GENERAL DISCUSSION

GENERAL DISCUSSION.

It is decided to discuss here separately the comparative account of the histological architecture and the mucosubstances in the different histological sites such as brush border and apical granules of the epithelial cells and gland cells, duct cells and bile wherever present in the gall bladder of birds and mammals.

The present histological studies on gall bladder of birds and mammals revealed variations in the thickness of the wall. The gall bladder wall is comparatively very thin in the gall bladder of bat, thin in myna and sea bird (birds) and hare and hare fetus (mammals). On the other hand, moderately thick walled gall bladder is present in fowl and duck (birds) and guinea pig, pig and guinea pig fetus (mammals). The thick walled gall bladder is present in ox and human fetus of age five months and six months. In all birds and mammals under the present investigation, the gall bladders are in contact with the liver.

The present investigation indicated the variation in the nature of mucosa. The gall bladder wall is smooth only in ox and guinea pig fetus. In the remaining vertebrates, the mucosa is folded. Variations are also seen in the number and the nature of folds in the gall bladder. The number of folds is many in sea bird, hare and pig. On the other hand, the number of folds is few in fowl, duck, myna, hare fetus, guinea pig and human fetus. The folds are thin and elongated in sea bird, hare and bat. In the remaining birds and mammals, the folds are short and broad.

TABLE NO.5 : Comparison between histological structure and mucosubstances in the gall bladder of birds and mammals.

Sr. No.	Animal	Histological Observations				Histochemical Observations					
						Epithelial Cells		Glands			
		Wall	Mucosa	Epithelial Cells	Glands	Brush Border	Apical Granules	Brush Border	Apical Granules	Duct	Bile
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
A) BIRDS:											
1.	Guinea fowl	Moderately thick	Folded - few folds	Cuboidal to low columnar	Present	N=P+Su=W	N=W	N=P+Su=WtoM	N=W to M	-	N=P+Su=W
2.	Duck	"	Few folds	Tall Columnar	Present	N=W+Su= P+Si=P	N=PtoW	N=W+Su= P+Si=P	N=W	N=W+Su= P+Si=P	N=P+S=P
3.	Myna	Thin	Few folds	Cuboidal	Present	N=W+Su=W	N=P	-	-	-	-
4.	Sea bird	Thin	Highly folded	Cuboidal	Absent	N=P+S= P+Si=P	N=P	-	-	-	-
B) MAMMALS:											
5.	Ox	Thick	Smooth	Cuboidal to low columnar	Present	Su=P+Si=P	N=W	Su=W+Si=P	-	-	-
6.	Hare	Thin	Highly folded	Tall columnar	Present	Su=M	N=W	N=M	-	-	-
7.	Hare fetus	Thin	Few Folds	"	Present	A.T = I	A.T = W	A.T = I	-	-	-
8.	Guinea pig fetus	Moderately thick	Smooth	Low columnar	Absent	A.T = I	A.T = I	-	-	-	-
9.	Guinea pig	"	Few folds	Cuboidal to low columnar	Present	Su=P+Si=W	N=P+Si= P+Si=P	N=P+Su=	-	-	-
10.	Pig	"	Highly folded	Tall columnar	"	Su=I	Su=I	Su=M	-	-	-
11.	Bat	Very thin	Few folds	Cuboidal to low columnar	Absent	Su=M	Su=M	-	-	Su=M	-
12.	Human fetus (five months)	Thick	Very few folds	Low columnar	Absent	Su=M	Su=PtoW	-	-	-	-
13.	Human fetus (six months)	"	"	"	"	Su=M	Su=PtoW	-	-	-	-

Abbreviations: N = Neutral mucosubstance; Su = Sulfomucins, Si = Sialomucins, I = Intense, M = Moderate, W = Weak, P = Poor, A.T=Atypical mucosubstances

The gall bladder epithelial cells are of cuboidal type in birds (myna and sea bird) and mammals (bat), cuboidal to low columnar in birds (guinea fowl) and mammals (ox, guinea pig), low columnar in guinea pig fetus and human fetus. On the other hand, tall columnar epithelial cells are present in the gall bladder of bird (duck only) and mammals (hare, hare fetus and pig). Similar variations in the thickness of wall, presence or absence of folds and the nature of epithelial cells have been reported by Patil (1985) in seven species of birds and twelve species of mammals.

The epithelium of the gall bladder in mammals in embryonic period is composed of stratified or irregularly pseudostratified columnar epithelium and becomes uniformly a simple high columnar epithelium in mouse (Mori, 1938), guinea pig (Ishikawa, 1950) and rabbit (Togari and Okada, 1957). Recently, Schiebler et al. (1975) studied the development of guinea pig gall bladder epithelium from 19th intrauterine day to 31st post-natal day. They observed that the epithelium was first pseudostratified one and transformed into columnar epithelium by 31st day. The present investigation revealed the mucosa composed of tall columnar epithelium in rabbit hare. On the other hand, the gall bladder of mucosa in guinea pig fetus and human fetus of age five and six months is composed of low columnar epithelial cells.

As stated earlier also, the question regarding the presence or absence of goblet cells in aveian and mammalian gall bladder is not settled yet. It has been pointed out by a number of investigators the presence of goblet cells in the gall bladder of different mammals. The presence of goblet cells has been demonstrated in the gall bladder of man (Aschoff, 1909; Aschoff and Bacmeister, 1909; Ito and Nagahiro,

1941), pig (Ott, 1937), guinea pig (Ischikawa, 1950; Jennings, 1958), sea cow (Caldwell et al., 1969), cattle (Bruveris, 1969), rabbit and rat (Jennings, 1958) and in goat (Patil, 1985). On the other hand, the presence of goblet cells in the gall bladder of a number of mammals have been denied in man (Sommer, 1909; Policard, 1914; Halpert, 1927; Pfuhl, 1932), dog (Policard, 1914; Seelinger, 1937), cat (Seelinger, 1937), mice (Mori, 1938) and in sheep, buffalo, musk shrew, squirrel, dog, monkey, man and four species of bat (Patil, 1985). In the present investigation, goblet cells are not found in any bird or mammal.

In the present investigation, the definite glands were observed in the gall bladder of birds (guinea fowl and duck) and mammals (ox, hare, hare fetus, guinea pig and pig). However, there is a controversy about the presence or absence of glands in birds and mammals. Yamada and Hoshino (1972) reported on the downgrowth of the superficial epithelial cells in the gall bladder of chick. The crowding of cells known as downgrowth of cells which react differently than the surface epithelial cells in chick may represent the group of cells which occasionally form gland-like formation, the so-called Rokitansky-Aschoff sinuses as reported in the hamster by Yamada (1959). Patil (1985) also reported the presence of glands in the gall bladders of kite, kingfisher and crow. According to him, in these birds, the glands have formed by invagination of the mucosal epithelium.

Among the mammals, the absence of glands have been reported in the gall bladder of guinea pig (Tusques et al., 1964), cattle (Bruveris, 1969), rabbit (Weinstock and Bonneville, 1971), cat (Varute and Nalavade, 1973) and Pteropus, Pipistrellus, musk shrew, squirrel and man (Patil, 1985). On the other hand, presence of glands

have been reported in the gall bladder of man (Gompher, 1951), sheep (Tusques et al., 1964; Patil, 1985), sea cow (Caldwell, et al., 1969), bat (Nalavade et al., 1974) and Rhinolophus, Tadarida, sheep, goat, buffalo and monkey (Patil, 1985). The present investigation revealed the absence of glands in the gall bladder of bat and human fetus but the presence of glands in the remaining animals.

Patil (1985) found the invagination of the mucosal epithelium in the gall bladder of Rhinolophus, Tadarida and monkey, which were separated from the main mucosa and hence, considered as glands. According to him, these invaginations represent the Rokitansky-Aschoff sinuses as described in the gall bladder of hamster by Yamada (1959). Similar structures are found in the gall bladder of guinea pig under the present investigation. Therefore, these points of discussion generate a controversy regarding the presence or absence of glands in the mammalian gall bladder. Whether the presence or the development of glands have any evolutionary significance, i.e. in the physiology of the mammals, cannot be established from the existing data at present.

The present investigation revealed the type or mode of secretion as merocine or eccrine in the gall bladder epithelial cells and gland cells in four species of birds and five species of mammals as well as the fetuses of hare, guinea pig and human at different developmental stages.

The histochemical studies revealed the absence of any atypical mucosubstances such as alcianophilic but not-metachromatic, metachromatic but non-alcianophilic, high pH alcianophilic C.I.-reactive but non-alcianophilic, AF-reactive but non-alcianophilic and sometimes,

sulfated sialomucins, etc., in the gall bladder of all birds and adult mammals under the present investigation. However, atypical mucosubstances (C.I. reactive but non-alcianophilic) are found in the fetus of guinea pig and hare.

The results obtained in the present investigation revealed species diversity with regard to the mucosubstances in different histological sites of gall bladder of birds and mammals. For example, the brush border in the gall bladder epithelial cells contain sulfomucins in mammals (hare, pig and human fetus), sulfomucins and sialomucins in ox and guinea pig, mixture of neutral mucosubstances and sulfomucins in fowl and myna and neutral mucosubstances, sulfomucins and sialomucins in duck and sea bird; atypical mucosubstances in hare fetus and guinea pig fetus. The apical granules of gall bladder epithelial cells contain neutral mucosubstances in all birds, ox and hare; only sulfomucins in guinea pig and C.I. reactive but non-alcianophilic atypical mucosubstances in guinea pig and human fetus. The gland cells elaborated a mixture of neutral mucosubstances and sulfomucins in guinea fowl, only neutral mucosubstances in hare, only sulfomucins in pig and a mixture of neutral mucins, sulfomucins and sialomucins in duck and guinea pig. The gland cells in hare fetus elaborated atypical mucosubstances like that of brush border in the same animal. The duct cells contained a mixture of neutral mucins and sulfomucins in duck while only sulfomucins in bat. The bile of sea bird contained a mixture of neutral mucins and sialomucins.

In the present investigation, the histological architecture and mucosubstances in the different histological sites of gall bladder in adults and fetus of hare and guinea pig and fetus of human at different

developmental stages were studied. These observations revealed variations in the structure of the gall bladder and the nature of mucosubstances in the gall bladder of adult and the gall bladder under develcpmental conditions. For example, the mucosa was highly folded in adult hare but only a few folds were present in the gall bladder of hare. The mucosa was almost smooth in guinea pig fetus, but was folded with few folds in adult guinea pig. Similarly, brush border of gall bladder epithelial cells reveal sulfomucins in hare and a mixture of sulfomucins and sialomucins in guinea pig. However, atypical mucosubstances (C.I. reactive but non-alcianophilic) are present in the fetus of these mammals. The gall bladders in the adult and fetus of hare revealed the presence of glands but in guinea pig, the glands were present only in adult. The mucosubstances in the apical granules of epithelial cells and gland cells of fetus and adults of these mammals also showed variations.

One of the aims behind undertaking the present investigation was to find out the relationship, if any, between the gall bladder of birds and mammals and their feeding habits. The histological and histochemical results on gall bladder of birds and mammals obtained in the present investigation have indicated that there is no relation between the presence or absence of goblet cells (though the goblet cells were not found in any animal under the present investigation), glands and the nature of mucosubstances in the brush border of apical granules of gall bladder epithelial cells, glands, duct cells (in duck and bat) and bile (in sea bird) and the feeding habits of the animals birds and mammals) under the present investigation.

Based on the circumstantial evidences, it is decided to ascertain some functions for the gall bladder epithelial cells, glands

and the mucosubstances in them. The existing literature shows that there is a controversy on the absorptive and secretory functions of the gall bladder. The epithelium of the gall bladder in mice (Mori, 1938), rabbit (Togari and Okada, 1951) and chick (Togari and Okada, 1951) is morphologically specialised for absorption and lacks a secretory picture. On the other hand, that of man (Togari and Okada, 1953), guinea pig (Ischikawa, 1950) and hamster (Yamada, 1959) show picturs of both capacities, absorptive and secretory. Weinstock and Bonneville (1971) also believed that the gall bladder epithelial cells in rabbit are endowed with the capacity to perform a dual function of absorption and mucous secretion. In the present investigation, it is hoped that wherever the goblet-cells and glands are absent, the epithelial cells can perform a dual function in absorption and secretion. The goblet cells and glands, in addition, perform mainly the secretory function. Greep (1966) also stated that the secretion of mucus in species is the function of glands.

The gall bladder epithelium involves in concentrating their contents of gall bladder lumen by absorption of water and electrolytes, principally sodium. The presence of Na^+, K^+-ATPase has been demonstrated in the gall bladder epithelial cells in some mammals (Van Os, 1970; Kharlamova, 1977; Yaremenko and Kharlamova, 1978), which may facilitate the ion transport across the epithelial cells. The sulfomucins elaborated by the gall bladder cells and those present in the brush border may perform similar functions in gall bladder. A general relationship between the ion accumulation and the acidic mucosubstances has been indicated in the work of Philpott (1964, 1965), Philpott and Goldstein (1967) and Morard (1967). The sulfomucins present in the brush border of

the epithelial cells may perform a similar function. This may be the reason why the sulfomucins occur in the brush border of all animals under the present investigation.

Due to a toxic substance in the gall bladder, the epithelial cells secreted more and more mucins and tried to protect the mucosa of the gall bladder. When the mucus secretion by epithelial cells became insufficient, then the mucus secretion by goblet cells and glands became evident. In this connection, the work of Dyban (1970) is important. In one experimental study, Dyban (1970) demonstrated that the injection of dimethyl-1-polysiloxane (SKTN-1) solidifies in 20 minutes and is toxic in the gall bladder of guinea pig. At daily intervals (1 to 100 days), the epithelium was studied. This experiment demonstrated a protective role of mucins, as shown to be elevated secretion by epithelial cells from 12th day formation of goblet cells and formation of glands by 20th day and occasional tubulo-alveolar glands by 43rd day. Esterly and Spicer (1968) have also observed increased number of goblet cells in human gall bladder under some pathological conditions.

The protective role of mucins by gall bladder in pathological condition is also explained by some other workers in man (Gompher, 1951). Adenocarcinoma may retain their epithelial secretory function and the demonstration of mucin is frequently useful in the evaluation of less or well-differentiated tumours (Henke and Lubarsch, 1929; Spicer, 1960; Spicer et al., 1962; Dobrogolski and Braunsten, 1963; Foster and Levine, 1963; Franke et al., 1964; Hukill and Vidone, 1965). Margi (1968) identified acidic carbohydrates in the normal gall bladder epithelium, the concentration of which decreased in the carcinoma of the gall bladder. Smith and LaMont (1985) identified mucin bilirubin complex

in human cholesterol gallstone.

Esterly and Spicer (1968) have demonstrated histochemically sulfomucins in the human gall bladder epithelium, whereas carboxy, neutral or their mixture in pathological conditions such as adenocarcinoma, cystic fibrosis and cholecystis. The increased number of goblet cells in the surface epithelium under pathological conditions showed staining reactions indicative of carboxymucins. An elevated amount of acid connective tissue polysaccharides such as haparin, dermatan sulfate and chondroitin-4 and 6-sulfates have also been demonstrated by Laitio and Terho (1975) in carcinoma of human gall bladder. Montella (1956) by histochemical studies demonstrated mucopolysaccharides and mucoproteins in the epithelium of the gall bladder in ten cases of lithasic cholecystis during phlogisis. Luciano et al. (1974) also demonstrated sulfated glycoproteins in human gall bladder epithelium in three cases of cholesterosis. Henke and Lubarsch (1929), however, reported that the secretion of mucus occur in chronic cholecystitis.

Gall bladder epithelial proliferation due to abnormal secretion and absorption of mucopolysaccharides in acute and chronic inflammation have been reported by Tool (1977) and in cholithasis in rabbit by Lee et al. (1986). Lee (1981) described three models of cholethiasis to find out the quantity and chemical composition of mucin produced in the normal and pathological conditions. The stone formation increased epithelial mucus production and it was incorporated as a part of the gallstone.

Lee et al. (1981) found a relationship between cholesterol and mucin in the gallstone formation in Prairie dog. They found an increase

in cholesterol content in the bile of cholesterol-fed dogs which stimulated mucus hypersecretion by the gall bladder and mucus gel was found nucleating agent for biliary cholesterol. Smith et al. (1983) have identified mucin bilirubin complex formation in human cholesterol gallstone. Koga (1985) identified lipid droplets in submucosa and epithelial cells of human gall bladder with cholesterosis by employing both light and electron microscopy. LaMont et al. (1983-a) found that the gallstone in mice is associated with increase in glycoprotein release from the neck of the gall bladder. In the latter study (1983-b) in man, they also observed the mucin glycoprotein of the gall bladder contributed the matrix of nucleus in gallstones.

The above review of the studies of gall bladder under pathological conditions may indicate the diagnostic value of the altered mucosubstances, such as carboxylated mucosubstances and sialic acid in adenocarcinoma, cystic fibrosis and cholecystitis. In addition, the increased level of mucins or the appearance of goblet cells and/or glands for increased levels of mucus secretion may indicate the protective role of the mucosubstances and the role of abnormal levels of mucosubstances in the gallstone formations, involving the nucleated agents of mucus. In dogs, it has been reported that aspirin may prevent the gallstone formation. However, such human bladder samples were not used in the present investigation and further studies are required to understand the role of gall bladder and bile mucosubstances in protection and in abnormal conditions, the gallstone formation. It should also be studied whether in human beings also, aspirin prevents the gallstone formation.

Based on the circumstantial evidences, only the functions such as protection, ion-exchange and absorption have been suggested to the gall bladder mucosubstances and further experimental studies should be carried out to confirm these functions of the gall bladder mucosubstances.