CHAPTER ONE

INTRODUCTION

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The accessory reproductive glands of the male are specialized for the formation of spermatic fluid, storage of spermatozoa, and their conveyance through an adequate vehicle to the exterior at proper time. These structures, in man, include multiple ductuli efferents, paired epididymis, vasa efferentia, seminal vesicle, ejaculatory duct, Cowper's glands and the prostate gland. The part of the seminal fluid emanating from the accessory glands forms a suitable medium and furnishes a vehicle for the conveyance of sperms and also provides an environment in which the sperms can retain their greatest fertilizing capacity (Turner and Bagnara, 1971). The major part of the seminal fluid is contributed by the prostatic secretion, which is a thin, milky, alkaline fluid containing citric acid, calcium, acid phosphatase and fibrinolysin (Guyton, 1971). The alkaline characteristic of the prostatic fluid is quite important for enhancing the motility and fertility of the sperms because the sperms do not become optimally motile until the pH of the surrounding fluids rises to approximately 6.0 to 6.5 (Turner, 1971) and it is the alkaline prostatic fluid which neutralizes the acidic metabolic end-products of the sperms coming from the vasa deferentia and similarly it probably neutralizes the acidic vaginal secretions after ejaculation, thus enhancing motility and the fertility of the sperms.

It is a well established fact in the physiology of reproduction

that all the accessory elements depend on androgenic hormones for their efficient functioning. The abnormal release of androgens, due to various disorders, also cause deterioration in the physiological functions of these glands. Among these, prostate is the major gland subjected to various pathological disorders. The critical evaluation of the literature shows that the major prostatic disorders include hyperplasia, atrophy and induced carcinomas (Turner and Bagnara, 1971, Guyton, 1971; Ashley, 1980).

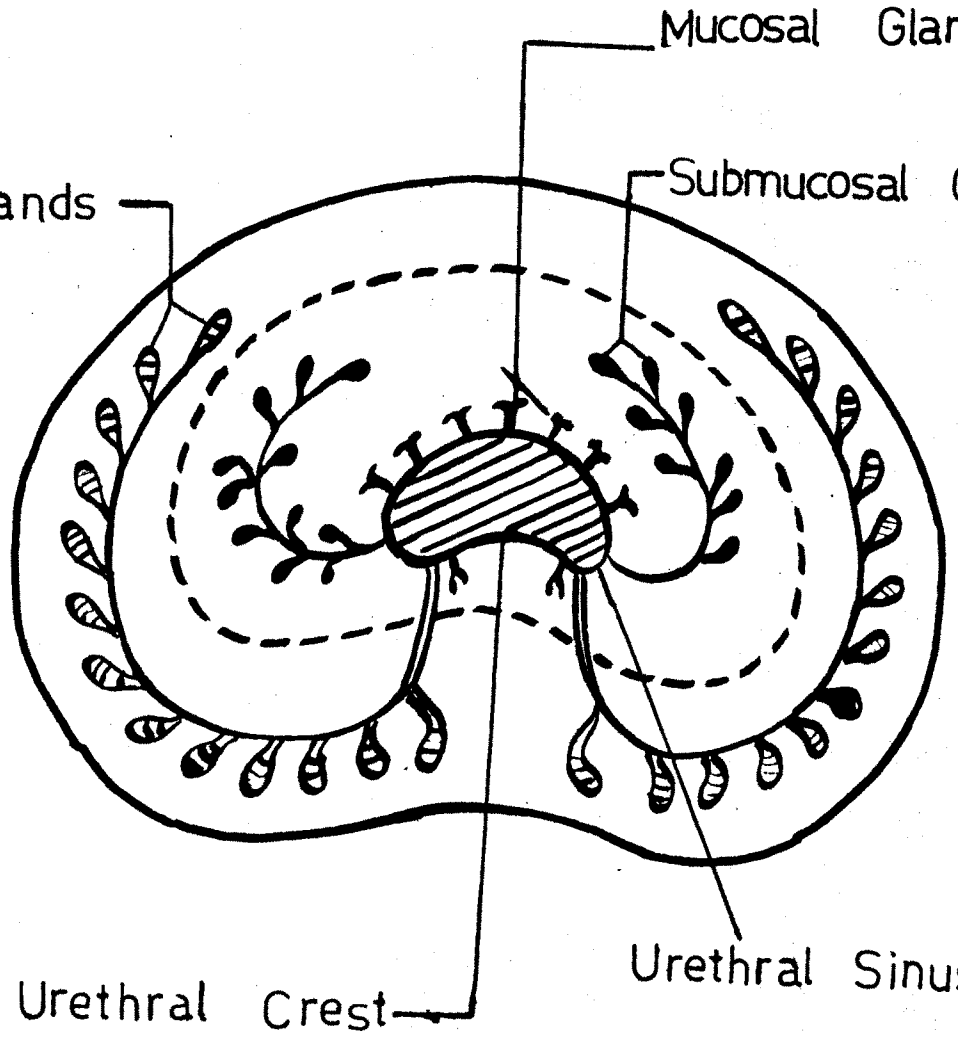
For the understanding of the work included in this dissertation, a precise and detailed information about the morphology and histology of the normal prostate and prostate under different pathological conditions, their chemistry and secretion, enzymatic components in the secretion, lipids, carbohydrates, inorganic ions, endocrine regulations and the functioning of the prostate glands is essential. It is with this view that a brief but critical review of the work done on prostate gland with reference to the the above mentioned parameters is given in subsequent pages.

1) The Gross Anatomy and Histology of the Prostate Gland:

a) Gross Anatomical Structure:

The human prostate consists of three types of glands (Ham, 1969; Ashley, 1980). (Fig. No. 1)

i) The Mucosal Glands: They are the smallest glands and are present



[Fig. No.1 : Gross Anatomical Structure Of Human Prostate (diagramatic) Drawn & Slightly modified from Histology by Arthur W. Ham.]

near the periurethral tissue. These glands open at various points around the lumen of urethra.

ii) The Submucosal Glands: They are present around the periurethral tissue and open into the posterior margins of the urethral sinuses.

iii) The Main Glands or True Glands of the Prostate: They are the largest glands and are present in the outer or peripheral region of the prostate. The main glands also open in the posterior margins of urethral sinuses.

The prostate gland is imperfectly divided into 3 lobes by the passage, through it, of the ejaculatory ducts. Each lobe is imperfectly subdivided into lobules. The ducts that drain the lobules of the bulk of the organ sweep backward to empty into the urethral sinuses. In the lobules, the ducts branch into tubulo-alveolar secretory units. (Ham, 1969)

b) Microscopic Structure of the Prostate:

The prostate is a large gland which surrounds the bladder neck and the first part of the urethra in the midline. The wall of the prostatic urethra is formed by the substance of the prostate gland (Wheater et al.,1982), In general the plan of organization of the prostate gland is similar to that of an exocrine gland. The microscopic observations show that the prostate contains glandular elements, involuntary muscles and fibrous tissue. The prostatic

acini and tubules are invested by limiting fibromuscular stroma propria that follows all the irregularities of the glandular elements. Immediately outside the stroma propria the extensive supporting framework of the prostate is continued as fibrous tissue containing irregularly interlacing strands and bundles of smooth muscles. The outer capsular layer consists of connective tissue and elastic tissue as well as smooth muscle fibres.

The adult prostatic alveoli vary in size and shape and their walls are often infolded into intra-acinar villous processes which some times branch to form secondary papillae. The acinar epithelium is composed of an inner layer of columnar or cuboidal cells separated from the stroma by a basal row of elliptical or elongated cells. The active epithelium is tall, granular, and pseudostratified containing nuclei placed at different levels in the cells. These tall cells have well developed Golgi network between the nuclei and free borders of the cells. In older subjects the columnar epithelium has basally set nuclei, pale to clear cytoplasm that forms an uniform, even, luminal border. Sometimes the cells are small and the epithelium is apparently crowded with irregularly stratified nuclei (Ashley, 1980). Bartsch (1980) carried out comparative LM and EM study of human, dog and rat prostate and found higher amount of ER in rat prostate than the normal human and dog prostate, but the amount of secretory granules was higher in dog and human prostate.

2) Histopathology:

Some idea of histopathological aspects of prostate can be had by a cursory glance through an article entitled 'Tumors of the Prostate Gland' from the book, Evans Histological Appearances of Tumors edited by David Ashley (1980) and also recent views and papers published during the last decade (Bartsch et al., 1979; Ohtsuki et al., 1982; Zirkin et al., 1984; Poulet, 1985; Alguacil et al., 1986; Young and Philip, 1987; Ashley, 1980). Critical evaluation of this literature shows histopathological changes in the case of BPH and Chronic prostatitis in which the stroma and epithelium are involved and these tissues cause a reasonably symmetrical and nodular localized enlargement of the prostate. The central portion of the gland is often affected and this middle lobe forms irregular spherical mass into the floor of the neck of the bladder (Moore, 1936). Enlargement is brought about by an increase in the amounts of the epithelial and fibromuscular tissues. The adjacent tissue is compressed into a false capsule from which the hyperplastic mass can be easily enucleated. Glandular elements predominate in most of the areas and are aggregated together into orderly spherical masses of varying sizes bounded by bands of fibrous tissue. Small cysts are common. Stromal type of hyperplasia may lead to the formation of one or more nodules composed almost entirely of fibromuscular tissue (Ashley, 1980). The glandular elements increase in size and number. Proliferation is regular and symmetrical and although they lie close together,

each acinar structure tends to remain discrete and distinct and retains the convoluted outline of normal acini. Foci of markedly adenomatous character are produced which consist of small tubules separated from each other by delicate strands of fibromuscular tissue. In hyperplasia, however, the pattern is more or less uniform in all directions. The stroma in hyperplasia closely irvests the acinar wall and it contains smooth muscle fibres. The acini show the presence of basal layer of cells, but such layer is usually absent in malignant acini (Ashley, 1980; Bratsch, 1979). Ohtsuki et al. (1982) observed fine structure of nuclear bodies in the human prostatic hyperplasia which varied from 0.3 to 1.8 µm in diameter. Zirkin et al. (1984) studied age-dependent hyperplasia in dog, in which the proliferation of prostatic glandular and stromal components increases the epithelial number and epithelial cell size leading to increase in the total weight of prostate. EM study showed that the volume densities of RER and secretion granules are significantly lower in epithelial cells of immature prostate than in the hyperplastic prostate of mature dog. No significant differences are seen in volume densities of RER, free ribosomes, secretion granules, SER and mitochondria in prostatic epithelial cells of dogs of 1.5 to 9 years of age. Poulet (1985) showed the storage of 5α-Dihydrotestosterone in dog prostate cells leading to hyperplasia. Alguacil et al. (1986) studied histology of 20 consecutive cases of tranceurethral prostectomy and found that the areas of chronic prostatitis usually show degenerated lymphocytes and stromal cells with signet ring appearance that occasion-

ally can mimic the carcinoma. Young and Philip (1987) studied the rate variant of benign prostatic hyperplasia analogous to sclerosing adenosis of the breast and found that the prostate chips have a proliferation of irregular crowded acini, small nests and isolated cells within the stroma. The acini are lined by columnar and focally by basal cells (Myoepithelium).

It is quite common to find small areas of inflammatory infiltration in the substance of prostate which is the site of adenomatous hyperplasia. These may consist either of collections of lymphocytes and plasma cells or of acute micro-abscesses showing polymorphonuclear leucocytes either in the stroma or within the lumina of dilated glands. In some cases vascular obstruction and small areas of infarction are found. In the areas of infarction there is frequently squamous metaplasia of the gland epithelium (Mostofi and Morse, 1951). Once the microabscesses are formed, the growth of fibromuscular tissue proceeds dominating the glandular elements (Franks, 1954 b).

Incidence of prostatic cancer in children is very rare (Kagan and Fox, 1959), but characteristically this form of malignant disease affects the males over 45 years of age and its incidence increases with the increase in age. Most commonly the tumor occurs in the subjects more than 60 years old (Mckenzie et al., 1957). Detailed histological examination of prostates removed at routine necorpsies has shown a significantly high frequency of unsuspected foci of malignancy which are structurally carcinomas (Franks, 1954 b).

It is very difficult to identify the prostatic cancers with the help of histological appearances, as the microscopic features of prostatic malignancy are not of regular consistant occurrence. Similarly, varieties of carcinomas represent their individual characteristic forms of histological appearance. The major groups of carcinomas occurring in human prostate are generally identified as adenocarcinomas of the prostate and are classified into ductal, tubular and tubulo-alveolar variants (Foot et al., 1950; Edwards et al., 1953). Both early and advanced cancers of prostate consist of well or poorly formed, small, medium or large acini and tubules. The glandular elements may be closely packed together with little intervening stroma and grouped in a nodular or linear arrangement (Ashley, 1980). Other adenocarcinomas are more obviously infiltrative and their branching tubules are dispersed irregularly in a disorderly radial fashion throughout a variable amount of fibromuscular stroma. The convoluted outlines found in normal glands are lacking in their malignant homologues. In the better differentiated adenocarcinomas, the glandular elements have a smooth, round or oval contour and their columnar or cuboidal cells often show a marked uniformity and have a granular, pale or clear cytoplasm with sharp borders and dark nuclei. Occasionally, intra-acinar proliferation results in secondary gland formation within the lumina of the glands and evaluation of a cribriform, laciform or multiacinar pattern. In the more poorly differentiated adenocarcinoma expansile nodules of closely packed, often imperfect, small, irregular and distorted acini may

be produced. The more advanced adenocarcinomas show infiltration of the neoplastic acini which penetrate in the surrounding stroma and separate or rupture the strands of muscles or burrow deeply into bundles of smooth muscles (Ashley, 1980).

Malignant acini usually consist of a single row of columnar and cuboidal cells and nearly always lack a basal layer of cells, even when the glandular elements seem to consist of more than one row of cells. Permeation of the lymphatic beneath the capsule is a common finding and involvement of lymphatic occurs regardless of size of cancer. Blood vessel invasion is observed in a significant number of prostatic cancers. Totten et al. (1953) claimed that certain nuclear features aid in the distinction of normal or hyperplastic cells from malignant cells. According to them the changes concerned with the presence of prominent, relatively large, complex and deeply staining nucleoli are most helpful. The chromatin gets concentrated at the nuclear membrane of malignant cells. Melicow (1966) suggested the histological criteria for the diagnosis of prostatic cancer should be microacini, often consisting of pale cells with a back to back arrangement, nuclear hyperchromatism and nucleolar prominance, foci of linear infiltration into the stroma and extraprostatic spread, a diffuse array of microacini and anaplasia. In general, when the acinar structures differentiated in the tumor are small, the nuclei are large and hyperchromatic and when the acini are large and tubuloalveolar, the cytoplasm is abundent and the nuclei are comparatively small and pyknotic.

A squamous cell carcinoma is an infrequent tumor of the prostate (Kahler, 1939). Islands of metaplastic squamous epithelium, however, can be misdiagnosed as squamous cancers, particularly when they are associated with infarction. Schubert et al. (1981) studied histological types of prostatic carcinoma and their relation with the hormones and showed that the hormone therapy induces reticularization and vacuolization of the nuclear chromatin. Manivel et al. (1986) showed, in cytosarcoma phyllodus of prostate, the stroma progressed to clearly sarcomatous appearance, whereas other tumors had cellular stroma that was mitotically inactive. The findings of Hayashi et al. (1987) suggested that prostatic carcinoma is multifocal in origin and that focal, well differentiated carcinomas are different in biological behaviour from invasive, poorly differentiated carcinomas.

3) Chemical Composition:

Prostate glands have been studied in different mammals for their chemical composition by applying various biochemical and histochemical methods. These studies have revealed that these glands mainly contain proteins, carbohydrates, various enzymes, lipids and inorganic ions. The secretion of prostate shows more or less similar chemical composition.

a) Proteins:

The important chemical constituent found in prostate secretion is protein which forms one of the major components contributing to the volume of semen in most of the mammalian species (Price, Williams, Ashman, 1961; Mann 1964). The secretion of the prostate in most species is found to be less viscid and proteinaceous than that of the seminal vesicle (Flickinger, 1974). They further demonstrated that most of the proteins are secreted by merocrine mode, while small amount accounts for apocrine.

The following is a brief review on proteins found in prostate secretion in normal as well as under different pathological conditions studied during recent years. Chen et al. (1982) isolated the prostate α-protein which is a major glycoprotein (mol. wt. 50,000) in the cytosol fraction of rat ventral prostate. Lee et al. (1985) studied proteins in rat prostate lobes of which six groups of proteins showed lobe-specific differences. Ponsette et al. (1981) showed that prostate secretion protein or estramustine binding protein is a major protein. This protein is found to be increased during puberty. Their study also suggested that it is androgen-sensitive protein as it decreases after castration and increases by administration. Age-specific proteins of rat prostate and their dependence on androgen level have been revealed by Shain et al. (1986). Sheth et al. (1981) detected the presence of high amount of inhibin in prostate which is a unique

feature of human prostate. They showed inhibin increase in benign prostatic hyperplasia (BPH) by ten fold in comparison to normal and cancerous tissue. Further, they (Sheth et al., 1985) showed that the concentration of inhibin is more in epithelial cells lining the healthy lobules than in those undergoing atrophy, whereas Tremblay et al. (1987) showed the presence of three major prostatic secretory proteins in the urine of normal men and patients with BPH and prostatic cancer. The proteins are prostatic acid phosphatase, prostate specific antigen and β-inhibin. Their results indicated that these proteins may be used as ordinary markers which provide an easy means to study the behaviour of primary prostatic tumor. The prostatic steroid binding protein which acts as carcinogen binding agent is reported in rat prostate (Soderkvist et al., 1987).

The review on recent literature regarding the proteins of prostate shows that the keratin protein and its association with different pathological conditions of human prostate is reported by a number of workers, such as in metaplastic squamous epithelium (Friedmann et al., 1985), in microcarcinoma and a typical hyperplasia (Battaglia et al., 1985), in normal and benign prostatic tissue (Kitajima et al., 1986), in normal, BPH and carcinoma (Kuwahara et al., 1987) and in histological sections of normal, hyperplastic and neoplastic prostate (Purnell et al., 1987).

b) Acid Phosphatase:

Lysosomes and acid phosphatase activity of prostate has received a great deal of attention in normal as well as in the different pathological conditions of prostate. It is well recorded that secretory fluids produced by normal prostate exhibit high activities of acid phosphatase (Price et al., 1961; Mann, 1964; Kent et al., 1969). Gutman and Gutman (1938) showed the presence of high acid phosphatase activity in prostate gland of dog, cat, rabbit, guinea pig and rat, and also in monkeys by Gutman (1939). Gomori (1941) showed that the epithelial cells of prostate gland give positive reaction when tested for acid phosphatase. Similar reports have been also made by various research workers (Bern, 1949; Kobat and Furth 1949; Wolf et al., 1949; Brandes, 1954; Takkar, 1969). Takkar et al. (1969) further demonstrated acid phosphatase in the nucleus and lysosomes. Changes in the distribution of acid phosphatase in mouse ventral prostate were described at various times after castration or after administration of estrogen and progesteron (Brandes and Bourne 1956; Brandes and Groth, 1961; Brandes et al., 1962). Hugin et al. (1967) and Schooness et al. (1972) demonstrated that primary prostatic secretion of dogs contained very high concentration of acid phosphatase. By exogenous testosterone treatment acid phosphatase concentration of primary prostatic fluid was significantly increased. In the normal animals reaction appeared in the form of cytoplasmic bodies (Brandes and Groth, 1961). Helminen and Ericsson (1970)

showed that mature secretion granules produced by epithelial cells in rat ventral prostatic lobe contained acid phosphatase and thus represent primary lysosomes. Serranon et al. (1976) investigated acid phosphatase at ultrastructural level in rat, dog, monkey and man prostate and observed that this enzyme is localised in secretory granules, Golgi cisternae, and Golgi vacuoles in prostatic epithelium. Buchanan and Andrew (1984) studied acid phosphatase activity in both human and young adult rat ventral prostate and showed that the intensity of reaction is greater in human prostate. De Vries and Sanders (1986) reported acid phosphatase in lysosomal and microsomal fractions of human prostatic epithelium. Goldford et al. (1986) studied prostatic acid phosphatase during different ages and showed that acid phosphatase which is high at birth, decreases at age of six months, reappears by the age of ten years and increases in puberty. Thus, the levels of prostatic acid phosphatase appear to follow the testosterone level suggesting hormonal dependence. Mori and Choei (1985) studied prostatic acid phosphatase in normal and hyperplastic prostate and revealed that acid phosphatase is uniform at the apical portion of glandular epithelium, but electron microscopic studies showed that the prostatic acid phosphatase is localized in microvilli lining, prostatic and vesicular bodies of apical cells. The neoplastic prostatic tissue revealed more intense and uniform staining of tumor cells and the glandular epithelium of well differentiated adenocarcinoma, whereas less intense and more variable staining is seen in neoplastic cells of moderately and poorly differentiated

adenocarcinoma. Further, in neoplastic cells the granules with less intensity are found between collagen fibres as well as in the adjacent endothelium of stromal capillaries in anaplastic tissue. Zundervan et al. (1986) demonstrated the localization of secretory prostatic acid phosphatase in human hyperplastic prostatic epithelium and showed acid phosphatase activity in columnar epithelial cells containing α-glucosidase and β-galactosidase. Loor et al. (1981) showed by specific immunochemical measurements the activity of prostate acid phosphatase in prostate cancer averaging about 25 per cent of that in normal prostate or benign prostatic hyperplasia. Noguchi (1984) studied acid phosphatase activity immunohistochemically and showed that all hyperplastic prostate glandular cells have uniform distribution of prostatic acid phosphatase (PAP). In cancerous prostate the distribution of PAP varies from case to case. Song et al. (1985) studied acid phosphatase in human hyperplastic prostate and reported that the columnar secretory epithelium of prostate gland show different intensity and distribution of immunostaining, but under electron microscope the secretory epithelial cells often showed electron dense reaction product in Golgi apparatus, Golgi secretory vesicles and Golgi vacuoles. Aumueller and Seita (1985) showed that acid phosphatase activity is confined exclusively to secretory vacuoles of glandular cells of metastatic prostatic cancer. This increased PAP may be due to the carcinomatous cells or leucocytes destroyed during the process of metastasis. Warhol et al. (1985) reported the PAP immunoreactivity and showed that this reactivity is localised in lysosomal granules

in both hyperplastic glands and adenocarcinoma secretory cells. Crignon and Michael (1985) studied three immunological markers viz., prostate specific antigen, PAP, and keratin to determine the effect of hormonal therapy, whereas Rubey (1985) studied the ratio of α-acid-glycoprotein/prealbumin referred to cancer serum.

c) Esterase and Other Enzymes:

Nachlas and Seligman (1949 a) demonstrated esterase activity in various tissues of dog and rat and observed significant amount of esterase in the prostate. Similar studies by Burrnett (1952 b) showed weak esterase activity in the prostate of dog, by employing indoxyl acetate method. Frost and Brandes (1967) observed cytoplasmic and droplet-like esterase activity in rat prostate. Markert and Hunter (1959) studied the mouse ventral prostate esterase by electrophoresis.

Non-specific esterase have been demonstrated by various methods in the human prostatic epithelial cells (Brandes and Bourne, 1956; Fleischhaurer, 1957), the reaction appeared in the form of granules of varying sizes concentrated in the supranuclear regions. Hyperplastic and carcinomatous glands showed similar positive reaction for esterase (Brandes and Bourne, 1956; Kirchheim et al., 1964). Together with alkaline and acid phosphatase, α-napthol acetate esterase was histochemically studied in different structures of human prostate which showed highest esterase activity in the prostatic acini and their ducts (Yurchenko, 1972). Seaman and Winell (1959) studied

esterase activity by employing different methods and observed that there was consistently high degree of activity in prostatic cancer cells of mature dogs. Their studies, by employing zymogram technique indicated that at least there are eight esterases of non-specific type in the dog prostate. Presence of non-specific esterase in the epithelium of prostate gland of common mormoset, Callythrix jacchus was observed by Miraglia et al. (1970). Takkar et al. (1969) reported that esterase activity is localized in intracytoplasmic granules of lysosomes in acinar epithelial cells of the rat prostate, whereas lipase in the cytoplasm of the epithelial cells of the prostatic lobes. Mountzing (1972) examined prostatic tissue from eleven species for acid phosphatase, alkaline phosphatase, non-specific esterases, leucine-amino peptidase and β-glucuronidase The enzymes were found in all the species but the amount varied from species to species. Astanasov (1973) obtained at least 12 bands with esterase activity in human prostatic homogenates by acrynimide disc electrophoresis.

The effect of castration on the non-specific esterase activity in the acinar epithelial cells of rat prostate has been studied by Harkonen et al. (1964). Their studies showed marked reduction in the esterase activity after castration. Dube et al. (1986) studied dog prostate arginine esterase and human PSA and showed that they are related with each other.

Mizutani et al. (1986, 1987) studied α-reductase in BPH and

prostatic cancer and observed 5 times higher 5 α-reductase activity in BPH than in the prostatic cancer, whereas 5 α-reductase activity in the interstitium of BPH was four times higher than that in the epithelium of BPH, but in prostatic cancer there was no significant difference in the 5 α-reductase activity in the epithelium and interstitium.

Ronquist et al. (1984) suggested a prostatic origin of fucosyl transferase activity studying healthy men and men with prostatic cancer. Two of the men with prostatic cancer displayed 50-90 per cent decreased activities. Antiandrogenic therapy in another man with cancer resuslted in the substantial reduction in seminal plasma contents of fucosyl transferase, ATPase, acid phosphatase and fructose, suggesting a role of testosterone in their secretion.

Hendriks et al. (1987) reported kininase-I enzyme activity in human fluids and tissues and showed that the enzyme value is similar in normal prostate, BPH and the prostatic carcinoma.

Kaburagi et al.(1987) determined aromatase in the homogenates of human prostatic tissue (3 BPH and 2 normal) and showed presence of an androgen-aromatase system in the human prostate.

Localization of prostate-specific antigen (PSA) and its use in the therapy of prostatic cancer was studied by different scientists. Warhol Michael (1985) showed that PSA immunoreactivity is

localized over ER, cytoplasmic vesicles, vacuoles and within the lumina of prostate glands. Fuse et al. (1986) determined PSA and prostatic acid phosphatase (PAP) with a citus test kit for prostatic cancer and others. The endocrine treatment decreased the elevated values of PAP and PSA in 60% of the cases, while most of the PAP and PSA levels in the cases controlled under endocrine therapy were within normal values. Guinan et al. (1987) showed that the ultimate role of PSA may be as the marker of choice to monitor therapy for prostatic carcinoma. Ahmann et al. (1987) showed that PSA is more sensitive and potentially more useful tumor marker than acid phosphatase measurement in the patients with metastatic prostatic carcinoma.

d) Lipids:

The seminal plasma of many species contains small globules, droplets or granules known as lipid bodies which are derived chiefly from the prostate in man, dog, cat and rabbit (Mann, 1964). Although Iversen (1953) observed lipids in the human prostate, no correlation was found between the lipid content and phosphatase activity. Lipids form a major component of prostatic epithelium and secretion in dog (Seaman, 1956). Acinar cell lipids were found to be predominantly neutral lipids and there were less amounts of phospholipids, CHO and CHO-esters. Most of the evidences indicated the presence of lecithin in the phosphatide fraction in acinar cells, but lecithin and

cholesterol were absent in lumen. Seaman and Studen (1960) observed lipids in prostate of dog and rat and noted a species difference. In both the species lipids are involved in lipid complexes. Human prostate has high contents of lipids and it was shown by Huggins and Webster (1948) that, following intramuscular injection of diethylstilbestrol, the cells of anterior portion of the prostate regress and lose their eosin staining lipid material much more promptly than the corresponding cells of the posterior region. Studies by Pretl (1948) on human prostate showed the presence of sudanophilic droplets, lipofuschin and acetyl phospholipids in epithelial cells. Moore et al., (1941) analysed samples of 12 human prostatic secretion and found up to 9.5 mg lipid phosphorus/100 g fluid with an average of 2.7 mg p/100 g or 67.5 mg phospholipid/100 g . Scott (1953) reported 186 mg total lipid/100 ml prostatic fluid and 179 mg phospholipid/100 ml prostatic fluid of human beings. Galli and Mori (1967) compared the lipid content in normal and adenomatous prostatic tissue of human. They estimated 48 mg/gm total lipids of which 80 per cent were TG, 9 per cent phospholipids and the remaining FFA and CHO in normal prostatic tissue. In adenomatous prostate tissue, the total lipids were reduced to 34 mg/gm of which TG were 65 per cent, but a significant increase was observed in phospholipids which constituted 24 per cent indicating enhanced biosynthetic activity in pathological tissue. Compbell et al. (1966) separated lipids from dog-prostate and showed that the amount of neutral lipids in the prostatic fluid

was more (94 per cent) than that in the serum (65 per cent). Triglycerides and free fatty acids found elevated but phospholipids depressed in prostate fluid than in serum. Levin et al. (1955) studied effects of testosterone on prostate of control and castrated rats and found increase in the phospholipid contents. Takkar and Kamboj (1971) demonstrated sudanophilic lipids in the cytoplasm of the epithelial cells of rat prostate complex. Progesterone and estrogen caused increase in the epithelial lipids in Golgi complex, subnuclear areas and in periacinal stroma of all the lobules. Ventral prostate was found to be more sensitive to hormones than the dorsal or lateral prostate. A high dose of estrogen (5 µm) resulted in overall reduction of lipids in cytoplasm except in ventral prostate. Umapathy et al.(1979) reported that administration of testosterone for 7 days elicited a significant rise in the total lipids, phospholipids, cholesterols and glycerides in the prostate, whereas progesterone and estrogen brought about a decrease in glycerides. Pawar (1976) studied seasonal variations in TL, NL and PL of bat prostate and showed increase in all these three components during active breeding period. Toro (1984) observed decrease in TL and NL and increase in PL in rats treated with Vinca alkaloid. Vitex negunda extract was shown to decrease TL, NL and PL in rat prostate (Sohni, 1984). Similarly, Shaha (1984) showed decrease in lipids in rats treated with Daucus seed extract, while Awati (1984) showed increase in TL, NL and PL in rats administered with Butea extract.

e) Inorganic-Ions:

The ionic composition studied in prostate tissue and its secretion indicated presence of Na, K, Cl, Ca, Mg, Cu, Fe, Zn, Cd and citrate. Among these the zinc has been paid more attention because of its important role in prostatic function. Zn alongwith DNA and RNA in typical canine hyperplasia is shown to increase than in the normal prostate (Tunn et al., 1979), whereas low levels of Zn are detected from the chronic prostatic diseases except in cases of adenoma (Leissner et al. 1980). Jafa et al. (1980) studied Zn, Cu, and Fe in prostate tissue and the plasma of benign prostatic hyperplasia, fibrous growth, chronic prostatitis and malignancy and showed the diagnostic significance of these metalic ions in prostatic diseases. Local injections of 20 per cent Cu-Zn-EDTA in the human hyperplastic prostate caused atrophy of acinar epithelium, dilation of the acinar cavity and fibrosis of the stroma (Hikosaka, 1981). Feustel et al. (1984) observed the highest concentration of Cd and the lowest levels of Zn in the poorly differentiated carcinomas, whereas Larue et al. (1985) showed that epithelium of human normal prostate is rich in Zn and in hyperplastic prostate it is bound to cytoplasmic citrate. Kauanagh (1985) observed that the ionic composition of human prostatic fluid varied greatly in individuals reflecting the secretory activity of the gland and presence or absence of inflammatory disease. In normal prostatic fluid the major component is citrate, while Cl concentration is lower. Their counter ions are

mainly Na and K with Ca, Mg, and Zn. The prostatic secretion of men with prostatitis comprises mainly Na and Cl. Candle et al. (1986) measured seminal zinc in normospermic and infertile patients by a new calorimetric method. The data suggested that the Zn is secreted mainly by the prostate while vesicular, epididymal and testicular secretions are devoid of Zn. Elevation of this metal, so, permits the diagnosis of potency of seminal pathways, but does not permit identification of prostatitis.

Review of literature during recent years on Zn in prostate shows that this metal is mainly studied in relation to testicular hormones. Timms et al.(1983) studied the effect of androgens on the ultrstructure and subcellular Zn distribution in prostatic epithelium of castrated rats and found that testosterone propionate is most effective, elevating Zn in lateral lobe. In ventral lobe both 5α DHT and testosterone propionate androgens caused an increase in subcellular Zn concentration. The yields of bound Zn are decreased by androgenic steroids which increase the free Zn levels in prostatic tissue (Larue et al., 1985). Further Sanada et al. (1985) reported that Zn is mainly secreted by the prostate and Zn concentration in seminal plasma is an excellent indicator of prostatic secretory function. The prostatic secretion has stimulatory effect on spermatozoan motility. Rul et al. (1986) reported that in old age the putrescine and prosta-glandin E are reduced, while Zn level is elevated showing possible

disturbance of prostatic function in the middle aged man. Chung et al. (1986) studied the presence of androgen receptors in ventral prostate glands of Zn deficient rats and showed that this metal is involved in androgen binding process in target cells.

f) Carbohydrates:

These organic chemical constituents have been mainly studied under the heading mucopolysaccharides or complex carbohydrates. Mucins are one of the important secretory products of the mucous cells found in the prostate gland. The light microscopic study has been made and histochemistry of prostate gland of mouse and rat have been extensively studied by Brandes (1966). The carbohydrate produced in the anterior lobe of prostate is chiefly fructose (Davies and Mann, 1947-a, Lutwak and Mann, 1951). Glycogen is found to be absent in the prostate of rat (Takkar and Kamboj, 1970) and bull (Sajonski et al., 1972). On the other hand the presence of glycogen has been reported in the prostate of dog (Gerber, 1961), rabbit (Schantz, 1964) and Wombat (Brooks et al., 1978). Rodger and Ian (1980) demonstrated the glycogen and N-acetyl glucosamine as prostatic carbohydrate of Australian and American marsupials and showed that prostatic secretion of the Dasyurid and Sarcophilus has a very high glycogen concentration as compared to other species of marsupials. Bischof and Gerhard (1982) showed age-dependent changes in the carbohydrate pattern of human prostate epithelium

and showed that the carbohydrate moiety responsible for peroxidase conjugated lectins binding presumably containing molecule, most likely a glycoprotein which is present in actively functioning prostatic epithelial cells. Histochemical studies showed the presence of PAS-positive and diastase-resistant material in the prostate of dog (Arcadi, 1952), ram (Aitken, 1954), boar (Aitken, 1960) and bull (Stallcup, 1969).

Arcadi (1952) reported protein glycogen complexes in some ovoid cells of dog prostate. Kanwar and Sheikher (1977) reported that the columnar epithelium in S.murinus is secretory and the secretion is rich in protein and polysaccharides. Ali et al. (1976) reported mucoprotein in camel prostate. Gupta and Yashwant Singh (1982) observed the saliva-resistant PAS-positive material in the intracellular region of goat prostate which was negative to Best's carmine reaction. Serus alveoli were AB-negative, whereas mucous alveoli revealed moderate alcianophilia.

The presence of acidic mucins in the prostate has been reported by the number of research workers. Acidic mucopolysaccharides have been studied in the prostate of dog (Arcadi, 1952), deer (Aughey, 1969), rat and man (Preto-Parvis et al., 1966; pasqualucci and Macha, 1968; Leutert and Jahn, 1970). Miraglia et al. (1970) in marmoset and Goyal and Mathur (1974) in H.auratus collaris prostate demonstrated the presence of sialic acid. Biochemical

studies revealed an average sialic acid content in the prostate ranged at 24.3 mg/100 ml in monkey (Bose and Kar, 1968) and 60.6 mg/100 ml in human prostatic fluid (Warren, 1959). Seasonal variations leading to depletion in the prostatic glycogen, sialic acid and mucopolysaccharides are shown in quiescent period of some bats (Pawar, 1976; Vibhute, 1980; Fartade, 1982). Sato and Fernc (1980) studied S^{35} distribution by organ culture method in mouse prostate tissue and its secretion and showed the presence of chondroitin-4-sulfate, dermatan sulfate, heparan sulfate and heparan sulfate oligosaccharides. Tsukise and Yamada (1984) studied the complex carbohydrates of secretory epithelium of goat prostate and showed three types of secretory epithelial cells which contained neutral and acidic complex carbohydrates, whereas Tsukise et al. (1986) observed weak to strong positive histochemical reactions for complex carbohydrates in the secretory epithelium of hamster prostate. The results showed more amount of neutral glycoproteins with various saccharide residues and small amount of acidic mucopolysaccharides. Golgi area of these secretory epithelium revealed strong positive reaction for neutral glycoproteins.

Singhal (1968) showed that testosterone increases glycogen content of prostate in rat. Hexestrol treatment revealed similar results in dog prostate (Chieregо and Fabris, 1951), whereas Bose and Kar (1968) reported that castration in monkey results an increase in prostatic sialic acid content. Gupta and Yashwant Singh (1982) observed the moderate increase in intracellular PAS-positive material

of goat prostate after castration. Reddy and Govindappa (1985) observed that prolactin administration resulted in the accumulation of prostatic glycolysis with increased glycogen content.

Cyproterone acetate administration resulted in decrease in sialic acid content of prostate in rat (Rajlaxmi, 1972). Chinoy and Sheth (1977) showed increased amount of glycogen with administration of $CaCl_2$ in rat prostate. Awati, Toro and Shaha (1984) demonstrated, histochemically, decrease in glycogen and sialic acid contents in acinar cells, interacinar cells and in secretion of prostate in albino rats after administration of Butea monosperma extract, Vinca rosea and Daucus carotid alkaloids respectively, whereas Sohani (1984) observed no significant alterations in these contents after Vitex negunda extract administration.

A review of literature on recent work related to prostate mucins shows that they have been mainly studied under different pathological conditions. Some mucicarminophilic material is present in a significant number of well-differentiated carcinomas of the prostate (Levine and Foster, 1964). The distribution and nature of the mucins in normal and cancerous prostatic tissue have been studied histochemically by Franks et al. (1964). In their studies they found that alcian blue positive mucins occur commonly in the cells and acini of both latent and overt prostatic cancers, but rarely in the normal gland or in benign nodular hyperplasia. These reactions are helpful in the recognization of well differentiated cancers. Similarly, in the

cases recorded by Sika and Buckley (1964) the acini contain variable amounts of PAS-positive and mucinocarminophilic substance, but no mucin can be demonstrated in the cells. Further Franks et al. (1964) showed that the colloid cancers produce large quantities of alcian blue positive, sialic acid containing mucins in which sialic acid residues are sulfated. These extensively mucinous tumors also contain nonsulfated mucins. Patel et al. (1981) studied a case of mucin secreting adenocarcinoma of prostate with very high acid phosphatase level. Sanefuji et al. (1982) reported synthesis and secretion of neutral and acidic mucosubstances in explant culture from carcinogenesis of human prostate. Villary and Napoli (1984) studied diagnostic value of acidic mucins in prostate neoplasm and reported that out of 38, nine cases of atypical epithelial hyperplasia showed weak positive material in the lumen of gland, but in the well differentiated adenocarcinoma this material was found more frequent and abundant. According to these research workers acid-mucin secretion is useful in confirming the presence of cancer in equivocal cases and for a quicker identification of microcarcinoma. Histochemistry of complex carbohydrates in prostate tumor was studied by Sugiyama (1985) which indicated the presence of neutral and acidic carbohydrates. Further with the help of immunohistochemical studies he showed that benign prostatic hyperplastic (BPH) cell basement membrane, the cytoplasm of the epithelium and cell interstitium contain chondroitin B and hyaluronic acid. 1,2 Glycol group of neutral complex carbohydrates

in the interstitium of the prostatic cancer are shown to exist in smaller amount than those in the BPH. In addition the prostatic cancer cell cytoplasm showed presence of chondroitin sulfate A, C and hyaluronic acid. Matson et al. (1986) in their histochemical studies showed that glandular parenchyma contain large amount of sialidase labile sialomucins as well as acic phosphatase and small quantities of alkaline phosphatase and proteins.

g) Hormone:

Hormonal excitation apparently initiates, maintains and controls the form and function of prostatic epithelium (Scott, 1953). It is clear that castration causes a decrease in the size of prostate and that similar atrophy followed hypophysectomies in animals. In hypophysectomised animals' prostatic atrophy, however, can be prevented by giving testosterone. The hormonal effect on the prostate is mainly studied with reference to therapeutic use in the prostate cancer and BPH. Rubio et al. (1986) showed that LHRH analogues with antiandrogens is a valuable tool for the fight against prostatic cancer. Purvis et al. (1985) observed that the cytosol from rat ventral prostate contains two estrogen binding components and they also showed that estrogen exerted direct effect on both stromal and epithelial tissue of prostate. Kume et al. (1986) studied effect of sex hormones on primary cultured cells of rat ventral prostate by using primary culture system and found that DHT have a stimulatory

effect, estradiol have an inhibitory effect while addition of cyproterone acetate to DHT results in little effect on cell growth. Phadake et al. (1987) observed a significant rise in inhibin levels in BPH as compared to age matched control group, whereas LH and FSH levels decrease significantly. After surgery the levels of inhibin and prolactin are reduced but no consistant change in LH, FSH and TSH levels is noted. Thus, these observations suggested that the changes observed in the hormonal levels in BPH patients are not related to age of the patient or size of the tumor. Benson et al. (1987) showed that nuclear receptor binding activity in the localized and metaplastic human prostatic cancer tissue is predictive of response to the hormonal manipulation. Hudson et al. (1987) compared nuclear α-reductase activities in the stromal and epithelial fractions of human prostatic tissue and the results suggested that differences in the conversion of testosterone to DHT help to explain the DHT levels seen in hyperplastic prostate. Bartsch et al. (1987) showed that the treatment with 5α- DHT or 3α -androstanediol combined with 17β estradiol not only induces prostatic overgrowth but also leads to prostatic hyperplasia of glandular type. However, the stereologic analysis of canine prostates following steroid administration showed that canine hyperplasia is primarily a glandular disease while human BPH shows more stromal activation.

4) Reasons for Undertaking the Present Study:

The above mentioned critical review of the work done on

the prostate gland gives clear understanding about the reasons why the present problem is undertaken. This review brings out the following significant facts:

A review of literature on the prostate gland mentioned in the earlier pages of this chapter shows that this gland has been studied in different mammals for the understanding of histological structure and secretion, presence of different chemical components in the gland and its secretion such as proteins, enzymes, lipids, various inorganic ions, carbohydrates etc. The review also shows that, though not in all, at least in some pathological disorders, this gland has been studied for understanding of histopathology in man. Few references also have been accumulated on the variations in the chemical components found in various prostatic disorders.

On the other hand mucopolysaccharides of the prostate gland and their variations (alterations) in different pathological disorders in man have not been fully investigated yet. Only in the recent years some information regarding the presence of neutral and acidic mucins in the normal prostate of man and some of the other mammals like rat, dog has been made available by few workers (Brandes, 1966; Takkar and Kamboj, 1970; Sajonski et al., 1972; Brooks et al., 1978; Rodger and Ian, 1980; Sato and Fernc, 1980; Tsukise and Yamada, 1984; Sugiyama, 1985; Matson et al., 1986).

An evaluation of the above given critical review stimulated

to undertake the present research project and our efforts were directed:

1 to study the histological structure of human normal prostate and the prostate under different pathological disorders;

2 to find out the histopathological differences in the prostate glands selected for the present investigation;

3 to find out the distribution and localization of various mucosubstances in normal and prostate under different pathological conditions in man.

4 to study the variations in the distribution and localization of various mucosubstances in the human prostate gland selected for the present investigation;

5 to find out whether these variations in the distribution and localization of mucosubstances bear any relationship with and significance in the early diagnosis of prostatic disorders.

5) Plan of the Proposed Work:

The present investigation is, therefore, undertaken to get an insight into the histopathology and histochemistry of mucopolysaccharides of prostate under various diseased conditions. Our efforts were directed towards:

i) the study of histological and histopathological characteristics of normal and diseased human prostate;

ii) the study of distribution of mucopolysaccharides of prostate tissue in normal and under pathological condition;

iii) the study of alterations, if any, in the mucopolysaccharides of normal and prostate under diseased condition.

a) Choice of the Tissue:

For the present investigation the human prostate was selected after careful examination of records, at Krishna Medical Research Institute, Karad on various disorders of reproductive organs in man. This examination showed that the prostatic disorders are of frequent occurrence. Therefore, the prostatic tissue was selected for the present investigation.

b) Choice of the Technique:

To achieve a technical and methodological perfection, standard and recent histological and histochemical techniques are selected for the study of histopathology and mucopolysaccharides.

i) As the present investigation aims at the detailed study of histopathological changes in human prostate under different pathological conditions, the usual haematoxylene eosine technique

is employed.

ii) A series of histochemical techniques involved reactivities of various complex carbohydrates towards PAS, alcian blue at pH-1.0 and 2.5, aldehyde fuschin, colloidal iron, etc., are worked out in the present investigation. The aim of selecting these methods is to characterise histochemically the nature of complex carbohydrates. These histochemical techniques are currently used to identify glycogen, neutral mucosubstances, acidic mucosubstances such as sialomucins and sulfomucins.

c) Critical Evaluation of the Dissertation:

The present research project entitled "Study of Mucopolysaccharides in the Prostate Gland of Man (Normal and under Pathological Conditions)" on histopathology and histochemistry of mucosubstances in human prostate is carried out to augment our knowledge about histology of normal human prostate, histopathological changes produced due to prostate disorders, nature of mucosubstances at different cellular sites of prostate, such as epithelium of acini, basement membrane, stroma, luminal secretion and smooth muscles and to compare the effects produced by these prostatic disorders on histology and nature of mucosubstances in prostate as well as to compare the results obtained in the present study with that of existing literature

the prostate of human being and other mammals also.

d) Presentation of the Dissertation:

It is decided to divide the present dissertation into four chapters. The first chapter includes introduction and review of literature on histology, histopathology and histochemistry of mucosubstances in prostate tissue and the reason that stimulated to undertake the present research work and the plan of proposed work. The second chapter gives the detailed description of material and methodology and the techniques employed in the present investigation. The third chapter describes all the observations in detail on histological, histopathological and histochemical observations on the nature and distribution of mucosubstances in prostate tissue; the text of observation is well supported by tabulated data and photomicrographs of histochemical observations. The fourth chapter deals with the discussion on the results obtained in the present investigation and the existing literature on the prostate of different mammals. This chapter also contains summary and concluding remarks on the present investigation.

The dissertation ends with a bibliography of exhaustive literature cited in this dissertation.