CHAPTER ONE

INTRODUCTION

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A critical survey of the existing literature clearly pointed out that the mucosubstances have been studied in several organ systems, such as alimentary tract, respiratory system, reproductive system along with non-gonadal organs in the reproductive system, salivary glands, lingual glands, kidney, muscle of the normal animals, and in some cases, under experimental conditions. However, scanty information is available on the mucosubstances in the gall bladder of vertebrates.

Harding (1931) has brought to the notice that "the description of the epithelium of the gall bladder given in standard textbooks of histology are woefully scanty, slightly inaccurate and sometimes, notably untrue". Histological observations have been carried out on the gall bladder of mammals like man, cat, dog, pig, guinea pig, mice, rabbit, and of birds like fowl and quail, but variations have been reported regarding the ultrastructure. Johnson et al. (1962), therefore, pointed out that very little attention has been paid to the ultrastructure of this organ.

The tenth edition of Schafer (1916) contains a drawing taken from Sommer (1909), showing the granules of mucus in the eplithelial cells of gall bladder but does not mention it in the text. However, Jordan (1927), Mann (1928) and Cajal and Raman (1933) have not mentioned the presence of any mucus granules in the cells of gall bladder. However, very good description

is given by Pfuhl (1932) in van Mollendarf's expensive textbook, but even this account contains several minor inaccuracies. This appears to be true at present also.

Though some of the earlier workers have demonstrated the existence of the mucopolysaccharides in the cells of gall bladder of vertebrates the problem dealing with its occurrence in the gall bladder of vertebrates is, at present, most disputable due to various techniques and to individual interpretation of the results obtained. While dealing with the problem of glycogen and the nature of acid mucopolysaccharides in the epithelium of the gall bladder mucosa under non-experimental conditions, Grzycki and Rzeszowska (1968) have mentioned that the morphology and physiology of the gall bladder epithelium has been extensively studied.

The problem regarding the presence or absence of goblet cells in the surface epithelium and the glands is not settled yet. The goblet cells and glands have been reported by some of the investigators in some animals while some have denied the presence of the goblet cells in the same animals. Increased number of goblet cells in the surface epithelium and the glands along their secretions under pathological conditions have been reported by some of the workers. On the other hand, Dyban (1970) reported that the goblet cells and the mucous glands do not occur in the gall bladder as its constituent parts, but these can be induced to be developed under some experimental conditions such as injections of dimethyl-1-polysiloxane in guinea-pig.

The gall bladder epithelium have been studied by some workers during the embryonic development of some animals. Schiebler et al. (1975) studied the histology as well as the nature of mucosubstances in the epithelial cells during the embryonic development in guinea pig.

The abovementioned views by several workers stimulated to undertake the present investigation **"Studies on Mucosubstance in Gall Bladder of some Birds and Mammals"** to study the histology of gall bladder in these vertebrates to find out presence or absence of goblet cells and glands, the nature of mucosubstances in the epithelial cells, goblet cells and glands of gall bladder in these vertebrates in relation with their dietary habits as well as to compare the histology and nature of mucosubstances in the developing and adult condition of some mammals. The following is a brief review on the gall bladder of birds and mammals with regard to the aforementioned points.

I) Review of the Existing Literature on Gall Bladder of Aves and Mammals:

i. Aves:

A critical survey of existing literature pointed out that little attention has been paid to the gall bladder of the submammalian vertebrates. Among the submammalian vertebrates, the aveian gall bladder is the most neglected organ. The anatomical observation by Bader (1965) on fowl and by Yamada (1970) on quail gall bladder reported the presence of epithelial cells. Yamada and Hoshino (1972) studied the histology of gall bladder and nature of mucosubstances

by employing a series of histochemical techniques in the gall bladder epithelial cells of fowl, Gallus domesticus and reported the presence of sulfated, carboxylated and neutral mucopolysaccharide-protein complexes. Recently, Patil (1985) studied the histology and nature of mucosubstances in different histological sites of gall bladder of seven birds and pointed out that there exists no relation with the nature of mucosubstances and the dietary habits of the birds.

Okada (1951) reported that the gall bladder epithelium in chick becomes high columnar from stratified to pseudostratified epithelium during the period of development.

Noble and Cannor (1984) studied lipid and fatty acid composition of gall bladder bile during the embryonic period of chick in the last week of incubation. Their studies indicated that there was a substantial proportion of cholesterol ester and high level of oleic acid and less than 50% of phospholipids of the total lipids in the bile during this period of development. Phospholipid contained a high level of arachidonic acid. This study showed considerable metabolism in chick embryo during last week of incubation.

ii. Mammals:

Existing literature available on mammalian gall bladder states that these have been studied from several points of views, such as histology, ultrastructure and metabolites, such as enzymes, lipids and mucosubstances. Mostly, these metabolites have been studied by histochemical methods; however, in some cases,

autoradiography, bioassay and electrophoretic separation methods have also been employed. Some workers have detected the muco-substances in bile in healthy persons as well as patients during various pathological states, cancer and during gallstone formation. The following is the brief summary of the existing literature on gall bladder of mammals.

The gall bladder epithelium has been studied extensively by a number of workers in several mammals from different groups by using both light and electron microscopes. However, all these studies revealed a little difference in the structure of epithelium, particularly in the presence or absence of cuticular or brush border. In relation with this, the histological observations have been carried out on the gall bladder of mice, pig and rabbit (Cabibbe, 1901, 1902); guinea pig (Nagahiro, 1938; Ischikawa, 1950; Togari and Okada, 1953); pig (Ott, 1937); dog (Cabibbe, 1901, 1902; Patil, 1985); cat (Varute and Nalavade, 1973); Monkey (Ralph, 1950; Patil, 1985); and man (steiner, 1892; Aschoff, 1905; Ralph, 1950 and Patil, 1985). Harding (1931) described that the mucosa of the gall bladder has a single layer of large columnar or prismatic cells thrown into folds of varying height according to the degree of distention of this organ. Chowdhari and Jain (1970) found few solitary cells in the epithelium of gall bladder of rabbit. Harding (1931) found no special difference in the cells of gall bladder in man, cat, dog, goat, sheep, pig and guinea pig. Recently, histology of gall bladder in man suffering from cholesterosis have been worked out under light as well as electron microscope by Koga (1989).

The study of Yamada (1962-a) on gall bladder of laboratory rodents indicated two peculiar epithelial cell types. Varute and Nalavade (1973) found special type of goblet cells interspersed in the columnar epithelial cells in gall bladder of cat. The goblet cells have also been reported by Patil (1985) in the gall bladder of goat. Nalavade et al. (1974) observed single layer of epithelial cells in the gall bladder of bat, but the presence of glands in the lamina propria just below the mucosa.

Caldwell et al. (1969) observed only three layers in the gall bladder of sea cow, Dugong dugong, viz. epithelium with lamina propria, fibromuscular layer and a perimuscular layer. They further reported the presence of goblet cells between the columnar cells and also the tubulo-alveolar glands below the mucosa.

Though the gall bladders of mammals have been studied extensively in relation with the presence or absence of goblet cells, there is no common agreement regarding this view. The goblet cells have been reported in the gall bladder epithelium of rat and rabbit (Jennings, 1958); cattle (Bruveris, 1969); sea cow (Caldwell et al. 1969); guinea pig (Ischikawa, 1950; Jennings, 1958); pig (Ott, 1937); goat (Patil, 1985); and man (Aschoff, 1909; Aschoff and Bacmeister, 1909; Ito and Nagahiro, 1941). On the other hand, the presence of goblet cells has been denied in the gall bladder of mice (Mori, 1938); cat (Seelinger, 1937); dog (Policard, 1914; Seelinger, 1937; Patil, 1985); buffalo, musk shrew, squirrel, goat, monkey (Patil, 1985) and man (Sommer, 1909; Policard, 1914; Halpert, 1927; Pfuhl, 1932; and Patil, 1985). Yamada (1959) found absence of goblet cells but the presence of scattered

cask-like cells and crayon-like cells in the gall bladder of hamster. On the other hand, transforming goblet cells from the epithelial cells or special type of goblet cells have been reported in gall bladder epithelium of sheep, guinea pig (Tusques et al., 1964); rabbit (Weinstock and Bonneville, 1971) and cat (Varute and Nalavade, 1973).

The problem concerning the presence or absence of glands in the gall bladder is not yet solved properly as there exists a controversy regarding this problem. Absence of glands is reported in the gall bladder of guinea pig (Tusque et al., 1964); cattle (Bruveris, 1969); rabbit (Weinstock and Bonneville, 1971); cat (Varute and Nalavade, 1973); and in frugivorus bat, Pteropus giganteous gitanteus; insectivorous, bat Pipistrellus, mimus mimus; musk shrew; squirrel; dog and man (Patil, 1985). However, glands have been reported in the gall bladder of man (Gompper, 1951), sheep (Tusques et al., 1964), , sea cow (Caldwell et al., 1969); bat Cynopterus sphinx (Nalavade et al., 1974); guinea pig (Geleft and Bock, 1985) and in goat, sheep buffalo, bats (Rhinolophus luctus), bedommei, Tadarida aegyptiaca and monkey, Macaca radiata (Patil, 1985).

One of the problems regarding the absorptive and/or secretory nature of the gall bladder epithelial cells deserve further attention. Jordan (1927), Mann (1928) and Cajal (1933) have mentioned absence of any mucous granules in the gall bladder epithelial cells. Similarly, Maximove (1931) stated definitely that the gall bladder does not secrete mucus. However, Harding (1931), while studying the secretion of mucus by the gall bladder epithelial cells in cat, dog, guinea pig, goat and sheep, mentioned that the description

of the epithelium of the gall bladder in standard textbooks is woefully scanty, in nearly all instances, slightly inaccurate, and sometimes, notably untrue. Recently, definite secretion of mucus by gall bladder epithelial cells have been reported by Varute and Nalavade (1973) in cat, Nalavade et al. (1974) in bat C.sphinx, Moori et al. (1984) in guinea pig, Patil (1985) in sheep, goat, buffalo and dog and by Geleff and Bock (1985) in guinea pig.

Wolf-Heiddegger et al. (1965) studied the ultra-structure of the gall bladder in cat and man. They are of the view that the mucosa of the gall bladder is active in secretory as well as absorptive capacities. However, to investigate morphologically whether the epithelium of the gall bladder has secretory or absorptive function is one of the most important problem. A number of authors are of the opinion that the gall bladder epithelium performs the only function of absorption and no secretory function. Mori (1938) in mice and Togari and Okada (1951) in rabbit; while, according to Ischikawa (1950) in guinea pig; Togari and Okada (1951) in man; and Yamada (1959) in hamster; the gall bladder epithelial cells have dual function of absorption and secretion. Furthermore, the view that the gall bladder epithelial cells perform the dual function of absorption and secretion is supported by Weinstock and Bonneville (1971) in rabbit and Patil (1985) in a number of mammals.

According to Hayward (1962), the apical cytcplasm of epithelial cells in gall bladder contains vesicles of two distinct types and they have been noted in the gall bladder of mouse. He further noted that the smaller granules may be formed by the pino-cytosis at the basis of microvilli. Blaisdell and Chandler (1927) and

Nylander (1961) demonstrated histochemically the role played by gall bladder epithelial cells in dog and carnivores in concentrating the bile.

The mode of secretion in the gall bladder epithelial cells in man and higher vertebrates is supposed to be eccrine (Shikinami, 1908; Nagahiro, 1938; Togari and Okada, 1953). On the other hand, some have believed that the mode of secretion is apocrine-type as in man, cat and other mammals (Jurisch, 1909) and in cat (Mathis, 1927).

Mustakallio (1954) examined the succinic dehydrogenase activity in the gall bladder of fasting-pregnant and fed-pregnant mice as well as the controlled mice. The lactic dehydrogenase activity in the gall bladder of normal human and hospital patients have been studied by Hsien and Bluementhal (1956). Turchini et al. (1960) reported decreased cholinesterase and lipase activities and unaltered glucose-6-phosphatase activity in the wall of gall bladder of Rhinolophus hipposiderus during hibernation. Madec (1965) analysed glucose-6-phosphatase, dehydrogenase, glutathione reductase, lactate dehydrogenase, fructose-1-6-diphosphate aldolase, piruvate kinase and catase in the extract of the sheep gall bladder. Similarly, Na^+, K^+-dependent Mg^{++}-dependent, and in some cases, ACO_3^--dependent ATPase enzymes activities have been reported in gall bladder of rabbit (Van Os and Slegers, 1970; Yaremenko and Kharlamova, 1978) and pig (Kharlamova, 1977). By employing Gomori's method No and Park (1971) demonstrated alkaline phosphatase and acid in the gall bladder mucosa and bile duct in dog, cat, rabbit, guinea pig and human fetus. Kyosola (1977) demonstrated acetyl cholinesterase activity in the

human gall bladder mucosa, nerve fascides of closely packed dense and single nerve fibers and small ganglia. He further reported that cholinergic innervation may be related to secretion, absorption and contractility of the gall bladder. Bennet et al. (1981) examined the activity of glycosyl transferase in the gall bladder of adult mice after injecting the galactose and sialic acid in the lumen of the gall bladder. Recently, Kohara et al (1985) have detected enzyme activities in human gall bladder of normal, cholecystities and carcinomas. They detected succinic dehydrogenase, acid phosphatase, β-glucoronidase and non-specific esterases. They found similar enzyme activities in human gall bladder of normal, cholecystitis and carcinomas. They detected succinic dehydrogenase, acid phosphatase, β-glucoronidase and non-specific esterases. They found similar enzyme activities in normal gall bladder and in cholecystitis. In carcinoma of the gall bladder, the succinic dehydrogenase activity was suppressed, but augmented the activities of lactate dehydrogenase and acid phosphatase.

According to Mori (1938) the gall bladder in mouse is devoid of lipid. But since the contribution by Virchow (1857) numerous studies have appeared on epithelial lipid of gall bladder in various vertebrates (Aschoff, 1906; Shikinami, 1908; Jurisch, 1909; Aschoff and Bacmeister, 1909; Policard, 1914; Halpert, 1927; Kusnetzowsky, 1923; Naunyn, 1923; Torinoumi, 1924; Kataka, 1928; Pfuhl, 1932; Hsano, 1935; Nagahiro, 1938; Togari and Okada, 1953; Yamada, 1953; Wallraff and Dictrich, 1957). In these studies, however, attempts have been made to elucidate the chemical composition of the lipid, except the contribution by Wallraff and Dietrich (1957) in human gall bladder.

Zorzoli and Bidone (1954) detected characteristic chromolipide pigment in the smooth muscle fibres of gall bladders affected by cholesterosis. Yamada (1960-b) studied histochemically the lipids in the gall bladder epithelium of mouse by Sudan-staining and extraction procedures, which revealed neutral fats and phospholipids as major components in addition to phosphatides. The ultrastructural studies by Yamada (1959) revealed fat droplets in basal and apical regions of gall bladder epithelial cells and cask like cells in the gall bladder of hamster. They consisted of neutral fats, cholesterol and PAS positive lipids. Onochi et al.(1984) analysed lipids and bile acids in the gall bladder bile and gall stone by HPLC. Their studies revealed lower concentration of cholesterol, phospholipids and total bile acids than in calcium billirubinate stones, wherein free bile acids are detected.

Ross et al. (1984) found cholesterol esters in all human bile samples and gall bladder mucosa. Tusque et al. (1964) studied the effect of estradiol implants in situ on the absorption and lipid release from the mucus membrane of the gall bladder of male and female dogs and made comparison with normal dogs. According to them, the estradiol caused intensification of absorption and excretion of cholesterol esters, while testosterone inhibited absorption and excretion of cholesterol esters but facilitated the absorption and secretion of triglycerides.

Badowski and Elzbieta (1978) analysed the bile in patients with cholelithasis. They noted ten-fold increase in cholesterol concentration and two- to four-fold increase in lecithins, bile salts,

total proteins, seromucoid and β-lipoprotein. According to Bennett (1976), the bile from a woman on oral contraceptives was more saturated with cholesterol than the control. Bondar et al. (1979) after analysing the bile from patients with cholecystitis reported that the level of bile acids, bilirubin and lipds were below normal and those of cholesterol above normal. The changes were correlated with the type of cholecystitis. Thus, bile analysis can be used in the diagnosis of biliary tract disease.

Paris et al. (1974) found reducing sugar in all samples of gall bladder bile obtained during surgery and of bile obtained from post-operative bile duct drainage. Prostaglandin release from guinea pig gall bladder was studied by Booker and LeMonte (1983) in the tissue sections incubated for one hour in Kreb's solution.

The intraduodenal administration of $MgCl_2$ stimulated pancreatic enzyme secretion and gall bladder emptying (Holtermuller et al. 1976). Structural changes in the gall bladder epithelial cells of mouse have been worked out by Wahlin et al. (1976) following in vivo and in vitro stimulation of the gall bladder with cholecystokinin and pancreozymin. By observing overall increase in size of secretory granules and increased secretory activity within 2-3 minutes after hormone administration, they concluded that cholecystokinin and pancreozymin apparently stimulates the secretion of glycoproteins from gall bladder epithelium. Effect of serotonin cretinin sulfate on active electrolyte transport was evaluated in vitro in epithelial sheets of rabbit gall bladder by Donowitz et al. (1980). Lamote et al. (1982) investigated the growth promoting effects of cholecystokinin octapeptide,

caerulein and pentagastrin on gall bladder mucosa of mice. Epithelial cells hyperplasia occurred after caerulein or cholecystokinin administration, whereas pentagastrin had no effect on epithelial population. Studies of Wingrave and Kay (1982) on 23,000 women using oral contraceptive and equal number of controls suggested that there is no overall increased risk of gall bladder disease in women on oral contraceptives. Spuzyak et al. (1984) studied the effect of insulin on the nature of gall bladder filling in dogs. According to them, the small doses of insulin stimulated bile production and secretion and accelerated gall bladder filling, whereas large doses has opposite effects.

Treatment of guinea pigs with progesterone inhibited the contractile response of isolated gall bladder strips to acetylcholine and cholecystokinin octapeptide (Ryan and Pellecchia, 1982). Effect of estrogen and progesterone on gall bladder motility in vitro has also been studied by Ryan et al. (1982). Similarly, the contractile function of the gall bladder in response to pancreozymin have been studied by Usubakanov (1982).

Roland (1978) studied the effects of gastrointestinal hormones on concetrating function and motility in the gall bladder of cat. According to him, intravenous injections of secretin abolishes the net absorption of Na^+ and HCO_3^- and decreased the net absorption of K^+ and Cl^- by gall bladder mucosa, however, it did not affect gall bladder motility. Cholecystokinin induced the gall bladder contraction, but did not affect H_2O transport across the gall bladder wall.

Presence of glycogen is shown in the epithelium of gall bladder of cat, dog (Seelinger, 1937), mice (Mori, 1938;

Hayward, 1962; Grzycki and Rzeszowski, 1968); guinea pig (Ischikawa, 1950) and rabbit (Togari and Okada, 1951); but the absence of glycogen has been reported in the epithelial cells of human gall bladder (Togari and Okada, 1953; Patil, 1985). Similarly, absence of glycogen has been reported in gall bladder epithelium in dog, bat, monkey, buffalo, squirrel, etc. (Patil, 1985).

Montella (1956) identified the substance in the gall bladder wall of man partly as mucopolysaccharides and partly as mucoproteins, after employing the method of Hotchkiss-McManus, after diastase and bacterial or testicular hyaluronidase. Chowdhari and Jain (1970) found deeply stained PAS postive apical border in the gall bladder epithelial cells of some vertebrates. Hakkinen and Laitio (1970) detected the glycoproteins in the gall bladder epithelial cells of human by immunological studies. Wolf-Heidegger et al. (1965) showed neutral mucosubstances in the vicinity of Golgi apparatus and in apical granules in the gall bladder epithelial cells of cat and man. Varute and Nalavade (1973) studied the nature of mucosubstances histochemically in gall bladder epithelium of cat. They stated that the columnar epithelial cells apparently contain protein-sulfomucin complexes which occur in the striated border and in granules grouped in apical regions. The sulfomucins were found in special goblet cells. Yamada and Hoshino (1976) demonstrated 1,2-glycol groups with different electron microscopic stains for carbohydrates in gall bladder epithelial cells of rabbit.

Acidic mucopolysaccharides or mucosubstances have been demonstrated in the gall bladder of mouse (Grzycki and Rzeszowski, 1968), rabbit (Yamada, 1974) and man (Margi, 1968). Similarly,

Terho and Laitio (1974) reported hyaluronic acid, heparane sulfate, dermatin sulfate and chondroitin 4 and/or 6-sulfate in the wall of human gall bladder. No and Park (1971) reported that the muco-substances in the gall bladder mucosa and bile duct in dog, cat, rabbit, guinea-pig and human fetus reacted positively towards PAS, AB pH 2.5, mucicarmine and AB pH 2.5-PAS. The gall bladder epithelial cells have been reported to contain sialomucins in cattle (Bruveris, 1969); sulfomucins in man (Esterly and Spicer, 1968); cat (Varute and Nalavade, 1973); cat and rabbit (Jennings, 1958) and in Pteropus Rhinolophus, Tadarida, Pipistrellus, sheep goat (Patil, 1985); mixture of neutral mucosubstances and sulfomucins in bat (Nalavade et al., 1974); guinea-pig (Tusque et al., 1964); man (Hakkinen and Laitio, 1970; Patil, 1985).

Warner (1953) reported on the presence of glyco-protein as the main constituent in the gall bladder epithelial cells of vertebrates which included hexosamine, galactose and methylpentose. Odin (1958) detected fucose amine, mucoproteins and sialic acid in the gall bladder epithelium of man. Weicker and Graesslin (1965) found xylose as the main sugar in the gall bladder mucosubstances in man. Lee et al. (1979) estimated 55-75% carbohydrate being galactose, fucose, N-acetylglucosamine, mannose, glucose and sialic acid in human bile and gall bladder mucosa. Civalleri and Fiotek (1981) extracted and identified the mucopolysaccharides in the gall bladder of mucosa of the pig as chondroitin sulfate and heparin sulfate as well as polysulfated mucopolysaccharides.

Some of the investigators have analysed pigments, bile acids, lipids and mucosubstances from the gall bladder bile.

Matsuhiro et al. (1970) isolated glycoproteins and sulfated glycoproteins from human bile. On the other hand, Imado (1970) fractionated and separated human white bile mucopolysaccharides by cellulose acetate electrophoresis. The bile contained galactose, fucose, glucoseamine, galactoseamine and sialic acid. Neiderhiser et al. (1971) isolated two distinct mucins as glycoproteins in the gall bladder bile of pig. Terho and Laitio (1974) isolated hyaluronic acid and chondritin 4- and/or 6-sulfate in gall bladder bile of human. They also found glucoseamine, galactoseamine as well as different amount of fucose, sialic acid and sulfate. Popa and Funduc (1979) analysed the composition of human bile both, hepatic and vesicular, which contained bile acids, bile pigments, cholesterol, phospholipids and metals. Lee et al. (1979) also analysed the composition of human hepatic and gall bladder bile and found galactose, fucose and N-acetylglucose amine, mannose, glucose, N-acetylgalactoseamine as major monosaccharides and sialic acid. They further stated that the sialic acid was present in large amounts in the bile of an ulcerated and inflammed gall bladder. They found similar carbohydrate constituents in the gall bladder with gallstone.

Kanaeva (1980) stated that in the patients with chronic cholecystitis, the bile contained higher concentration of sialic acid and further added that the detection of sialic acid is suitable for the differential diagnosis of cholecystitis and biliary tract dyskinesis. Hong and Meng (1982) identified bilirubin, cholesterol, phospholipids and total bile acids in the gall bladder bile of different human races. The bile components were comparable in Chinese and Japanese, which were different in Swedish people. Caldwell et al. (1969) reported on the presence of these modern

C_{24} acids, including cholic, deoxycholic and chenodeoxycholic acids in the bile of sea cow. The concentration of cholesterol, bile acids and lecithin was found to be lower than in other species. According to Smith and LaMont (1983), mucin-billirubin complexes function as nucleating centers for cholesterol gall stones as well as gall bladder mucin and billirubin are frequently found at the center of human cholesterol gall stones.

The epithelium of the gall bladder in higher vertebrates during embryonic period is composed of stratified or irregularly pseudostratified columnar epithelial cells and becomes uniformly simple columnar epithelium in mice (Mori, 1938); guinea pig (Ischikawa, 1950); and rabbit (Togari and Okada, 1951). Observations have also been reported in guinea pig (Schiebler et al., 1975). They further reported that the epithelium contains glycogen upto 57 days, neutral and carboxylated mucosubstances were demonstrable after 30th day and sulfomucins visualized after 48 days. In similar studies, Bruveris (1969) found increased level of mucopolysaccharides in the gall bladder of cattle in the fetal stage. In the epithelium of gall bladder acid mucopolysaccharides were detected at 2.5 to 3 months of age and sialomucins in the goblet cells. He detected neutral mucins, hyaluronic acid and sulfomucins in the submucosa of gall bladder. The metachromatic properties were stronger in younger animals.

II) Reasons for undertaking the Present Investigation:

An insight into the reasons as to why the present problem was undertaken for investigation can be obtained from the

above critical review of the work done on gall bladder of birds and mammals. This review brings out the following reasons, which led to the selection of the present study:

1) The existing literature pointed out that the types of cells and/or mucosubstances in them, have been studied extensively in connective tissue, respiratory tract, nervous system, alimentary tract and associated glands, reproductive system along with glands and non-gonadal sex organs, kidneys, muscles, neoplasms, etc., but scanty literature is available on gall bladder of vertebrates.

2) There are few reports on the gall bladder of vertebrates. However, the laboratory animals and mammals were considered in most cases. Comparatively less attention has been paid to the sub-mammalian vertebrates like birds.

3) In literature, controversy is generated regarding the presence or absence of goblet cells. There are some reports on the presence of goblet cells, but these have not been reported in all the animals studied.

4) In an identical manner, the problem of presence or absence of mucous glands in the gall bladder is not settled yet. These glands have been reported in some animals only. Their presence has any significance with feeding habits of animals is yet to be decided.

5) As reviewed earlier, the problems regarding the presence of cuticular border or brush border and the nature of the

epithelial cells of the gall bladder, whether secretory or absorptive, are not answered satisfactorily.

6) The existing literature revealed diverse types of mucosubstances in the epithelial cells, goblet cells and/or glands in the gall bladder. For example, the gall bladder mucosubstances have been reported to be PAS positive or reactive with mucicarmine or AB positive, etc., without characterising the nature of mucosubstances.

7) Some of the authors have reported on the presence of acidic mucosubstances in the gall bladder of a given type of vertebrate, without characterising further the nature of acidic mucosubstances.

8) There are few reports only on the gall bladder of vertebrates during the embryonic development.

9) In some of the earlier researches, the histology and nature of mucosubstances have been studied in the gall bladder of some animals during the embryonic time. However, such a study has not been carried out in the gall bladder of fetus and adult simultaneously.

10) Based on the aforementioned points of evaluation of the present information, it was decided to study and to get an insight into the histological architecture and characterisation of mucosubstances in the cells and/or glands of the gall bladder of birds and mammals and to compare the results obtained in the present investigation with those of the existing literature.

III) Plan of the Proposed Work:

a) Choice of the Animals:

For the present investigation, adults of four species of birds, five species of mammals and fetus of guinea pig, rabbit and human were selected. Most of the animals locally available were collected. The gall bladders of human fetus were supplied by Dr. Mane from Satara, while the fetus of guinea pig and rabbit were obtained from the pregnant uterus of these animals. Care was taken to select the animals differing in their dietary habits whenever possible.

b) Choice of Techniques:

In the present investigation, standard histological techniques and a series of histochemical techniques, which are well established and recommended, are employed. The main aim of selecting these methods is to study the histology as well as to characterise histochemically the nature of mucosubstances. These histochemical techniques are currently used to identify glycogen, neutral mucosubstances, acidic mucosubstances such as sulfomucins, sialomucins, hyaluronic acid and some atypical mucosubstances.

c) Critical Evaluation of Observations:

The present investigation on gall bladder of some birds and animals was carried out to augment our knowledge about histology, nature of mucosubstances in epithelial cells and in goblet cells and glands, if present, species diversity, if any, difference in gall bladder mucosubstances due to variation in the dietary habits of the animals and to compare the results

obtained in the present investigation with the existing literature on gall bladder of birds and mammals.

d) Presentation of Dissertation:

It was thought to divide the present Dissertation into four Chapters, the First Chapter being on introduction giving the existing literature and reasons that stimulated to undertake the present investigation. The Second Chapter deals with material and methods employed in the present work. Chapters Third and Fourth will include histological and histochemical observations on the nature and distribution of mucosubstances in the gall bladders of birds and mammals respectively. Each of these Chapters will also include discussion on the results obtained in the present investigation and the existing literature on gall bladder of a particular class. It will be followed by general discussion giving comparative account on the gall bladders of birds and mammals. The general discussion will be followed by Summary and Concluding Remarks. The references cited in the above Chapters will be listed at the end of the Dissertation.

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