CHAPTER - ONE

INTRODUCTION

REVIEW OF THE EXISTING LITERATURE ON BULBOURETHRAL OR COWPER'S GLANDS, REASONS FOR UNDERTAKING OF THE PRESENT INVESTIGATION AND PLAN OF THE PROPOSED RESEARCH

I N T R O D U C T I O N

I. Review of the existing literature on bulbourethral or Cowper's glands :

The male reproductive system in mammals consists of paired gonads (testes), epididymis, vas deferens and sex-accessory glands such as ampullary glands, seminal vesicles, coagulating glands, prostate, urethral glands (glands of Littré), bulbourethral glands (Cowper's glands) and preputial glands. The secretions from these glands in general, provide internal millieu for viability, maturation and transport of the sperms. Some variations have been reported for the presence or absence of the glands and their size and number. For example, ampullary glands are present in few animals. Seminal vesicles may be absent in some mammals. When prostate is small, the seminal vesicles are greatly developed and when seminal vesicles are small or absent then prostate is enlarged and so on.

A. Presence or absence of Cowper's glands :

Retif (1949) reported on the absence of Cowper's glands in lower vertebrates. These glands are also absent in dog (Zuckerman and Mc Keown, 1938), bear and dog (Beyler and Zaneveld, 1982), Dugong (Hill, 1955) and Cetacea, Sirenia and few carnivores (Price and Williams- Ashman, 1961). The Cowper's glands are present in most of the mammals such as rat, mouse, horse, hamster, guinea pig, boar, cat, bats, mole, squirrel, elephant, hyena, shrew, bull, goat, field vole, camel, monkey, man etc, the literature on which is reviewed by Eckstein and Zuckerman (1962). Generally single pair of these glands is

present in these animals. They further described that three pairs of Cowper's glands are present in opossum viz. Cowper's I, II and III, the I being distinct and largest. Three pairs of glands (Cowper's A, B and C) are also present in Philander opossum (Nogueira et al., 1984) and hairy-nosed wombat, Lasiorhinus latifrons (Brooks et al., 1978; Barbour, 1981).

Anatomy :

Cowper's glands are well developed, compact, tabular or tubulo - alveolar or acinar in nature. They lie on either side of the muscular urethra. The glands vary in shape such as bean-shaped, oval, flattened-oval, pyriform etc. in different mammals. These glands are very large in squirrel (Mossman et al., 1932) and boar (Eckstein and Zuckerman, 1962). In Stallion, the ducts from bulbourethral glands join the urethra at the base of the penis (Weichart, 1958). Three independent pairs of ducts from three pairs of Cowper's glands open into the muscular urethra in opossum and wombat. In other mammals, the Cowper's glands are small, covered by striated muscles and generally true capsule is absent in bats (Pawar, 1976; Vibhute, 1980; Fartade, 1981; Murthy, 1981) and other animals (Eckstein and Zuckerman, 1962). In monotremes, Cowper's glands are the only accessory reproductive glands (Weichart, 1958). Mossman et al. (1932) considered these glands as " bulbar glands " or " glanduli bulbi " . Courrier (1927) considered Cowper's glands as external prostate. Perk (1962) designated these glands as " Glandulae Bulbourethrales ".

B. Histology :

The bulbourethral or Cowper's glands are surrounded by

striated muscles but true capsule is absent. The glandular secretory units appear in the form of simple tubules, tubulo-alveolar or acinar in nature. The secretory cells are generally tall columnar but they appear as low cuboidal in non-breeding or sexually quiescent period. The connective tissue between the tubules, alveoli or acini is represented by collagen fibers and few elastic fibers. The myoepithelial cells surrounding secretory units are absent. In the functionally active glands the lumina of tubules or alveoli or acini are filled with yellowish, homogenous and gelatinous fluid. In general similar histological architecture is reported for the Cowper's glands in camel (Perk, 1962; Ali et al., 1976), bats (Krutzsch et al.,1976; Pawar, 1976; Vibhute, 1980; Fartade, 1981; Murthy, 1981), cat (Nogueira,1970), boar (Nielsen et al.,1977), P.opossum (Nogueira et al., 1984), squirrel (Reddi and Prasad, 1968), wombat (Brooks et al.,1978) and boar(Aitken, 1960).

C. Ultrastructure of Cowper's glands :

In few mammals, the ultrastructure of glandular secretory cells in the Cowper's glands has been studied. The cells possess microvilli at the apical plasma membrane, no foldings at basal plasma membrane, numerous mitochondria, free and membrane bound ribosomes, scanty lysosomes, a typical Golgi apparatus and secretion granules at the supranuclear cytoplasm. This fine structure has been described for the cells in the Cowper's glands of rat (Grzycki and Latalski, 1969; Nielsen, 1976) hamster (Feagans et al., 1963; Nittinger, 1973) and cat (Wrobel, 1969; Nogueira, 1970).

D. Histochemical studies on Cowper's glands :

Acid phosphatase containing granules have been reported in the cells of bulbourethral glands of boar (Aitken, 1960). Alkaline phosphatase is absent in these glands. Wrobel (1969) reported a strong 5-nucleotidase activity in the intercellular canaliculi between the bulbourethral gland cells in cat. The glandular parenchyma is exceptionally strong for unspecific esterase and rich in β - D - gulcuronidase, aldolase, glycerophosphatase, lactate dehydrogenase, alcohol dehydrogenase, NAD - dependent isocitrate dehydrogenase, succinic dehydrogenase and cytochrome oxidase. Wrobel (1970) demonstrated β - glucuronidase, β - D - galactosidase, esterase and leucine aminopeptidase in the Cowper's glands of goat. Kind (1974) found decreased levels of β - glucuronidase and acid phosphatase in the bulbourethral glands of castrated rats. Ali et al. (1976) studied acid phosphatase, alkaline phosphatase, adenosine triphosphatase, adenosine - 5 - monophosphatase, succinic dehydrogenase and lactate dehydrogenase in the bulbourethral glands of camel. Sinowatz et al. (1976) observed weak β - glucuronidase activity in the bulbourethral glands of bull. β - glucuronidase has been demonstrated in the Cowper's glands of bats, Hipposideros speoris, Cynopterus sphinx sphinx (Baile, 1976) and Megaderma lyra lyra (Kanase, 1979) and non-specific esterases in these glands of C.sphinx sphinx (Mote, 1979). Pawar et al.(1986) reported on the presence of acid and alkaline phosphatases, succinic dehydrogenase, lactate dehydrogenase and NADP - dependent diaphorase in the bulbourethral glands of ram.

ii) Lipids :

Rajalakshmi et al. (1973) detected polyglycerol

phosphatide in the accessory organs but not in the testes. The lipid analysis revealed phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine and sphingomyelin in the accessory organs of the golden hamster. Ali et al. (1976) demonstrated trace amount of neutral lipids in the bulbourethral glands of camel. Recently, Pawar et al. (1986) demonstrated sudanophilic lipids in the bulbourethral glands of ram.

iii) Ascorbic acid :

Dvorak and Podany (1971) found highest ascorbic acid level in the testes but decreasing amounts in the Cowper's glands and other accessory glands in the large white boars. Krutzsch et al. (1976) observed that the testicular hypertrophy was accompanied with increased levels of ascorbic acid in the testes and all the accessory glands including Cowper's glands in the bat, Macrotus waterhousii.

iv) Glycogen and Mucosubstances :

Glycogen has been reported to be absent in the Cowper's glands of ram (Aitken, 1959), boar (Aitken, 1960), bull (Stallcup, 1969; Sajonski et al., 1972) and camel (Ali et al., 1976). Histochemically, presence of glycogen has been demonstrated in the Cowper's glands of hamster(Feagans et al., 1961); bats, M.lyra lyra, Taphozous kacchensis, Taphozous longimanus longimanus, Pteropus giganteus giganteus, Rousettus leschenaulti, Hipposideros speoris, Hipposideros fulvus fulvus, Hipposideros lankadiva, Tadarida aegyptiaca, Pipistrellus ceylonicus chrysothrix and Pipistrellus mimus mimus (Vibhute, 1980), Taphozous theobaldi (Fardade, 1981), B and C Cowper's glands in wombat

(Barbour, 1981), intermediate and medial Cowper's glands in P.opossum (Nogueira et al., 1984) and ram (Pawar et al., 1986).

Geuze and Slot (1978) demonstrated PAS positive neutral glycoprotein granules in the columnar cells at the origin of the duct in the bulbourethral glands of the rat. Presence of neutral mucosubstances has been reported in the Cowper's glands of rat (Marois and Salessas, 1968), muskrat (Halbhuber, 1969), cat (Nogueira, 1970), boar (Nielsen et al., 1977), type B Cowper's in wombat (Barbour, 1981), ram (Pawar et al., 1986) and bats, Scotophilus temmincki and C.sphinx sphinx (Pawar, 1976), T. theobaldi (Fartade, 1981) and the bats studied by Vibhute (1980) and Vibhute and Nalavade (1980) except T.kacchensis and T.longimanus longimanus.

Based on few histochemical reactions, some investigators demonstrated only the presence of acidic mucosubstances in the Cowper's glands of hamster (Feagans et al.,1961, 1963), rat (Marois and Salessas, 1968; Geuze and Slot, 1976, 1978), bull, sheep, chinchilla, bat and Okapis (Halbhuber K.J., 1969), muskrat (Halbhuber, K.J. 1969), boar (Nielsen et al., 1977) and cat (Nogueira, 1970).

Halbhuber and Geyer (1965) identified sialomucins in the bulbourethral glands of man, mice, rat, and guinea pig. Their presence is probable in golden hamster. Sialic acid containing mucosubstances are also secreted by the Cowper's glands in cat (Wrobel, 1969), goat (Wrobel, 1970), some glandular units in camel (Ali et al., 1976), P.opossum (only medial glands) (Nogueira et al., 1984), type C Cowper's in wombat (Brooks et al., 1978; Barbour, 1981) and bats, S.temmincki and C.sphinx sphinx.

(Pawar, 1976), T.theobaldi (Fartade, 1981) and the bats studied by Vibhute (1980) except T. kacchensis, T.longimanus longimanus and M.lyra lyra.

Bioassay studies have also revealed the presence of sialic acid, the quantity of sialic acid varies in the Cowper's glands of boar (Hartree, 1962), rat (Rajalakshmi and Prasad, 1968), bat P.giganteus giganteus (Rajalakshmi and Prasad, 1970), golden hamster (Rajalakshmi et al., 1973), Suncus murinus and Hemiechinus aurithus collaris (Goyal and Mathur, 1974) and bull and boar (Beyler and Zaneveld, 1982).

In some mammals the acidic mucosubstances have been identified as sulfomucins in the Cowper's glands of ram (Aitken, 1959), boar (Aitken, 1960), bull (Stallcup, 1969), cat (Wrobel, 1969), camel (Perk, 1962; Ali et al., 1976), rat (Nielsen, 1976), intermediate Cowper's in P.opossum (Nogueira, et al., 1984) and bats, S.temmincki, C.sphinx sphinx (Pawar, 1976), T.theobaldi (Fartade, 1981) and bats studied by Vibhute (1980) except M.lyra lyra, T.kacchensis and T.longimanus longimanus. In last two species of bats, the Cowper's glands secrete atypical mucosubstances known as sulfated sialomucins (Vibhute, 1980).

E. Seasonal variations in Cowper's glands :

Perk (1962) reported that in camel during rutting season, the tubuli of Cowper's glands compose of tall prismatic cells and show increased secretion. During the inactive period the epithelial cells of the tubuli reduce in height and become cuboidal and secretion is nearly absent. Krutzsch et al.(1976) studied sex-cycle of the bat, M.waterhousii. The spermatogenic cycle

was initiated in June. Sperms were available in August. Regression of testicular cycle began in September and testes were involuted in early December. The plasma testosterone level declined at this time. They found that Cowper's glands underwent an annual cycle in synchrony with testicular cycle.

Pawar (1976) observed cyclic variations in histology and mucosubstances in Cowper's glands of two species of bats, S.temmincki and C.sphinx sphinx during their sex-cycle/s. He found very poor staining for neutral mucosubstances, sialomucins and sulfomucins during sexual quiescence. These mucosubstances gradually increased from prebreeding period and attained maximum levels during the active breeding period and gradually depleted from post-breeding period onwards. This was based on the gradual increase in the staining intensities for these mucosubstances from prebreeding period, maximum intensities during active breeding period and a gradual reduction in the staining intensities during the post-breeding period.

Similar cyclic variations in the Cowper's glands were observed according to the quiescent period, prebreeding period, active breeding and post-breeding periods for glycogen and neutral mucosubstances in the bat, M.lyra lyra; glycogen and sulfated-sialomucins in bats, T.kacchensis and for glycogen, sialomucins and sulfomucins in P.giganteus giganteus, R.leschenaulti, H.speoris, H.fulvus fulvus, H.lankadiva, T.aegyptiaca and P.ceylonicus chrysothrix (Vibhute, 1980). He also studied Cowper's glands of a continuous breeding bats, P.mimus mimus, wherein no variations were observed for glycogen, sulfomucins and sialomucins and

T.longimanus longimanus for glycogen and sulfated sialomucins.

In similar histochemical studies, Fartade (1981) found only poor staining for glycogen in the Cowper's glands of another bat, T.theobaldi during sexual quiescence. The staining for glycogen and neutral mucosubstances gradually increased during prebreeding period. During the active breeding period the glands elaborated maximum amounts of glycogen, sulfomucins and sialomucins as intense staining intensities were noted for these mucosubstances. The staining intensities for these mucosubstances gradually reduced during post-breeding period leading to only residual glycogen in the subsequent sexual quiescence.

II. Effects of castration and steroid hormones on the Cowper's glands.

As with other male accessory organs the development and maintenance of the bulbourethral or Cowper's glands are under the control of androgens (Heller, 1932; Grzycki and Latalski, 1969; Beyler and Zaneveld, 1982). The androgen dependency of Cowper's glands has been demonstrated by castration and castration followed by androgen administration studies.

Atrophy of the Cowper's glands after castration has been reported in cattle (Schneidermuhl, 1888), pig (Backer, 1928), rabbit (Leydolph, 1929) and guinea pig and rat (Barrington, 1953; Heller, 1932; Tschopp, 1936) and sheep (Srivastava et al., 1981). Restoration of castration induced atrophy in Cowper's glands following administration of androgens in rat has been reported by Heller (1932) and Tschopp (1936) and Sheep (Tryphonas et al., 1979). Dinkar et al. (1974) reported that microquantities

(45-70 µg/capsule/day) of 5-α-dihydrotestosterone released from 4-8 implants of silastic capsules over a period of 3 months in castrated rhesus monkeys caused increase in the wt. of accessory organs except caput epididymis.

Some investigators have showed that androgens and some steroid hormone anologs not only stimulate the increase in the wt. of Cowper's glands and reverse the atrophic changes (height of the glandular cells) after castration but also increase some of the metabolites secreted by these glands.

Chinoy et al. (1973) showed that subcutaneous injection of testosterone propionate in castrated pigs reversed castration induced decrease in enzyme activities in accessory sex-glands (prostate, bulbourethral glands, seminal vesicles). Succinic dehydrogenase activity was more androgen dependant than acid phosphatase and alkaline phosphatase.

Rajalakshmi and Prasad (1968) reported that castration in rat was followed by decrease in sialic acid level in Cowper's glands and testosterone administration reversed this effect. Moreover, sialic acid concentration exceeded than the control values. Manjula and Kadam (1980) found in castrated slender loris, Loris tradigradus lydekkerianus that castration resulted in decrease in wt. and secretory activity of epididymis, ductus deferens and accessory glands. Testosterone propionate (250 µg/ day) and 5-α-dihydrotestorone maintained the wt. and secretory activity in accessory glands. The androgen doses were different in different organs. Cowper's glands required 500 µg androgens to

increase sialic acid concentration. Srivastava et al. (1981) found decrease in levels of glycogen, sialic acid and activities of maltase, acid and alkaline phosphatases in castrated rats. However, these effects were reversed by testosterone propionate.

Lauwers (1984) found that diethylstilbestrol (DES) caused increase in the wt. of bulbourethral glands in barrows, increased secretory activity in these glands and induced sloughing of epithelium in the collecting ducts. Lauwers et al. (1984) also showed that increase in wt. and secretory activity in the bulbourethral glands of castrated male pigs. Lauwers et al. (1981) treated castrated barrows with DES alone or with trenbolone or with methyltestosterone and studied morphological changes in bulbourethral glands and vesicular glands. These steroids caused enlargement in the bulbourethral glands and vesicular glands and desquation of collecting ducts. DES and methyltestosterone combination increased mucous activity in the bulbourethral glands. The effects of testosterone, estrogen and tomoxifen alone or in combinations were studied in control and castrated rabbits by Orgebin - Crist et al. (1983). Their observations indicated that testosterone increased the wts. of bulbourethral glands both in intact and castrated rabbits. The responses of estrogen were characterized by significant increase in wts. of glands in intact and castrated rabbits. On the other hand, tomoxifen (which is an anti-estrogen) given together with estrogen reduced estrogen mediated wt. increase in the bulbourethral glands.

III. Reasons for Undertaking of the Present Investigation

A glance at the survey of existing literature on the presence or absence, anatomy, histology, histochemistry, bioassay

studies and effects of castration and hormone replacement therapy on mammalian bulbourethral or Cowper's glands reveals that still there are several avenues open for investigators in the aforementioned fields. Therefore, in the present investigation mucosubstances in the Cowper's glands of six species of mammals have been investigated based on several histochemical methods. Some of the reasons for undertaking of the present investigation are listed below :

1) Extensive researches have been carried out on the testes of the animals, their ultrastructure, histology, seasonal variations, and androgen secretion. Among the accessory glands, prostate has received more attention. This may be due to several diseases and pathological variations in the prostate. In this regard, Cowper's glands and other accessory glands in the male reproductive system have received comparatively little attention by investigators in this field.

2) Practically nothing is known about the nature of mucosubstances elaborated by the Cowper's glands of the animals under present investigation except rabbit and white rats. In this regard, rabbits were investigated earlier by Leydolph (1929) to find out effects of castration on Cowper's glands (only histological studies). White rats can easily be maintained in captivity and several experiments are carried in white rats. Hence to some extent, Cowper's gland mucosubstances in white rats have been studied but different investigators reported different results.

3) Earlier researches on Cowper's glands were concerned with the presence or absence of Cowper's glands, their number

(1 to 3 pairs), anatomy, morphology etc. In comparison less attention has been focused on mucosubstances elaborated by Cowper's glands. Bioassay studies are still meagre, wherein some of the investigators have estimated sialic acid quantities from the Cowper's glands of rat, giant fruit bat, golden hamster, bull, boar, S.murinus and H.aurithus collaris.

4) In the horse (Bharadwaj and Calhoun, 1962) the bulbourethral glands are serous glands, whereas in rest of the animals, they are mucous in nature. Wrobel (1970) identified sialomucin - secreting cells (mucous cells) and protein secreting cells (serous cells) in the bulbourethral glands of the goat. Geuze and Slot (1978) identified PAS positive mucous granules containing cells at the origin of the duct in the bulbourethral glands of the rat. In the wall of the gland some small acini with serous cells were observed. In most of the mammals, the Cowper's glands contain only mucous cells. In the animals selected for the present investigation, nothing is known about the number of cell types in the Cowper's glands except white rat.

5) Some of the investigators studied Cowper's glands of single animal and hence comparative account on these glands of the mammals belonging to the different orders is scanty. In this regard Pawar (1976) studied mucosubstances in two bats (one megachiropteran and one microchiropteran). Vibhute (1980) studied Cowper's glands of two megachiropteran and nine microchiropteran bats. Beyler and Zaneveld (1982) estimated sialic acid contents of bulbourethral glands of bull and boar. For similar studies Goyal and Mathur (1974) used Cowper's glands of S.murinus and H.aurithus collaris.

6) Some investigators have demonstrated only PAS positive material in the Cowper's gland without further identification of the nature mucosubstance/s. For example, Geuze and Slot (1978) demonstrated PAS positive granules in columnar cells at the origin of the duct in the bulbourethral glands of rat. Likewise there are reports on presence or absence of glycogen in these glands of different mammals. In this regard nothing is known about the Cowper's glands of the animals selected for the present investigation. Based on some histochemical methods, some investigators have reported on presence of acidic mucopolysaccharides or mucosubstances in Cowper's glands of some mammals without characterization of mucosubstances.

7) The effects of castration on the Cowper's glands have been studied in limited number of animals such as white rat, pig, slender loris and rhesus monkey. Therefore house rat is selected in the present investigation. House rats are continuous breeders and one has not to wait until breeding season unlike seasonal breeders.

8) The castration induced alterations were shown to be reversed by androgens in white rat, pig, bull, rabbit and guinea pig. In the present investigation effects of testosterone propionate are studied in the normal intact and castrated house rats.

9) A critical analysis of the existing literature pointed out that several authors have obtained different results in the bulbourethral or Cowper's glands of same animal. This is evident from the following tabulated representation.

	Animal	Mucosubstances	Authors
1.	Rat	Acidic Mucosubstances	Geuze and Slot (1976, 1978).
2.	Rat	Neutral and Acidic mucosubstances	Marois and Salessas (1968).
3.	Rat	Sialomucins	Halbhuber and Geyer (1965).
4.	Rat	Sialomucins	Rajalakshmi and Prasad (1968).
5.	Rat	Sulfomucins	Nielsen (1976).
1.	Ram	Sulfomucins	Aitken (1959).
2.	Ram	Sialomucins	Wrobel (1970).
3.	Ram	Glycogen and Neutral mucosubstances	Pawar et al.(1986).
1.	Cat	Neutral and Acidic mucosubstances	Nogueira (1970).
2.	Cat	Sulfomucins and Sialomucins	Wrobel (1969).
1.	Boar	Neutral and Acidic mucosubstances	Nielsen et al.(1977).
2.	Boar	Sialomucins	Hartree (1962); Beyler and Zaneveld (1982).
3.	Boar	Sulfomucins	Aitken (1960).
1.	Giant fruit bat	Sialomucins	Rajalakshmi and Prasad (1970).
2.	Giant fruit bat	Glycogen, Sialomucins and Sulfomucins	Vibhute (1980); Vibhute and Nalavade (1980).

1.	Bull	Acidic mucosubstances	Halbhuber (1969).
2.	Bull	Sialomucins	Beyler and Zaneveld (1982).
3.	Bull	Sulfomucins	Stallcup (1969); Sajonski et al.(1972)
1.	Hamster	Acidic mucosubstances	Feagans ét al.(1963).
2.	Hamster	Glycogen	Feagans et al.(1961).
3.	Hamster	Probable presence of Sialomucins	Halbhuber and Geyer (1965).
4.	Hamster	Sialomucins	Rajalakshmi et al.(1973).
1.	Wombat	Type C Cowper's Sialomucins	Brooks et al.(1978).
2.	Wombat	Type A Cowper's - Proteins Type B Cowper's - Glycogen and Neutral mucosubstances Type C Cowper's - Glycogen and Sialomucins	Barbour (1981).

Some of the aforementioned animals will be studied for Cowper's gland mucosubstances for post - M.Phil. research for confirmation of the results. From the animals listed above white rats are used in the present investigation.

10) Although the mucosubstances have been studied in the Cowper's glands of white rats, these were also used in the present investigation to find out whether similarities or differences occur in Cowper's gland mucosubstances in closely related species such as white rat and house rat. In similar M.Phil.Dissertation, Bargaje (1989) found differences in cell types and mucosubstances

in the seminal vesicles of Rattus norvegicus (white rat) and Rattus rattus (house rat). The Cowper's gland mucosubstances in closely related species exhibit similarities as well as differences. These are represented below :

Animal	Mucosubstances	Authors
1. T.kacchensis 2. T.longimanus longimanus	Glycogen and Sulfated sialomucins	Vibhute (1980); Vibhute and Nalavade (1980).
3. T.theobaldi	Glycogen, Sialomucins and Sulfomucins	Fartade (1981).
1. H.speoris 2. H.fulvus fulvus 3. H.lankadiva	Glycogen, Sialomucins and Sulfomucins	Vibhute (1980); Vibhute and Nalavade (1980).
1. P.ceylonicus chrysothrix 2. P.mimus mimus	Glycogen, Sialomucins and Sulfomucins	Vibhute (1980) ; Vibhute and Nalavade (1980).

The bioassay studies, seasonal variation (in seasonal breeders) and effects of castration and androgen administration in intact and castrated animals used in the present investigation and some additional animals will be studied for post M.Phil. research.

IV. Plan of the Proposed Research :

a) Choice of animals

Six species of mammals belonging to different orders such as Chiroptera, Lagomorpha, Rodentia and Artiodactyla were used in the present investigation. As mentioned earlier, practically very

little is known about the presence or absence of Cowper's glands, their number, cell types, if any, and the nature of mucosubstances elaborated by them, except white rat. Secondly, these animals are available throughout the year except bats of this species Rhinolophus luctus beddomei are found in a single pair during breeding season only. After mating, the sexes are separated and these bats migrate to other places. The material from he bufallos or even from bull can be obtained any time from slaughter house. Closely related species viz. white rat and house rat are selected for the present studies to know similarities or differences in the histology, cell types, if any, and the nature of mucosubstances elaborated by them.

b) Choice of Histological and Histochemical Methods :

The routine histological staining procedures viz. Hemaloxylin - Eosin and Mallory's Triple staining technique were employed for histological observations of testes and Cowper's glands. Several histochemical staining procedures including PAS, modifications in PAS following α-amylase and phenylhydrazine pretreatment, alcianophilia at different pH levels, colloidal iron, alcian blue and colloidal iron followed by PAS, aldehyde fuchsin alone or with AB pH 2.5 step afterwards, azure A at different pH levels, sulfation followed by azure A, critical electrolyte concentration, mild and active methylations and methylations followed by saponification and alcian blue staining, acid hydrolysis and sialidase, hyaluronidase and pepsin digestions followed by alcian blue staining were employed in the present investigation. According to the chemicals, reagents and stains available in this laboratory, the aforementioned methods are standardized and used in the

present investigation. At present these methods are recommended for the demonstration of glycogen, neutral mucosubstances, sialomucins, sulfomucins, hyaluronic acid and some atypical mucosubstances in the cells of various tissues and their secretions.

c) Presentation of the Dissertation :

It was decided in the beginning to divide the present dissertation into four chapters, the first being on the introduction which includes review of the existing literature on Cowper's glands, reasons for undertaking of the present investigation and plan of the proposed research. The second chapter will be devoted to material and methods. This chapter also includes nomenclature of complex carbohydrates and a brief survey of the advances in histochemical techniques for detection of mucosubstances. The third chapter will include histological observations on testes and histological and histochemical observations on Cowper's glands together with effects of castration and testosterone propionate on intact and castrated animals. The fourth chapter will concern with discussion in relation to the results obtained in the present investigation and existing data. This chapter will be followed by summary and concluding remarks. The bibliography of the literature cited will be given at the end of the dissertation.

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