CHAPTER - FOUR

DISCUSSION
ON
COWPER'S GLANDS

The reproductive system in the male vertebrates consists of paired primary sex-organs (male gonads or testes) and vas deferens. Among submammalian vertebrates, the testes are always abdominal and accessory glands are not developed except slight enlargement of the vas deferens in some birds. The mammals exhibit variations in the position of the testes such as abdominal (bats), external permanently in the scrotal sacs (rat, dog, monkey, man etc.) and in some mammals the testes on both sides descend in the scrotal sacs through the inguinal canal during breeding season (squirrel).

The peculiarity of mammalian male reproductive system is the high degree of development of accessory glands such as ampullary glands, seminal vesicles, coagulating glands, prostate, bulbourethral or Cowper's glands, urethral glands and preputial glands. Several variations were noted in the size, sphape and secretions by these glands. In few animals, some of these glands may be totally absent. In such conditions the other glands show enlargement. For example, when seminal vesicles are absent (or small), the prostate glands are greatly developed. On the other hand, when prostate glands are absent or small the seminal vesicles are greatly enlarged. The other accessory glands may be totally absent. Weichert (1958) reported that the Cowper's glands are the only accessory reproductive glands in Monotremes.

The Cowper's glands have been variously known as bulbourethral glands, Mery glands, external prostate, bulbar glands, glandli bulbi and glandulae bulbourethrales. The Cowper's glands secrete a fraction of the seminal plasma. The contribution of the

Cowper's glands to the seminal plasma varies in different mammals. In boar, the Cowper's glands contribute 10-25 % of the seminal plasma (Nalbandov, 1970).

Most of the earlier researches on Cowper's glands were restricted to their presence or absence, number, size, shape, histology and in few mammals cyclic histological variations according to the sex-cycle/s and effects of castration and androgen administration in castrated animals. Comparatively little attention has been paid to the metabolites present and secreted by the cells of the Cowper's glands. In last one or two decades few investigators have demonstrated enzymes, lipids, ascorbic acid and mucopolysaccharides or mucosubstances in the Cowper's glands. With regard to the mucosubstances several investigators have reported different results for the Cowper's gland of the animals belonging to the same species. Others have found similarities and in some cases differences in the mucosubstances of the Cowper's glands of closely related species. These points are described in detail in the introductory chapter.

Therefore the present investigation was undertaken with a view to augment the nature of mucosubstances secreted by the Cowper's glands of few mammals. Moreover, nothing is known about histology, cell types, if any, and mucosubstances in the Cowper's glands of the animals employed in the present investigation to compare the results in closely related species i.e.house rats. The effects of castration have been studied in few mammals such as white rat, pig, slender loris, rhesus monkey and hence continuous breeding house rats are used for the effects of castration and androgen administration.

1. Presence or absence of Cowper's glands :

The Cowper's glands are absent in dog (Zucherman and Mc Keown, 1938), Dugong (Hill, 1955), Cetacea, Sirenia and few carnivores (Price and Williams- Ashman, 1961), and bear and dog (Beyler and Zaneveld., 1982). In an extensive review, Eckstein and Zuckerman (1962) described the presence of these glands in stallion, guinea pig, boar, mole, squirrel, elephant, hyena, shrew, bull, rhesus monkey and man. Others have reported their presence in rat, mouse, hamster, cat, several species of bats, goat, field vole, opossum, camel and wombat.

Initially commonly available animals such as white rats, cats and dogs were also used for the present studies. The efforts to trace the Cowper's glands were failed and from the literature, later on it came to be known that these glands are absent in the dogs. Therefore, the dogs were not employed for the present investigation. Since Nogueira (1970) studied the histology and mucosubstances of the bulbourethral glands in cat, these were not selected in the present studies. Although the Cowper's glands of white rat have been studied earlier, the white rats are also included in the present investigation for comparision with the results in closely related species, house rat or black rat. The six species of mammals selected for present studies viz. horse-shoe bat, rabbit, house rat, white rat, squirrel and he buffalo posses paired Cowper's glands lying one on each side of the muscular urethra. In all these mammals the glands are abdominal in position.

2. Shape and size and number of Cowper's glands :

As with most other accessory glands, there is extensive variation in the shape, size and number of the Cowper's glands among different species. Eckstein and zuckerman (1962) described the shape of Cowper's glands as oval (in rhesus monkey), ovoid (Stallion), flattened oval (elephant), pyriform (hyena), compact (Shrew), bean-shaped (rat) and very large (boar).The observations in the present investigation revealed variations in the shape of Cowper's glands. These glands were oval (bat), strip-shaped (rabbit), bean-shaped (house rat) spherical (white rat), irregular mass (squirrel) and oval or slightly oblong (he buffalo).

In an extensive review, Eckstein and Zuckerman (1962) included the size of the Cowper's gland reported by some earlier investigators. The sizes of the glands range 125 mm.X 65 mm. (elephant), 3 mm. in length (shrew), 2.5 cm. X 4 cm. (stallion) and nearly 10 mm (rhesus monkey). Mossman et al.(1932) reported 12 mm. X 15 mm. size of the bulbourethral glands in squirrel. Hunt (1924) obtained 5 mm. length of Cowper's glands in rat. In the present investigation the measurements of the Cowper's glands were 1.5 mm. X 1 mm.(bat), about 2 mm. X 3 mm. (rabbit), 5 mm. X 3 mm. (house rat), 2 to 3 mm. (White rat), 12 mm. X 10 mm. (squirrel) and 4-5 X 3 cm. (he buffalo).

A single pair of Cowper's glands are present in most of the mammals (Eckstein and Zuckerman, 1962). Chase (1939) and Rubin (1944) reported that in opossum, bulbourethral glands are well developed and consist of three pairs. The first and largest of these (Cowper's I) are distinct, but the other two (Cowper's II and III) are much smaller, enclosed in a common capsule of striated muscles. Nogueira et al. (1984) also found three pairs of bulbourethral glands in *P.opossum viz*. lateral, intermediate and medial glands. Barbour (1981) and Brooks *et al*. (1978) also noted three pairs of Cowper's glands (Cowper's A, B and C) in hairy-nosed wombat, *L.latifrons*. The animals studied for the present investigation posses in each a single pair of Cowper's glands.

3. Histology of Cowper's glands :

The bulbourethral or Cowper's glands are surrounded by a layer of striated muscles. The true connective tissue capsule is absent surrounding each Cowper's glands in all the animals studied so far. The secretory units may be tubular or tubuloalveolar or acinar in different animals. The cells lining the tubules or alveoli or acini vary from flat cells, prismatic cells, cuboidal cells, low columnar cells or tall columnar cells. In a given animal, the cells vary in height according to the breeding and non-breeding seasons (in seasonal breeders). In few animals the Cowper's glands are partitioned by connective tissue septae into multilobulated glands. The secretion is drained into the main duct on each side which carries the secretion to the urethral lumen. In the functionally active glands, the lumini of the tubules, alveoli or acini are filled with yellowish or browhish, homogenous and gelatinous fluid or secretion. In some animals, the Cowper's gland secretion is temporarily stored in a central cavity or cystic dilation. Except variations in the thickness of the striated muscle capsule, height of the glandular epithelial cells, multiple lobulation of the glands and central cavity, at a gross level the histological structure described above has been reported in the Cowper's glands in bats (Gopalkrishna, 1948; Choudhari, 1968; Patil, 1968; Madhavan, 1968; Baile, 1976;

Pawar 1976; Krutzsch et al., 1976; Pawar, 1978; Mote, 1980, Vibhute, 1980; Vibhute and Nalavade, 1980; Fartade, 1981; Murthy, 1981), rat (Hunt, 1924), Squirrel (Mossman et al., 1932; Reddi and Prasad, 1968), opossum (Nogueira et al., 1984), cat (Nogueira, 1970), boar (Aitken, 1960; Nielsen et al., 1977), wombat (Brooks et al., 1978; Barbour, 1981) and camel (Perk, 1962; Ali et al., 1976).

Histological architecture of the Cowper's glands of the animals investigated resembled to the general description given above for these glands in other animals except some minor variations. The Cowper's glands are multilobulated in most of the animals except bat. The glands are acinar in these animals except tubular or tubulo-acinar in squirrel. The glandular epithelial cells are cuboidal in shape in the Cowper's glands of bat and squirrel, cuboidal to low columnar in rabbit, house rat and white rat and tall columnar in he buffalo. Two type of glandular epithelial cells are present in the Cowper's glands of house rat and he buffalo.

4. Histochemical reactivities of Cowper's gland mucosubstances :

Glycogen, neutral mucosubstances, sulfomucins and sialomucins each alone or in various combinations in the Cowper's glands of the animals under present investigation showed staining reactivities towards PAS, modifications in PAS by phenylhydrazine and α-amylase, alcianophilia at different, pH levels, colloidal iron, aldehyde fuchsin, sequential stainings such as AB pH 1.0-PAS, AB pH 2.5- PAS, C.I.- PAS, AF - AB pH 2.5, metachromasis with azure A at different pH levels, induced metachromasia, critical electrolyte concentration, methylastions and methylations followed by saponification, acid hydrolysis and enzyme digestions similar to the aforementioned polysaccharides or mucosubstances in other organ-systems including reproductive system.

Occasionally in the cells of some organ-systems, some unusual or atypical mucosubstances have been detected such as C.I.-reactive but AB-unreactive, AF-reactive but AB-unreactive, alcianophilic after saponification, alcianophilic but non-azurophilic, azurophilic but non-alcianophilic, sialidase resistant sialomucins, sulfated-sialomucins (sulfosialoglycoproteins) etc., the literature on which is extensively reviewed by Nalavade(1975). No such atypical mucosubstances are present in the Cowper's gland of the animals studied. Recently Vibhute (1980) studied histology, mucosubstances and their seasonal variations in gonads and accessory glands in eleven species of bats. He identified atypical sulfated sialomucins in the Cowper's glands of two bats, T.kacchensis and T.longimanus longumanus.

5. Mucosubstances in the Cowper's of the animals under present investigation :

The histochemical results obtained in the present investigation on mucosubstances in the cells and secretion in the Cowper's glands are represented below in a tabulated form :

Animal	Glandular epithelial cells	Secretion
Horseshoe bat	N (poor), G (poor)	N (moderate), G (poor).
Rabbit	N (weak), Si(trace)	N (trace), Si(poor to weak).

Animal	Glandular epithelial cells	Secretion
House rat	Type-I cells- Su (moderate), Si (poor). Type-II cells-Si (weak).	Su (weak to moderate), Si (trace).
White rat	Su (moderate), Si (trace).	Su (trace), Si (trace).
Squirrel	N (poor), G (poor).	N (weak), G (poor).
He buffalo	Type-I cells -Su (moderate), N (poor). Type-II cells -Si (poor to weak) N (trace).	N (poor), Su (moderate), Si (poor).

G = Glycogen, N = Neutral mucosubstances, Su = Sulfomucins, Si= Sialomucins.

9. Comparative distribution of mucosubstances in Cowper's glands of mammals :

This point of discussion concerns with the comparison between the results obtained in the present investigation and the Cowper's gland mucosubstances in other animals reported by other investigators. An attempt is made here to find out similarities of differences, if any, in the Cowper's glands of the animals under present investigation and the existing literature on these glands of other mammals.

The glandular epithelial cells of the alveoli and ampulla of the Cowper's glands have been described as mucous (Stieve, 1930) or mucous - like (Bloom and Fawcett, 1968). Presence of mucous droplets in the apical cytoplasm in the secretory cells of the Cowper's glands has been reported by Heller (1930, 1932), Ortiz

(1953), Feagahs et al. (1961) and Jeffery et al. (1967).

Absence of glycogen in the cells of the Cowper's glands has been reported in ram (Atken, 1959), boar (Aitken, 1960), bull (Stallcup, 1969; Sajonski et al.,1972), camel (Ali et al. 1976) and bats, S.temmincki and C.sphinx sphinx (Pawar, 1976). The histochemical results in the present investigation also indicate absence of glycogen in the glandular cells of these glands in rabbit, house rat, white rat and he buffalo.

Histochemically, presence of glycogen has been demonstrated in the Cowper's glands of hamster (Feagans et al.,1961; 1963), eleven species of bats (Vibhute, 1980; Vibhute and Nalavade, 1980), another bat, T.theobaldi (Fartade, 1981) and ram (Pawar et al.,1986). Barbour (1981) demonstrated considerable protein in the Cowper's glands A in wombat and glycogen in Cowper's B and C glands. In similar histochemical studies Nogueira et al. (1984) identified glycogen in the intermediate and medial Cowper's glands in P.opossum. The present investigation revealed the presence of glycogen in the Cowper's glands of horseshoe bat and squirrel.

PAS reactive neutral glycoproteins or neutral mucosubstances are present in the Cowper's glands of rat (Marois and Salessas, 1968; Geuze and Slot, 1976, 1978), muskrat (Halbhuber, K.J. 1969), cat (Nogueira, 1970), boar (Nielsen et al.,1977) ram (Pawar et al., 1986), bats S.temmincki and C.sphinx sphinx (Pawar, 1976),bats studied by Vibhute (1980) and Vibhute and Nalavade (1980) except T.Kacchensis and T.longimanus longimanus; only type B Cowper's in wombat (Barbour, 1981) and all the three

Cowper's glands (lateral, intermediate and medial) in wombat (Nogueira et al., 1984). Results obtained in the present studies also show the presence of neutral mucosubstances in the Cowper's glands of horseshoe bat, rabbit, squirrel and he buffalo.

Based on some histochemical reactions, presence of acidic glycoproteins or acidic mucosubstances has been reported in the Cowper's glands of hamster (Feagans et al., 1961, 1963), rat (Marois and Salessas, 1968; Geuze and Slot, 1976, 1968) muskrat, bull, sheep, chnchilla, bat and Okapis (Halbhuber K.J., 1969), cat (Nogueira, 1970) and boar (Nielsen et al., 1977).

In the present investigation by employing several histochemical staining methods, the acidic mucosubstances have been identified as sialomucins and sulfomucins in combination or with neutral mucosubstances. Some of the earlier investigators identified acidic mucosubstances in the Cowper's glands as sialomucins in man, mice, rat, guinea pig and their probable presence in golden hamster (Halbhuber and Geyer, 1965), goat (wrobel, 1970), type C Cowper's in wombat (Barbour, 1981) and medial glands in wombat (Nogueira et al., 1984) and sulfomucins in ram (Aitken, 1959) bull (Stallcup, 1969), camel (Perk, 1962), rat (Nielsen, 1976) and intermediate glands in wombat (Nogueira et al., 1984).

Bioassay studies by some investigators confirmed the presence of sialic acid in the Cowper's glands. The quantities of sialic acid estimated are 25.2 % (dry wt.) in boar (Hartree, 1962), 955.6 ± 31.1 μ moles/100 g in rat (Rajalakshmi and Prasad, 1968), bat, P.giganteus giganteus (Rajalakshmi and Prasad, 1970) and 254.9 ± 15.7 μ moles/100 g in golden hamster

(Rajalakshmi et al., 1973). High concentration of sialic acid has been reported in the Cowper's glands of S.murinus and H.aurithus collaris (Goyal and Mathur, 1974) and bull, boar (Beyler and Zaneveld, 1982).

In the present investigation, only sialomucin (except type-II cells in house rat) or only sulfomucin secreting cells are not present in the Cowper's glands of six mammals, but these mucosubstances occurred in various combinations such as neutral mucosubstances and sialomucins (rabbit and type-II cells in he buffalo), neutral mucosubstances and sulfomucins (type-I cells in the buffalo) and sulfomucins and sialomucins (white rat and type-I cells in house rat). In similar histochemical studies, Ali et al. (1976) identified sialomucins in some secretory units and sulfomucins in some secretory units in the Cowper's glands of camel. Nogueira et al. (1984) demonstrated sialomucins in the medial Cowper's glands and sulfomucins in the intermediate Cowper's glands in wombat. Wrobel (1969) found PAS reactive sialomucin containing and sulfomucin containing secretion granules in the same glandular cells in the bulbourethral glands of cat.

Pawar (1976) characterized histochemically sulfomucins, sialomucins together with neutral mucosubstances in the Cowper's glands of bats, S.temmincki and C.sphinx sphinx. Sulfomucins and sialomucins occur together with glycogen in the bats, T.theobaldi (Fartade, 1981); P.giganteus giganteus, R.leschenaulti, H.speoris, H.fulvus fulvus, H.lankadiva, T.aegyptiaca, P.ceylonicus, chrysothrix and P.mimus mimus (Vibhute, 1980; Vibhute and Nalavade, 1980). The latter investigators identified glycogen and atypical sulfated sialomucins in the Cowper's glands of T.kacchensis and T.longimanus longimanus.

The aforementioned comparative data clearly indicates that only in very few mammals are similar in their Cowper's gland mucosubstances but these mucosubstances occur in diverse combinations in the Cowper's glands of different mammals. These combinations include the following categories.

1) Neutral mucosubstances + glycogen.
2) Neutral mucosubstances + acidic mucosubstances.
3) Glycogen + acidic mucosubstances.
4) Neutral mucosubstances + sialomucins.
5) Neutral mucosubstances + sulfomucins.
6) Sialomucins.
7) Sulfomucins.
8) Sialomucins + sulfomucins (in the same cells).
9) Neutral mucosubstances + sulfomucins + sialomucins.
10) Glycogen + sulfomucins + sialomucins.
11) Sulfated - sialomucins (atypical).
12) Sialomucins and sulfomucins (in separate secretory units).
13) Neutral mucosubstances (Cowper's B), glycogen (Cowper's B and C) and sialomucins (Cowper's C)
14) Glycogen (medial and intermediate Cowper's), sialomucins (medial Cowper's) and sulfomucins (intermediate Cowper's).

A critical survey of the literature makes the comparison difficult because several investigators identified different mucosubstances in the same species. This point is elaborately represented in a tabulated form (Chapter-I, point III-9).

7. <u>Cowper's gland mucosubstances in closely related species:</u>

In the present investigation house rat and white rat are studied for their Cowper's gland mucosubstances to find out whether their mucosubstances are similar or differ from each other. These rats are closely related and belong to the same genus (Rattus). Earlier, Vibhute (1980) and Vibhute and Nalavade (1980) found similarity in mucosubstances viz. glycogen and sulfated - sialo-mucins in Cowper's glands of closely related bats, T.kacchensis and T.longimanus longimanus; glycogen, sialomucins and sulfomucins in H.speoris, H.fulvus fulvus and H.lankadiva and glycogen, sulfomucins and sialomucins in P.ceylonicus chrysothrix and P.mimus mimus.

In closely related species differences also occur. For example, Fartade (1981) demonstrated histochemically the presence of glycogen, sialomucins and sulfomucins in the Cowper's glands of T.theobaldi. These results are different than the glycogen and atypical sulfated-sialomucins found in the Cowper's glands of closely related species, T.kacchensis and T.longimanus longimanus described above.

The present investigation reveals difference in the cell types in the Cowper's glands of house rat and white rat. Type-I cells elaborate a mixture of sulfomucins and sialomucins and type-II cells only sialomucins in these glands of house rat. On the other hand the single type of glandular cells secret a mixture of sulfo-mucins and sialomucins in white rat.

8. Secretion :

Most of the earlier investigators have reported on muco-substances in the cells of the Cowper's glands. Some of the investigators studied the secretions in the lumini of the tubules or alveoli or acini of these glands and central cavit in addition to the cells. The nature of secretion varies in the Cowper's glands of different mammals. Barbour (1981) demonstrated considerable amounts of proteins in the secretion of Cowper's glands A in wombat, glycogen and neutral glycoproteins in secretion are contributed by Cowper's B and glygogen and sialomucins by Cowper's C. Protein secreting cells are also present in the Cowper's glands of goat (Wrobel, 1970). Secretion in the bulbourethral glands contain sulfomucins in ram (Aitken, 1959),boar (Aitken, 1960) and bull (Stallcup, 1969).

The secretion in the Cowper's glands contain neutral mucosubstances and glycogen in harseshoe bat and squirrel. This is in agreement with the composition of secretion in M.lyra lyra (Vibhute, 1980; Vibhute and Nalavade, 1980). The secretions of these glands contain neutral mucosubstances and sialomucins in rabbit and a mixture of sulfomucins and sialomucins in house rat and white rat. The results in the present investigation show that the secretion in these glands contain neutral mucosubstances, sulfomucins and sialomucins. Similar composition of secretion is present in the Cowper's glands in the bats (Vibhute, 1980 , Vibhute and Nalavade, 1980), except M.lyra lyra, T.kacchensis and T.longimanus longimanus. The latter two bats contain in their secretion only glycogen and sulfated-sialomucins. The Cowper's glands secretion consists of glycogen, sulfomucins and sialomucins in T.theobaldi (Fartade, 1981).

Geuze and Slot (1978) reported that copulation in rat forced the mucous cells in the bulbourethral glands to accelerate their glycoprotein secretion. The glands lost their secretion within four hours after start of capulation. The secretion of mucous granules occurred in an exocytotic fashion. Refilling of glands was slow and took more than one week. Previously, Geuze and Slot (1976) using ^{14}C - leucine and ^{3}H-galactose labeling at time intervals showed depletion in glycoproteins in the Cowper's glands of rat after capulation. The act of copulation induced the release of glycoproteins.

9. Seasonal alterations in Cowper's glands :

Seasonal changes in the Cowper's glands have been studied in limited mammals, pasticularly bats.

Perk (1962) reported that the tubuli of Cowper's glands contain cuboidal cells and absence of secretion in camel during sexually inactive period. During rutting season, the tubuli of the glands are composed of tall prismatic cells showing increased secretion.

Krutzsch *et al*. (1976) studied the sex-cycle of the bat *M.waterhousii* - together with, changes in the testis and accessory glands. In this bat the spermatogenic cycle is initiated in June, sperms are available in August, regression began in September and the testes are involuted in December (plasma testosterone declines at this time). Cowper's glands undergo an annual cycle in synchrony with the testicular cycle.

Pawar (1976) identified glycogen, sulfomucins and sialomucins in varied levels (based on very faint staining) in the Cowper's glands of bats, *S.temmincki* and *C.sphinx sphinx* and during sexual quiescence. These mucosubstances started gradually increasing during prebreeding period, attained maximum in concentration during active breeding period and followed by gradual depletion from post-breeding period onwards. Similar results were obtained in the Cowper's glands during various phases of the sex-cycle in P.giganteus giganteus, R.leschenaulti, H.speoris, H.fulvus fulvus, H.lankadiva, T.aegyptiaca and P.ceylonicus chrysothrix (Vibhute, 1980). He also noted gradual increase in glycogen and neutral mucosubstances in the Cowper's glands of M.lyra lyra from pre-breeding period, reaching maximum amounts during active breeding period, followed by a gradual reduction during post-breeding period reaching to minimal levels (or traces) in sexually inactive bats. Similar cyclic alterations were also observed by him for glycogen and sulfated-sialomucins in the Cowper's glands of T.kacchensis.

Fartade (1981) noted very poor amount of glycogen in the Cowper's glands of the bat, T.theobaldi during sexual quiescence, increase in the glycogen and neutral mucosubstances during pre-breeding period, glycogen, sulfomucins and sialomucins during active breeding period followed by their gradual depletion from post-breeding period onwards.

Although the animals used in the present investigation (seasonal breeders) have not been studied throughout the year, the aforementioned cyclic variation will help to understand the sexual status of the animals according to the testicular histology and the histology and nature of mucosubstances in the cells and secretion of the Cowper's glands. It appears that the androgens (chief androgen in mammals is testosterone) are at very low level during the sexual quiescence, the hormone titer gradually increase during pre-breeding period, attains peak during active breeding followed by depletion during the post-breeding period. This is witnessed by the observations in *M. waterhousii* (Krutzsch *et al*., 1976) who noted maximum level of plasma testosterone during breeding season and declined level when the testes involuted.

10. Sexual status of the animals under present investigation :

Pawar (1976), Vibhute (1980) and Fartade (1981) studied the histology of the testes and accessory glands in bats during various phases of their sex cycle. They reported the following histology of the testes in bats.

Sexual quiescence :- Testes in regressed condition were reduced in size with no spermatogenesis, reduction in the diameter of the seminiferous tubules, increase in the interstitial connective tissue, tubules with single layer of germinal epithelium and one or two layers of resting spermatogonia and hypotrophied Leydig cells.

Pre-breeding period :- The testes gradually increased in size, reduction in the thickness of the intertubular connective tissue due to increase in the diameter of the seminiferous tubules, spermatogenic stages with spermatogonia, spermatocytes and in late pre-breeding period, spermatids and slightly enlarged Leydig cells than the sexual quiescence.

Active breeding period :- Testes attained maximum size during this period, seminiferous tubules enlarged further in the diameter, intense spermatogenesis showing sperm bundles (few of them attached with Sertoli cells) and sperms in the lubular lumina and hypertrophied Leydig cells mostly aggregated in groups.

Post-breeding period :- During this period the size of the testes started reduction in the size, rate of spermatogenesis gradually declined, cell debris in the lumini of seminiferous tubules, the tubules consisted of germinal epithelium and one or two layers of spermatogonia. The Leydig cells were smaller in size than the active breeding period. The histology of the testes in continuously breeding bats resembled to that described for the testes during the active breeding period in seasonally breeding bats.

Based on the aforementioned histology of the testes during various phases of the sex-cycle and the histology of the testis of the animals studied in the present investigation, the rabbits and squirrels were considered to be at late pre-breeding period or early active breeding period. The bats were considered in the active breeding period and he buffalos in post-breeding period. Since house rats and white rats are continuous breeders, they are always in active breeding phase once they attain puberty. In a recent report on sex-cycle of roe-buck, Short and Mann (1960) described the inactive testis of non-rut with a low testosterone content and small interstitial cells. The active testis of the rutting animals reflected in high testosterone content with hypertrophied eosinophilic interstitial cells. Inns (1982) studied accessory glands and plasma testosterone levels in tammar wallaby, *Macropus eugenii* every two months after maturity. He noted significant increase in the size of prostate and Cowper's glands and in peripheral testosterone concentration during the breeding season.

11. Effects of castration on Cowper's glands :

The Cowper's glands are under the control of androgens

(Heller, 1932; Grzycki and Latalski, 1969; Beyler and Zaneveld, 1982). The androgen dependency of the Cowper's glands like other accessory glands can be showed by castration. After bilateral castration, the Cowper's glands become atrophic with reduction in size, weight and secretory activity in cattle (Schneidermuhl, 1888), pig (Backer, 1928), rabbit (Leydolph, 1929), guinea pig and rat (Barrington, 1913; Heller, 1932; Tschopp, 1936) and sheep (Srivastava et al., 1981).

The results obtained in the present investigation also show that the castration in house rats reflected aterations in the Cowper's glands in reduced size, shrinkage, acinar size reduction, height of the cells in acini or tubules, significant depletion in the secretion and dereased staining intensities of the glandular epithelial cells. These observations are in good agreement with earlier reports in other animals.

12. Effects of testosterone propionate on the Cowper's glands of castrated house rats :

The androgen dependency of the Cowper's glands can be confirmed by androgen administration in castrated animals. The atrophic changes in Cowper's glands induced by castration were reversed by androgen treatment in rat (Heller, 1932; Tschopp,1936) and sheep (Tryphonas et al.,1979). Dinkar et al.(1974) showed that 45-70 µg/capsule/day release of 5 , α - dihydrotestosterone from 4 - 8 implants of silastic capsules over a period of 3 months in castrated rhesus monkeys maintained the wts. of accessory organs (except caput epididymis) at the same levels as in the intact controls.

The results in the present studies also show that testosterone propionate administration (40 mg/100 g body wt.) in castrated house rats increase the overall size of the glands than in the castrated rats and attained the size as in the control rats. Testosterone induced increase in the diameter of the acini, height of glandular cells, size of the central cavity and amount of secretion of sulfomucins and sialomucins by type-I cells and sialomucins by type-II cells.

In similar studies, testosterone administration increased enzyme activities in castrated pigs (Chinoy et al., 1973), sialic acid in castrated rats (Rajalakshmi and Prasad, 1968), slender loris (Manjula and Kadam, 1980) and glycogen, sialic acid and enzymes in castrated rats (Srivastava et al., 1981). Manjula and Kadam (1980) further reported that 250 μg /day administration of testosterone propionate and 5 α - dihydrotestosterone maintained the wt. and secretory activity in the accessory glands in castrated slender loris. The androgen doses were different in different accessory organs. The Cowper's glands required 125 μg/day testosterone or 50 μg/day 5 α - dihydrotestosterone to increase sialic acid concentration.

Lauwers (1984) studied the effects of DES on the bulbourethral glands and vesicular glands of castrated pigs. He observed an increase in the wt. and secretory activity in these glands after DES treatment. In earlier study Lauwers et al.(1981) teated the castrated barrows with DES alone or in combination with tenbolohe or with methyltestosterone to find out morphological changes in the bulbourethral and vesicular glands. They found that the steroids caused enlargement in these accessory glands and desquamation in the collecting ducts in the bulbourethral glands. DES + methyl-testosterone stimulated mucous secretory activity in the bulbourethral glands. Orgebin - Crist et al. (1983) demonstrated increase in the wts. of bulbourethral glands of intact and castrated rabbits by testosterone. Estrogen administration also gave similar results. On the other hand, tomoxifen (an anti-estrogen) given together with estrogen tended to reduce wt. increase in the bulbourethral glands caused by estrogen.

Histological and histochemical studies were carried out by Sikorski and Kmiec (1983) on the bulbourethal glands of rats following prostatectomy and seminal vesiculectomy. They found changes in the contents of neutral and acid mucosubstances, in particular increase in sialomucins as compared to the contents of these mucosubstances in sham-operated animals.

13. Effects of androgens and other hormones on Cowper's glands:

Like the other male accessory reproductive organs, post-natal development, maintenance and secretary activity in the bulbourethral or Cowper's glands are under the control of androgens (Heller, 1932; Feagans et al., 1963; Gryzcki and Latalski, 1963; Beyler and Zaneveld, 1982). Srivastava et al. (1981) demonstrated marked increase in the levels of glycogen, sialic acid, maltase and alkaline phosphatase in rat Cowper's glands after testosterone administration in the intact (non-castrated) animals.

The results obtained in the present investigation with administration of testosterone propionate in intact normal house rats also witness slight increase in the size of the glands than the control rats, increase in the height of the glandular cells, amount of secretion and slight increase in sulfomucins and sialomucins in the cells and secretion.

Gardner and Adams (1986) administered 6-12 mg of zeranol in Merino wethers and another group was treated with 150 mg.testosterone and were allowed to graze together with untreated sheep at two different sites of low estrogen pasture. Bulbourethral gland wts. were found to increase both by zeranol and estrogenic pasture. They concluded that testosterone protected against, whereas zeranol exacerbated the influence of estrogenic pasture. Hendry et al. (1985) reported on estrogen receptor system in the accessory glands of immature rabbits which is regulated in organ-specific manner. They further reported that vesicular glands were most responsive to the estrogen treatment and bulbourethral glands, the least responsive. Szumowski (1980) found that methyl trienolone injections to swine increased wts. of seminal vesicles 103.8 - fold and Cowper's glands 26.9 fold. Low doses of estradiol and nor-testosterone decreased the effects of methyl trienolone.

14. Effects of other chemicals on Cowper's glands :

Rajalakshmi et al. (1970) showed that Cis - Clomiphene citrate in rats (500 µg/Kg/day) for 60 days decreased sialic acid levels in Cowper's glands and also in epididymis and a gradual recovery to control levels occurred following cessation of treatment. The decrease in sialic acid content in rat Cowper's glands is also caused by cyproterone acetate alone or in combination with ORF -1616 (Rajalakshmi and Prasad, 1971).

15. The role of Cowper's gland mucosubstances in reproduction :

This point of discussion concerns with the functional role of Cowper's gland mucosubstances in reproduction based on circumstantial evidences. Bishop (1961) opined that the seminal plasma secreted by accessory glands may be involved to supply nutrients as energy source to sperms, vehicle for transport of sperms and to maintain adequate buffering capacity.

The principal sugar of the semen is usually free fructose secreted mainly by seminal vesicles which plays an essential role in the survival and motility of sperms (Mann, 1946; Mann and Lutwak - Mann, 1948). The mammalian semen is practically devoid of glucose except man (Hornstein, 1961; Elliason, 1965) and few other species (Mann, 1964). In addition to fructose, seminal plasma contains sorbitol (King and Mann, 1959) which is held possible intermediary in the conversion of glucose to seminal fructose (Hers, 1960). Trace amounts of carbohydrates, amino - sugars, saccharides etc. have been described in some cases. (Kubicek and Santavy, 1958; d'Arcangelo, 1962; Mann and Rottenberg, 1966).

The occurrence of seminal glucose is of special interest as this sugar is actively used by spermatozoa more quickly than fructose when both are present (Mann, 1964). In the Chinese hamster and golden hamster, the glucose may be an essential nutrient in the absence of seminal fructose. The glycogen in the cells and secretion in few animals under present investigation and in some in some animals studied by other investigators may be the source of glucose in the Cowper's glands secretion. This is only a suggestion which should be confirmed by further studies. In this connection, it is of interest to consider the observations by Srivastava <u>et al</u>.(1981) who found testosterone administration in prepubertal rats markedly increased glycogen (also sialic acid) and maltase enzyme activity in the Cowper's glands.

Dasgupta et al.(1966) demonstrated sialic acid in the genital organs of the male rat and opined that the exact role of sialic acid in reproduction is not known. It has been suggested that sialic acid may be involved in the capacitation of spermatozoa.

Weichart (1958) described that during sexual excitement in stallion, the Cowper's glands secrete a clear vicid fluid which facilitates sexual act by means of its lubricating properties. Beyler and Zaneveld (1982) reported that the bulbourethral glands in mammals function in supplying lubricant to the urethra and the tip of the penis, facilitating ejaculation and intromission. Weichart (1958) also described that in stallion, the Cowper's gland secretion has a slight alkaline reaction. This secretion tends to neutralize any acid which may be present in the urethra after the previous passage of urine and also helps to neutralize any acid conditions in the vagina. It can be further suggested that the neutral mucosubstances secreted by the Cowper's glands may neutralize acid conditions in the urethra and vagina.

Semen coagulation or the formation of seminal gel is essential for the sperm transport in the female reproductive tract and results from the interaction of specific male sex accessory gland secretions (Blandau, 1945; Greenstein and Hart, 1964). The coagulating glands secrete a heat-labile enzyme termed " vesiculase" which coagulates a basic protein secreted by the seminal vesicles (Gotterer et al., 1955; Manyai, 1964; Notides and Williams - Ashman, 1967). In contrast to the vesiculase, Cowper's glands secrete a heat-stable sialic acid - rich complex which coagulates a vesicular protein fraction that is electrophoretically distinct from the substrate of vesiculase (Hart and

Greenstein, 1964, 1968).

In boar, the bulbourethral glands secrete extremely viscious, almost rubber- like, white secretion distinguished by high sialio-protein. This plays an important part in the process of " gelation " which occurs in the seminal plasma at the time of ejaculation and immediately afterwards (Mann, 1964). Boursnell et al.(1970) isolated a fraction from boar Cowper's gland mucin which could be digested by neuraminidase indicating sialic acid is involved in the seminal gel formation. According to Eadia (1948), the coagulating agent is produced by Cowper's glands and a coagulable substance secreted by the prostate gland in Insectivora.

Beyler and Zaneveld (1982) reported that a heat-labile, non dialysable glycoprotein from bulbourethral glands causes clotting of vesicular basic protein by means of a non-enzymatic and non-covalent mechanism. Vesiculase from the coagulating gland potentiates this clot formation by formation of covalent cross-linkages. Hart (1969) demonstrated that the rat coagulating secretion at pH 5.9 displayed minimal activity in coagulating vesicular substrate but potentiated the coagulation reaction involving Cowper's and seminal vesicle secretion. This effect could not be mimicked by prostate, ampullary or epididymal secretions.

Hart (1968) demonstrated <u>in vitro</u> interaction of rat Cowper's and vesicular gland secretions to produce a coagulant by a non-enzymatic mechanism. The effect of ionic strength on the reaction suggests that ionic interactions are involved in coagulation. The inhibitory effect of versene indicated the involvement of metal ions in the reaction. Recently, Boursnell and Butler (1974) devised a method to study boar seminal gel enabling investigations of chemical and physical properties of gel formed in vitro from the bulbourethral gland mucin and either vesicular of the haemagglutinin (protein H) in boar vesicular secretion combines with the mucin to form a gel. The content of Zn, Mg and citrate of the natural gel is less than that of the seminal plasma.

One of the striking physiological properties of the seminal plasma in some though not in all the animals is the ability to coagulate which leads to the formation of vaginal plug which prevents the backflow of semen from the vagina and thereby assists the passage of spermatozoa from cervix to the uterus and then to fallopian tubes. The formation of vaginal plug has been reported in bats (Rhinolophidae and Vespertilionidae) by Courrier (1927). Hart and Greenstein (1968) have isolated a glycoprotein fraction from Cowper's glands of rat, mouse and hamster (but not in guinea pig) which coagulated vesicular secretion. They further opined that the secretions from Cowper's glands may have a hitherto unrecognised role in the formation of the copulatory plug and subsequent fertility in these species. In pig, the Cowper's gland secretion is the last fraction of the ejaculate and is believed to occlude cervix to prevent backflow of semen (Nielsen et al., 1977).

Mann (1964) suggested that sialomucins from bulbourethral gland secretions are possibly involved in the mechanism by which the spermatozoon is attached to the egg in the process of penetration and fertilization.

Some of the aforementioned physiological roles such as supplying nutrients to spermatozoa (fructose or glucose), neutralization of acid conditions in urethra or vagina, lubrication, formation of seminal gel and vaginal plug etc. may be performed by the polysaccharides and mucosubstances from the Cowper's glands in the animals studied in the present investigation. This is only a suggestion which requires further studies to establish definite roles. At present some efforts are being made in these directions such as standardization of chromatographic technique for isolation and identification of fructose, glucose, bioassay studies on quantitation of sialic acid and collection of animals, wherein no work has been done on Cowper's glands e.g. hare, mongoose, jackel etc. It is of interest to note a report by Krutzsch _et al_. (1976) who identified fructose in the Cowper's glands of _M.waterhousii_.

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