CHAPTER 1

INTRODUCTION

The male reproductive system is composed of the testes, genital ducts, accessory glands, and penis. The prostate gland is the largest accessory genital gland in the male reproductive tract. It functions with other accessory genital glands (seminal vesicles and bulbourethral glands) and genital ducts to produce secretions. These secretions provide nutrients for spermatozoa while they are confined to the male reproductive tract. Spermatozoa and the secretions of the accessory glands and genital ducts make up semen, which is introduced into the female reproductive tract through the penis. Semen also makes the vaginal canal less acidic (Junqueira *et al.*, 1998; Chan and Ho, 1999; National Kidney and Urologic Diseases Information Clearing house, 2000 [online]).

Comparative anatomy of prostate gland (Reviewed by Blandy and Lytton, 1986)

The most mammalian prostates have paired secretory glands. There are many variations in the characteristics of this organ from one species to another, and even within the same species and it sometime is difficult to identify. For example, the prostate secretory gland of goat, sheep, hippopotamus, whale and some marsupials are

appearance as a diffuse type. This type is a sleeve of glandular tissue surrounding the urethra within its muscular wall and dose not separate into caudal, cranial, inner or peripheral zones (Fig. 1). However, in dog and man, the prostate forms a body, which in turn may be either compact and solid or composed of distinct lobes, as found in rodents. In pig, the prostate is mainly composed of transversely oriented striated muscle fibers surrounding a tiny prostate.

The primate prostate more closely resembles the prostate of man. It seems to be made up of two pairs of glands, the cranial and the caudal prostate glands. Each gland arises from the dorsal (posterior) aspect of the urethra, they may enlarge and fold round on either side of the urethra, and the caudal prostate is usually larger than the cranial one. In shape, each gland is like an unbuttoned collar, open in front, and these two glands (cranial and caudal) are separated by a cleft through which the vas deferens and the duct of the seminal vesicle pass to enter the urethra (Fig. 2).

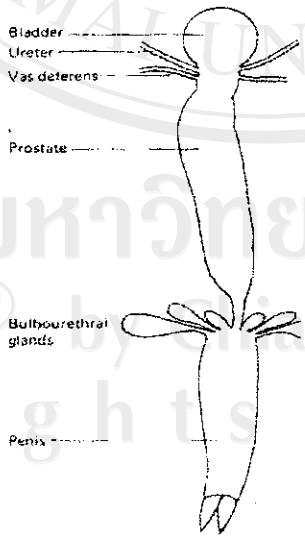

Figure 1 Diagram of the prostate in the male opossum (Blandy and Lytton, 1986).

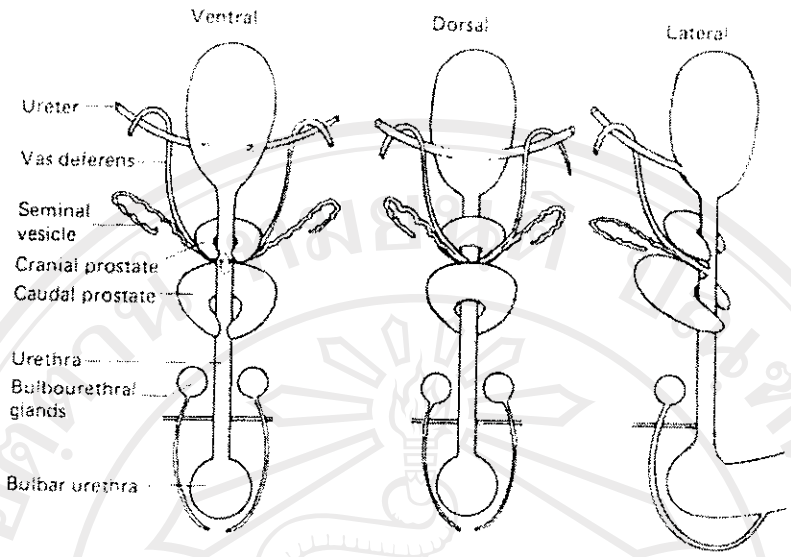

Figure 2 Diagram of the primate prostate from 3 different views, ventral, dorsal and lateral . There are two distinct prostates, a cranial and a caudal gland (Blandy and Lytton, 1986).

Some authors describe many variation on the primate prostate, it may be entirely confined to the dorsum of the urethra (in *Callicebinae*, the titi monkeys and *Aotes*, the night monkey). The prostate may be small and difficult to find, limited to the flat plate on either side of the midline (*Alouatta*, the howler monkey). In some species it is easy to make out the distinction between the cranial and caudal prostates, for example, in *Indriidae*, *Daubentonia*, *Samiri*, the squirrel monkey; *Cebus*, the Capuchin monkey (Fig. 3). In others, one cannot make this distinction as easily (*Cercopithecus ascanius*, the black-cheeked whith-nosed monkey; Fig. 4)

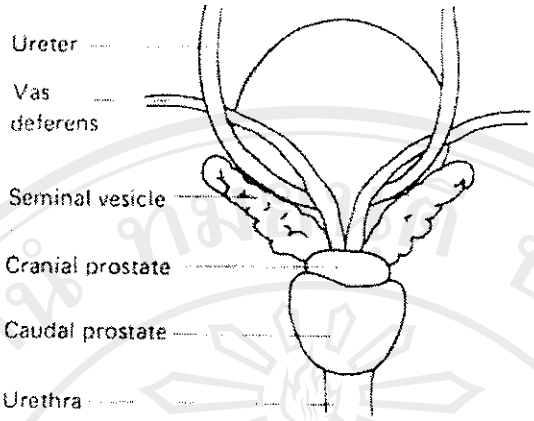

Figure 3 Prostate in *Cebus capucinus* (Blandy and Lytton, 1986).

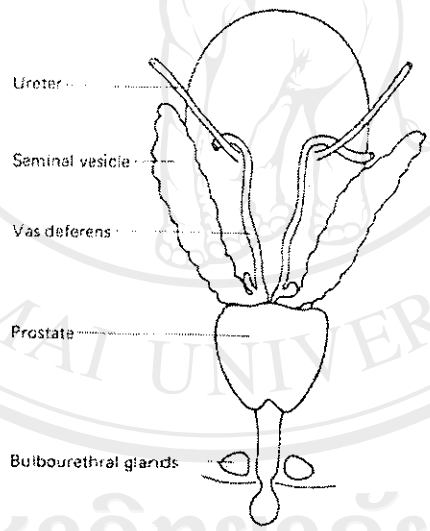

Figure 4 Prostate in *Cercopithecus ascanius ascanium* (Blandy and Lytton, 1986).

Gross anatomy of rat prostate gland

The rat prostate is a large gland, which surrounds the proximal urethra and is composed of several distinct lobes: the ventral, dorsal, lateral, and anterior (coagulating gland). Each lobe is different in morphology, secretion, response to hormones, and position. The ventral prostate consists of paired right and left lobes arising from the ventral aspect of the urethra immediately below the bladder. It constitutes about half of the mass of the entire prostatic complex. The dorsal prostate emerges from the posterior aspect of the urethra. It is located below and behind the attachment of the seminal vesicle and the coagulating gland inferoposterior to the bladder and posterior to the lateral prostate. The lateral prostate is located just below the seminal vesicle and coagulating gland. It extends ventrally to partially overlap the ventral prostate and blends dorsally with the dorsal prostate. The coagulating gland consists of paired right and left lobes attached to the inner concave surface of the seminal vesicle (Hauke *et al.*, 1989; Chan and Ho, 1999).

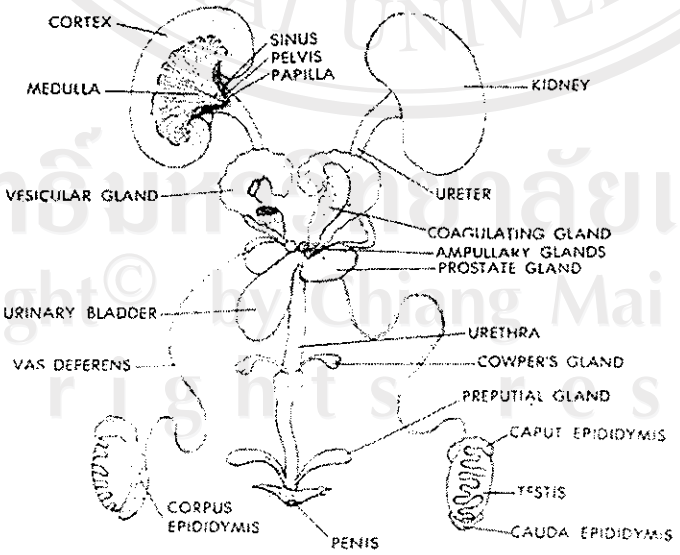

Figure 5 Diagram of the male pelvic organs of the rat (Chiasson, 1975).

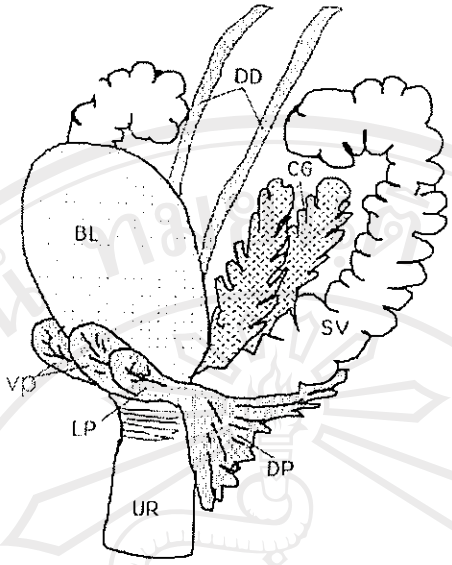

Figure 6 Diagram of the lateral view of the rat male pelvic organ. CG, coagulating gland; VP, ventral prostate; LP, lateral prostate; DP, dorsal prostate; BL, urinary bladder; SV, seminal vesicle; DD, ductus deferens; UR, urethra (Cunha *et al.*, 1987).

Histology of prostate gland

The rat prostate is a compound ductal gland completely lacking of true acini. Individual ducts are lined by a pseudostratified columnar secretory epithelium, whose height varies, while in the functional state of the gland, the microvilli can be investigated at the luminal surface. An electron microscope shows many rough endoplasmic reticulum in cytoplasm and secretory granules in the apical cytoplasm of epithelial cells. Nonsecretory basal epithelial cells are interspersed along the basement membrane. The significance of these basal cells in the normal prostate and during prostatic pathogenesis is unknown. It has been suggested that the reserved or stem cells are possibly capable of differentiating into columnar secretory cells (Harkin, 1961; Cunha *et al.*, 1987; Hayashi *et al.*, 1991; Junqueira *et al.*, 1998).

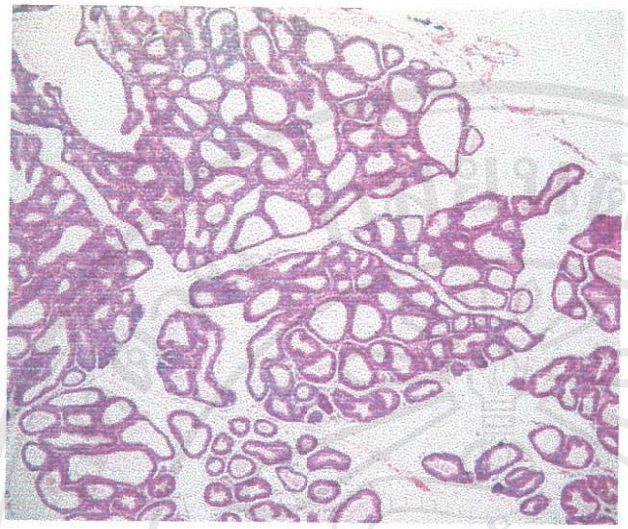

Figure 7 Histology of rat prostate gland (x 50)

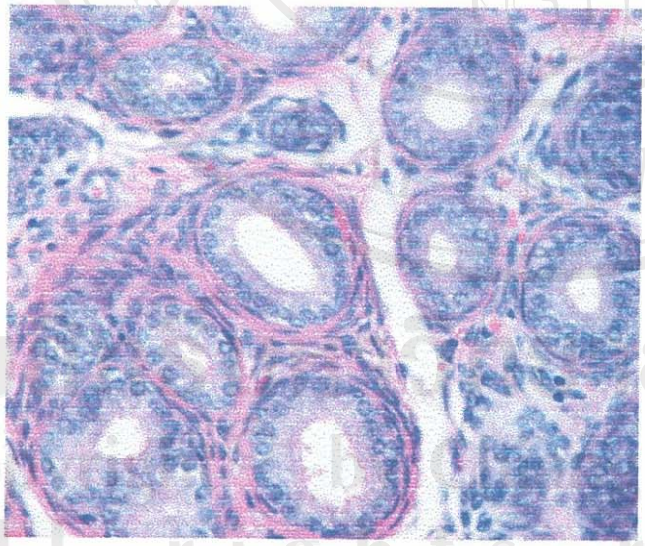

Figure 8 Histology of rat prostate gland (x 400)

Glycoconjugates and lectins

Glycoconjugates are compounds in which one or more monosaccharide or oligosaccharide units (the glycone) are covalently linked to a noncarbohydrate moiety (the aglycone). A glycoprotein is a glycoconjugate in which a protein carries one or more oligosaccharide chains covalently attached to a polypeptide backbone, usually via N-or O-linkages. A glycolipid is an oligosaccharide usually attached via glucose or galactose to the terminal primary hydroxyl group of the lipid moiety ceramide, which is itself composed of a long chain base and a fatty acid (Varki *et al.*, 1999).

Glycoconjugates occur from the variable portion of the molecule comprising sugar chains or glycans. Most of the enzymes involved in glycan biosynthesis are glycosyltransferase, which act by adding monosaccharides to specific position. The biosynthesis of glycan is primarily determined by these sequentially acting enzymes, which assemble monosaccharides into linear and branched sugar chains. Many, but not all, of these enzymes are found within the ER-Golgi pathway for export of newly synthesized glycoconjugates (Varki *et al.*, 1999).

Glycoconjugates are located both intra and extracellularly and also at the cell surface which can be secretory or structural. Inside the cells, glycoconjugates are located in diffuse cytoplasmic, granular cytoplasmic (secretory granule), endosomes or lysosomes and their contents, endoplasmic reticulum, Golgi apparatus, nucleus and nuclear membranes. For the cell surface and extracellular matrix, glycoconjugates are located in glycocalyx of the cell membrane, secretion, intercellular junction, basement membrane, and extra cellular matrix (glycosaminoglycans, collagen, and matrix glycoproteins) (Brooks *et al.*, 1997).

The carbohydrate residues of glycoconjugates are important in activities such as maintaining protein conformation and solubility, stabilizing the polypeptide against uncontrolled proteolysis, mediating biological activity (e.g. immunogenic recognition, non-immunogenic phagocytosis and receptor mediated endocytosis), intracellular sorting and the secretion of glycoprotein and embryonic development and differentiation.

The glycoconjugates patterns can be investigated by using lectin. Lectin is a specific carbohydrate binding protein of nonimmune origin that agglutinates cells or precipitates polysaccharides or glycoconjugates, and that has been utilized extensively as a biochemical tool to isolate glycoconjugates, glycolipids, and polysaccharides and as a probe to investigate the sugar residues of the cell surface. They are especially useful in studies of the quality, distribution, assembly and turnover of glycoconjugates in normal and pathological tissues, as well as during embryonic differentiation (Chan and Wong, 1992; Chan and Ho, 1999).

In secretory epithelial cells, the Golgi is most highly developed. It has three important roles, the modification of complex molecule (such as protein) by the addition of sugar, proteolysis of peptide molecules, which makes them become active and sorting of molecule for either (transport out of the cell, incorporation in the cell membrane, or transport to another part of the cell) (Golgi apparatus [online], 2002). The Golgi complex reduces metal salts, such as salts of osmium and silver, which may be stained with these compounds. Such staining methods were responsible for the discovery of the Golgi complex (Weiss, 1988; Cook, 1974).

However, Golgi apparatus functions with rER and the main function of rER is to segregate proteins from the cytosol that are destined for export or intracellular use.

Additional functions include the initial glycosylation of glycoproteins, the synthesis of phospholipid, the assembly of multichain proteins, limited proteolysis of the signal sequence of newly synthesized proteins, and certain post-translational modifications of newly formed polypeptides. In summary, the Golgi has a system of postal worker, called vesicle, which will pick up packages from the ER and take them to the edge of the cell membrane after the Golgi's work is done.

The prostate produces many substances for the seminal composition, so that the rER and Golgi of secretory epithelial cells are well developed.

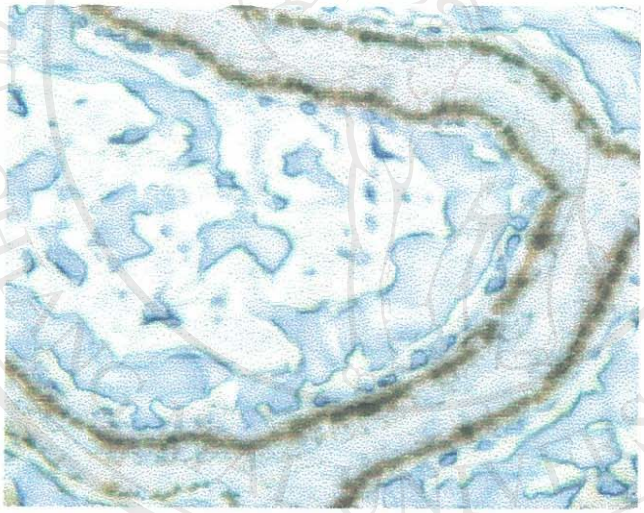

Figure 9 The McDonald's modification of Lascano's technique staining showing Golgi apparatus zone in rat prostate gland.

In the past, there were some reports on the morphology difference of the secretory epithelium of the rat (Harkin, 1961) and human prostate glands (Sö derström, 1987) in various ages. However, little of lectin binding patterns in each lobe of prostate gland has been elucidate (Chan and Ho, 1999). Moreover, there were no reports about glycoconjugate patterns among different age groups. Therefore, the present study is designed to compare the expression of glycoconjugates in prostatic secretory cells by investigating the patterns of lectin binding in the prostate at different ages of rats.

LITERATURE REVIEW

Chan and Ho (1999) studied, characterized and compared the expression of glycoconjugates in three different lobes (ventral, lateral, and dorsal) of the Noble rat prostate gland by lectin histochemistry using 30 different lectins. Different prostatic lobes elaborate and secrete different glycoconjugates. Dorsal prostatic epithelium secretes the secretion which is rich in Man, GlcNAc, Gal/GalNac, Fuc, NeuAc (α 2, 6) Gal, and oligosaccharides, as they were also stained by S-Con A, LCA, PWA, UEA-II, RCA-I, DBA, ECA, WFA, Jacalin, MPA, SJA, LTA, UEA-I, PHA-E and PHA-L. The epithelium of lateral prostate showed weak or negative reaction to lectins, suggesting that it may express fewer glycoconjugates as compared to the other two lobes. The epithelial Golgi region of the ventral prostate contained abundant GlcNAc, α / β -GalNAc and NeuAc (α 2,6) Gal residues, as shown by its intense reactions to S-WGA, DBA, SBA, HAA, HPA, WFA and SNA. GS-I-B4 reacted specifically with

the basal epithelial cells in all three lobes, indicating that glycoconjugates with terminal α-Gal/GalNAc were commonly expressed in these basal cells.

Gerhardt and colleague (1983) studied the function of the ventral lobe, lateral lobe, dorsal lobe, and the coagulating gland of prostate in Sprague Dawley rats by using biochemical parameters. These included spermine, citric acid, acid phosphatase, fructose and zinc, as well as the pattern of proteins in both cytosols and secretions, which were determined using one dimentional SDS, polyacrylamide gel electrophoresis. The results suggest that several parameters can be determined including: ventral lobe -spermine or prostatein, lateral lobe – zinc or a 15000 dalton protein, dorsal lobe – spermine or a 6000 dalton protein, and coagulating gland – fructose.

McNeal and colleague (1987) studied the histochemical staining with ten lectins demonstrating differences in lectin binding patterns between the seminal vesicle, prostatic central and peripheral zones, and foci of prostate intraductal dysplasia, a putative premalignant lesion. Lectin binding patterns of seminal vesicle and central zone of the prostate were identical except for a single lectin, supporting the concept that these two structures have a common embryologic origin from the wolffian duct. Three of the lectins that were bound to the central zone were not bound in the peripheral zone, indicating a biologic difference between these two origins of the prostate. Dysplasia foci showed markedly reduced binding with all lectins, consistent with impaired processing of glycoconjugates. Lectin binding patterns appear to have value as sensitive markers of differences in terminal differentiation of closely related tissues and of early impairment of differentiated function in lesions that are precursors to carcinoma.

Hauke and colleague (1987) studied the postnatal development of carbohydrate constituents in the rat ventral prostate by using five Fluorescein-isothiocyanate (FITC) labeled lectins. Binding sites for Con A, WGA, PNA, and DBA were found from day 10 to 13 post partum onwards. Each lectin showed a characteristic localization. Binding sites for the lectins used changed to different extents during the following two weeks. After the 24th day post partum no further changes in the lectin-binding pattern could be found. The development of the lectin binding properties showed that the changes in carbohydrate containing constituents of the prostate correlate with the beginning of prostatic secretion and to prostatic epithelial differentiation.

Chan and Wong (1992) studied glycoconjugates of the lateral prostate by using lectin-gold histochemistry method. The results showed that the rough endoplasmic reticulum (rER) was rich in glycoproteins with mannosyl residues while the Golgi cisternae, secretory granules and microvilli were less so.

Avtsyn and colleague (1981) studied histological structure of the human prostate and its zinc content at various ages. In the prepubertal group whose histological appearance was inactive, there was lesser zinc content in the dry tissue of the gland (48.10 ± 9.10 vs 64.10 ± 6.10 mg/kg) and this index tended to be less in the older group.

HYPOTHESIS

As reviewed, the activity of secretory cells of the prostate is relatively low at the infant stage but significantly increases after puberty. It is possible that the modificaton of prostatic secretory cells would be related to the expression patterns of glycoconjugate of such cells at particular age intervals.

OBJECTIVE

1. To study the glycoconjugate patterns of secretory epithelial cells in each lobe; ventral, dorsal, lateral, and anterior lobes (coagulating gland) of a normal rat prostate gland at various ages (2 weeks, 1 month, 3 months, and 14 months).
2. To analyze and find association between the sugar residue in each lobe and the growth development of the rat prostate gland.