CHAPTER THREE

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON MUCOSUBSTANCES IN THE GALL BLADDER OF BIRDS AND DISCUSSION

CHAPTER THREE / HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON MUCOSUBSTANCES IN THE GALL BLADDER OF BIRDS AND DISCUSSION.

In the present investigation, four species of birds, N.meleagris (guinea fowl), A.strepera (duck), A.tristis (Indian myna) and L.argentatus (sea bird) differing in their feeding habits were used. The histological and histochemical observations on the gall bladder of these birds are presented hereafter.

OBSERVATIONS

A) Histological Observations:

The histological observations were carried out on H.E.stained sections of gall bladder in guinea fowl (Figs.1,2), duck (Figs.9,10,11), myna (Figs.17,18) and sea bird (Fig.23). The wall of the gall bladder was moderately thick in guinea fowl and duck; however, it was comparatively thin in myna and sea bird. The thickness of the gall bladder wall was mainly due to the amount of submucosal connective tissue. The mucosa was folded almost in all birds. The folds were numerous in guinea fowl (Fig.1), duck (Fig.9) and sea bird (Fig.23), but very few in myna (Figs.17,18). In guinea fowl and duck the folds were short and broad but they were elongated and narrow in myna and sea bird. In sea bird, particularly, the mucosa was highly folded and the folds were elongated. The core of the mucosal folds consisted of the lamina propria which was formed by the extension of the submucosal connective tissue. The epithelial cells were of single type in all birds. These cells were cuboidal with centrally placed nuclei in myna and sea bird,

CAPTIONS TO FIGURES

Fig.1 : T.S. of gall bladder of N.meleagris, H. E. Staining X 100.

Fig.2: A magnified view of T.S. of gall bladder of N.meleagris, showing the epithelial cells and glands. H. E. staining X 450.

Fig.3 : T.S. of gall bladder of N.meleagris, PAS staining X 150.

Fig.4: T.S. of gall bladder of N.meleagris, P-PAS staining X 200.

Fig.5 : T.S. of gall bladder of N.meleagris, D-PAS staining X 200.

Fig.6 ; T.S. of gall bladder of N.meleagris AB pH 1.0 staining X 150.

Fig.7 : T.S. of gall bladder of N.meleagris, AB pH 2.5 staining X 150.

Fig.8 : T.S. of gall bladder of N.meleagris, AB pH 1.0-PAS staining. X 100.

Fig.9 : T.S. of gall bladder of A.strepera, H. E. staining X 300.

ABBREVIATIONS

A	=	Apical granules.
B	=	Brush border.
BL	=	Bile.
E	=	Epithelial cells.
GL	=	Glands.
S	=	Submucosa.

CAPTIONS TO FIGURES

Fig.10 : T.S. of duct of A.strepera, H. E. staining X 450.

Fig.11 : T.S. of duct of A.strepera, PAS staining X 450.

Fig.12 ; T.S. of gall bladder of A.strepera, PAS staining X 450.

Fig.13 ; T.S. of gall bladder of A.strepera, D-PAS staining X 300.

Fig.14 : T.S. of gall bladder of A.strepera, AB pH 1.0 staining X 300.

Fig.15 ; T.S. of gall bladder of A.strepera, AB pH 2.5 staining X 300.

Fig.16 : T.S. of gall bladder of A.strepera, C.I. staining X 200.

Fig.17 : T.S. of gall bladder of A.tristis, H. E. staining X 200.

Fig.18 : T.S. of gall bladder of A.tristis, H. E. staining X 300.

ABBREVIATIONS

A = Apical granules.
B = Brush border.
E = Epithelial cells.
GL = Glands.
S = Submucosa.

CAPTIONS TO FIGURES

Fig.19 : T.S. of gall bladder of A.tristis, PAS staining X 300.

Fig.20 : T.S. of gall bladder of A.tristis, AB pH 1.0 staining X 300.

Fig.21 : T.S. of gall bladder of A.tristis, AB pH 1.0-PAS staining X 300.

Fig.22 : T.S. of gall bladder of A.tristis, AB pH 2.5-PAS staining X 300.

Fig.23 : T.S. of gall bladder of L.argentatus, H. E. staining X 450.

Fig.24 ; T.S. of gall bladder of L.argentatus, PAS staining X 300.

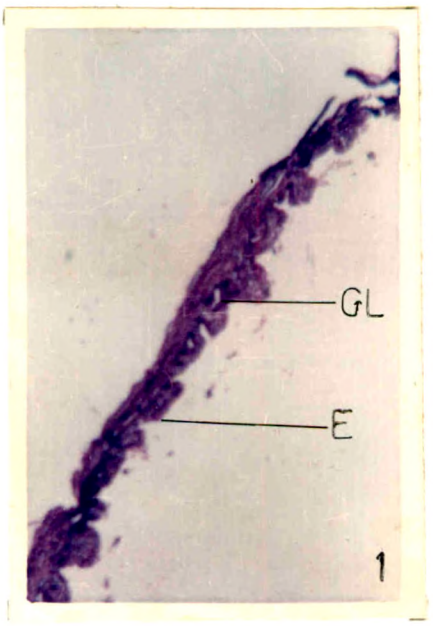
Fig.25 ; T.S. of gall bladder of L.argentatus, AB pH 1.0 staining X 200.

Fig.26 : T.S. of gall bladder of L.argentatus, C.I. staining X 200.

Fig.27 ; T.S. of gall bladder of L.argentatus. AB pH 2.5-PAS staining X 300.

ABBREVIATIONS.

A = Apical granules.
B = Brush border.
BL = Bile.
S = Submucosa.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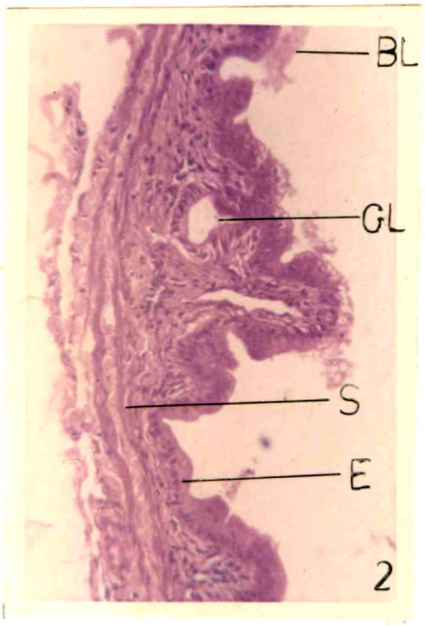
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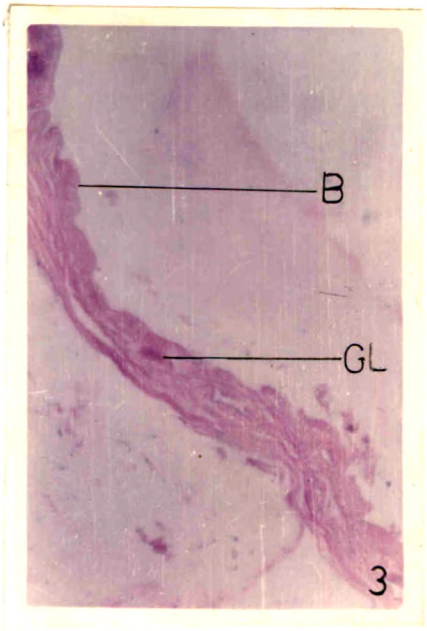
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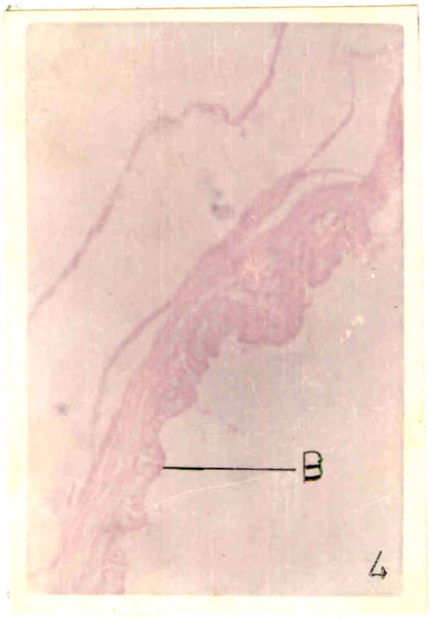
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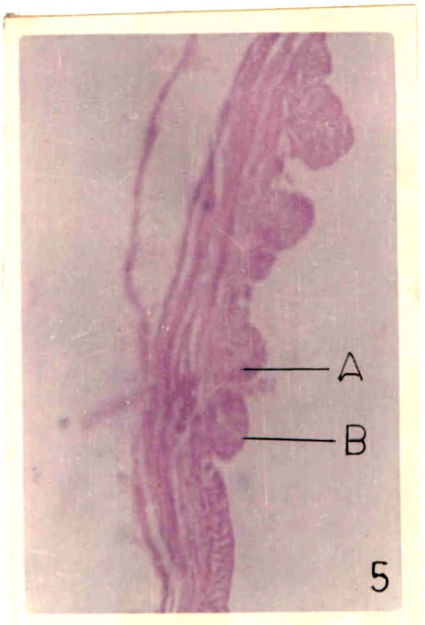
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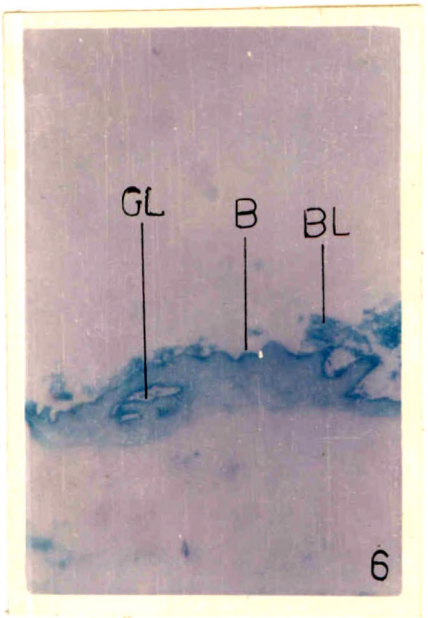
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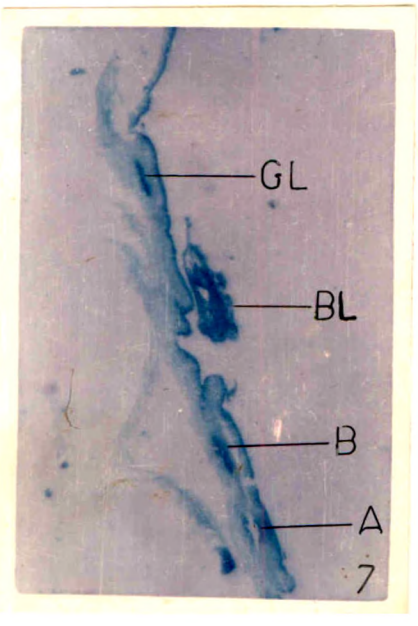
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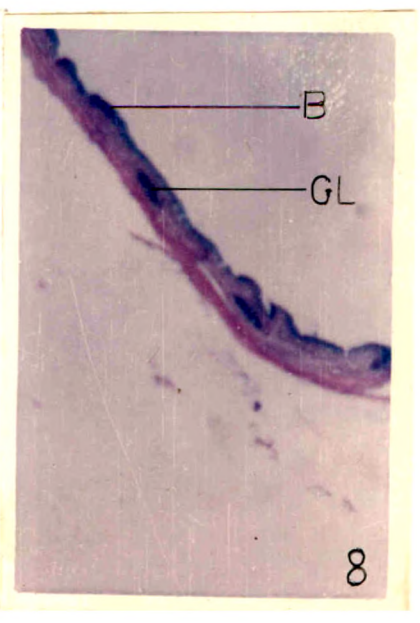
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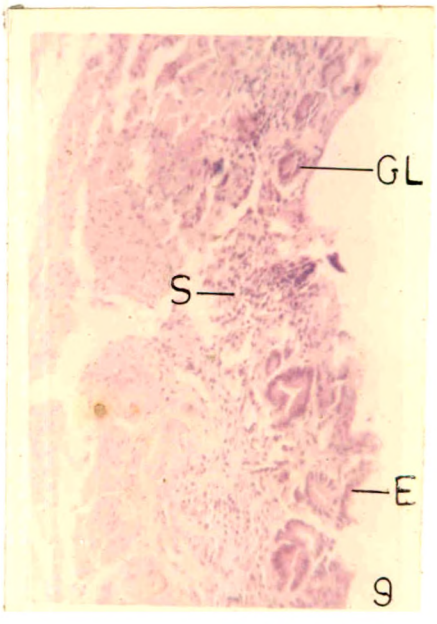
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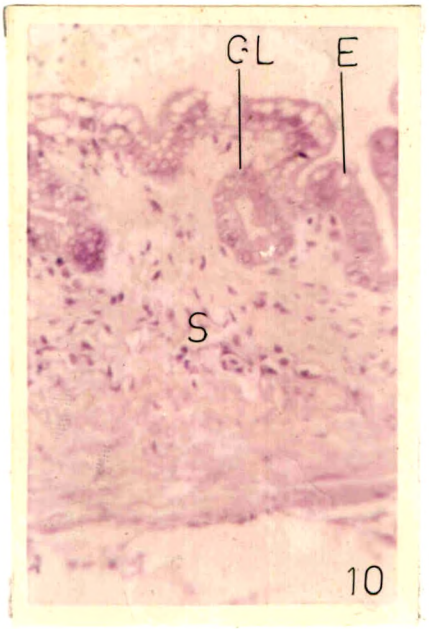

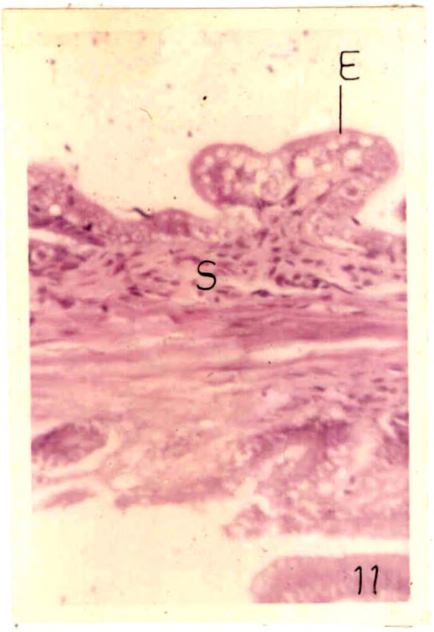

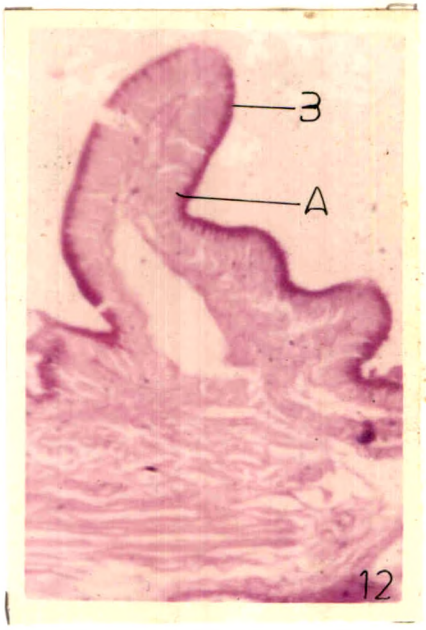

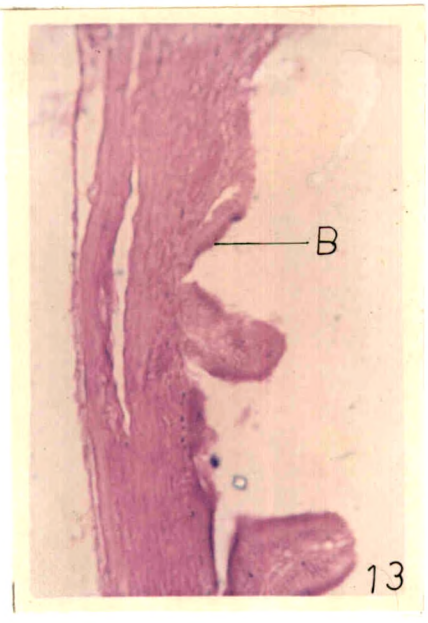

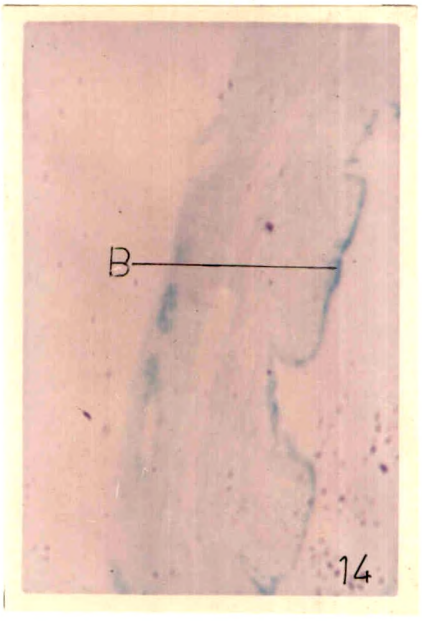

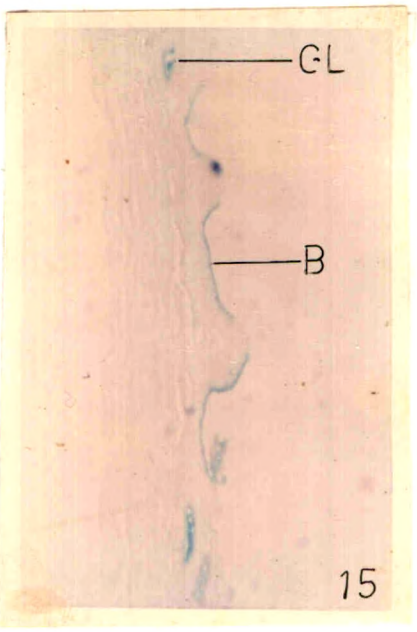

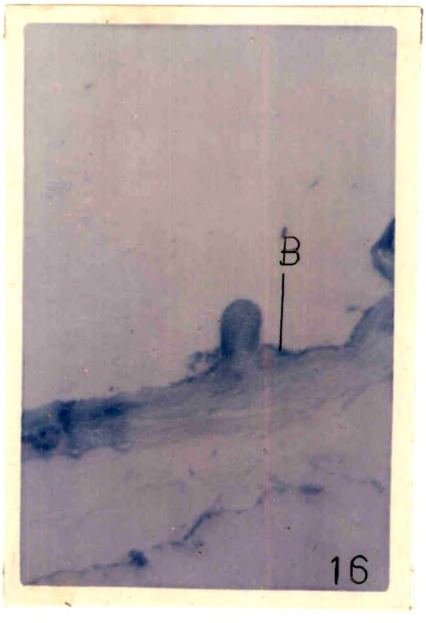

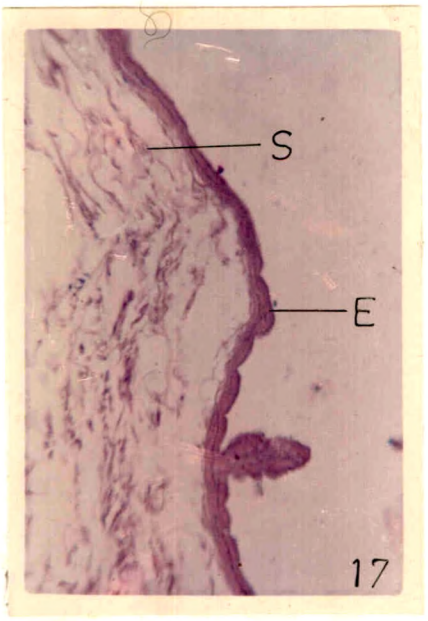

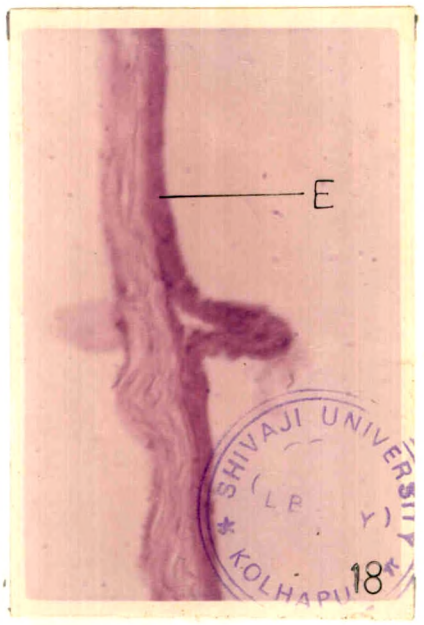

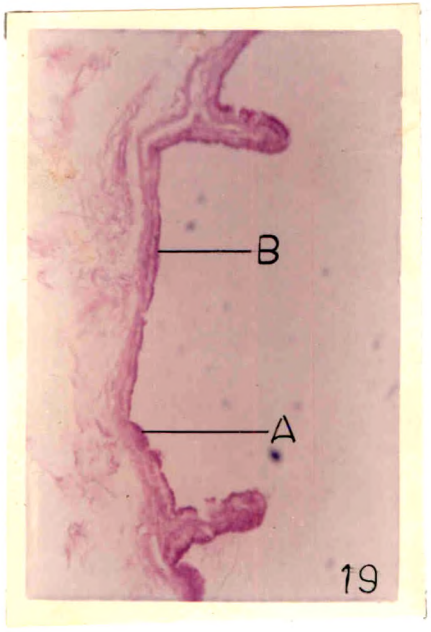
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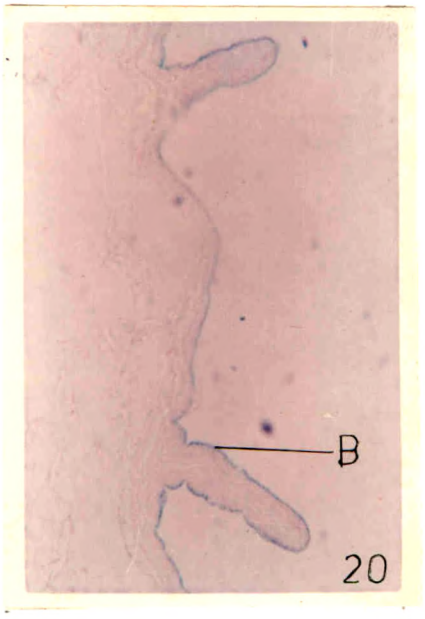
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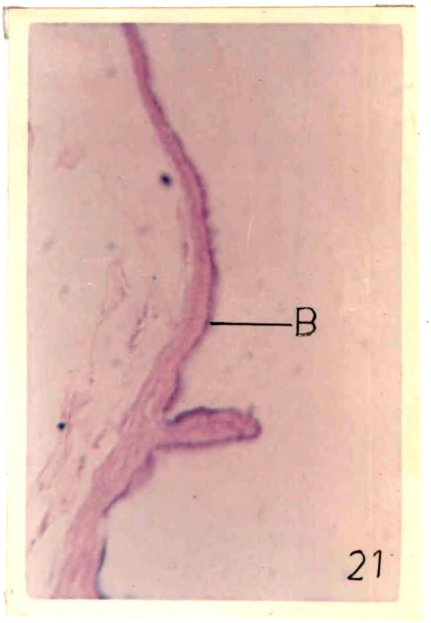
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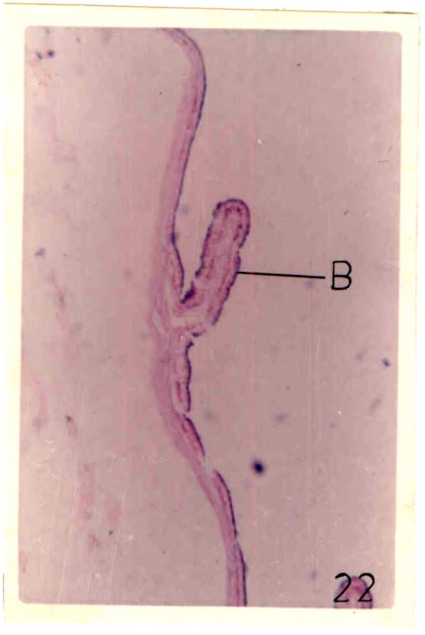
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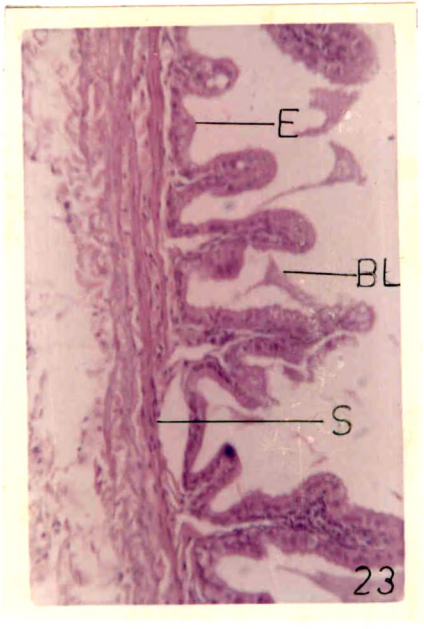
E
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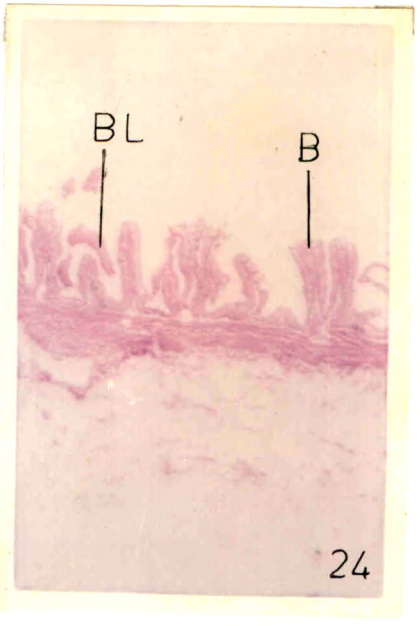
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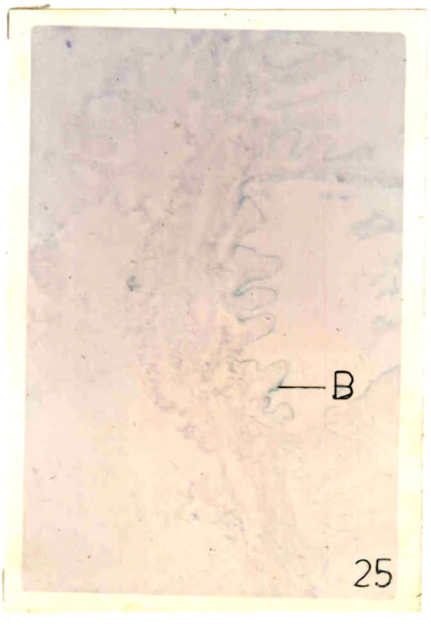
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B
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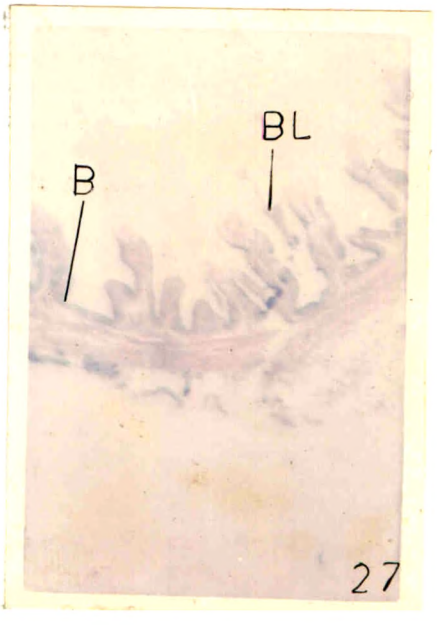
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cuboidal to low columnar in guinea fowl, while tall columnar with basally situated nuclei in duck. The submucosal glands were observed only in guinea fowl (Figs.1,2) and duck (Fig.9) but were found absent in the remaining two birds (Figs.17,18,23). The glands were formed by simple invagination of the mucosa. The muscularis made up of scattered circular smooth muscles in all these birds. A clear muscularis was seen in the gall bladder of them. The outermost free surface of ther gall bladder was surrounded by mesothelial serosa. The duct was found along with gall bladder only in duck. The mucosa of the duct also appeared folded (Fig.10). Moreover, the submucosal glands were also present in the duct (Fig.10).

B) Histochemical Observations:

The histochemical observations on the mucosubstances in the gall bladder of birds are recorded in Table No.2. The histochemical reactivities of the mucosubstances in the brush border and apical granules in the epithelial cells, gland cells and duct cells (only in duck) and bile (only in sea bird) arerecorded according to the visually estimated intensities and shades with ++++ representing intense reactivity, +++ representing moderate reactivity, ++ representing weak, + poor, ± trace and - representing absence of reactivity. The histochemical distribution of mucosubstances in the above histological sites is illustrated in photomicrographs (guinea fowl - Figs.3,8; duck - Figs.11-16, myna - Figs.19-22 and sea bird - Figs.24-27). The histochemical results requiring further description and considerations are presented hereafter along with their interpretations.

TABLE NO.2 : Comparative histochemical reactivities of mucosubstances in the gall bladder of birds.

Sr. No.	Histochemical Reactions.	Epithelium							Glands				Duct		Bile	
		Brush Border				Apical Granules			Brush Border		Apical Granules		in A.strepera			
		N.meleagris	A.strepera	A.tristis	A.argetatus	N.meleagris	A.strepera	A.tristis L.argetatus	N.meleagris	A.strepera	N.meleagris	A.strepera	Duct Cells	Gland Cells	N.meleagris	L.argetatus
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.
1.	PAS	+++ P	++++ P	++++ P	+++ P	++ P	++ P	+ P	++++ P	++++ P	+++ P	++ P	++++ P	++++ P	+++ P	++ P
2.	P-PAS	++ P	++ P	++ P	++ P	-	-	-	+++ P	++ P	-	-	++ P	++ P	++ P	++ P
3.	D-PAS	+++ P	++++ P	++++ P	+++ P	++ P	++ P	+ P	++++ P	++++ P	+++ P	++ P	++++ P	++++ P	+++ P	++ P
4.	AB pH 1.0	++ B	+ B	++ B	+ B	-	-	-	+++ B	+ B	-	-	+ B	+ B	++ B	+ B
5.	AB pH 1.0-PAS	+++PB	++++PB	++++BP	+++BP	++ P	++ P	+ P	++++PB	++++BP	+++ P	++ P	++++BP	++++BP	+++PB	++BP
6.	AB pH 2.5	++ B	++ B	++ B	++ B	-	-	-	+++ B	++ B	-	-	++ B	++ B	++ B	+ B
7.	AB pH 2.5-PAS	+++PB	++++BP	++++BP	+++BP	++ P	++ P	+ P	++++PB	+++PB	+++ P	++ P	++++PB	++++PB	+++PB	++BP
8.	C.I.	++ B	++ B	++ B	++ B	-	-	-	+++ B	++ B	-	-	++ B	++ B	++ B	+ B
9.	C.I.-PAS	+++PB	++++BP	++++BP	+++BP	++ P	++ P	+ P	++++PB	++++PB	+++ P	++ P	++++PB	++++PB	+++PB	++BP
10.	AF	++ P	+ P	++ P	+ P	-	-	-	+++ P	+ B	-	-	+ P	+ B	++ P	+ P
11.	AF-AB pH 2.5	++ P	++ BP	++ P	++ BP	-	-	-	++++ P	++BP	-	-	++BP	++BP	++ P	+ P
12.	Azure A pH 1.5	++ M	+ M	++ M	+ M	-	-	-	++ M	+ M	+ O	-	+ M	+ M	++ M	+ M

Table No.2 (contd.)

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.
13.	Azure A pH 3.0	++ M	++ M	++ M	+± M	+ O	+ O	± O	++± M	+± M	+ O	++ M	+± M	+± M	++ M	+ M
14.	Azure A pH 4.5	++ M	++ M	+++ M	++ M	++ O	+± O	+ O	++± M	++ M	++± O	++ O	++ M	++ M	++ M	+ M
15.	Sulfation Azure A pH 1.5	+++ M	++++ M	++++ M	+++ M	++ M	+± M	+ M	+++± M	++++± M	++± M	++ M	++++ M	+++± M	+++ M	++ M
16.	CEC + 0.1 M Mg^{++}	++ B	+ B	++ B	+ B	-	-	-	++± B	+ B	-	-	+ B	+ B	++ B	+ B
17.	CEC + 0.2 M Mg^{++}	++ B	+ B	++ B	+ B	-	-	-	++± B	+ B	-	-	+ B	+ B	++ B	+ B
18.	CEC + 0.4 M Mg^{++}	++ B	-	++ B	-	-	-	-	++± B	-	-	-	-	-	++ B	+ B
19.	CEC + 0.6 M Mg^{++}	+ B	-	+ B	-	-	-	-	+± B	-	-	-	-	-	+ B	-
20.	M 37 - AB pH 2.5	++ B	+ B	++ B	+ B	-	-	-	++± B	+ B	-	-	+ B	+ B	++ B	+ B
21.	DM 37 - AB pH 2.5	++ B	++ B	++ B	+ B	-	-	-	++± B	+ B	-	-	++ B	+ B	++ B	+ B
22.	M 60 - AB pH 2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
23.	DM 60 - AB pH 2.5	-	+ B	-	+ B	-	-	-	-	-	-	-	+ B	-	-	-
24.	Acid Hydrolysis - AB pH 2.5	++ B	+ B	++ B	+ B	-	-	-	++± B	+ B	-	-	+ B	+ B	++ B	+ B
25.	Sialidase - AB pH 2.5	++ B	+ B	++ B	+ B	-	-	-	+++ B	+ B	-	-	+ B	+ B	++ B	+ B
26.	Hyaluronidase - AB pH 2.5	++ B	++ B	++ B	++ B	-	-	-	++± B	-	-	++ B	++ B	++ B	++ B	+ B
27.	Pepsin - AB pH 2.5	++ B	++ B	++ B	++ B	-	-	-	++± B	++ B	-	-	++ B	++ B	++ B	+ B

I) Epithelial Cells:

i) Brush Border:

(a) Guinea Fowl (N.meleagris).

The brush border of the gall bladder epithelial cells in guinea fowl showed moderate PAS reactivity (Fig.3) which was resistant to diastase digestion (Fig.5), but could slightly be reduced, following phenylhydrazine treatment (Fig.4). These results indicated the presence of neutral and acidic mucosubstances but the absence of glycogen.

The brush border of gall bladder epithelial cells in this bird was weakly stained with AB at pH 1.0 (Fig.6) and the alcianophilia was not enhanced at pH 2.5 (Fig.7). The C.I. reactivity of the brush border was same as that of alcianophilia of pH 2.5. However, the brush border appeared purple-blue (the blue tinge was slightly more than pink tinge) with combined histochemical techniques such as AB pH 1.0-PAS (Fig.8), AB pH 2.5-PAS and C.I.-PAS.

Only weak purple staining with AF and AF-AB pH 2.5 sequence, weak metachromasia with azure A at pH 1.5 and persistant alcianophilia in CEC techniques upto the presence of 0.6 M $Mg.^{++}$ concentration indicated the presence of only sulfomucins as acidic mucosubstances in the brush border of gall bladder epithelial cells of this bird. These sulfomucins were resistant to mild methylation and hyaluronidase digestion, but active methylation removed their alcianophilia and subsequent saponification failed to restore it.

Partial reduction of PAS staining intensity by phenylhydrazine pretreatment indicated the presence of neutral mucosubstances alongwith sulfomucins in this site. This conclusion was further

strengthened by purple-blue combined staining (purple or pinkish tinge was there but it was poor) with AB pH 1.0-PAS (Fig.8), AB pH 2.5-PAS and C.I.-PAS sequential staining procedures and enhancement in metachromasia with azure A (moderate staining) at pH 1.5 after sulfation.

The results obtained with histochemical staining techniques, thus, revealed the presence of neutral mucosubtances (poor) and sulfomucins (weak) in the brush border of the gall bladder epithelial cells in guinea fowl.

(b) Duck (A.strepera).

The brush border of the gall bladder epithelial cells in duck showed intense PAS reactivity (Fig.12). The PAS reactivity of this site was resistant to diastase digestion (Fig.13) and the intensity of PAS was dminished by phenylhydrazine pretreatment. These initial histochemical results revealed the absence of glycogen but the presence of neutral and acidic mucosubtances.

The brush border showed only poor alcianophilia at pH 1.0 (Fig.14) and the alcianophilic blue staining was slightly enhanced at AB pH 2.5 (Fig.15) and C.I. (Fig.16) staining. From these observations, it was concluded that the brush border of these cells contained sulfomucins and carboxymucins.

The presence of sulfomucins in this histological site was further characterised by only poor purple staining within AF but blue-purple staining with AF-AB pH 2.5 sequence, metachromatic pink staining in CEC techniques in the presence of graded concentrations with Mg^{++} upto 0.2 M. The presence of sulfomucins was also confirmed by active methylation which removed alcianophilia and

subsequent saponification failed to restore it (it was restored but poorly). These sulfomucins were resistant to mild methylation and hyaluronidase digestion and there was no enhancement in the alcianophilia by prior pepsin digestion.

Together with sulfomucins, carboxymucins were also identified in the brush border of gall bladder epithelial cells of this bird. The presence of carboxymucins was inferred from slight increase in alcianophilia at pH 2.5 (Fig.15) than at pH 1.0 (Fig.14), blue staining with C.I. (Fig.16), which was identical to that with AB pH 2.5, blue-purple staining with AF followed by AB pH 2.5 staining, enhanced metachromatic pink staining with azure A at pH 3.0 and above, partial loss of alcianophilia in CEC techniques by addition of 0.1 M Mg^{++} concentration and only poor restoration of alcianophilia by both mild and active methylation followed by saponification. These carboxymucins were further identified as sialomucins since acid hydrolysis and sialidase digestion slightly reduced the alcianophilia in this histological site of the gall bladder of duck.

The presence of neutral mucosubstances in this site was inferred by reduction in the PAS staining reactivity by phenylhydrazine pretreatment, blue-purple staining with AB pH 1.0-PAS, AB pH 2.5-PAS and C.I.-PAS sequential staining procedures and increased metachromatic pink staining with azure A at pH 1.5 following the sulfation.

The aforementioned histochemical reactivities, thus, lead to the conclusion that the brush border of gall bladder epithelial cells of duck contained neutral mucins (weak), sulfomucins (poor) and sialomucins (poor).

(c) Myna (A.tristis).

The brush border of gall bladder epithelial cells in myna resembled in histochemical reactivities (Figs.19-220 to the brush border of similar cells in the gall bladder of guinea fowl. The only difference was that it reacted intensely with PAS in this bird. Therefore, it was concluded that the brush border of gall bladder epithelial cells in myna contained a mixture of neutral mucosubstances (weak) and sulfomucins (weak).

(d) Sea Bird (L.argentatus).

The brush border of gall bladder epithelial cells in this bird showed more or less identical staining reactivities (Figs.24-27) as the brush border of gall bladder epithelial cells in duck. The only difference was that the PAS staining intensity was moderate. Therefore, it was concluded that the brush border of gall bladder epithelial cells in sea bird contained neutral mucins (poor), sulfomucins (poor) and sialomucins (poor).

ii) Apical Granules:

The apical granules in the gall bladder epithelial cells of all these birds exhibited varied PAS reactivity. Their PAS reactivity was weak in guinea fowl (Fig.3), poor to weak in duck (Fig.12) and poor in myna (Fig.19) and sea bird (Fig.24). Their respective PAS reactivities could completely be blocked by pretreatment with phenylhydrazine but resisted to diastase digestion. These initial histochemical results indicated the presence of only neutral mucosubstances but the absence of glycogen in the apical granules of gall bladder epithelial cells of all four species of birds.

The absence of acidic mucosubstances in them was inferred from their negative staining with AB pH 1.0 (Figs.6,14 and 25), AB pH 2.5 (Figs.7,15 and 26), C.I. (Fig.16) and AF. The alcianophilia was also not evident in these apical granules even after pepsin digestion.

The earlier conclusion that the apical granules contained only neutral mucosubstances was also supported by their rspective PAS reactivities only with combined histochemical staining techniques, viz. AB pH 1.0-PAS (Fig.8), AB pH 2.5-PAS (Fig.27) and C.I.-PAS, only blue orthochromatic staining with azure A at all pH levels (the intensities of which gradually increased with higher pH values) and varied metachromatic pink staining (Table No.2) after sulfation.

Therefore, it was concluded that the apical granules in the gall bladder epithelial cells contained only neutral mucosubtances in weak amount in guinea fowl in poor to weak concentrations in duck and only in poor quantities in myna and sea bird.

II) Glands:

The glands were found in the gall bladder of guinea fowl and duck only. These were found separate from the mucosal epithelium, but in some regions, deep invaginations could be seen. It appeared that the glands might have been developed by the invagination of the mucosa. Moreover, the gland cells in their histological and histochemical results resembled with their mucosal epithelial cells. The lumen of the glands also contained secretion. The following conclusions were drawn for the gland cells since their staining reactivities were identical to the mucosal epithelial cells of these birds.

i) Brush Border:

(a) Guinea fowl (N.meleagris).

The brush border of gland cells in the gall bladder of guinea fowl (Figs.3-8) exhibited more or less identical staining reactivities to that of brush border in the mucosal epithelial cells of this bird. The only difference was that the PAS reactivity was moderate to intense. These histochemical reactivities, thus, indicated the presence of neutral mucins (poor) and sulfomucins (weak to moderate) in the brush border of gland cells in the gall bladder of guinea fowl.

(b) Duck (A.strepera).

The brush border of gland cells in the gall bladder of duck also exhibited identical histochemical staining reactivities (Figs.12-16 , which exhibited by the brush border of mucosal epithelial cells of this bird. However, the PAS reactivity was slightly less.

These histochemical observations thus indicated the presence of neutral mucins (poor to weak), sulfomucins (poor) and sialomucins (poor) in the brush border of gland cells in the gall bladder of duck.

ii) Apical Granules:

(a) Guinea fowl (N.meleagris).

The apical granules in the gland cells of gall bladder of this bird showed weak to moderate PAS staining which was resistant to diastase digestion but could completely be blocked by prior phenylhydrazine treatment indicating the absence of

glycogen and any acidic mucosubstances but the presence of only neutral mucosubstances in them.

The negative reaction of the apical granules in these birds with AB pH 1.0, AB pH 2.5 (Fig.15), C.I. and AF revealed the absence of acidic mucosubstances. This was further confirmed by pepsin digestion which did not exhibit any alcianophilia.

The presence of only neutral mucosubstances in the apical granules was also substantiated by their only PAS reactivity in sequential staining procedures such as AB pH 1.0-PAS, AB pH 2.5-PAS and metachromatic staining only after sulfation of the sections.

The aforementioned histochemical observations revealed the presence of only neutral mucosubstances (weak to moderate) in the apical granules of gland cells in guinea fowl.

(b) Duck (A.strepera).

The apical granules in the gland cells of the gall bladder in this bird showed identical staining reactivities to those of apical granules in the gland cells of guinea fowl. The only difference was that the PAS staining reactivity was weak.

Therefore, it was concluded that the apical granules in the gland cells of this bird also contained only neutral mucosubstances in weak quantities.

III) Duct Cells.

The duct was observed only in duck. The duct mucosa was also thrown into the folds, which were more numerous but short and broad

(Fig.10). The duct cells were cuboidal to low columnar. However, unlike the gall bladder epithelial cells in this bird, the brush border and apical granules could not be distinguished from each other. The glands were also found in the submucosa (Fig.10). The gland cells in duct also resembled to the gland cells of the gall bladder. The following conclusions were drawn from the histochemical results:

1) The duct cells in this bird showed histochemical reactivities with various histochemical techniques, practically identical to their brush border of the gall bladder epithelial cells. Therefore, it was concluded that the duct cells in duck also elaborated a mixture of neutral mucins (weak), sulfomucins (poor) and sialomucins (poor).

2) The gland cells present in the duct also showed practically identical histochemical reactivities to those exhibited by gland cells of the gall bladder of this bird. Therefore, it was concluded that the gland cells in the duct of the duck also elaborated neutral mucins (poor to weak), sulfomucins (poor) and sialomucins (poor).

IV) Bile:

1) The bile in the gall bladder of guinea fowl showed practically identical histochemical staining reactivities like that of brush border of gall bladder epithelial cells of this bird. Therefore, it was concluded that the bile in the guinea fowl also contained a mixture of neutral mucosubstances (poor) and sulfomucins (weak).

The bile in the gall bladder of sea bird reacted weakly with PAS (Fig.24). The PAS reactivity was resistant to diastase digestion and the intensity of PAS was slightly diminished by pheylhydrazine

pretreatment. These initial histochemical results revealed the absence of glycogen but a partial presence of neutral mucosubstances.

Only poor alcianophilia with AB pH 1.0 (Fig.25) which was not enhanced at pH 2.5 indicated the presence of acidic mucosubstances which were sulfomucins but absence of carboxymucins in the bile of sea bird. The presence of sulfomucins in the bile was also characterized by purple-blue staining with AB pH 1.0-PAS, poor purple staining with AF and AF-AB pH 2.5 combined histochemical procedure, poor pink metachromatic staining with azure A even at lower pH level (pH 1.5), which was not enhanced with increasing pH levels. These sulfomucins wre resistant to mild methylation but active methylation-saponification procedures effected an irreversible loss of alcianophilia. These sulfomucins wre hyaluronidase resistant and there was no enhancement in the alcianophilia by prior pepsin digestion.

The presence of neutral mucosubstances in the bile was inferred by slight reduction in the PAS reactivity by phenylhydrazine pretreatment, purple-blue staining with AB pH 1.0-PAS, AB pH 2.5-PAS and C.I.-PAS sequential staining procedures and ehnanced metachromatic pink staining with azure A at pH 1.5 following sulfation.

The aforementioned histochemical reactivities lead to the conclusion that the bile in the gall bladder of sea bird contained neutral mucosubstances (poor) and sulfomucins (poor).

DISCUSSION

An insight into the existing literature has pointed out that the aveian gall bladder is the most neglected organ, particularly from histochemical point of view. Upto some extent, the ultrastructure of the gall bladder epithelial cells has been reported in fowl (Bader, 1965) and quail (Yamada, 1970).

Patil (1985) has comparatively studied the gall bladders in seven species of birds, viz. sparrow, koel, pond heron, hornbill, crow, kite and kingfisher. He found a thick gall bladder wall in the pond heron and kite than in other birds. The present investigation also revealed variations in the thickness of the gall bladder walls. It was thin in myna and sea bird, while moderately thick in guinea fowl and duck. The epithelial cells in the gall bladder mucosa in all the four birds were of only one type (goblet cells were absent). Patil (1985) also reported only a singular type of epithelial cells in all the seven species of birds that he had studied. The epithelial cells were cuboidal in myna and sea bird, cuboidal to low columnar in guinea fowl, while tall columnar in duck. Similar results were reported by Patil (1985). He found tall columnar epithelial cells in the gall bladder of koel, kite and kingfisher, but cuboidal in the rest of the birds.

Yamada and Hoshino (1972) reported the absence of glands in the gall bladder of fowl. Okada (1951) reported that the gall bladder epithelial cells in chick during the development become high columnar from the stratified to pseudostratified epithelium in chick. Patil (1985) has reported the absence of the glands in sparrow, koel, pond heron and hornbill. On the other hand, for the first time, he reported the presence of glands in the gall bladder of crow, kite and kingfisher.

The present investigation also revealed the absence of glands in myna and sea bird, but the presence of glands in guinea-fowl and duck.

At present, except for the report from Patil (1985) on the gall bladders of crow, kite and kingfisher, the glands have not been reported in the gall bladder of birds. On the other hand, Yamada and Hoshino (1972) reported on the down-growth of the epithelial cells in the gall bladder of chick. According to him, this may be the earlier stage in the formation of the glands.

The histochemical observations revealed the heterogenous distribution of neutral mucosubstances, sulfomucins and sialomucins in the various histological sites in the gall bladder of the present birds. The brush border contained a mixture of neutral mucosubstances (poor) and sulfomucins (weak) in guinea fowl, a mixture of neutral mucosubstances (weak) and sulfomucins (weak) in myna and a mixture of neutral mucins (poor) sulfomucins (poor) and sialomucins (poor) in the sea bird, while a mixture of neutral mucosubstances (weak) and sialomucins, both in poor amount in duck. The apical granules in the gall bladder epithelial cells contained varied quantities of only neutral mucosubstances in all the four present birds.

The brush border of gland cells in guinea fowl contained a mixture of neutral mucosubstances (poor) and sulfomucins (weak to moderate) and in duck, a mixture of neutral mucins (poor to weak), sulfomucins (poor) and sialomucins (poor). These results resembled to the brush border of gall bladder epithelial cells in these birds. The apical granules in the gland cell contained only neutral mucins (weak to moderate in guinea fowl and only weak in duck). However,

the glands were found to be absent in myna and sea bird.

The bile duct was seen along with the gall bladder in duck. Like the brush border of the gall bladder epithelial cells, the duct cells also elaborated a mixture of neutral mucins, sulfomucins and sialomucins. The bile in the gall bladder of guinea fowl and sea bird contained a mixture of neutral mucosubstances (poor in guinea fowl and sea bird), and sulfomucins (weak in guinea fowl and poor in sea bird). Yamada and Hoshino (1972) also reported the sulfated, carboxylated and neutral mucopolysaccharide-protein complexes in the gall bladder epithelial cells of fowl. In a similar histochemical study, Patil (1985) has also reported the heterogenous distribution of sulfomucins, sialomucins and neutral mucosubstances in the various histological sites like the brush border and apical granules in the gall bladder epithelial cells, gland cells, duct cells and in the bile in different birds that he has worked on.

The aforementioned points of discussion indicate species diversity in relation with the presence or absence of glands as glands were found only in two birds from the four used in the present investigation. However, all birds possess only single type of gall bladder epithelial cells, as goblet cells have been found to be absent in these birds. The goblet cells have also not been reported by any one in the gall bladder epithelial cells of any bird previously.

The birds used in the present investigation possess different dietary habits such as graminivorous (guinea fowl), omnivorous (myna), vegetarian (duck) and carnivorous (sea bird). The present investigation revealed varied quantities of neutral mucosubstances and sulfomucins

in brush border of gall bladder epithelial cells and gland cells in guinea fowl (graminivorous), myna (omnivorous) and sea bird (carnivorous) and a mixture of neutral mucosubstances, sulfomucins and sialomucins in these histological sites in duck (vegetarian - mixed surface feeder). Furthermore, the apical granules in epithelial cells and gland cells contain only neutral mucosubstances but in varied amounts in all the four birds though they are having different dietary habits. Therefore, the present ivnestigation reveals that there is no relationship between the presence or absence of glands and the nature of mucosubstances in the different histological sites of gall bladder and the dietary habits of the birds.

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