CHAPTER - THREE

OBSERVATIONS

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON MUCOSUBSTANCES IN THE COWPER'S GLANDS OF MAMMALS AND EFFECTS OF CASTRATION AND TESTOSTERONE PROPIONATE

The male reproductive system in mammals consists of paired gonads (testes), epididymis, vas deferens and several accessory glands such as ampullary glands, seminal vesicles (vesicular glands or vesiculae seminales), prostate glands, coagulating glands, urethral glands (glands of Littre'), bulbourethral glands (Cowper's glands) and preputial glands. Several variations occur in the presence or absence, number, size, seasonal alterations (in seasonal breeding animals) and the secretions elaborated by the accessory glands. For example, the seminal vesicles are greatly developed in some animals when prostate is small and vice versa. In some of the mammals belonging to the lower orders such as Monotremata, the Cowper's glands are the only accessary glands present which contribute the seminal plasma. The Cowper's glands contribute 10-25 % of the seminal plasma (Nalbandov, 1970).

The literature on presence or absence, number, position, size, shape, anatomy and morphology of the Cowper's glands is very elaborately reviewed by Eckstein and Zuckerman (1962). The literature on some aspects such as histology, seasonal alterations, effects of castration and subsequent steroid hormones (androgens and other hormones) and enzymes, lipids, and mucosubstances in the Cowper's glands of mammals is reviewed by Baile (1976), Pawar (1978), Kanase (1979), Mote (1980), Vibhute (1980) and Fartade (1981).

The following is a brief account on the histological and histochemical observations on Cowper's glands of the mammals employed in the present investigation. These animals were collected irrespective of their active breeding period (in case of seasonal

breeding animals) and hence the observations also include the testicular histology of the corresponding animals. At present it is decided that chromatographic separation and identification of simple sugars, bioassay studies on mucosubstances, seasonal variations and effects of castration and castration followed by androgen administration on Cowper's glands of some animals employed in the present investigation and some additional animals will be investigated for Post- M.Phil.research and efforts are going on in these directions.

O B S E R V A T I O N S

1. Horseshoe bat:

A) Histological observations :

a) Testis :

The sections of the testis stained with H-E (Fig.1) consisted of enlarged seminiferous tubules and hence the inter-tubular connective was very narrow. The wall of each seminiferous tubule was multilayered and contained germinal epithelium, spermatogonia, spermatocytes, spermatids and spermatozoa. Occasionally Sertoli cells could be identified. The interstitial or Leydig cells were found to be hypertrophied. This histological architecture revealed that the bats of this species were in active breeding phase of the sex-cycle. In late July, August and early September, the male and female in a single pair colony remained together. In some colonies, the sexes separated in early September.

b) Cowper's glands :

A single pair of Cowper's glands were observed, in this bat. They were abdominal in position lying one on each side

CAPTIONS TO FIGURES

Fig.1 Testis of the bat stained with H-E to show an intentense spermatogenesis in a seminiferous tubule X 400.

Fig.2 Cowper's gland of the bat stained with H-E to show lumina of acini and central cavity filled with secretion X 150.

Fig.3 A magnified view of Fig.2 to show cuboidal glandular cells, secretion and covering of striated muscles X 300.

Fig.4 Cowper's gland of the bat stained with M-T. Note secretion faint to dark blue and muscles - red X 150.

Fig.5 Cowper's gland of the bat stained with PAS to show weak staining in the cells and moderate to intense staining in the cells and moderate to intense staining in the secretion X 150.

Fig.6 Cowper's gland of the bat stained with Ph- PAS. Note absence of staining in the glandular cells and secretion. Some of the acinar lumini showed weak staining even after phenylhydrazine treatment X 150.

Fig.7 Cowper's gland of the bat stained with PAS after α-amylase digestion. Note slight reduction in staining itensity in all the histological sites X 150.

Fig.8 Cowper's gland of the bat stained with AB pH 1.0 - PAS. Note absence of alcianophilia and only PAS reactivity as in Fig.5 X 150.

ABBREVIATIONS

C - Glandular epithelial cells.

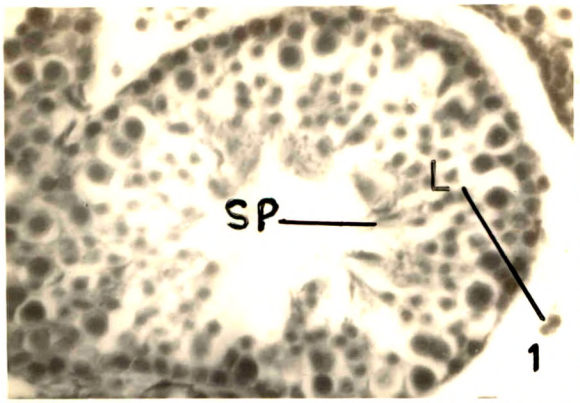
CC - Central cavity.

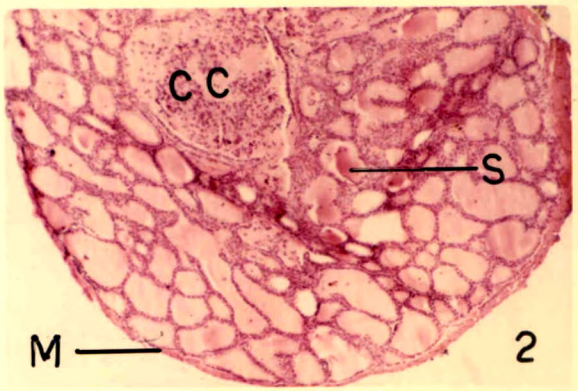
L - Leydig cells.

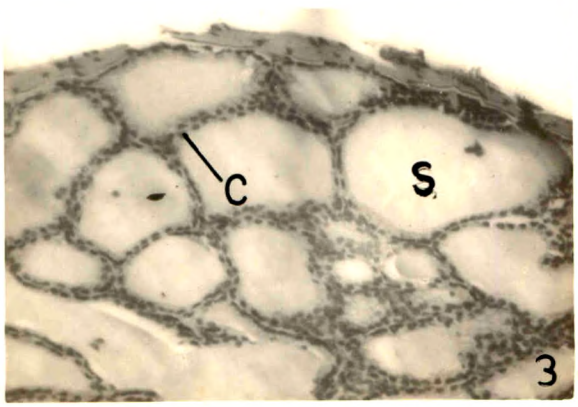
M - Muscles.

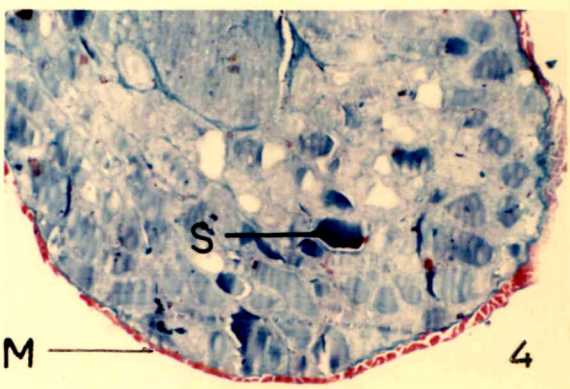
S - Secretion.

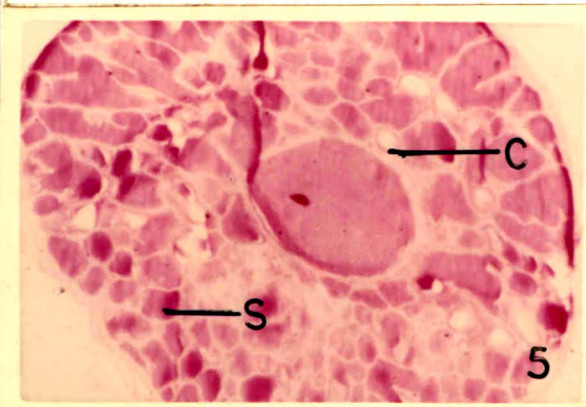
SP - Spermatozoa.

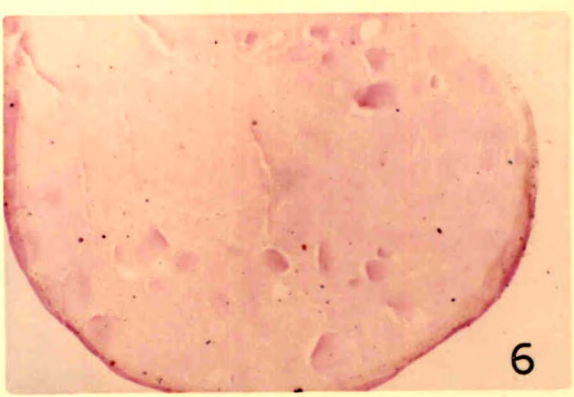

SP
L
1

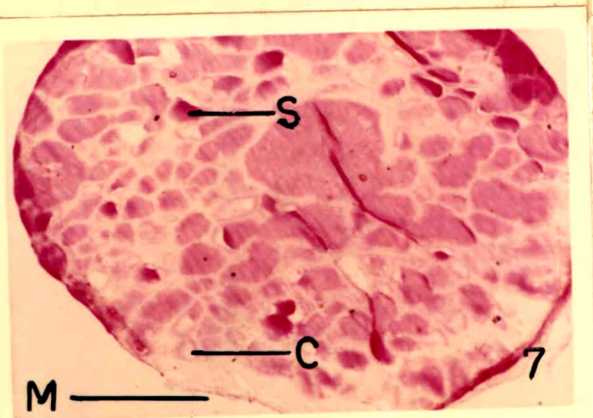

C C
S
M
2

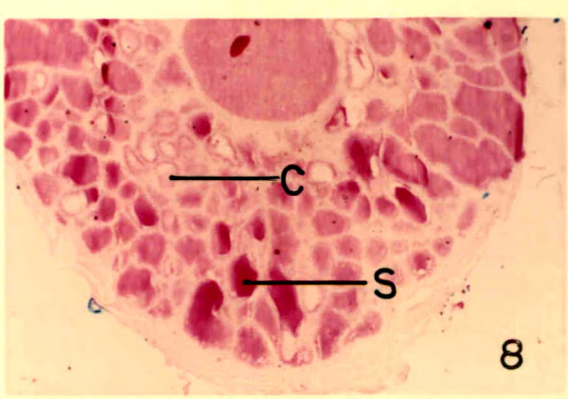

C
S
3


S
M
4


C
S
5


6


S
C
M
7


C
S
8

distal to the prostate and opened independently in the lumen of the muscular urethra. Each gland was oval in shape and attained the size of 1 to 1.5 mm. in length and 1 mm. in diameter. The glands consisted of several acini of variable size. The acini were not partitioned into lobules.

H-E stained preparations (Fig. 2, 3) revealed that the acini were lined by a single layer of cuboidal epithelial glandular cells. True connective tissue capsule was absent. Each gland was covered with a thin layer of striated muscles. Due to the enlargement of the acini, the interacinar connective tissue was reduced in the thickness. The lumini of the acini were full of homogenous secretion. The secretion was first collected in a central cavity (Figs.2, 4, 5, 8) and then carried to the urethral lumen.

With H-E staining, the nuclei appeared blue, cytoplasm of the cells and the secretion in the acinar lumina and in the central cavity was stained pink. M-T staining procedure stained nuclei-red, cytoplasm- faint blue and secretion - dark blue (Fig.4). Since the bats were in active breeding phase of the sex-cycle, most of the secretion was released in the acinar lumen and also appeared in the central cavity, hence the cells exhibited comparatively less staining than the secretion.

B) Histochemical observations :

The histochemical results on mucosubstances in the Cowper's glands of the bat are recorded in Table No.2 according to the visually estimated intensity of staining and shade with ++++ representing an intense staining. The histochemical results are illustrated in photomicrographs (Figs. 5 to 8). The histochemical results requiring further description and consideration are

Table No.2: Histochemical staining reactivity of mucosubstances in the Cowper's glands of horseshoe bat.

Sr. No.	Histochemical Reaction	Glandular Epithelial Cells	Secretion
1	PAS	++P	++++P
2	Ph-PAS	-	-
3	α-amylase-PAS	+P	+++P
4	AB pH 1.0	-	-
5	AB pH 1.0 - PAS	++P	++++P
6	AB pH 2.5	-	-
7	AB pH 2.5-PAS	++P	++++P
8	C.I.	-	-
9	C.I.-PAS	++P	++++P
10	AF	-	-
11	AF-AB pH 2.5	-	-
12	Azure A pH 1.5	-	+0
13	Azure A pH 3.0	+0	++0
14	Azure A pH 4.5	++0	++++0
15	Sulfation-Azure A pH 1.5	++M	++++M
16	CEC - 0.0 M Mg^{++}	-	-
17	CEC - 0.1 M Mg^{++}	-	-
18	M-37°C-AB pH 2.5	-	-
19	M-37°C-S-AB pH 2.5	-	-
20	M-60°C-AB pH 2.5	-	-
21	M-60°C-S-AB pH 2.5	-	-
22	Acid hydrolysis - AB pH 2.5	-	-
23	Sialidase - AB pH 2.5	-	-
24	Hyaluronidase - AB pH 2.5	-	-
25	Pepsin - AB pH 2.5	-	-

The secretion remain unstained with AB at both the pH levels, C.I. and AF. Azure A stained secretion only blue, the intensity of this orthochromatic staining increased gradually from pH 1.5 to 5.0, thus indicating the absence of acidic mucosubstances in the secretion.

The presence of neutral mucosubstances (moderate amounts) and glycogen (poor quantities) in the secretion was further supported from its only pink or magenta colouration with AB pH 1.0 - PAS (Fig.8), AB pH 2.5 - PAS, C.I. - PAS staining procedures and an intense pink metachromasia after sulfation only.

2. Rabbit

A) Histological observations :

a) Testis :

H-E stained preparations (Figs.9, 10) revealed very thin connective tissue between the seminiferous tubules. This may be due to the enlargement in the diameter of the tubules. Each seminiferous tubule consisted of germinal epithelial cells which rested on the basement membrane, spermatogonia, spermatocytes, spermatids and few spermatozoa. The Leydig cells occurred in groups. They may be considered slightly hypertrophied and started secreting androgens. This conclusion was based on the histology of the Cowper's glands (Fig.12), wherein most of the acinar lumini were filled with secretion. According to the testicular histology, the male rabbits were considered in the late prebreeding phase of the reproduction. This phase was not considered as sexual quiescence as the lumen of each seminiferous tubule was not empty. The rabbits were not in the post-breeding phase, since cell debris of residual spermatozoa was not observed in any of the

CAPTIONS TO FIGURES

Fig.9 Testis of the rabbit stained with H-E. Note the absence of clear lumina in the seminiferous tubules, stages of spermatogenesis and interstitial Leydig cells X 150.

Fig.10 Enlarged view of Fig.9 to show few spermatozoa X 500.

Fig.11 Cowper's gland of the rabbit stained with H-E to show two prominent lobules partitioned by prominent connective tissue. Note several acini in each lobule with secretion in their lumini X 150.

Fig.12 A magnified view of Fig.11. Note the cuboidal cells lining in each acinus and secretion in the lumen X 450.

Fig.13 Cowper's gland of the rabbit stained with M-T to show thin inter-acinar connective tissue, prominent inter-lobular connective tissue, glandular epithelial cells and luminal secretion X 200.

Fig.14 Cowper's gland of the rabbit stained with AB pH 1.0. Note absence of staining in the cells and secretion. Alcianophilia is evident only in the mast cells X 200.

Fig.15 Cowper's gland of the rabbit stained with C.I. Note poor or trace C.I. reactivity in the cells, and poor to weak staining in the luminal secretion X 200.

Fig.16 Cowper's gland of the rabbit stained with C.I.-PAS to show weak purple-blue staining in the cells and weak to moderate staining in the luminal secretion X 200.

ABBREVIATIONS

C - Glandular epithelial cells.

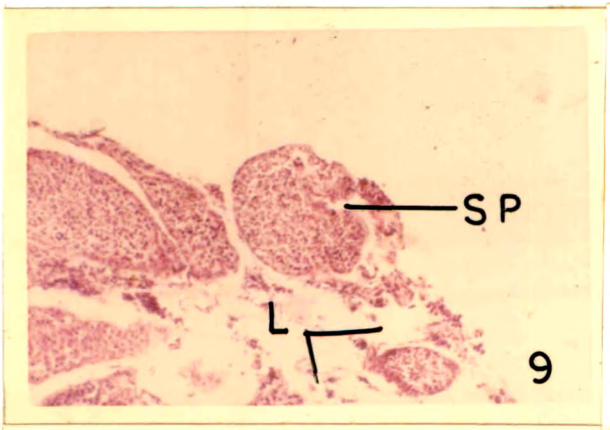
L - Leydig cells.

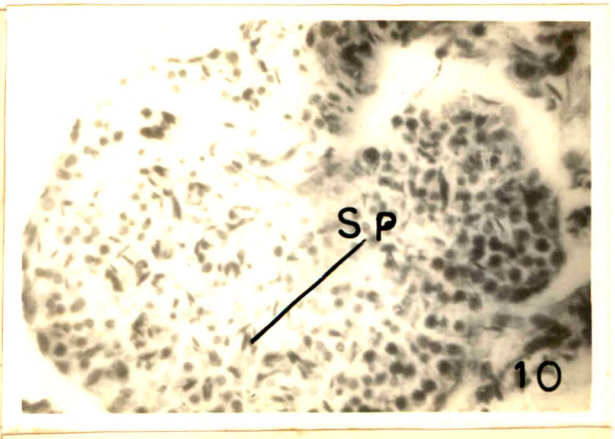
S - Secretion.

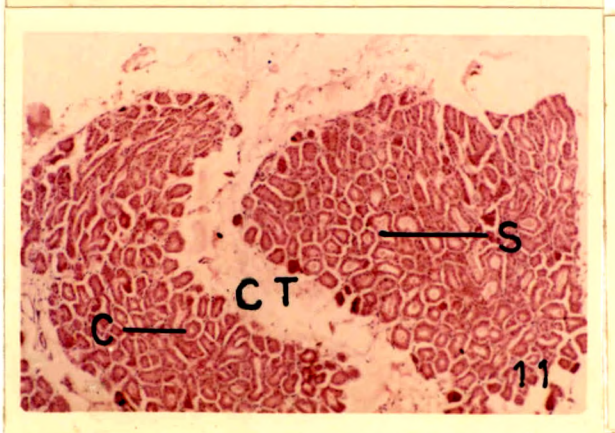
CT - Connective tissue.

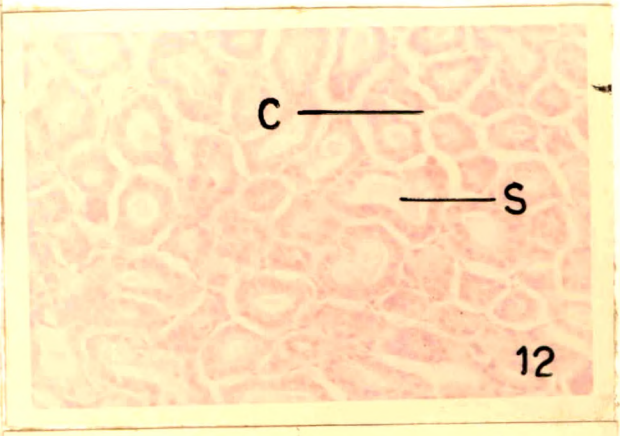
MC - Mast cells.

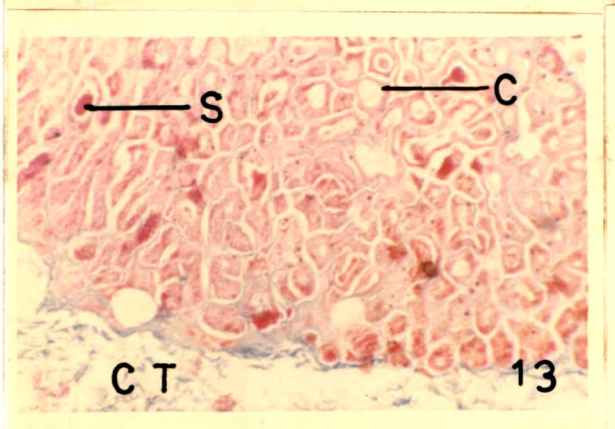
SP - Spermatozoa.

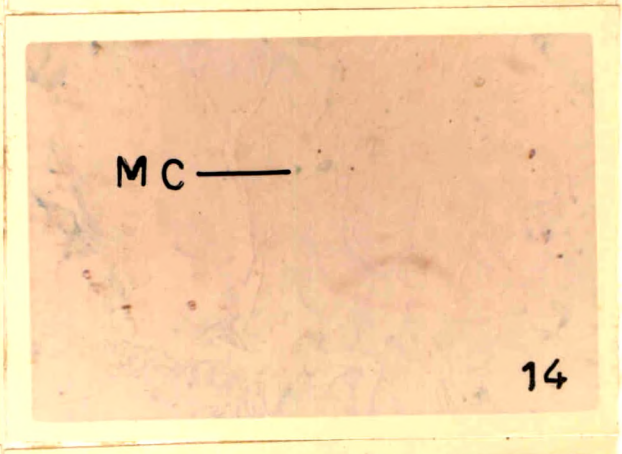

SP
L
9

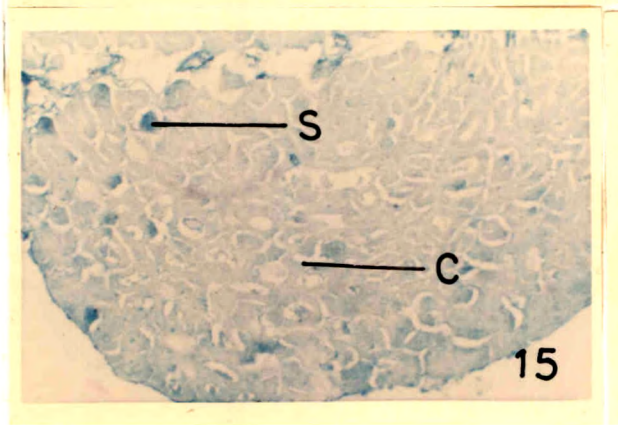

SP
10

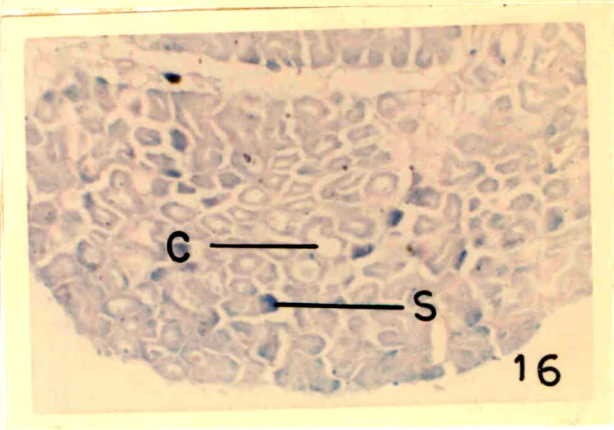

S
CT
C
11


C
S
12


S
C
CT
13


MC
14


S
C
15


G
S
16

seminiferous tubule. Moreover, spermotogenesis in these tubules was not very intense or rapid. From these observations it was concluded that the male rabbits were in late prebreeding phase or in early active breeding phase of the reproductive cycle.

b) Cowper's glands :

Paired Cowper's glands were observed in the rabbits, lying one on each side of the muscular urethra. They were abdominal in position and each gland opened in the muscular urethra by three ducts. The glands were yellow in colour. The glands measured 2.3 mm. in length and 1.5 mm. in the breadth. Histology of the glands in H-E stained sections revealed that the glands were multilobulated (Fig.11) and covered by a thin layer of striated muscles. The interlobular connective tissue was prominent (Figs.11, 13) than the thin connective tissue layer between the acini. Each lobule consisted of numerous acini filled with homogenous secretion. Some acini contained little amount of secretion in their lumini (Figs. 12, 13). The acini were lined by cuboidal to low columnar epithelial cells with basal nuclei.

The nuclei of the glandular epithelial cells stained blue and cytoplasm pink in H-E staining procedure. The secretion appeared faint pink. In M-T staining method (Fig.13), the nuclei stained red, cytoplasm - slight blue to orange, secretion - faint to dark orange, connective tissue - faint blue and muscles - pink.

B) Histochemical observations :

The histochemical data on Cowper's glands of rabbits are recorded in Table No.3 and illustrated in photomicrographs (Figs. 14-16).

Table No.3 : Histochemical staining reactivities of mucosubstances in Cowper's glands of rabbit.

Sr. No.	Histochemical Reaction	Glandular Epithelial Cells	Secretion *
1	PAS	++±P	++±P
2	Ph-PAS	±P	++P
3	α-amylase-PAS	++±P	++±P
4	AB pH 1.0	-	-
5	AB pH 1.0 - PAS	++± P	++±P
6	AB pH 2.5	±B	++B
7	AB pH 2.5-PAS	++±BP	++±PB
8	C.I.	±B	++B
9	C.I.-PAS	++±BP	++±PB
10	AF	±P	++P
11	AF-AB pH 2.5	±B	++B
12	Azure A pH 1.5	-	+0
13	Azure A pH 3.5	±M	++M
14	Azure A pH 4.5	±M	++M
15	Sulfation-Azure A pH 1.5	+++M	++±M
16	CEC + 0.0 M Mg^{++}	++±B	+++B
17	CEC + 0.1 M Mg^{++}	-	-
18	CEC + 0.2 M Mg^{++}	-	-
19	M-37°C-AB pH 2.5	-	-
20	M-37°C-S- AB pH 2.5	±B	++B
21	M-60°C-AB pH 2.5	-	-
22	M-60°C-S-AB pH 2.5	±B	++B
23	Acidhydrolysis - AB pH 2.5	-	-
24	Sialidase - AB pH 2.5	-	-
25	Hyaluronidase - AB pH 2.5	±B	++B
26	Pepsin - AB pH 2.5	±B	++B

* Secretion in the lumini of some acini exhibited slightly moderate staining.

a) Glandular epithelial cells :

These cells reacted weak to moderately with PAS reaction. Phenylhydrazine pretreatment almost blocked their PAS staining except very poor (trace) staining in these cells but α-amylase digestion had no effect on their PAS reactivity. These results indicated the presence of neutral mucosubstances (weak amounts) but absence of glycogen in the cells. The cells remain unstained with AB pH 1.0 (Fig.14) indicating the absence of sulfomucins. This conclusion was further supported by absence of purple or purple blue or blue-purple staining in AF - AB pH 2.5 sequence, absence of metachromatic staining with azure A at lower pH (pH 0.5 to 2,0) and absence of staining in CEC technique at and above 0.2 M Mg^{++} concentration.

These cells exhibited traces of alcianophilia at pH 2.5, C.I. reactivity (Fig.15) blue staining in AF - AB pH 2.5 sequence, very faint metachromasia with azure A at and above pH 3.0 and extinction of alcianophilia in CEC technique after addition of 0.1 M and higher concentrations of $MgCl_2$. Both mild and active methylations blocked the traces of alcianophilia in these cells and subsequent saponification restored it. These reactivities indicated the presence of carboxymucins (in traces) in these cells which were identified as sialomucins as acid hydrolysis and sialidase digestion eliminated the alcianophilia in these cells.

The presence of neutral mucosubstances (weak in amounts) was inferred from bluish-purple staining in AB pH 2.5 - PAS and C.I. - PAS (Fig.16) sequences and weak to moderate metachromasia with azure A following sulfation. The aforementioned results, thus

indicated the presence of neutral mucosubstances (weak) and traces of sialomucins in the cells.

b) Secretion :

Secretion in the lumen of each acinus showed weak PAS reactivity which was resistant to α-amylase digestion and slightly reduced by phenylhydrazine pretreatment. Therefore it was concluded that the secretion contained neutral mucosubstances in traces but not glycogen.

The secretion was not stained with AB pH 1.0 but showed poor to weak alcianophilia at pH 2.5, C.I. reactivity (Fig.15), purple-blue staining with C.I. - PAS (Fig.16), blue staining with AF - AB pH 2.5, metachromasia with azure A at and above pH 3.0, loss of alcianophilia in CEC staining with 0.1 M and higher concentrations of Mg^{++} and reversible blockade of alcianophilia in methylation and methylation followed by saponification. These results revealed the presence of carboxymucins, which were identified as sialomucins as the alcianophilia of the secretion was labile to acid hydrolysis and sialidase digestion.

The neutral mucosubstances were also characterized from the tinctorial affinities of the secretion in which it appeared purple-blue in AB pH 2.5 - PAS and C.I.- PAS (Fig.16) and metachromasia after induced sulfation. These results were unaffected by pepsin digestion. Thus, the secretion contained traces of neutral mucosubstances and poor to weak sialomucins.

3. House rat :

A) Histological observations :

a) Testis :

The house rats are continuous breeders. Therefore, after

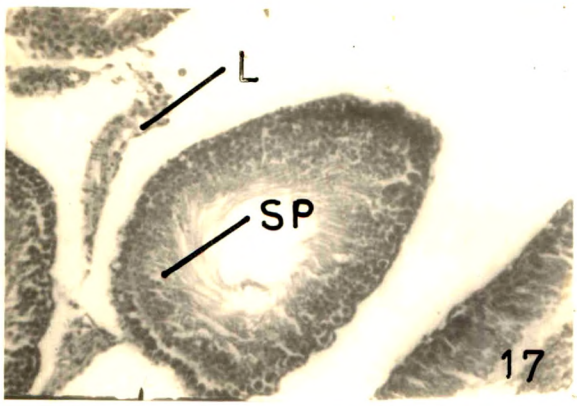
attaining maturity an intense spermatogenesis is found in the seminiferous tubules.

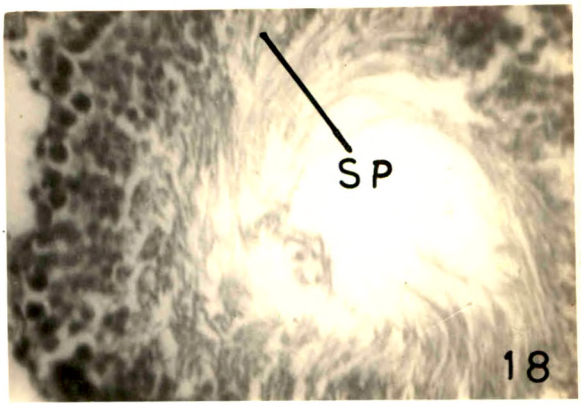
The sections of the house rat testis stained with H-E, contained several stages of spermatogenesis (Figs. 17, 18). Numerous spermatozoa were seen towards the lumen of each tubule. The Leydig cells were found aggregated in groups in the interstitial regions.

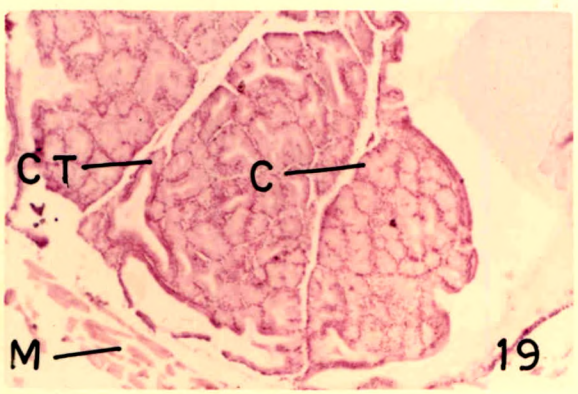
b) Cowper's glands :

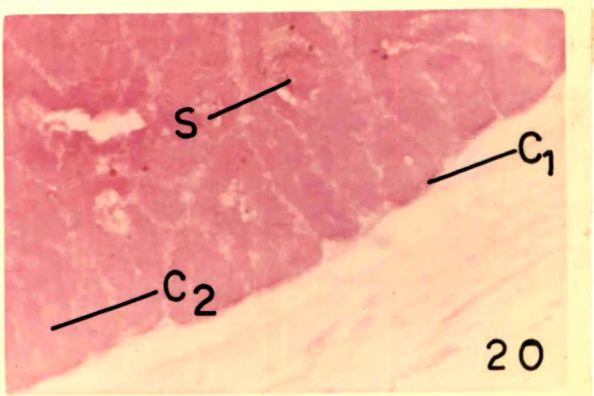
The Cowper's glands were paired, one on each side of the muscular urethra. Each gland has a separate duct which opened in the muscular urethra. The glands were white in colour and ranged from 4-5 mm. in length and 2-3 mm. in diameter.

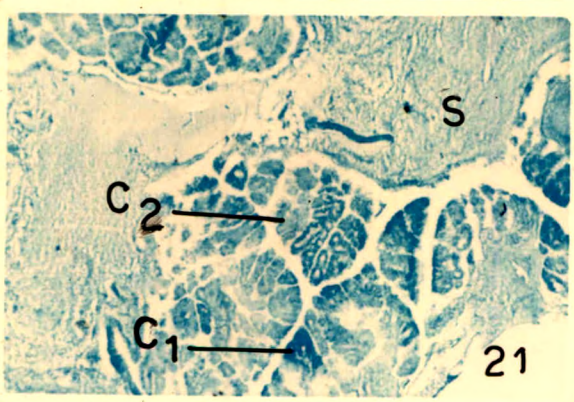
Histological observations with H-E (Fig.19) and M-T staining revealed that the glands were multilobulated and each gland was covered by slightly thick layer of striated muscles. The true connective tissue capsule was absent. The connective tissue was more prominent between the lobules. The glands were acinar type and each acinus was lined by cuboidal to columnar glandular epithelial cells. Although cell types could not be distinguished in histological staining procedure, two type of cells could clearly be distinguished in histochemically stained preparations (Figs.21, 24, 26 to 30). Some of the cells were intensely stained (referred to them as type-I cells) and others weakly stained (referred to them as type-II cells). The secretion was first collected in a large central cavity (Figs.21, 23, 29) and then drained to the lumen of the urethra. In H-E staining, nuclei appeared blue and cytoplasm and the secretion pink. M-T

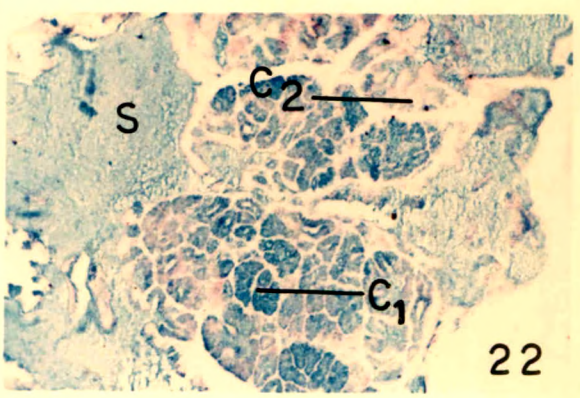

L
SP
17

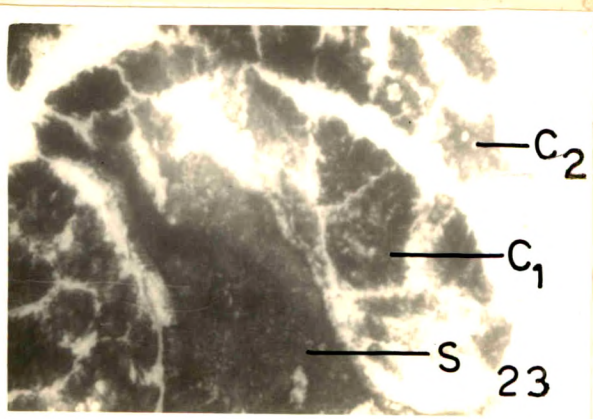

SP
18

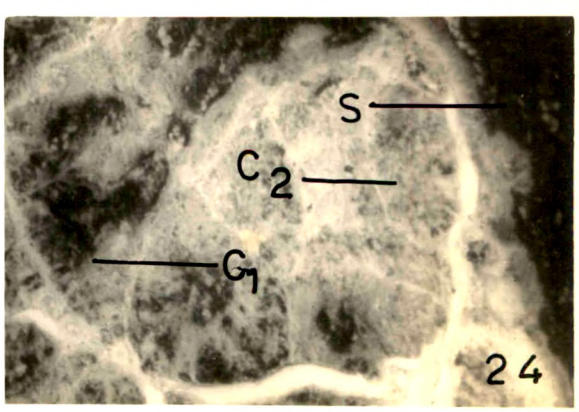

CT
C
M
19


S
C1
C2
20


S
C2
C1
21


S
C2
C1
22


C2
C1
S
23


S
C2
C1
24

CAPTIONS TO FIGURES

Fig.25 Cowper's gland of the house rat stained with C.I. to show type-I cells, type-II cells and secretion X 250.

Fig.26 Cowper's gland of the house rat stained with AF to show an intense staining in type-I cells, weak staining in type-II cells and moderate staining in secretion X 350.

Fig.27 Cowper's gland of the house rat stained with AF. Note a clear distinction between type-I and type-II cells. X 200.

Fig.28 Cowper's gland of the house rat stained with AF-AB pH 2.5 to show cell types and secretion. X 350.

Fig.29 Cowper's gland of the house rat stained with AF-AB pH 2.5. Note blue-purple type-I cells, blue type-II cells and purple or blue-purple stained secretion with some only blue patches X 200.

Fig.30 Another view of the Cowper's gland in house rat to show predominent peripheral type-I cells and few type-II cells. Note abundant secretion in the central cavity X 200.

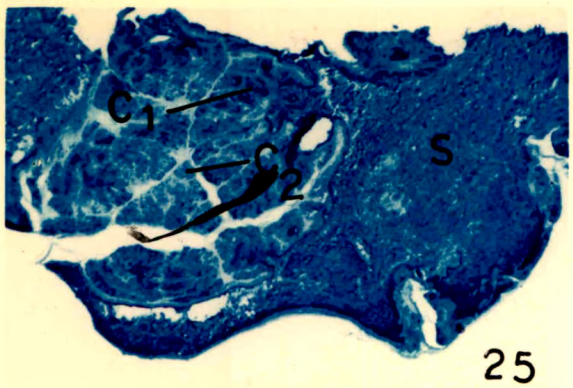
Fig.31 Cowper's gland of house rat stained with AB pH 2.5 after mild methylation to show slightly reduced alcianophilia (than control AB pH 2.5) in type-I cells and complete blockade of alciano philia in type-II cells X 200.

Fig.32 Cowper's gland of house rat stained with AB pH 2.5 after acid hydrolysis. Note reduction in alcianophilia in type-I cells and unstained type-II cells X 200.

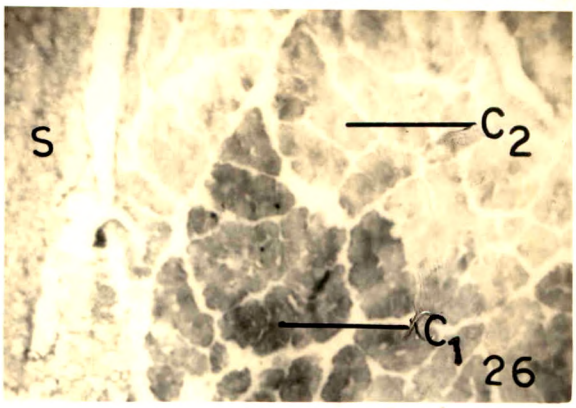
ABBREVIATIONS

C_1 - Type-I cells. C_2 - Type-II cells.

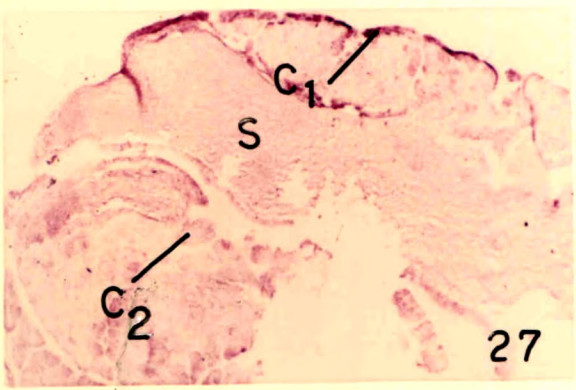
S - Secretion.



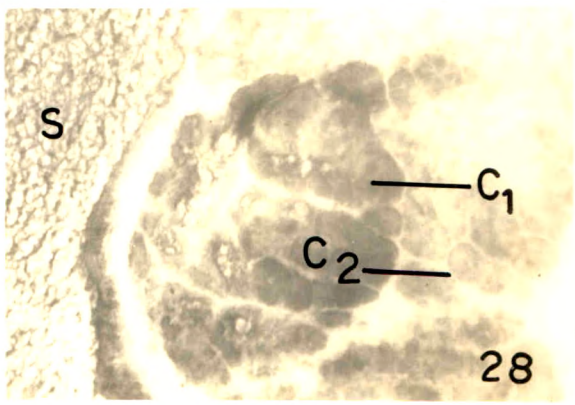
25



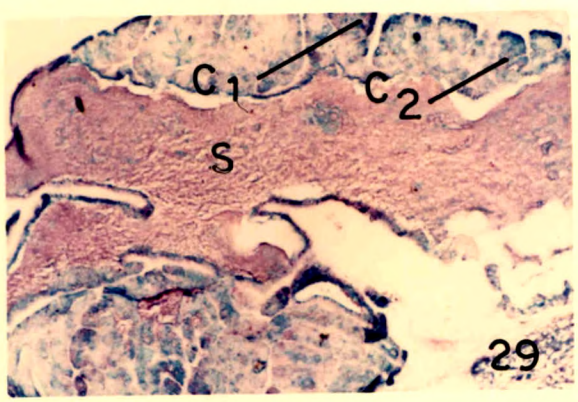
26



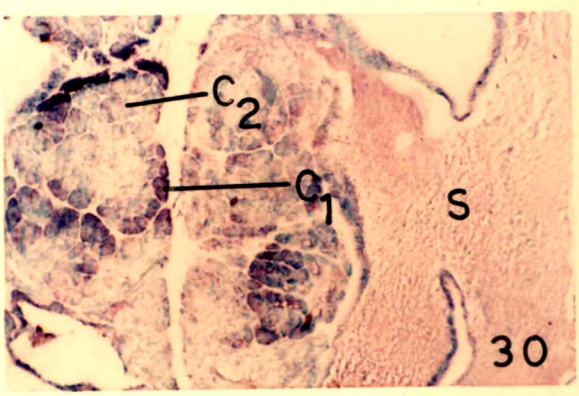
27



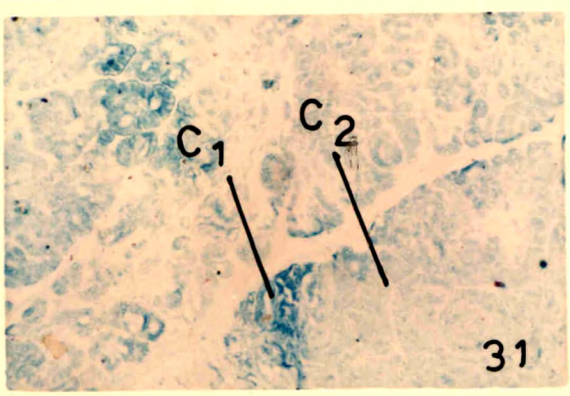
28



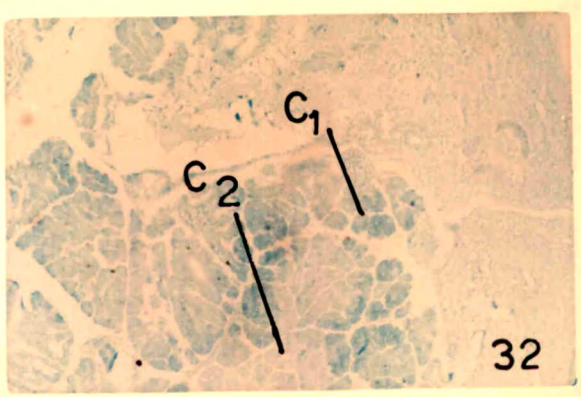
29



30



31



32

staining procedure stained nuclei red, secretion moderate blue and the cytoplasm dark-blue (type-I cells) to faint-blue (type-II cells).

B) Histochemical observations :

The histochemical data on Cowper's glands of house rat are represented in Table No.4 and illustrated in photomicrographs (Figs.20 to 32).

a) Glandular epithelial cells :

i) Type-I Cells :

These cells were intensely stained with PAS (Fig.20). Their PAS reactivity was resistant to both phenylhydrazine pre-treatment and α-amylase digestion indicating the absence of neutral mucosubstances and glycogen.

These cells exhibited moderate alcianophilia at pH 1.0 (Fig.21) which was slightly enhanced at pH 2.5 (Fig.23) and exhibited C.I. reactivity (Figs.24, 25). These results indicated the presence of sulfomucins (predominent) and carboxymucins (traces). The presence of sulfomucins in these cells was also inferred from their stainings purple-blue with AB pH 1.0- PAS (Fig.22), intense purple with AF (Figs.26, 27), blue-purple with AF - AB pH 2.5 (Figs.28 to 30), moderate metachromasia with azure A even at low pH (pH 1.5), persistant alcianophilia in CEC technique upto 0.6 M Mg^{++}, resistant alcianophilia after mild methylation (Fig.31) and irreversible loss with active methylation and methylation followed by saponification. These sulfomucins were resistant to acid hydrolysis (Fig.32) and sialidase and hyaluronidase digestions.

Table No.4 : Histochemical staining reactivities of mucosubstances in the Cowper's glands of house rat, white rat and squirrel.

Sr. No.	Histochemical Reaction	House rat			Shite Rat		Squirrel	
		Glandular Epithelial Cells		Secretion	Glandular Epithelial Cells	Secretion	Glandular Epithelial Cells	Secretion
		Type-I	Type-II					
1	2	3	4	5	6	7	8	9
1	PAS .	++++P	++P	+++±P	++++P	++±P	++P	+++P
2	Ph-PAS	++++P	++P	+++±P	++++P	++±P	-	-
3	α-amylase-PAS	++++P	++P	+++±P	++++P	++±P	+P	++P
4	AB pH 1.0	+++±B	-	++± B	+++B	++B	-	-
5	AB pH 1.0-PAS	++++PB	++P	+++±PB	++++PB	++±BP**	++P	+++P
6	AB pH 2.5	++++B	++B	+++±B	++++B	++±B	-	-
7	AB pH 2.5	++++B	++B	+++±B	++++B	++±B	++P	+++P
8	C.I.	++++B	++B	++++B	++++B	++±B	-	-
9	C.I.- PAS	++++B	++B	+++±B	++++B	++±B	++P	+++P
10	AF	++++P	++P	+++±P	+++±P	+++P	-	-
11	AF-AB pH 2.5	++++BP	++B	+++±P*	++++BP	++±BP	-	-
12	Azure A pH 1.5	+++±M	++0	+++M	+++M	++M	-	±0
13	Azure A pH 3.0	++++M	++M	+++±M	++++M	++±M	+0	++0
14	Azure A pH 4.5	++++M	++M	+++±M	++++M	++±M	++0	+++0

1	2	3	4	5	6	7	8	9
15	Sulfation- Azure A pH 1.5	++++M	++M	+++±M	++++M	++±M	++M	+++M
16	CEC + 0.1 M Mg^{++}	++++B	++B	+++B	++++B	++B	++B	+++B
17	CEC + 0.1 M Mg^{++}	+++±M	-	++±B	+++B	++B	-	-
18	CEC + 0.2 M Mg^{++}	++++B	-	++±B	+++B	++B	-	-
19	CEC + 0.4 M Mg^{++}	++±B	-	++B	++B	±B	-	-
20	CEC + 0.5 M Mg^{++}	++B	-	+B	+B	-	-	-
21	CEC + 0.6 M Mg^{++}	+B	-	±B	-	-	-	-
22	CEC + 0.8 M Mg^{++}	-	-	-	-	-	-	-
23	M 37°C - AB pH 2.5	+++±B	-	++B	+++B	-	-	-
24	M 37°C-S-AB pH 2.5	++++B	++B	+++B	++++B	+B	-	-
25	M 60°C - AB pH 2.5	-	-	-	-	-	-	-
26	M 60°C-S-AB pH 2.5	+B	++B	++B	+B	+B	-	-
27	Acid hydrolysis-AB pH 2.5	+++B	-	++±B	+++B	+B	-	-
28	Sialidase - AB pH 2.5	++±B	-	++±B	+++B	+B	-	-
29	Hyaluronidase- AB pH 2.5	++++B	++B	+++±B	++++B	++±B	-	-
30	Pepsin - AB pH 2.5	++++B	++B	+++±B	++++B	++±B	-	-

* Some patches of secretion stained blue (Fig.29).
** Secretion in the lumini of acini exhibited blue-purple staining, wherein PAS staining was more.

Slight enhancement in alcianophilia of these cells at pH 2.5 (Fig.23) than at pH 1.0 (Fig.21) indicated the presence of carboxymucins. This conclusion was based on their stainings blue-purple with AF - AB pH 2.5 (Figs.28 to 30), enhanced metachromasia with azure A at and above pH 3.0 and reversible blockade of alcianophilia with methylations (Fig.31) and methylations followed by saponification. Slight reduction in their alcianophilia after acid hydrolysis (Fig.32) and sialidase digestion confirmed the carboxymucins as sialomucins.

ii) Type-II Cells :

These cells exhibited weak PAS reactivity which was resistant to prior phylhydrazine treatment and α-amylase digestion. Furthermore the cells remain unstained with AB pH 1.0 (Fig.21) but stained weakly with AB pH 2.5 (Fig.23). These initial results revealed the absence of neutral mucosubstances, glycogen and sulfomucins but presence of carboxymucins in these cells. This conclusion was substatiated by their weak C.I. reactivity (Figs. 24, 25) only blue or blue-green staining with AB 2.5 - PAS and C.I.- PAS, weak purple staining with AF (Figs.26, 27), only blue staining with AF- AB pH 2.5 (Figs.28 to 30), weak metachromasia with azure A at and above pH 3.0, extinction of alcianophilia in CEC procedure at and above 0.1 M Mg^{++} and reversible blockade of alcianophilia in methylations (Fig.31) and methylation saponification procedures. The carboxymucins were characterized as sialomucins as their alcianophilia was completely eliminated by acid hydrolysis (Fig.32) and sialidase digestion.

iii) Secretion :

The secretion was weak to moderately stained with PAS

and phenylhydrazine treatment and α-amylase digestion had no effect on its PAS reactivity. The secretion exhibited weak to moderate alcianophilia at pH 1.0 (Fig.21), moderate alcianophilia at pH 2.5 (Fig.23), C.I. reactivity (Fig.24), blue-purple staining with AF - AB pH 2.5 (Fig.29), weak metachromasia with azure A at pH 1.5 and moderate metachromasia at pH 3.0 and above, persistant alcianophilia in CEC staining method upto 0.6 M Mg^{++} and partial restoration of alcianophilia after saponification of previously methylated sections. Alcianophilia of secretion was partially reduced by acid hydrolysis and sialidase digestion. These histochemical results revealed the presence of sulfomucins (moderate) and sialomucins (traces) but absence of neutral mucosubstances and glycogen in the secretion of the Cowper's glands of house rat.

4. White rat :

A) Histological observations :

a) Testis :

White rats are also continuous breeding animals after attaining maturity. Therefore H-E stained sections of the testis (Figs.33, 34) showed histological picture (spermatogenesis and aggregated groups of Leydig cells) identical to that of house rat testis.

b) Cowper's glands :

A single pair of Cowper's glands was present in white rats. Each gland measured 3-4 mm. in length and 2-3 mm. in breadth. The glands were white in colour. The gland on each side opened in the urethra by a single duct.

CAPTIONS TO FIGURES

Fig.33 Testis of the White rat stained with H-E to show an intense spermatogenesis and Leydig cells X 300.

Fig.34 A part of the seminiferous tubule in Fig.33 is magnified to show several bundles of spermutozoa and free luminal spermatozoa X 600.

Fig.35 Cowper's gland of the white rat stained with H-E to show part of two adjacent lobules and striated muscle covering X 250.

Fig.36 Cowper's gland of the white rat stained with H-E to show few acini containing columnar glandular epithelial cells with basal nuclei and eosinophilic cytoplasm X 400.

Fig.37 Cowper's gland of the white rat stained with M-T to show glandular epithelial cells, interlobular connective tissue and striated muscle covering X 150.

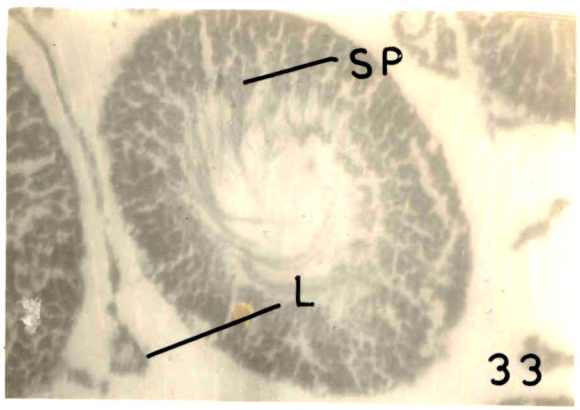
Fig.38 Cowper's gland of the white rat stained with PAS to show intensely stained glandular cells and weakly stained secretion X 150.

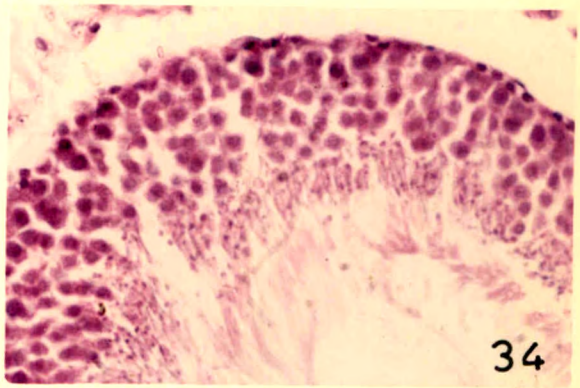
ABBREVIATIONS

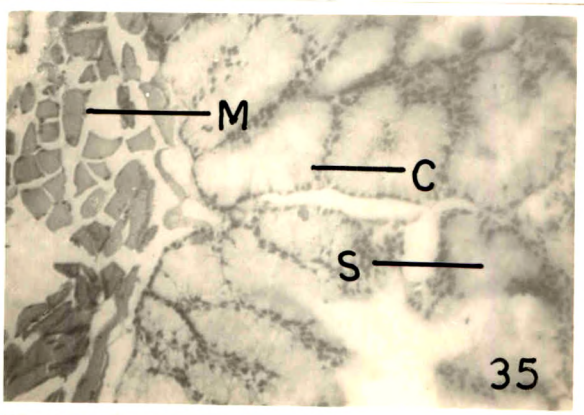
C - Glandular epithelial cells. L - Leydig cells.

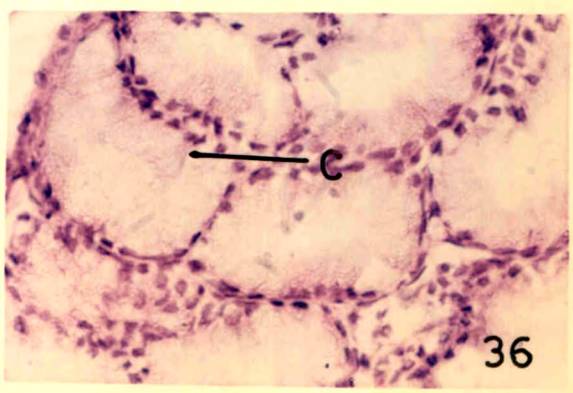
M - Muscles. S - Secretion.

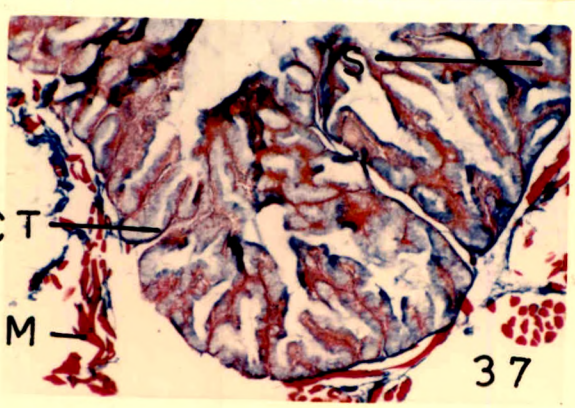
SP - Spermatozoa.

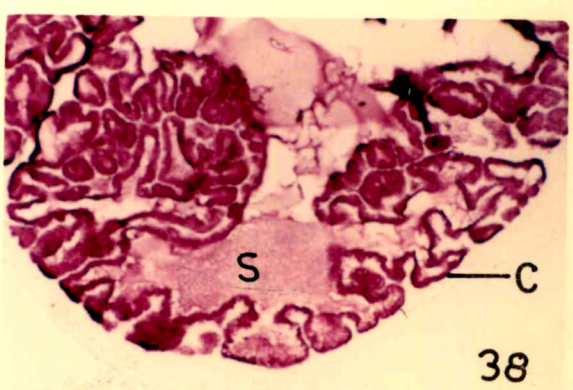

SP
L
33


34


M
C
S
35


C
36


S
C T
M
37


S
C
38

CAPTIONS TO FIGURES

Fig.39 Cowper's gland of the white rat stained with AB pH 1.0. Note moderate alcianophilia in the glandular cells and weak staining in the secretion X 150.

Fig.40 Cowper's gland of the white rat stained with AB pH 1.0 - PAS to show purple - blue staining in the glandular cells. The secretion is shows slightly less staining intensity X 150.

Fig.41 Cowper's gland of the white rat stained with AB pH 2.5 to show intensely stained glandular cells and weakly stained secretion X 250.

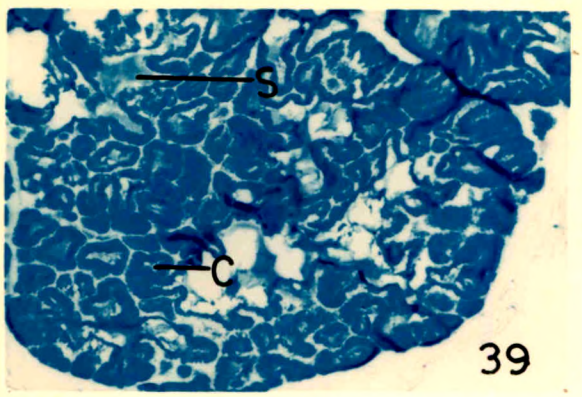
Fig.42 Cowper's gland of the white rat stained with AF to show an intense purple staining in the glandular cells X 150.

Fig.43 Cowper's gland of the white rat stained with AF-AB pH 2.5 to show glandular cells (intense blue purple) and secretion (weak blue-purple) X 150.

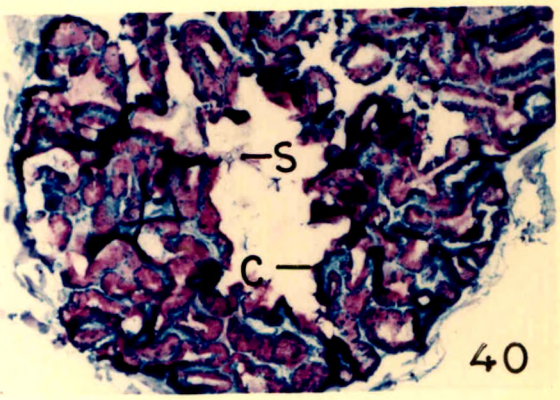
Fig.44 Cowper's gland of the white rat stained with AB pH 2.5 after mild methylation. Note reduced alcianophilia in the glandular cells and secretion than in Fig.41 X 150.

ABBREVIATIONS

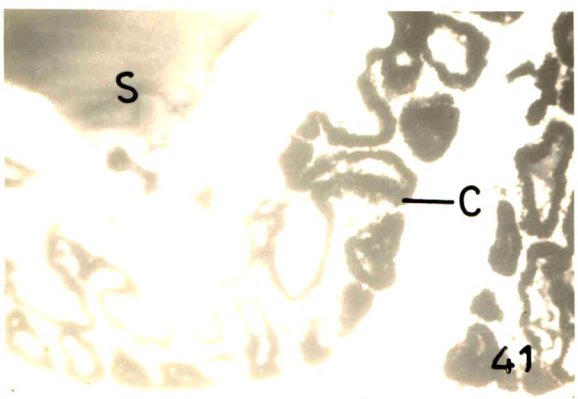
C - Glandular epithelial cells. S - Secretion.



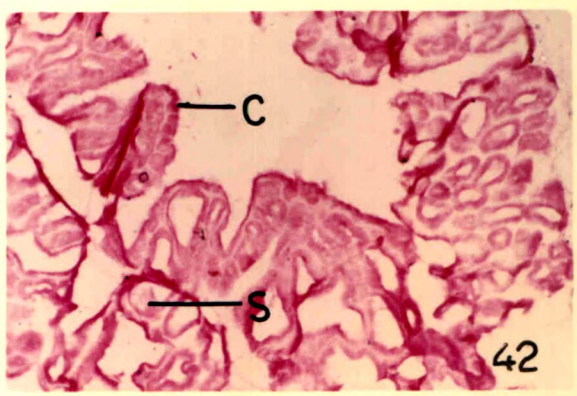
39



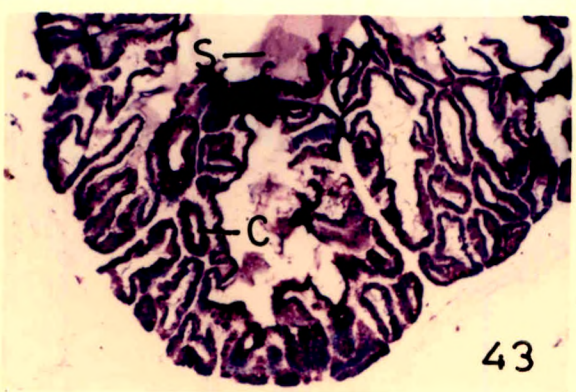
40



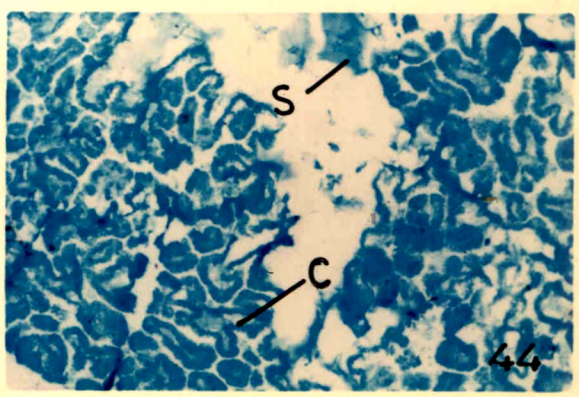
41



42



43



44

The histological observations of the Cowper's gland sections stained with H-E (Figs.35, 36) and M-T (Fig.37) showed that the Cowper's glands in white rat resembled to these glands in house rat. The glandular epithelial cells were only one type unlike house rat.

B) Histochemical observations :

The histochemical results on Cowper's glands of the white rat are recorded in Table No.4 and illustrated in photomicrographs (Figs.38 to 44).

a) Glandular epithelial cells :

PAS staining was intense in these cells (Fig.38) which was unaffected by phenylhydrazine pretreatment and α-amylase digestion. Therefore it was concluded that neutral mucosubstances and glycogen were absent in these cells.

These cells exhibited moderate to intense alcianophilia at pH 1.0 (Fig.39) and AB pH 1.0 - PAS (Fig.40) indicating sulfomucins (predominent). This conclusion was further supported by their purple staining with AF (Fig.42), blue-purple staining with AF-AB pH 2.5 (Fig.43), metachromasia with azure A at pH 1.5, extinction of alcianophilia above 0.6 M Mg^{++} concentration in CEC staining procedure, resistant moderate alcianophilia after mild methylation (Fig.44) and irreversible loss by active methylation and subsequent saponification. The sulfomucins were hyaluronidase resistant.

Only pink to magenta staining in AB pH 1.0-PAS sequence (Fig.40) and enhanced alcianophilia at pH 2.5 (Fig.41) than at pH 1.0 indicated that these cells also elaborate little amounts of

CAPTIONS TO FIGURES

Fig.45 Testis of the squirrel stained with H-E to show intense spermatogenesis, bundles of spermatozoa still attached with Sertoli cells and interstitial Leydig cells X 400.

Fig.46 Cowper's gland of the squirrel stained with H-E to show adjacent part of the lobules, interlobular connective tissue, tubules and acini, cuboidal glandular cells and secretion in the lumini of few acini X 200.

Fig.47 Another H-E stained section of Cowper's gland to show collecting duct in the lobule and a thin striated muscle covering X 200.

Fig.48 Cowper's gland of the squirrel stained with PAS. Note weak staining in the glandular cells and weak to moderate staining in the secretion X 200.

Fig.49 Cowper's gland of the squirrel stained with PAS after phenylhydrazine treatment to show blockad of staining in the cells and secretion. The secretion in the lumen of acinus showed slight PAS staining X 200.

Fig.50 Cowper's gland of the squirrel showing reduced PAS staining after α-amylase digestion X 200.

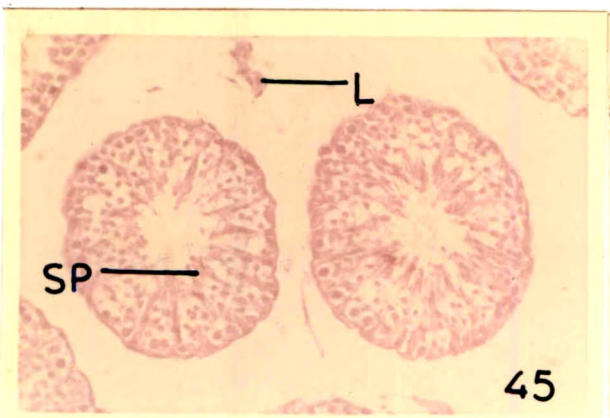
Fig.51 Cowper's gland of the squirrel stained with C.I. to show unstained glandular cells X 200.

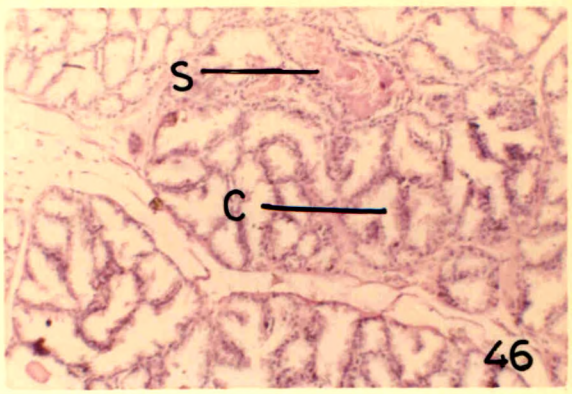
Fig.52 Cowper's gland of the squirrel stained with C.I. - PAS. Note only PAS reactive glandular cells and secretion X 200.

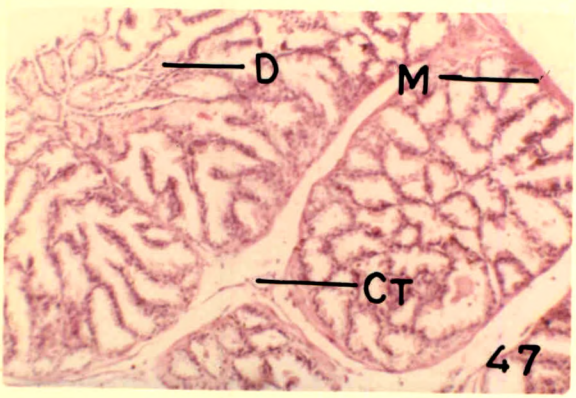
(The C.I. and C.I.-PAS stained sections showed some nuclear staining. The reason is not known.)

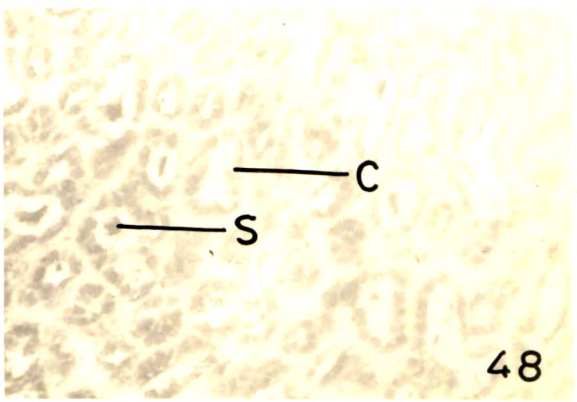
ABBREVIATIONS

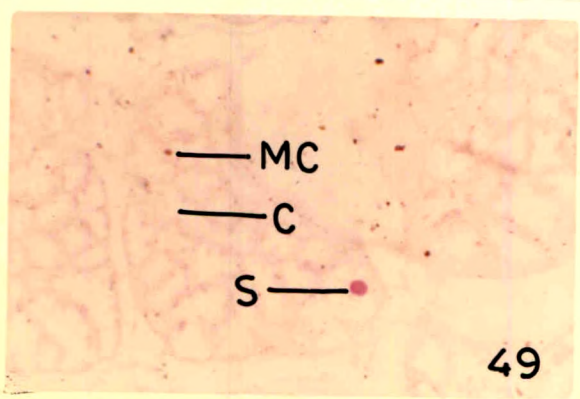
C - Glandular epithelial cells.
CT - Connective tissue.
D - Collecting duct.
L - Leydig cells.
MC - Mast cells.
S - Secretion.
SP - Spermatozoa.

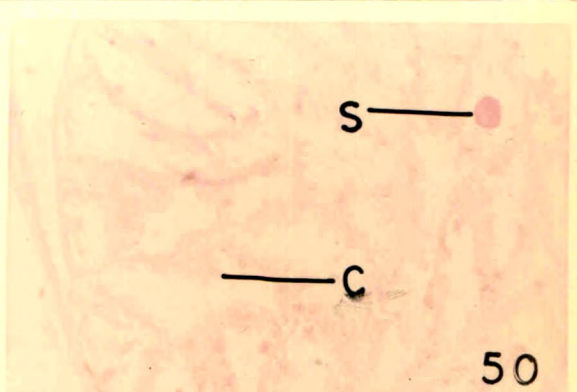

L
SP
45

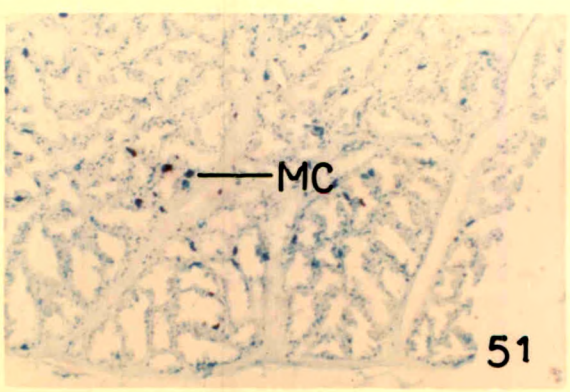

S
C
46

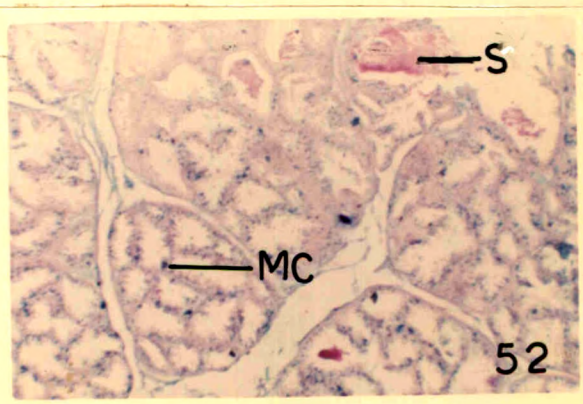

D
M
CT
47


C
S
48


MC
C
S
49


S
C
50


MC
51


S
MC
52

cells might have just started secreting androgens in very small amounts evidenced by meagre amount of secretion in few acini of the Cowper's glands (Figs.46, 48, 52).

b) Cowper's glands :

Paired Cowper's glands were present in the squirrel. They were yellowish in colour, abdominal in position and the glands opened in the urethra by separate ducts. These glands were largest than in the other mammals selected for the present investigation except he buffalo. Each gland measured about 12 mm. in length and 10 mm.in breadth. The glands were slightly irregular in shape.

Histologically the glands were multilobulated (Figs.46, 47), and covered by very thin layer of striated muscles (Fig.47). The glands were tubular or tubulo-acinar in nature. The glandular epithelial cells were cuboidal in shape. In some of the tubules and acini very little amount of secretion accumulated (Figs.46, 48, 52). Each lobule had a separate collecting duct (Fig.47) to drain the secretion in the main duct.

B) Histochemical observations :

Table No.4 includes the histochemical data on Cowper's glands of squirrel and the staining reactivities are illustrated in photomicrographs (Figs.48 to 52).

a) Glandular epithelial cells :

The cells were weakly stained with PAS reaction (Fig.48). Their PAS reactivity was completely blocked by prior treatment with phenylhydrazine (Fig.49) and partly reduced after α-amylase digestion (Fig.50). These preliminary tinctorial affinities and

modifications in the PAS reactivity indicated the presence of neutral mucosubstances (poor) and glycogen (poor).

The cells remain unstained with AB pH 1.0, AB pH 2.5, C.I. (Fig.51), AF and exhibited only blue orthochromatic staining with azure A at higher pH levels. These results could not be modified by pepsin digestion, thus indicating the absence of acidic mucosubstances.

The presence of neutral mucosubstances including glycogen in these cells was inferred from their only PAS staining in AB pH 1.0- PAS, AB pH 2.5 - PAS and C.I. - PAS (Fig.52) sequential staining methods and weak metachromasia with azure A at pH 1.5 after induced sulfation.

b) Secretion :

The secretion in the lumini of few tubules and/or acini (Figs.48-52) also exhibited tinctorial affinities identical to those described above for the glandular epithelial cells except that the intensity of staining with PAS was slightly higher than the cells. Therefore, it was concluded that the secretion contained weak amounts of neutral mucosubstances and poor amounts of glycogen.

A peculiarity of Cowper's glands of the squirrels was the presence of abundant mast cells in the connective tissue between the tubules and/or acini. The mast cells were very intensely stained with C.I. (Fig.51) and C.I. - PAS (Fig.52).

6. He buffalo :

A) Histological observations :

a) Testis :

Multilayerd seminiferous tubules were observed in the H-E stained sections of the buffalo testis (Fig.53). These layers consisted of spermatogonia, spermatocytes, spermatids and spermatozoa. The lumen of the tubule also contained few free spermatozoa. Aggregated masses of Leydig cells were observed in the interstitial regions. From the histology of the testis and meagre amount of residual secretion in the acini of Cowper's glands (Fig.55) it was considered that the he buffalos were in post-breeding phase of reproduction. There are also other possibilities that the he buffalos might have aged or their testes might have crushed with pestle (The latter possibility may be true because in villages, the farmers generally crush the testes in bulls and he buffalos at very young age and they are employed to pull the plough, bullock cart etc.). The servents in the slaughter house did not know about the age of the he buffalos.

b) Cowper's glands :

Single pair of Cowper's glands was observed in the he buffalos. They opened independently by separate duct on each side into the muscular urethra. Each gland was about 4 to 5 cm. in length and 3 cm. in diameter.

The glands consisted of several lobules (clearly seen in Figs.58, 60) and each lobule contained several acini. The glandular cells were tall columnar in shape (Figs.54, 55). The lumen of each acinus had little amount of secretion. Connective tissue was prominent between the acini and lobules. The nuclei appeared blue and cytoplasm pink in the glandular cells in H-E stained sections. The secretion was stained pink. M-T staining gave

CAPTIONS TO FIGURES

Fig.53 A section of he buffalo testis stained with H.E. Note sperm bundles in a part of the seminiferous tubule and interstitial Leydig cells X 500.

Fig.54 Cowper's gland of he buffalo stained with H-E to show few acini lined by columnar glandular epithelial cells with basal nuclei X 300.

Fig.55 Cowper's gland of he buffalo stained with M-T to show columnar cells, secretion, inter-acinar and interlobular connective tissue X 450.

Fig.56 Cowper's gland of he buffalo stained with PAS to show type-I cells (intensely stained and type-II cells (weak to moderately stained) X 150.

Fig.57 Cowper's gland of he buffalo stained with PAS after phenylhydrazine treatment to show reduction in the staining both in type-I and type-II cells X 150.

Fig.58 Cowper's gland of he buffalo stained with AB pH 1.0 to show alcianophilia only in type-I cells X 150.

Fig.59 Cowper's gland of he buffalo stained with AB 2.5 - PAS to show type-I cells (intense blue-purple) and type-II cells (weak to moderate blue-purple) X 150.

Fig.60 Cowper's gland of he buffalo stained with AF-AB pH 2.5. Note only purple staining in type-I cells and blue staining in type-II. Blue-purple stained secretion is seen at the top on left hand side X 150.

ABBREVIATIONS

C - Glandular epithelial cells.

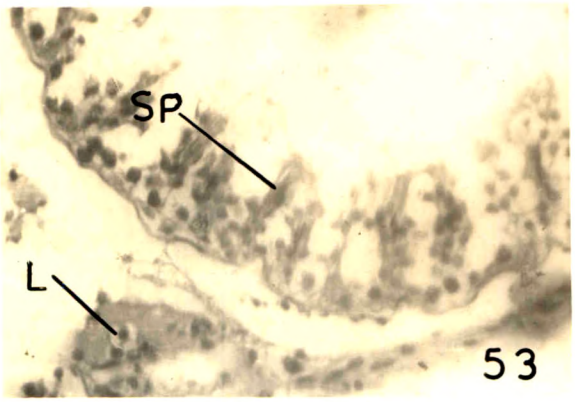
C_1 - Type-I cells.

C_2 - Type-II cells.

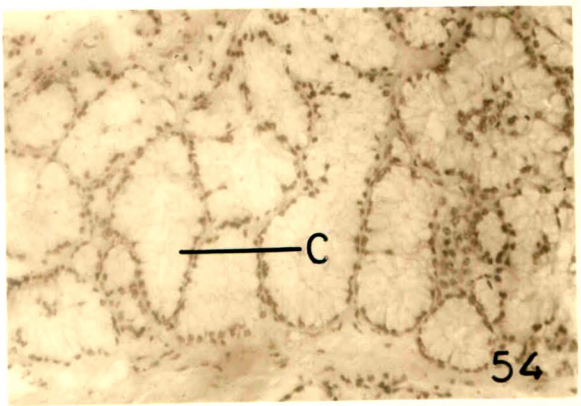
L - Leydig cells.

S - Secretion.

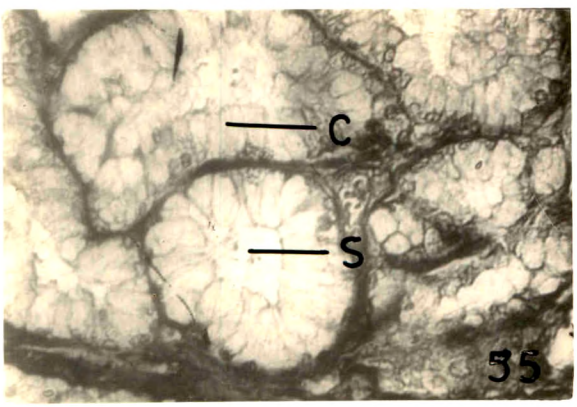
SP - Spermatozoa.



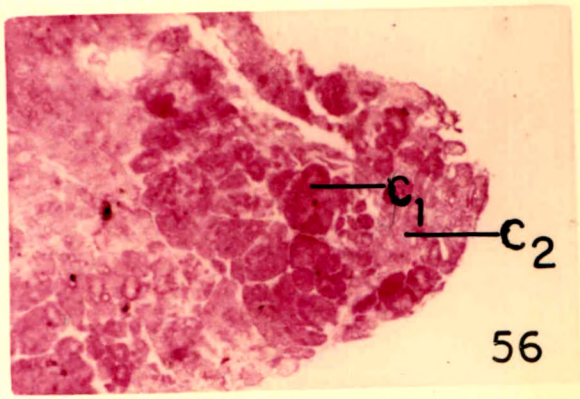
53



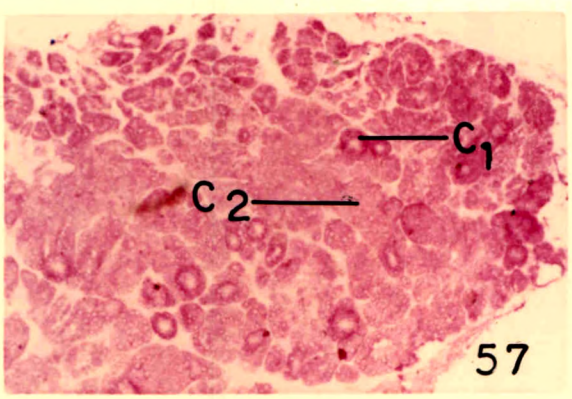
54



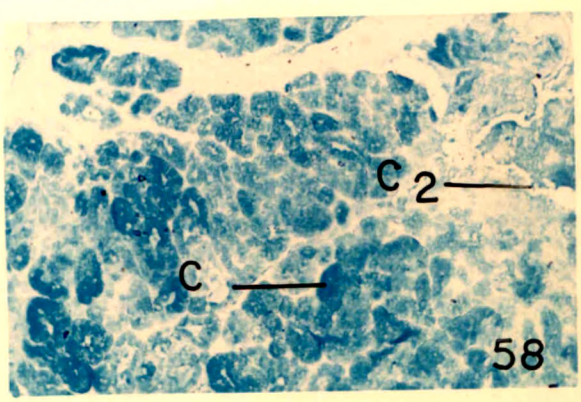
55



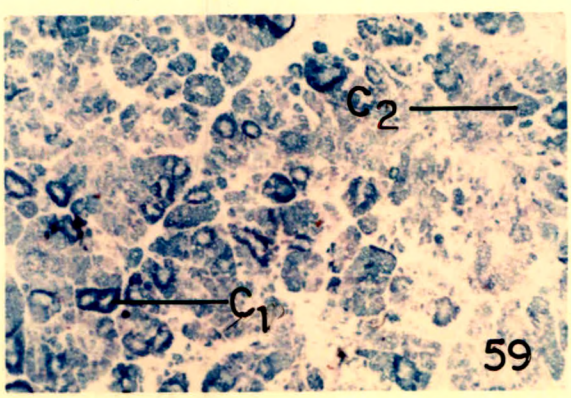
56



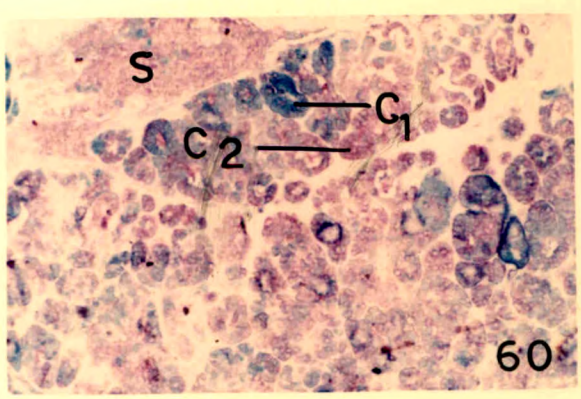
57



58



59



60

nuclei-red, cytoplasm in type I cells - faint blue and in type II cells - orange, secretion-orange blue, connective tissue-dark blue and muscles-pink. The two type of glandular cells could clearly be distinguished in histochemically stained sections (Figs.56 to 60).

B) Histochemical observations :

The histochemical results are recorded in Table No.5 and illustrated in photomicrographs (Figs.56 to 60)

a) Type-I cells :

These cells were intensely stained with PAS (Fig.56) even after x-amylase digestion (Fig.57) but was slightly reduced after phenylhydrazine treatment. These observations revealed the absence of glycogen but presence of neutral mucosubstances (less amount) in these cells.

Moderate alcianophilia was observed in these cells at pH 1.0 (Figs.58) which was not enhanced at pH 2.5 which lead to the conclusion that the cells elaborated acidic mucosubstances which were sulfomucins but not carboxymucins. Some of the histochemical staining results such as purple-blue staining in AB pH 1.0 - PAS, AB pH 2.5- PAS (Fig.59), C.I.-PAS staining sequences, purple staining with AF and AF- AB pH 2.5 (Figs.60), metachromasia with azure A at pH 1.5, 0.5 M Mg^{++} as critical concentration for extinction of alcianophilia and irreversible loss of alcianophilia in methylation - saponification procedures confirmed sulfomucins in these cells.

The neutral mucosubstances in these cells were identified from slight pinkish tinge in AB pH 1.0 - PAS, AB pH 2.5 - PAS (Fig.59) and C.I.-PAS sequences and enhanced metachromasia with

Table No.5 : Histochemical staining reactivities of mucosubstances in the Cowper's glands of he buffalo.

Sr. No.	Histochemical Reactions	Glandular Epithelial Cells		Secretion
		Type-I	: Type-II	
1	PAS	++++P	+±P	++++P
2	Ph-PAS	+++P	+±P	+++P
3	α-amylase-PAS	+++P	+±P	+++P
4	AB pH 1.0	+++B	-	+++B
5	AB pH 1.0- PAS	++++PB	+±P	++++PB
6	AB pH 2.5	+++B	+±B	++++B
7	AB pH 2.5-PAS	++++PB	+±PB	++++PB
8	C.I.	+++B	++B	++++B
9	C.I.- PAS	++++PB	+±PB	++++PB
10	AF	++++P	++P	++++P
11	AF-AB pH 2.5	+++P	++B	++++PB
12	Azure A pH 1.5	+++M	+0	+++M
13	Azure A pH 3.0	+++M	+±M	++++M
14	Azure A pH 4.5	+++M	+±M	++++M
15	Sulfation-Azure A pH 1.5	++++M	+++M	++++M
16	CEC-0.0 M Mg^{++}	+++B	++B	++++B
17	CEC-0.1 M Mg^{++}	+++B	-	+++B
18	CEC - 0.2 M Mg^{++}	+++B	-	++±B
19	CEC - 0.4 M Mg^{++}	++B	-	++B
20	CEC - 0.5 M Mg^{++}	+B	-	+B
21	M-37°C-AB pH 2.5	+++B	-	+++B
22	M-37°C-S-AB pH 2.5	+++B	+±B	++++B
23	M-60°C -AB pH 2.5	-	-	-
24	M-60°C-S-AB pH 2.5	-	+±B	+B
25	Acidhydrolysis - AB pH 2.5	+++B	-	+++B
26	Sialidase- AB pH 2.5	+++	-	+++B
27	Hyaluronidase- AB pH 2.5	+++B	+±B	++++B
28	Pepsin - AB pH 2.5	+++B	+±B	++++B

azure A at pH 1.5 after sulfation.

b) Type-II cells :

These cells showed weak to moderate PAS staining (Fig.56) which was resistant to α-amylase digestion (Fig.57) but phenylhydrazine treatment slightly reduced the staining intensity. Presence of neutral mucosubstances but absence of glycogen was inferred from these results.

These cells were not stained with AB pH 1.0 (Fig.58) and showed poor to weak staining with AB pH 2.5. Some of the important staining reactivities C.I. (blue staining) AB pH 1.0 - PAS, AB pH 2.5 - PAS (Fig.59), C.I.- PAS (purple-blue staining), AF-AB pH 2.5 (blue staining) (Fig.60) and methylation- saponification (reversible blockade of alcianophilia) revealed the presence of carboxymucins in type-II cells. The carboxymucins were identified as sialomucins as alcianophilia in these cells was completely eliminated by acid hydrolysis and sialidase digestion.

The presence of neutral mucosubstances in these cells was inferred from their purple-blue staining in sequential staining with AB pH 2.5 - PAS (Fig.59) and metachromasia with azure A at pH 1.5 after sulfation.

c) Secretion :

The secretion, in general gave the histochemical results more or less identical to those described for sulfomucins and neutral mucosubstances in the type-I cells (Table No.5) and for sialomucins in Type-II cells. Therefore it was concluded that

neutral mucosubstances, sulfomucins and sialomucins were present in the secretion.

7. Results obtained in experimental studies :

As described earlier in Chapter II, the house rats were housed in three cages and each group consisted of three rats. The first group of rats was castrated and kept in separate cages. The second group of rats was castrated and administered with testosterone propionate and kept in separate cages. The third group of normal intact rats was administered with testosterone propionate and then they were kept in separate cages. These rats were sacrificed after 10 days and their Cowper's glands were studied for histology and mucosubstances histochemically.

Nalbandov (1970) described that prostate and seminal vesicles act as sensitive indicators of androgen level in the body. Post-castration changes are found within 2 days in the prostate and after 4 days in the seminal vesicles. Recently, Bargaje (1989) also found atrophic changes in the seminal vesicles of the house rats. He further described that some investigators studied castration induced changes in the seminal vesicles after 5, 10, 20, 30 and 90 days.

Initially in the present investigation, castration effects on Cowper's glands in house rats were studied after 4 days. Significant changes were not observed in the Cowper's glands of such castrated rats. Rajalakshmi and Prasad (1968), found atrophic changes in the Cowper's glands of white rats after 10 days and testosterone administration reversed the castration effects. Therefore the castration induced alterations, castrated rats

receiving testosterone propionate and normal rats treated with testosterone propionate were studied for their Cowper's glands after 10 days. The histological and histochemical alterations and only conclusions drawn in the experimental studies are described hereafter.

I. Effects of castration on Cowper's glands of rats :

The results obtained after castration are represented in Table No.6 and shown in Figs.61 to 64.

As compared to the Cowper's glands of the normal house rats, castration resulted in overall size of these glands, shrinkage of the glands, reduction in the diameter of the acini, reduction in the height of the glandular epithelial cells, reduction in the size of the acinar lumini, and the central cavity and depletion in the rate of secretory activity by the glandular cells.

Histochemically, the overall staining intensities of the glandular epithelial cells and secretion in the Cowper's glands of castrated rats were reduced. The results indicated decrease in the staining for sulfomucins and sialomucins in the type-I cells and secretion and sialomucins in the type-II cells.

II. Effects of testosterone propionate on Cowper's glands of castrated rats :

The results obtained with testosterone propionate administration in castrated house rats are recorded in Table No.6 and illustrated in Figs.65 to 70.

The Cowper's glands of castrated rats after hormone

Table No.6 : Histochemical staining reactivities of mucosubstances in the Cowper's glands of castrated, castrated with testosterone propionate and normal intact house rats with testosterone propionate administration.

Sr. No.	Histochemical Reaction	Castrated rats			Castrated rats + Testosterone propionate			Normal rats + Testosterone propionate		
		Glandular Epithelial Cells		Secretion	Glandular Epithelial Cells		Secretion	Glandular Epithelial Cells		Secretion
		Type-I	Type-II		Type-I	Type-II		Type-I	Type-II	
1	2	3	4	5	6	7	8	9	10	11
1	PAS	++±B	+P	+++P	++++P	++P	+++P**	++++P	++P	+++P
2	Ph-PAS	++±P	+P	+++P	++++P	++P	+++P	++++P	++P	+++P
3	∝-amylase-PAS	++±P	+P	+++P	++++P	++P	+++P	++++P	++P	+++P
4	AB pH 1.0	+±P	-	++B	+++B	-	++B	+++B	-	++B
5	AB pH 1.0 - PAS	+++PB	+P	+++PB	++++PB	++P	+++PB	++++PB	++P	+++PB
6	AB pH 2.5	++±B	+B	+++B	++++B	++B	+++B	++++B	++B	+++B
7	AB pH 2.5- PAS	++±B	+B	+++B	++++B	++B*	+++B*	++++B	++B	+++B
8	C.I.	++±B	+B	+++B	++++B	++B	+++B	+++B	++B	+++B
9	C.I.-PAS	++±B	+B	+++B	++++B	++B	+++B	++++B	++B	+++B
10	AF	++±P	+P	+++P	++++P	++P	+++P	++++P	++P	+++P
11	AF - AB pH 2.5	+++BP	+B	+++BP	++++BP	++B	+++BP	++++BP	++B	+++BP
12	Azure A pH 1.5	++M	-	++M	+++M	+±0	++M	+++M	+±0	++M

1	2	3	4	5	6	7	8	9	10	11
13	Azure A pH 3.0	++±M	+M	+++M	++++M	++M	+++M	++++M	++M	+++M
14	Azure A pH 4.5	++±M	+M	+++M	++++M	++M	+++M	++++M	++M	+++M
15	Sulfatio-Azure A pH 1.5	++±M	+M	+++M	++++M	++M	+++M	++++M	++M	+++M
16	CEC + 0.0 M	++±B	+B	+++B	++++B	++B	+++B	++++B	±B	+++B
17	CEC + 0.1 M	±B	-	++B	+++B	-	-	+++B	-	++±B
18	CEC + 0.2 M	±B	-	++B	+++B	-	++B	+++B	-	++B
19	CEC + 0.4 M	+B	-	+B	++±B	-	±B	++±B	-	±B
20	CEC + 0.5 M	-	-	-	++B	-	+B	++B	-	+B
21	CEC + 0.6 M	-	-	-	+B	-	±B	+B	-	±B
22	CEC + 0.8 M	-	-	-	-	-	-	-	-	-
23	M 37°C -AB pH 2.5	±B	-	++B	+++B	-	±B	+++B	-	++B
24	M 37°C-S-AB pH 2.5	++±B	+B	+++B	++++B	++B	+++B	++++B	++B	+++B
25	M 60°C - AB pH 2.5	-	-	-	-	-	-	-	-	-
26	M 60°C-S-AB pH 2.5	+B	+B	+B	+B	++B	+B	+B	++B	+B
27	Acid hydrolysis-AB pH 2.5	±B	-	++B	+++B	-	++B	+++B	-	++B
28	Sialidase-AB pH 2.5	±B	-	++B	+++B	-	++B	+++B	-	++B
29	Hyaluronidase-AB pH 2.5	++±B	+B	+++B	++++B	++B	+++B	++++B	++B	+++B
30	Pepsin- AB pH 2.5	++±B	+B	+++B	++++B	++B	+++B	++++B	++B	+++B

* Type-II glandular epithelial cells (Figs.67 & 69) and secretion also showed pink to magenta x staining.

** The secretion in the central cavity exhibited moderate PAS staining (Fig.66), in addition intensely stained coagulum or patch like regions were observed.

CAPTIONS TO FIGURES

Fig.61 Cowper's gland of castrated house rat stained with H-E. Note overall reduction in the size of the acini X 200.

Fig.62 Cowper's gland of castrated rat stained with PAS to show reduced staining in the cells and secretion X 400.

Fig.63 Cowper's gland of castrated rat stained with AB pH 1.0 to show reduced alcianophilia in type-I cells. Type-II cells are unstained as in control animals X 200.

Fig.64 Cowper's gland of castrated rat stained with AB pH 1.0 - PAS. Note reduced staining intensities in the cells and secretion X 400.

Fig.65 Cowper's gland of the testosterone treated castrated rat stained with H-E. Note increased in size of the lobule and acini X 200.

Fig.66 Cowper's gland of the testosterone treated castrated rat stained with PAS. Note increase in PAS staining intensities in the cells and secretion than castrated rat (Fig.62) X 200.

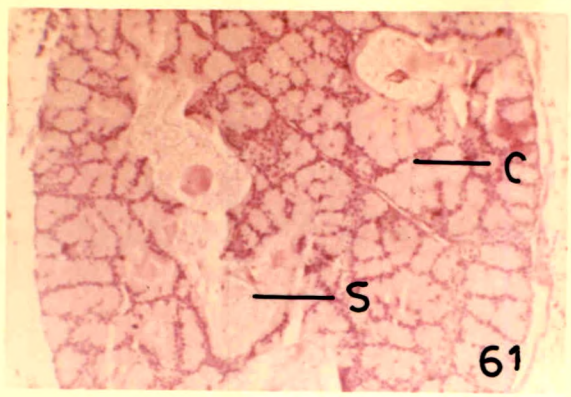
Fig.67 Cowper's gland of the testosterone treated castrated rat stained with AB pH 1.0 - PAS. Note increase in the staining intensities than castrated rat (Fig.64) X 200.

Fig.68 Cowper's gland of the testosterone treated castrated rat stained with AB pH 2.5 to show varied alcianophilia in the cells as in the glands of the control rat X 200.

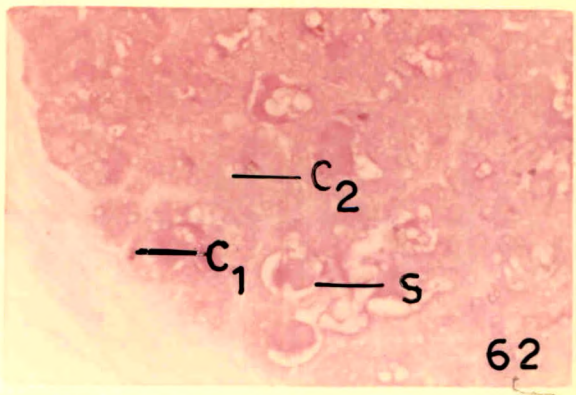
ABBREVIATIONS

C_1 - Type-I cells. C_2 - Type-II cells.

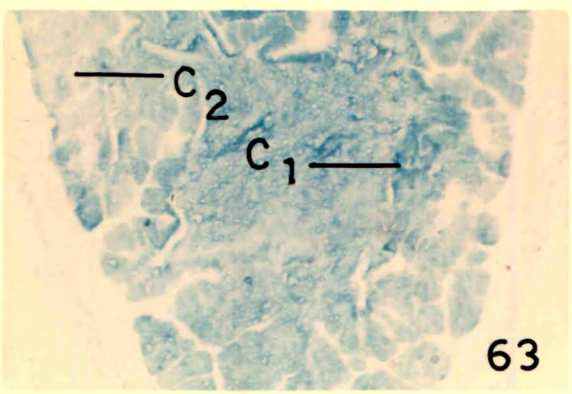
S - Secretion.



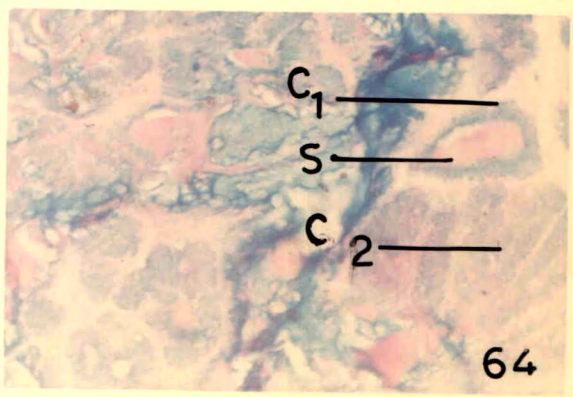
61



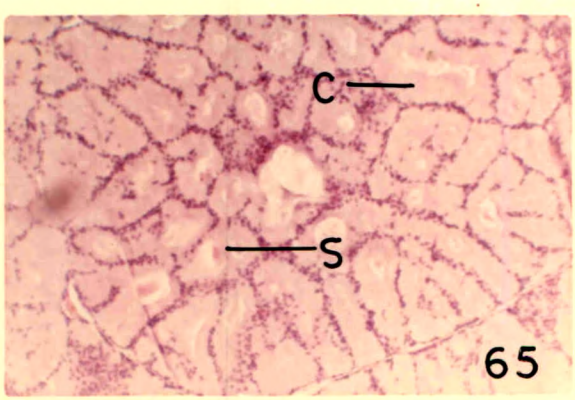
62



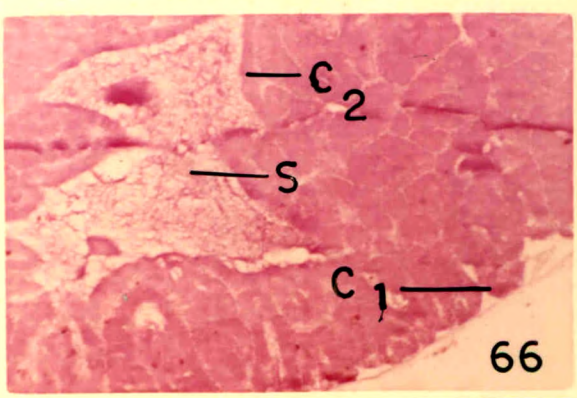
63



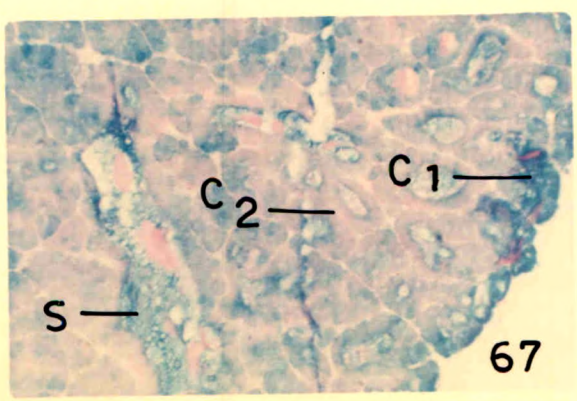
64



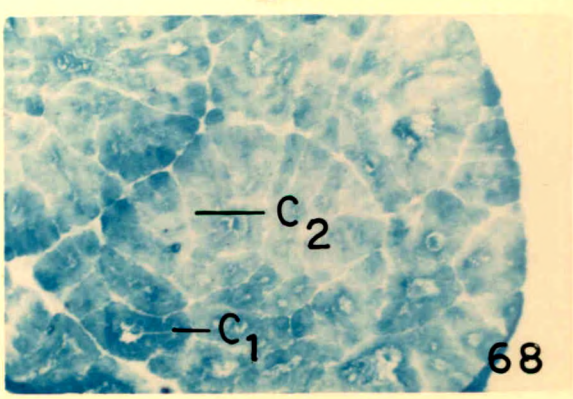
65



66



67



68

CAPTIONS TO FIGURES

Fig.69 Cowper's gland of the testosterone treated castrated rat stained with AB pH 2.5 - PAS to show enhanced staining in the cells and secretion than the castrated rat X 200.

Fig.70 Cowper's gland of the testosterone treated castrated rat stained with AF to show reversal of castration induced changes in the secretory activity of the cells and amount of secretion X 200.

Fig.71 Cowper's gland of normal rat after testosterone administration. Note PAS staining in cells or secretion as in control rat X 200.

Fig.72 Cowper's gland of normal rat after testosterone treatment stained with AB pH 1.0. The overall staining is similar to the glands of control rat X 200.

Fig.73 Cowper's gland of normal rat after testosterone treatment. Note AB pH 2.5 staining as in these glands of control rat X 200.

Fig.74 Cowper's gland of normal rat after testosterone treatment stained with AB pH 2.5 - PAS. Note similar staining as in the gland of control rat X 200.

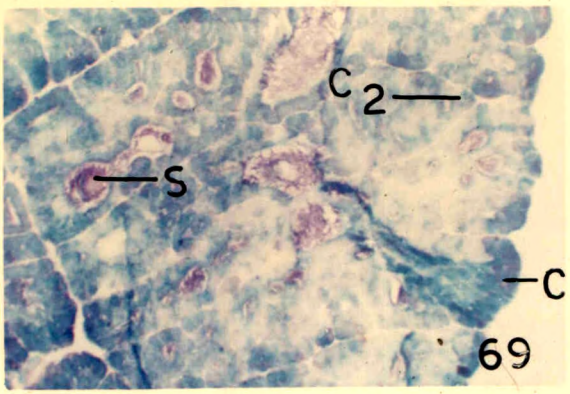
Fig.75 Cowper's gland of normal rat with testosterone administration. The overall C.I.- PAS staining is seen as in the gland of control rat X 200.

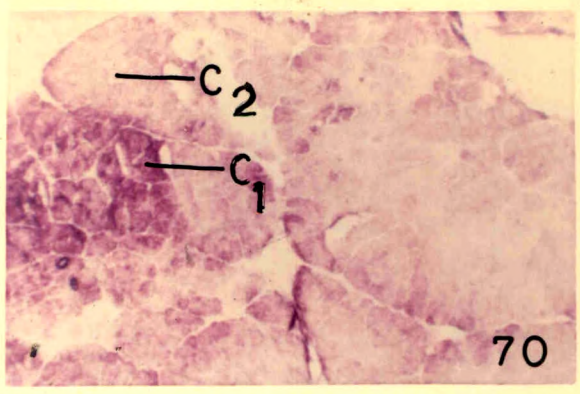
Fig.76 Cowper's gland of normal rat after testosterone administration. Note AF reactivity in two type of cells and secretion as in the gland of control rat X 200.

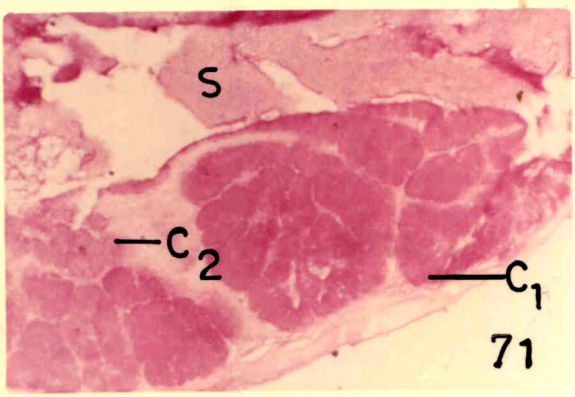
ABBREVIATIONS

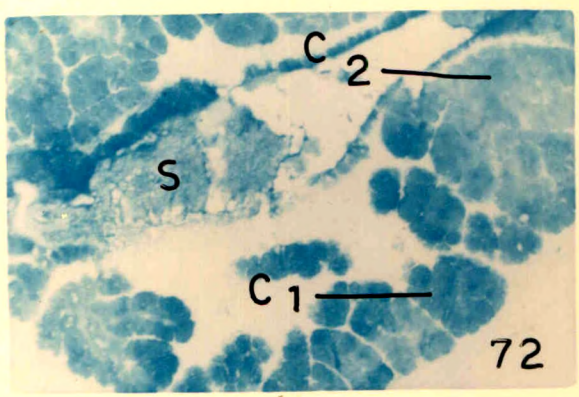
C_1 - Type-I cells. C_2 - Type-II cells.

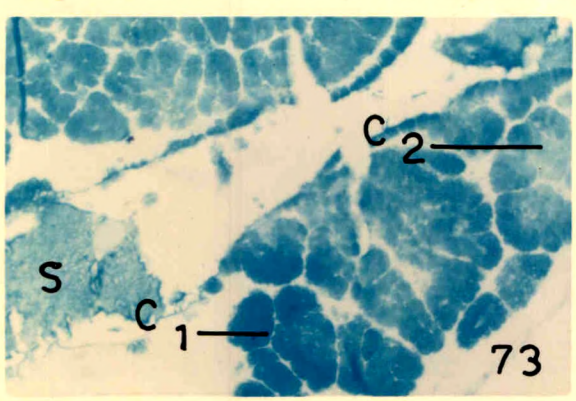
S - Secretion.

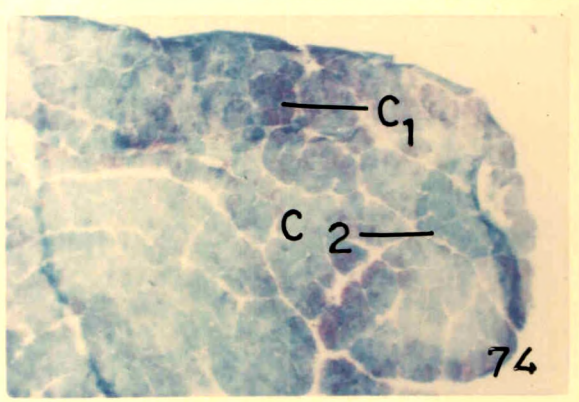

C 2
S
C
69

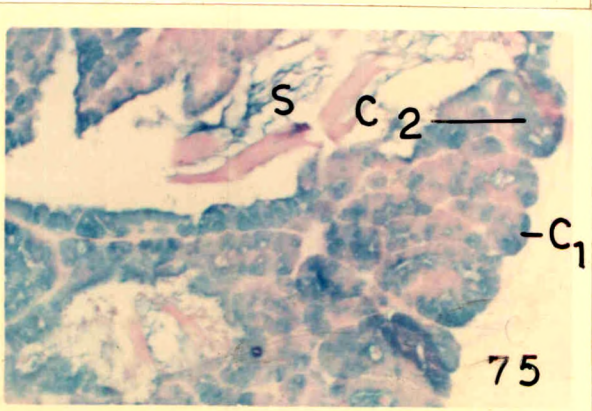

C 2
C 1
70

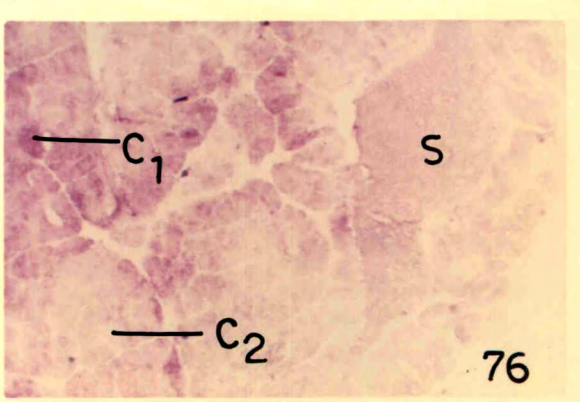

S
C 2
C 1
71


C 2
S
C 1
72


C 2
S
C 1
73


C 1
C 2
74


S
C 2
C 1
75


C 1
S
C 2
76

treatment showed increase in overall size of each gland and attained the size of the glands as found in normal rats. There was increase in the diameter of the acini, height of the glandular epithelial cells and the size of the central cavity (c.f.Figs. 61 and 66). The glandular cells augmented the secretary activity (c.f.Figs.62 and 66) and amount of secretion which is clearly seen in the central cavity.

The histochemical results indicated increase in the synthesis and secretion of sulfomucins by the type-I cells and sialomucins by the type-II cells than the respective cells in the Cowper's glands of castrated rats. Therefore it was concluded that testosterone propionate administration reversed the castration induced effects.

III. Effects of testosterone propionate on Cowper's glands of normal intact rats :

The results obtained are included in Table No.6 and shown in Figs.71 to 76.

No significant changes were noted in the Cowper's glands of rats treated with testosterone propionate. The glands slightly surpassed in size as compared to the galnds in normal animals. The height of the glandular cells was also slightly increased. There was increase in the amount of secretion in the central cavity.

The histochemical results revealed the presence of sulfomucins and sialomucins in the type-I cells and secretion and sialomucins in the type-II cells as in the control animals.