ferret. Birgele (1969) demonstrated neutral mucosubstances and acidic mucosubstances in the pyloric

glands of cow and Carvalho et al. (1973) showed neutral polysaccharides and carboxymucins in pyloric glands of armadillo. Recently Tsujimura (1976) demonstrated glycogen (small amount), neutral mucosubstances (predominant) and traces of sulfo and sialomucins in the pyloric glands of dog. Thus in general it appears that the neutral mucosubstances are produced in the pyloric stomach of various vertebrates. Neutral mucosubstances may act as buffer against the cardiac acidic chyme. Mogilnaya et al. (1978) suggested that in amphibians, the protection of mucosa is connected with the presence of neutral mucosubstances.

#### 4) Duodenum

The part of the small intestine which immediately follows stomach and receives the ducts from liver and pancreas is called as duodenum. The duodenum in the E. systoma was circular in outline. The mucosa was thrown into numerous finger-like projections lined by single layered epithelium. The epithelial cells were identified as columnar epithelial cells and goblet cells. The duodenal or Brunner's glands were found absent in this frog. The submucosa, muscularis and serosa layers were thin.

As in the previous organs studied, the histological and histochemical results on duodenum of the frog investigated showed absence of sexual dimorphism. In this connection it is interesting to note that Shackleford and Wilborn (1978) described sex dimorphism in duodenal glands of hamsters. They reported that the glands in males contained double the amount of acidic mucosubstances than the females and PAS reactivity was stronger in the duodenal glands of the females than the males.

The distribution of the mucosubstances in the various

cellular sites and layers in the duodenum of E. systoma slightly differed from that of the stomach. The columnar epithelial cells which were not observed in the pyloric stomach can be seen in this region. The columnar epithelial cells contained only neutral mucosubstances. The mucosal goblet cells contained sulfomucins (predominant) and sialomucins (traces). The connective tissue in the submucosa contain neutral mucosubstances and hyaluronic acid as in the previous organs but in addition contained sulfomucins. The sulfomucins have also been reported in the connective tissue of the other organs (Jackson, 1964, Thompson, 1966). Mancini (1955) and Mancini et al. (1956) confirmed the presence of sulfomucins in the connective tissue by autoradiography. Most of the literature on connective tissue mucosubstances has been recently reviewed by Nalavade (1975). The muscle layers of the duodenum contained glycogen, whereas the serosa contained neutral mucosubstances.

Mucosubstances have mainly been studied in the duodenum of the mammals. The literature on this aspect is already reviewed in the introductory chapter (chapter - one). The duodenal or the Brunner's glands were found to be absent in E. systoma, but the mucosubstances in the Brunner's glands have been described in chapter - one and by Krause (1973). Therefore the present comparative discussion is restricted to the duodenal epithelial mucosubstance.

More and Bayle (1972) identified sulfomucins in the duodenal caliciform cells of the rabbit. Carvalho et al. (1972) demonstrated only sialomucins in the duodenal goblet cells of the goat. Silva et al. (1973) reported the presence of neutral mucopolysaccharides and sulfated polysaccharides in the mucosal

goblet cells in the duodenum of the cat. Uspenskii (1972) demonstrated sialomucins and sulfomucins in the duodenal goblet cells. On the other hand Poddar and Jacob (1979) reported variations in staining reactivities in goblet cells of the duodenum in ferret. The majority of the surface epithelial cells and some deep cells contain sialomucins, whereas few superficial cells and occasional deep cells contain sulfated mucosubstances. Sinitsina (1966) demonstrated neutral mucosubstances, carboxymucins, sialomucins and sulfomucins in human duodenal goblet cells. Hoskins and Zamcheck (1963) identified only sialomucins in human duodenal mucosa.

The present investigation showed the presence of neutral mucosubstances (columnar cells in origin) and sulfomucins and sialomucins (goblet cells in origin) in the duodenum of the E. systoma. These mucosubstances may be protecting the mucosa of the duodenum. More and Bayle (1972) considered that the duodenal mucins may act as buffer against the gastric acidic chyme. Uspenskii (1972) found that in subjects with chronic increase of gastric acidity, the morphological changes in the duodenal mucosa are accompanied by increase of more active sialomucins and sulfomucins in the goblet cell mucin.

##### 5) Small intestine

The small intestine of E. systoma was thin walled and circular in cross section. The villi were more numerous than the duodenum. The single layered mucosa contained columnar epithelial cells and goblet cells. Herter (1941) also described in other amphibians that the epithelial surface itself is formed primarily of columnar cells with striated border, richly interspersed

with mucoid goblet cells. Presence of columnar epithelial cells and goblet cells in the epithelium has also been reported in toads (Loo and Wong, 1975; Mc Avoy and Dixon, 1978). The glands and crypts were found to be absent in the intestine of the frog investigated. Biederman (1911) and vonk (1941) also described that elaborate multicellular digestive glands appear lacking in most amphibians, but crypts of Lieberkuhn have been described in Salamandra and certain other urodeles. The submucosa, muscularis and serosa were as in the other amphibians reported by Reeder (1964) and Patt and Patt (1969).

Histological and histochemical observations on the small intestine of E.systema revealed the absence of sexual dimorphism.

The mucosubstances in different cellular sites from mucosa to the serosa exhibited tinctorial affinities identical to the similar mucosubstances in other tissues or organ-systems. In the small intestine of this frog also there is no indication of the presence of any atypical mucosubstances.

The present histochemical studies also show the heterogeneous distribution of mucosubstances in the various cell types and layers in the small intestine of the frog. The results obtained indicate the presence of neutral mucosubstances in the columnar epithelial cells, sulfomucins (predominant) and sialomucins (traces) in the goblet cells, neutral mucosubstances, sulfomucins and hyaluronic acid in the connective tissue, glycogen in muscularis and neutral mucosubstances in the serosa. In similar studies Loo and Wong (1975) demonstrated sulfated mucins in the goblet cells and in granules of columnar epithelial cells in intestine of the toad.

The next point of discussion is the comparison of the mucosubstances in the intestine of the frog investigated and the intestinal mucosubstances in the other vertebrates. Bucke (1971) found PAS and AB reactive cells in the intestinal epithelium of the fish E. lucius. Reifel and Travill (1979) studied the intestinal mucosubstances in ten teleost fishes. Their results indicated the presence of sialidase resistant sialomucins and some weakly acidic sulfomucins in the intestinal mucous cells in A. rupestris, L. macrochirus, M. salmoides, P. nigromaculatus, N. crysoleucas, P. promelas, I. nebulosus and P. flavescens. The mucous cells in E. americanus contained only sialomucins, whereas those in E. lucius contained sialomucins and neutral mucosubstances. The presence of acidic mucoproteins has been reported in the goblet cells of the ileum of two Egyptian lizards (Anwar and Mohmoud, 1975). On the other hand Loo and Swan (1978) identified sulfated mucins in the intestine of one Australian lizard.

Among the mammals, the intestinal goblet cells have been reported to elaborate and secrete only neutral mucosubstances in sheep and cattle (Skorodinskii et al., 1970) and man (Subbuswamy, 1971), a mixture of neutral mucosubstances and sialomucins in man (Filipe and Fenger, 1979), sulfomucins and sialomucins in ferret (Poddar and Jacob, 1979) and a mixture of neutral and acidic (sulfated) mucopolysaccharides in several vertebrates (Kim, 1972). Lev and Spicer (1965) also identified PAS reactive sialomucins and sulfated mucins in the human intestinal epithelial mucins. Bescol-Liversac and Guillam (1972) confirmed the sulfo-mucin synthesis in intestinal calciform cells by autoradio-

graphic studies.

Hauri et al. (1977) demonstrated the incorporation of C<sup>14</sup> - glucosamine in brush border glycoproteins in human intestinal mucosa. Chromatographic studies by Srinivas et al. (1971) revealed the presence of equimolar proportion of uronic acid, hexosamine and sulfate (25 %) in mucosal scrapings of pig intestine. After isolation of mucins from pig intestine by chromatography, Degand et al. (1972) estimated 16 % sialomucins and 78 % sulfomucins. The present histochemical results also indicated sulfomucins (predominant) and sialomucins (traces) in the mucosal goblet cells of intestine in the frog.

These intestinal epithelial mucins may protect the intestinal mucosa. This conclusion is based on some circumstantial evidences. Mitjavila et al. (1968) consider that mucins in rat intestine may fix tannin present in the medium and form an insoluble complex. This shows a protective barrier function against the toxic effect of tannin. Fox (1979) reported that mucus close to the cell surface in gastrointestinal tract may block the binding of lectins or attachment of the micro-organisms to the membrane receptors. Mnatsakayan (1967) demonstrated that glycoproteins slightly increased in rat intestine when milk is given but greatly increased (particularly sialomucins) when bread or meat is given. These observations indicated that the intestinal glycoproteins have protective function when proteolytic enzymes are active and possibly when proteolytic enzymes of micro-organisms are acting.

#### 6) Large intestine

Large intestine in E. systoma is wider in the diameter

than the small intestine. Reeder (1964) also described that the hindgut in amphibians is usually differentiated from the small intestine by abruptly increased cross section. In E. systoma, histologically this part showed lack of high slender folds, instead occasionally very short and low folds can be seen. The mucosa consisted of abundant goblet cells and very few columnar epithelial cells. Reeder (1964) also described that mucosal lining contained columnar epithelium with interspersed goblet cells, which become more densely distributed posteriorly. Loo and Wong (1975) observed columnar epithelial cells and goblet cells in the cloaca of the toad. The submucosa, muscularis and serosa in large intestine of E. systoma are similar to that in other amphibians as described by Patt and Patt (1969).

Histological and histochemical observations on the large intestine of E. systoma also indicated the absence of sex dimorphism.

The mucosubstances in the various histological sites of the large intestine resembled in their histochemical reactivities to those exhibited by identical mucosubstances in other organ systems. In the large intestine of this frog also there is no indication of the presence of atypical mucosubstances.

A heterogenous distribution of the mucosubstances is evident in the large intestine of this frog. The results obtained in the present investigation revealed the presence of neutral mucosubstances in the columnar epithelial cells, goblet cells, connective tissue and serosa, sulfomucins only in the goblet cells, hyaluronic acid in the connective tissue and glycogen only in the muscularis. Loo and Wong (1975) also identified histochemically



sulfomucins and small amount of neutral mucins in the goblet cells and granules in the columnar epithelial cells of the cloaca of the toad.

Scanty literature on mucosubstances in large intestine of other vertebrates also shows some similarities in their distribution. Reifel and Travill (1979) studied mucosubstances in the rectal or distal intestine of ten teleost fishes. Their histochemical results indicated the presence of sialidase resistant sialomucins and weakly acidic sulfomucins in the mucous cells of A. rupestris, L. macrochirus, M. salmoides, P. nigromaculatus, N. crysoleucas, P. promelas, E. americanus and P. flavescens, only neutral mucosubstances in E. lucius and only sulfomucins in I. nebulosus. Loo and Swan (1978) identified only sulfomucins in the mucosal epithelium of the cloaca of the Australian lizard. Subbuswamy (1971) reported the presence of acidic mucins in the goblet cells of human large intestine. Masuda et al. (1977) showed PAS and AB reactive cells in the mucosa of large intestine in rabbit and man. Kim (1972) demonstrated neutral and sulfated mucopolysaccharides in the goblet cells of large intestine in several vertebrates.

The large intestine participates in no principal digestive function, although it is possible here the bacterial breakdown of some inaccessible compounds such as cellulose. Some products of this hydrolysis may be absorbed here. Water and salts are absorbed in the large intestine (Reeder, 1964). The secreted mucus here may have lubricating function and may possibly a role in the prevention of dehydration.

