Chapter 1 Introduction

1. Introduction

The endocrine function of the gonads is primarily concerned with perpetuation of the species. The endocrine system is made up of specialized cells, tissues, and organs that create and secrete chemicals in blood called hormones, which then regulate other kinds of cells in the body. Each hormone affects particular cells through its "receptors" present perticularly for it. A small amount of a hormone attaches to a "receptor" and the hormone-receptor pair then initiates a cascade of chemical changes, often with major and far-reaching consequences in remote parts of the body. Thus, hormones act as chemical messengers, sending chemical signals that control various functions.

1.1 Reproductive process

The survival of any species depends on the integrity of its reproductive system. Reproduction is a complex process that requires interactions among multiple physiological systems. In addition, the two individuals or couple makes up the reproductive unit. The reproductive process is not limited to reproductive organs, but is largely governed by neuroendocrine influence.

1.2 Endocrine system

The endocrine system is a control system of ductless glands that secrete chemical messengers called hormones that circulate within the body via the bloodstream to affect distant cells within specific organs.

The endocrine system provides an electrochemical connection from the hypothalamus of the brain to all the organs that control the body metabolism, growth, development, and reproduction. There are three classes of hormones secreted in the endocrine system: (1) steroidal (2) protein based hormones (3) Biogenic amines. Feedback mechanism is the main phenomenon of regulation where an increase in hormone and its action, decreases its own production, by neuronal circuit (hypothalamus and pituitary). Other means of regulation of hormonal action is also desensitization and down regulation. These interfere with normal physiological processes. Apart from above mentioned phenomenon, immune system and other factors also contribute as control

factors. Thus, all factors together in coordination help in maintainence of constant levels of hormones.

1.3 Neuroendocrinology

Homeostasis, growth development and reproduction are regulated by the interactions of the endocrine and nervous systems. Almost all endocrine secretions are controlled directly or indirectly by the brain and virtually all hormones influence brain activity. Neurons provide an organized network of point-to-point connections as the basic unit of the nervous system. The basic unit of the endocrine system, the secretory cell, provides the regulatory influence through circulation. Nerve cells have a secretory function and the capacity to propagate action potential and endocrine cells have electric potential as well as secretory capacity. Neurons in common with endocrine glands activate target cells through chemical mediators that react with specific cell receptors. Any neuronal secretory product from a nerve ending can serve either as a neurotransmitter or a neuromodulator. Neurotransmitters are released into the synaptic cleft and stimulate (or inhibit) postsynaptic neurons. The distinctions between a neurotransmitter and a neuromodulator are not absolute, but neuromodulator tend to have a longer latency before response. Incase of male, hypothalamus, pituitary along with testis are important in neuroendocrinology.

1.4 Male reproductive physiology

In simple terms, reproduction is the process by which organisms create descendants. In human reproduction, two kinds of sex cells or gametes are involved. Sperm, the male gamete, made by male reproductive system and an egg or ovum, the female gamete produce by female should meet in the female reproductive system to create a new individual. While both the female and male reproductive systems are involved with producing, nourishing and transporting either the egg or sperm, they are different in shape and structure. The male has reproductive organs, or genitals, that are both inside and outside the pelvis, while the female has reproductive organs entirely within the pelvis.

The male reproductive system consists of a pair of testis, surrounded by a series of ducts and glands. Sperm are produced in the testis and are transported through the reproductive ducts. These ducts include the epididymis, ductus deferens, ejaculatory duct and urethra. The reproductive glands produce secretions that become part of semen, the fluid that is ejaculated from the urethra. These glands include the seminal vesicles, prostate gland, and bulbourethral glands.

1.5 Testis

The main function of the testis is to produce sperm and the hormones that regulate male sexual life. Regulations of both functions are under complex feed back control of hypothalamic-pituitary axis. Biosynthetic function and regulation of the testis are similar to the ovaries and adrenals, only difference is that the major secretory hormone of the testis is testosterone which serves as a circulating prohormone for other two classes of steroid hormones, 5α-reduced androgens (Dihydroxy testosterone; DHT) and estrogens (Estradiol, estriols). These products mediate most of the action of the testosterone. The functions of the testis differ depending on the phase of life in which it develops, from early gestation through old age. Although these biological effects differ, the mechanism of action and the regulation of testicular hormone production are similar at all different stages of life.

The testis contains a network of tubules for the production and transport of sperm to the excretory-ejaculatory ducts (Figure 1) and a system of interstitial cells (Leydig cells) that synthesize androgens (Fawcett, 1975). The functional complexity of the tissue is illustrated in transverse section of the testis (Figure 2). Figure 3 shows the scanning electron micrograph of transverse section of a rat seminiferous tubule. Sertoli cells (Se) serve to nuture germ cell stages from spermatogonia (Sg), through spermatocytes (Sc) and spermatids (Sd) to fully differentiated spermatozoa. Spermatozoa's tails (Ta) can be seen in the tubule lumen. Leydig cells would lie in the spaces between tubules along with blood vessels and lymphatics.

Spermatogenic tubules are composed of germ cells and Sertoli cells. Tight junctions between the Sertoli cells separate the spermatogonia from the primary spermatocytes and form a diffusion barrier that divides the testis into two functional compartments basal and adluminal. The barrier between these two compartments has limited permeability to macromolecules, analogous to the blood-brain barrier and other epithelial barriers. The adluminal compartment consists of the inner two thirds of the tubules, including primary spermatocytes and cells in more advanced stages of spermatogenesis. The basal compartment consists of the Leydig cells, the boundary tissue of the tubule including peritubular myoid cells, and the outer layers of the spermatogenic tubules that contain the spermatogonia.

The structure and function of the Sertoli cell are closely linked (Fawcett, 1975). The base of the cell is adjacent to the outer basement membrane of the spermatogenic tubule, whereas the inner portion consists of an arborized cytoplasm containing large gaps or lacunae, analogous to the branches of a tree. The mechanism by which the spermatogonia pass through the tight junctional complexes between the Sertoli cells as spermatogenesis commences is not known, but the arborized cytoplasm of the Sertoli cell encompasses the differentiating spermatocytes and spermatids so that spermatogenesis takes place within the Sertoli cell cytoplasm network. Sertoli cells synthesize hormones such as inhibin, activin, and prodynorphin as well as factors essential for spermatogenesis such as transferrin (Griswold et al., 1998).

The lipid droplets responsible for the foamy appearance of Leydig cell cytoplasm are composed largely of esterified cholesterol, derived in part from circulating lipoproteins and in part from locally synthesized cholesterol (Saez et al., 1994). Cholesterol serves as precursor for the testosterone synthesis. The amount of testosterone stored in the Leydig cell is small because newly synthesized testosterone diffuses promptly into the testicular venous blood. (Maddocks et al., 1993)

Figure 1. **The network of testis.**

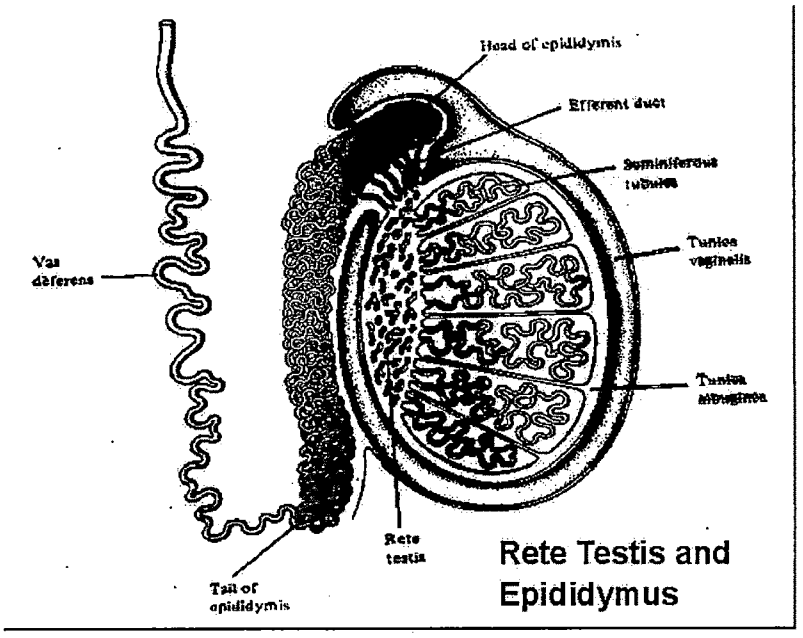


Figure 2. Transverse section of testis

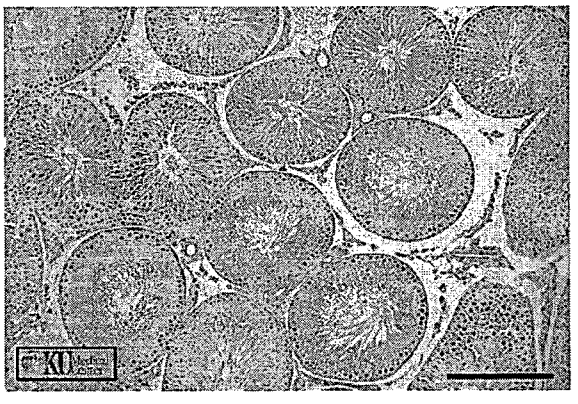
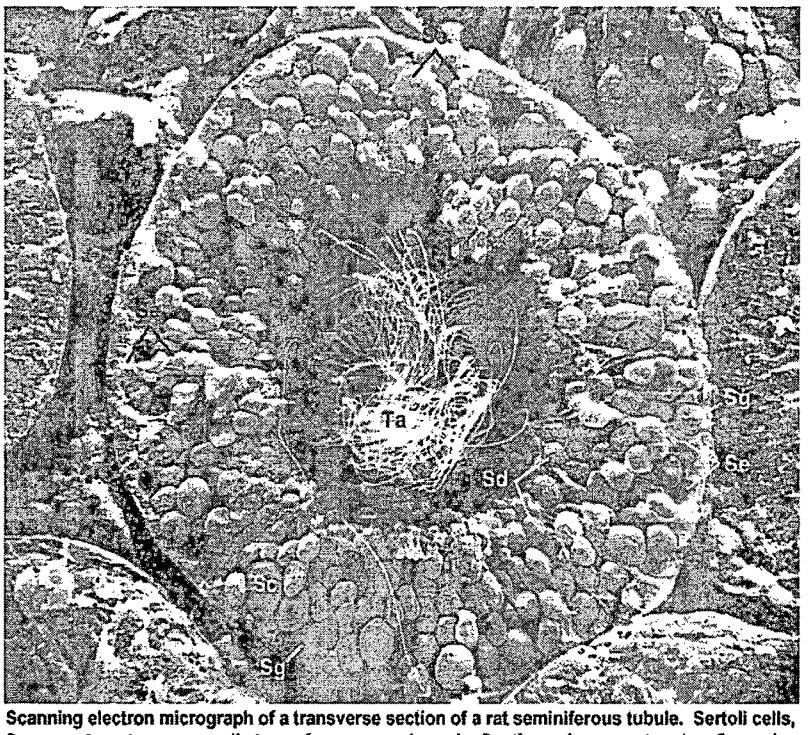


Figure 3. Scanning electron micrograph of a transverse section of testis



Scanning electron micrograph of a transverse section of a rat seminiferous tubule. Sertoli cells, Se, serve to nuture germ cell stages from spermatogonia, Sg, through spermatocytes, Sc, and spermatids, Sd, to fully differentiated spermatozoa whose tails, Ta, can be seen in the tubule lumen. Leydig cells would lie in the spaces between tubules along with blood vessels and lymphatics. Image by Kent Christensen, University of Michigan.

1.6 Hypothalamic-Pituitary-Testicular Axis

Hypothalamic Hormones

The hypothalamus is connected to the pituitary gland both by a portal vascular system and by neural pathways. The median eminence of the hypothalamus is the site at which anterior pituitary regulating hypothalamic neurons release their secretions into the capillaries of the primary plexus of the hypophyseal portal system. The median eminence has three components that are neural, vascular and epithelial. The portal vascular system provides a mechanism for the delivery of hypothalamic (releasing) hormones from the brain to the pituitary gland. This is considered as the major system by which the brain controls anterior pituitary function. Also pituitary hormones can be reaching the brain through hypophyseal-portal circulation (Oliver et al., 1993). The preoptic area and the mediobasal hypothalamus contain important centers

for control of gonadotropin secretion. Peptidergic neurons in this region secrete GnRH in a pulsatile fashion. (Silverman et al., 1979) Neurons from other regions of the brain terminate in this area and influence both the frequency and the amplitude of GnRH secretory pulses through catecholamine-related, dopamine-related, and endorphin-related mechanisms. GnRH, a decapeptide, like other neuropeptides is synthesised at the arcuate nucleus in hypothalamus as part of a large hormone that is cleaved enzymatically and further modified within secretory granules. Seven different forms of GnRH have been demonstrated in different species. All are 10 aminoacid containing peptides (decapeptide) and all have at least 50% homology to mammalian GnRH. The half life of GnRH is short (2 to 4 min) and its metabolic clearance rate is 800L/m^2 body surface area/day. The fact that LH and FSH secreted in short pulsatile bursts lead to the assumption that GnRH release is also pulsatile. GnRH is secreted in a pulsatile fashion at intervals of 70 to 90 min.

Episodic secretion of GnRH into the hypophyseal-portal system (Neill et al., 1977) is under the control of hormonal milieu. Testosterone slows the rate of discharge which is the main mechanism by which testosterone inhibits gonadotropin release. Neural control of GnRH secretion is mediated by signals from all four classes of neurotransmitters. Excitatory factors include factors like norepinephrine, neuropeptide Y, galanin, substance P, glutamic acid, NO, transforming growth factor α and prostaglandin E2. Catecholamines like epinephrine and norepinephrine increase GnRH release, while dopamine, serotonin are inhibitory for release of GnRH. Central neurons tonically suppress GnRH secretion. GnRH secretion is also reduced by corticotropin releasing hormone (CRH) and vasopressin. Other hormones in particular gut related peptide hormones also modulate GnRH release.

Pituitary Hormones

LH and FSH are the primary pituitary hormones that regulate testicular function. These hormones were named on the basis of their ovarian effects before their roles in testicular function were recognized. LH and FSH are secreted by the same basophilic cells in the pituitary. Gonadotropins belong to glycoprotein hormone family made up of 2 subunits α and β. α subunit is common to both LH and FSH. Prohormone is synthesized as 116 amino acids and mature molecule having 92 amino acids with two N-terminal oligosaccharide units. Both subunits are required for full biologic activity.

The disappearance of exogenous LH from blood is described by two linear exponentials with half-life of 40 and 120 minutes, and the metabolic clearance rate is approximately 25 mL/minute (Veldhuis et al., 1984). Only a small fraction of secreted LH appears in the urine. The turnover of FSH is also slower, the metabolic clearance rate being about 14 mL/minute, (Coble et al., 1969) and the disappearance of FSH from blood is described by two exponentials with half-life of 4 and 70 hours, respectively. (Yen et al., 1970).

LH secretion is under negative-feedback control by gonadal steroids at the level of the hypothalamus and the pituitary gland (Figure 5). Both testosterone and estradiol can cause this inhibition. Testosterone can be converted to estradiol in the brain and pituitary, but the two hormones are thought to act independently in the CNS (Morishima et al., 1995; Smith et al., 1994). One major effect of androgen in the CNS is to slow the hypothalamic pulse generator and consequently decrease the frequency of LH pulsatile release. LH is controlled by the negative feedback of gonadal steroids at the hypothalamic level (Plant et al., 1984). Acute infusions of estradiol also lowered LH levels associated with an increased frequency and decreased amplitude of the LH pulses (Santen et al., 1975). The fact that dihydrotestosterone (DHT), which cannot be converted to estrogen, exerts a negative-feedback control on LH secretion indicates that testosterone does not require aromatization to inhibit LH secretion (Santen et al., 1975). Testosterone also appears to have a negative-feedback action on LH secretion directly at the pituitary level because

administration of exogenous testosterone to GnRH-deficient men caused a decrease in mean plasma LH levels and in LH pulse amplitude (Sheckter et al., 1989). Hyperprolactinemia suppressed LH secretion, probably by inhibiting the pulsatile secretion of GnRH. (Nieschlag et al., 1997).

The negative-feedback control of FSH secretion involves peptide and steroid hormones from the testis. Serum FSH concentrations increase in proportion to the loss of germinal elements in the testis. Inhibin, activin, and follistatin were first identified as gonadal hormones that could exert selective effects on follicle-stimulating hormone (FSH) secretion without affecting luteinizing hormone (LH) (Barker et al., 1976). Although the primary sources of inhibin remains the gonad, both activin and follistatin are produced in extragonadal tissues and can exert effects on FSH through an autocrine-paracrine mechanism. These proteins can effect the regulation of the gonadotropins at many levels. First, activin can directly stimulate FSH biosynthesis and release from the gonadotrope cells of the pituitary gland. Second, activin up-regulates gonadotropin-releasing hormone receptor (GnRHR) gene expression, leading to alterations in the synthesis and release of both gonadotropins in response to GnRH. Third, activin can stimulate GnRH release from GnRH neurons in the hypothalamus and thereby affect FSH and LH secretion. Both inhibin and follistatin can negatively regulate these effects by preventing activin binding to the activin receptor at the cell membrane and blocking activation of downstream signal transduction pathways.

1.7 Spermatogenesis

A spermatozoon or spermatozoan (pl. spermatozoa), derived from the ancient Greek words, seed and living being. The entire process of sperm formation and maturation takes about 9-10 weeks. The first division is done by mitosis, and ensures a constant supply of spermatocytes, each with the diploid number of chromosomes. In second division spermatocytes then undergo a series of two cell divisions during meiosis to become secondary spermatocytes. During third division secondary spermatocytes finally become spermatids.

Spermatids, which are haploid cells, mature slowly to become the male gametes, or sperm. The sperm is the main reproductive cell in males. The tail flagellates, which we now know propels the sperm cell (at about 1-3 mm/minute in humans) by rotating like a propeller, in a circular motion, not side to side like a whip. The cell is characterized by a minimum of cytoplasm.

The epididymis is a tortuously coiled structure topping the testis, it receives immature sperm from the testis and stores it for several days. When ejaculation occurs, sperm is forcefully expelled from the tail of the epididymis into the ductus deferens. Sperm travels through the ductus deferens and up the spermatic cord into the pelvic cavity, over the ureter to the prostate behind the bladder. Here, the vas deferens joins with the seminal vesicle to form the ejaculatory duct, which passes through the prostate and empties into the urethra. Sperm cells become even more active when they begin to interact with the fertilizing layer of an egg cell. They swim faster and their tail movements become more forceful and erratic. This behavior is called "hyper activation." A recent discovery links hyper activation to a sudden influx of calcium ions into the tails. The whip-like tail (flagellum) of the sperm is studded with ion channels formed by proteins called CatSper. These channels are selective, allowing only calcium ion to pass. The opening of CatSper channels is responsible for the influx of calcium. The sudden rise in calcium levels causes the flagellum to form deeper bends, propelling the sperm more forcefully through the viscous environment. It takes sperm about 4 to 6 weeks to travel through the epididymis. The seminal vesicles and prostate gland produce a whitish fluid called seminal fluid, which mixes with sperm to form semen when a male is sexually stimulated. (www.wikibooks.com)

1.8 Biosynthesis of steroid hormones (testosterone)

The enzymes involved in the biosynthesis of active steroid hormones from cholesterol in gonads are illustrated in Figure 4. Synthesis of steroid hormones occur by action of FSH and LH as described earlier. The cholesterol derived from esters by the action of estrase, is transported across mitochondria

by a mitochondrial membrane protein called steroid acute regulatory protein (StAR). Following the transport, two different class of enzymes namely cytochrome P450 (heme-containing proteins) and hydroxysteroid dehydrogenases (HSD) plays an important role in steroidogenesis.

1.8.1 Cytochrome P450s

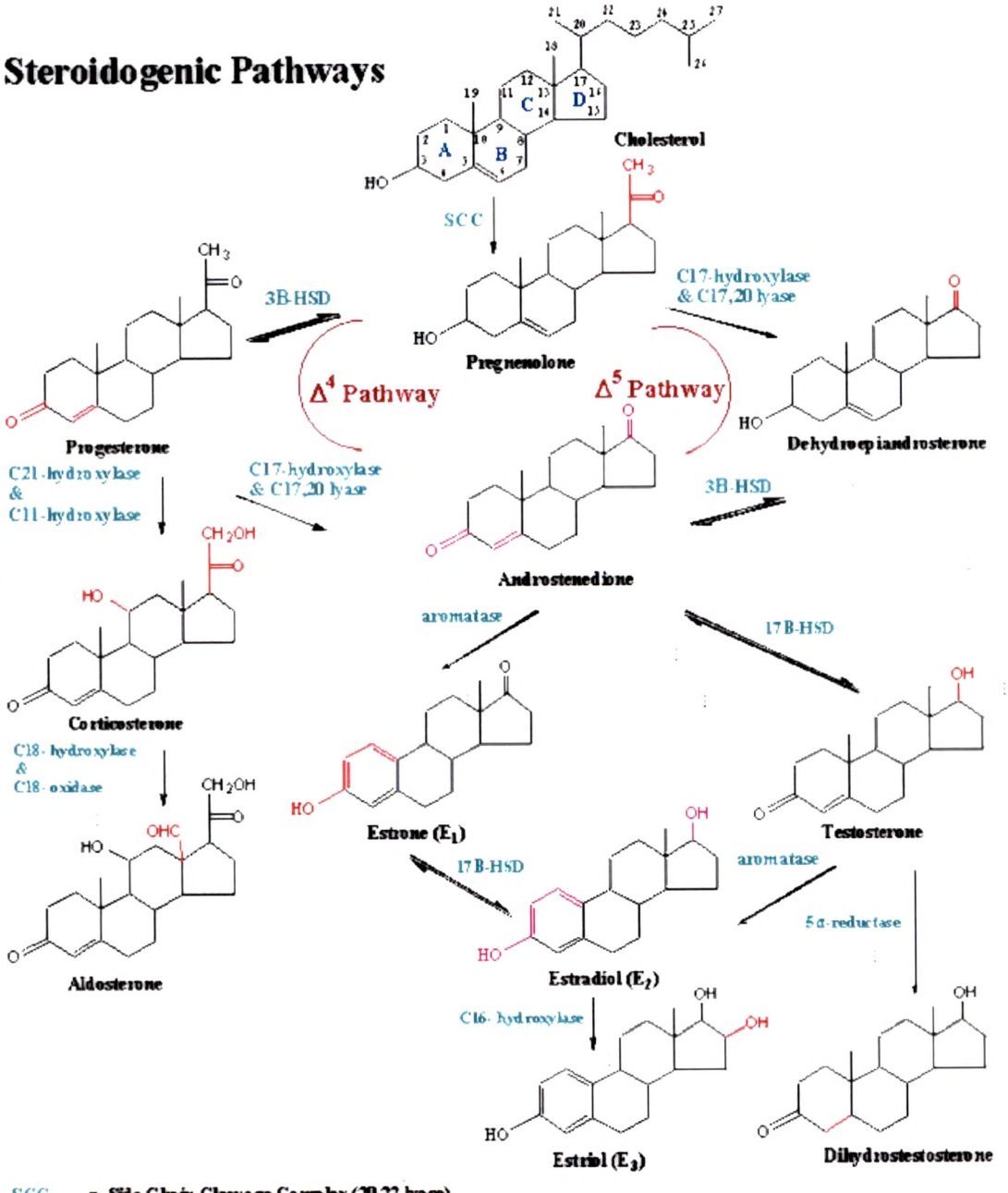
The P450 enzymes involved in steroid hormone biosynthesis are membrane-bound proteins associated with either the mitochondrial membranes CYP11A, CYP11B1, and CYP11B2, or the endoplasmic reticulum (microsomal) CYP17, CYP19, and CYP21. These P450 enzymes are members of a superfamily of heme-containing proteins found in bacteria, fungi, plants, and animals (Nelson et al., 1996). In the biosynthesis of steroid hormones from cholesterol, cytochrome P450 enzymes catalyze the hydroxylation and cleavage of the steroid substrate. They function as monooxygenases utilizing reduced nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor for the reduction of molecular oxygen. The general reaction is

$$RH + O_2 + NADPH + H^+ \quad ROH + H_2O + NADP^+$$

CYP11A (SCC)

CYP11A (P450scc) catalyzes the first and rate-limiting enzymatic step in the biosynthesis of all steroid hormones (Figure 4). The reaction requires three molecules of oxygen, three molecules of NADPH, and the mitochondrial electron transfer system described above. CYP11A catalyzes three sequential oxidation reactions of cholesterol with each reaction requiring one molecule of O_2 and one molecule of NADPH. The first reaction is hydroxylation at C22, followed by hydroxylation at C20 to yield 20,22R-hydroxycholesterol that is cleaved between C22 and C20 to yield the C21 steroid pregnenolone and isocaproaldehyde (Boyd et al., 1968; Burstein et al., 1976). Isocaproaldehyde is then oxidized to isocaproic acid (Schulster et al., 1976). The electrons required for the reaction are transferred from NADPH to ferredoxin reductase, to ferredoxin, and finally to CYP11A (Simpson et al., 1979).

Figure 4.


Steroidogenic Pathways
Cholesterol
SCC
Pregnenolone
3B-HSD
C17-hydroxylase & C17,20 lyase
Progesterone
Dehydroepiandrosterone
Δ4 Pathway
Δ5 Pathway
C21-hydroxylase & C11-hydroxylase
C17-hydroxylase & C17,20 lyase
3B-HSD
Androstenedione
Corticosterone
aromatase
17B-HSD
C18-hydroxylase & C18-oxidase
Estrone (E1)
Testosterone
17B-HSD
aromatase
5α-reductase
Aldosterone
Estradiol (E2)
C16-hydroxylase
Estriol (E3)
Dihydrostestosterone
SCC = Side Chain Cleavage Complex (20,22 lyase)
3B-HSD = 3β hydroxysteroid dehydrogenase/Δ4-Δ5 isomerase
17B-HSD = 17β hydroxysteroid dehydrogenase
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CYP17

CYP17 (P450c17) catalyzes two mixed function oxidase reactions utilizing cytochrome P450 oxidoreductase and the microsomal electron transfer system. The two reactions catalyzed by P450c17 are the 17α-hydroxylation of the C21 steroids, pregnenolone (Δ^5 steroid) or progesterone (Δ^4 steroid), followed by the cleavage of the C17-20 bond to produce the C19 steroids, dehydroepiandrosterone (DHEA) or androstenedione, respectively (Figure 4). Each reaction requires one molecule of NADPH and one molecule of molecular O2. In this two-step reaction, 17α -hydroxypregnenolone or 17α - hydroxyprogesterone is formed as an intermediate.

CYP19

CYP19 (P450 aromatase) catalyzes the conversion of the C19 androgens, androstenedione and testosterone, to the C18 estrogens, estrone and estradiol, respectively. The reaction involves the microsomal electron transfer system, cytochrome P450 reductase, and three molecules each of oxygen and NADPH. The first two oxygen molecules are involved in the oxidation of the C19 methyl group by standard hydroxylation reactions, whereas the third oxygen molecule is used in a reaction postulated to be a peroxidative attack on the C19 methyl group combined with elimination of the 1β hydrogen to yield a phenolic A ring and formic acid (Simpson et al., 1994; Graham-Lorence et al., 1991).

1.8.2 Hydroxysteroid dehydrogenases

The hydroxysteroid dehydrogenases, which include the 3βHSDs and the 17βHSDs, belong to short-chain alcohol dehydrogenase reductase superfamily (152). They are involved in the reduction and oxidation of steroid hormones requiring $NAD^+/NADP^+$ as acceptors and their reduced forms as donors of reducing equivalents. One of the major differences between the P450 enzymes and the hydroxysteroid dehydrogenases is that each of the P450 enzymes is a product of a single gene, whereas there are several isoforms for the 3βHSDs and several isozymes of the 17βHSDs, each a product of a distinct gene. The number

of isoforms or isozymes varies in different species, in tissue distribution, catalytic activity (whether they function predominantly as dehydrogenases or reductases), substrate and cofactor specificity, and subcellular localization.

3β-Hydroxysteroid dehydrogenase/isomerase

3βHSD/isomerases are membrane-bound enzymes and are distributed to both mitochondrial and microsomal membranes depending on the type of cell in which they are expressed. During the past decade, multiple isoforms of 3βHSDs have been isolated and characterized in human (Simard et al., 1996), mouse (Abbaszade et al., 1997; Payne et al., 1997), and rat (Simard et al., 1993; de Launoit et al., 1992). The different isoforms are numbered in the order in which they were identified.

Rat 3βHSD I catalyze the conversion of the Δ^5-3β-hydroxysteroids, pregnenolone, 17β-hydroxypregnenolone and DHEA to Δ^4-3-ketosteroids, progesterone, 17β-hydroxyprogesterone and androstenedione respectively. Two sequential reactions are involved in the conversion of the Δ^5-3β-hydroxysteroid to Δ^4-3-ketosteroid. The first reaction is the dehydrogenation of the 3β-equatorial hydroxysteroid (requiring the coenzyme NAD^+) to Δ^5-3-keto intermediate and reduced NADH. The reduced coenzyme, NADH, then activates the isomerization of the Δ^5-3-ketosteroid to yield the Δ^4-3-ketosteroid (Figure 4) (Thomas et al., 1995; Thomas et al., 2003).

17β-Hydroxysteroid dehydrogenases

Like 3βHSDs, the 17βHSDs play essential roles in steroidogenesis. These enzymes catalyze the final step in the biosynthesis of active gonadal steroid hormones, estradiol, and testosterone. 17βHSDs are not involved in the biosynthesis of adrenal steroids. The 17βHSDs convert inactive 17-ketosteroids into their active 17β-hydroxy forms. Human 17βHSD1 has substrate specificity for estrogens, whereas the rodent enzyme can utilize both estrogens and androgens; NADPH is the preferred cofactor for conversion of estrone to estradiol (Luu et al., 2001).

1.9 Steroid hormone function and biotransformation

The steroid hormones act both on peripheral target tissues and the central nervous system (CNS). An important function of the steroid hormones is to coordinate physiological and behavioral responses for specific biological purposes, e.g. reproduction. Thus, gonadal steroids influence the sexual differentiation of the genitalia and of the brain, determine secondary sexual characteristics during development and sexual maturation, contribute to the maintenance of their functional state in adulthood and control or modulate sexual behaviour. It has been discovered that in addition to the endocrine glands, the CNS is also able to form a number of biologically active steroids directly from cholesterol (the so-called "neurosteroids").

Despite their relatively simple chemical structure, steroids occur in a wide variety of biologically active forms. This variety is not only due to the large range of compounds secreted by steroid-synthesizing tissues, but also to the fact that circulating steroids are extensively metabolized peripherally, notably in the liver, and in their target tissues, where conversion to an active form is sometimes required before they can elicit their biological responses.

Liver plays an important role in maintaining homeostasis in all vertebrates. Changed bioavailability of steroids, through alteration of steroidogenesis or biotransformation rates, leads to changes in endocrine function. Steroid hormones lose their receptor reactivity in most cases when they are bound to binding proteins, while metabolic conversion can result in either active or inactive metabolites. Hydroxylation by cytochrome P450 (CYP) enzymes (Wilson et al., 1998) and conjugation with glucuronide and sulfate by use of UDPGT and sulphurylase (de Bethizy and Hayes, 1994) are among the major hepatic pathways of steroid inactivation, producing a more water-soluble product that can be excreted in urine. Oxido-reduction of testosterone to androstenedione, dihydro-testosterone and androstanediol is another hepatic biotransformation pathway that influences circulating concentrations of testosterone and other androgens. The expression of these biotransformation enzymes can be induced by many xenobiotics. Drug or xenobiotics metabolizing

enzymes (DMEs or XMEs) play central roles in the metabolism, elimination. Most of the tissues and organs in our body are well equipped with diverse and various DMEs including phase I, phase II metabolizing enzymes and phase III transporters, which are present in abundance either at the basal unstimulated level, and/or are inducible at elevated level after exposure to xenobiotics. Consequently, this homeostatic response of the body plays a central role in the protection of the body against "environmental" insults such as those elicited by exposure to xenobiotics. Biotransformation of steroids in hypothalamus and pituitary is demonstrated by following reaction.

Progesterone ———> 5α-pregnane 3,20 dione (5α reductase)

5α-pregnane 3, 20 dione ———> 3α-hydroxy 5α-pregnane 3,20 dione (3α HSDH)

1.10 Control of spermatogenesis

Spermatogenesis does not occur in the hypophysectomized state, and restoration of spermatogenesis after hypophysectomy and its initiation at puberty require LH and FSH. FSH acts directly on the spermatogenic tubule, whereas LH enhances spermatogenesis indirectly by increasing testosterone formation in Leydig cells. (Setchell et al., 1982). FSH binds to receptors on the surface of Sertoli cells and spermatogonia and stimulates adenylate cyclase, resulting in increased intracellular cAMP levels, activation of protein kinases, and phosphorylation of a variety of proteins. (Setchell et al., 1982).

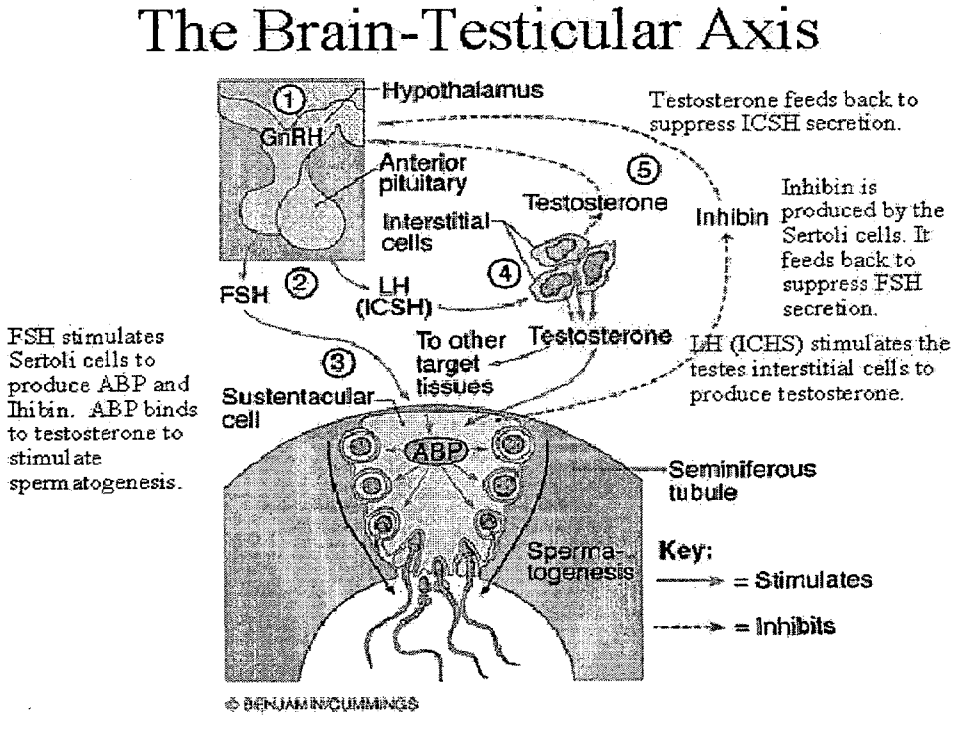
The ability of testosterone to maintain spermatogenesis in hypophysectomized animals has been confirmed by a number of investigators. Evidence has been obtained for a direct effect of testosterone, as well as a number of other C19 steroids on spermatogenesis (Joel, 1945) The some what puzzling observation that small doses of testosterone cause atrophy of the seminiferous epithelium in intact rats, while large doses do not (Zahler, 1944) was re-evaluated by Ludwig (Ludwig, 1950). Ludwig demonstrated that low doses of testosterone produce testicular damage by an indirect mechanism that involves suppression of pituitary gonadotropins. High doses of testosterone also exert a suppressive action on pituitary gonadotropins but are capable of affecting the spermatogenic process directly. Thus in spite of markedly

diminished gonadotropin levels, spermatogenesis occurs. Maintenance of spermatogenesis by testosterone in animals with estrogen induced suppression of pituitary gonadotropins has also been confirmed (Hohlweg et al., 1961).

The observation that treatment with testosterone started several weeks after hypophysectomy fails to reinitiate spermatogenesis (Woods and Simpson, 1961). Boccabella demonstrated that spermatogenesis can be reinitiated in animal's hypophysectomized for 67-70 days by 3-6 mg/day of testosterone proportianate for 90-110 days (Boccabella, 1963). However, the regeneration of the germinal epithelium was observed only in 33 % of the experimental animals and occurred only in a limited number of seminiferous tubules. Although the recovery of spermatogenesis was "patchy," some animals were fertile.

Evaluation of several 19-non steroids revealed that these compounds also possess a spermatogenesis-inhibiting property related to their ability to suppress pituitary gonadotropins and at high doses a capacity to maintain spermatogenesis related to their androgenic potency (Patanelli and Nelson, 1959).

Figure 5.



1.11 Mechanism of action of GnRH, gonadotropins and prolactin.

GnRH

GnRH interacts with high-affinity cell surface receptors coupled to G proteins on the plasma membrane of pituitary gonadotrophs. Binding of GnRH to specific cell surface receptors, leads to activation of a specific G protein (Gq/11), stimulation of multiple phospholipase activities in the plasma membrane, differential modulation of inositol 1,4,5 triphosphate and diacylglycerol signals and cytoplasmic calcium response. The acute administration of GnRH stimulates the release of both LH and FSH by the same mechanism as second messengers (Hawes and Conn, 1993). GnRH probably also acts long-term to enhance gonadotropin synthesis. The amounts of LH and FSH released in response to GnRH depend on age and hormonal status. The rate at which GnRH pulses are administered alters the pattern of LH and FSH, Slow-frequency GnRH pulses favor FSH secretion, whereas frequent pulses favor LH secretion (Marshall and Griffin, 1993). GnRH has been also implicated in sexual drive in rats, but not in monkeys and probably not in humans. Other potential sites of action correspond to the distribution of GnRH receptors and include human ovary, prostate, testis and lymphocytes. Although, GnRH effects can be demonstrated in testis, but it does not have a direct effect on the human leydig cell (Rajfer et al., 1987).

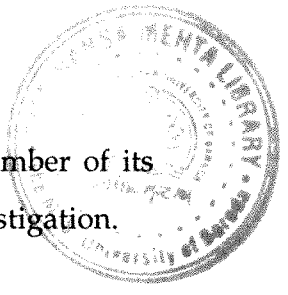
Gonadotropins

The LH receptor on the plasma membrane of Leydig cells is a member of the superfamily of G protein coupled, seven-transmembrane domain receptors (GPCRs). (Dufau, 1998). A large leucine rich extracellular domain is the basic structural characteristics of GPCRs. The leucine rich repeats are thought to be important for glycoprotein hormone binding. The binding of LH to the receptor activates signal transduction by both the cAMP and phospholipase C inositol 1,4,5-triphosphate systems. In the testis, receptor activation is coupled primarily a to Gs protein which leads to stimulation of adenylate cyclase and formation of cAMP, ultimatly causing regulatory subunit dissociation and catalytic subunit

activation of protein kinase A. The activated protein kinase A operates through unidentified steps to stimulate the synthesis of the enzymes of testosterone biosynthesis (Payne and Youngblood, 1995). The signal is terminated by endocytosis and degradation of the LH-receptor complex (LaPolt et al., 1991). In the intact testis and in cultured Leydig cells, the number of LH receptors decreases after administration of LH or hCG (LaPolt et al., 1991). This down-regulation of receptor number is associated with desensitization to subsequent LH administration.

The decrese response of LH/hCG to its receptors could be due to the higher estrogen levels of testis. This is suggested by the findings that the testicular estradiol level is elevated within 30 minutes after hCG administration. Also the estrogen antagonists can block hCG-induced desensitization (Cigorraga et al., 1980). The long-term in vivo administration of hCG causes an elevated plasma testosterone level within 2 hours in both rats and humans (Padron et al., 1980). On hCG injection, plasma testosterone level declines after the initial rise and then starts to increase again by 48 hours, while plasma estradiol levels peaks at 24 hours (Padron et al., 1980). Mechanism of this temporary desensitization involves inhibition of the 17, 20-lyase reaction. Concept is supported by the results of higher 17-hydroxyprogesterone after 24 hours followed hCG injection and then declines at 48 hours while testosterone levels continue to rise (Smals et al., 1980).

FSH receptors also belong to the large superfamily of G-protein coupled receptors (GPCRs). Plasma membrane of sertoli cells is the the primary site of action for FSH, where the hormone binds to the FSH receptor [45]. The second messenger is cAMP, which is also linked to the activation of protein kinase and stimulation of the synthesis of proteins such as androgen-binding protein and the aromatase that converts testosterone to estradiol (Means et al., 1976). FSH stimulates sertoli cells to produce androgen binding protein (ABP) and inhibin. ABP binds to testosterone to stimulate spermatogenesis. FSH plays an indirect role in androgen biosynthesis during development, (Levalle et al., 1998) but does not play a major role in the control of Leydig cell function in adults (Young et al.,



2000). Like LH and other peptide hormones, FSH regulates the number of its own receptors, but the significance of this phenomenon is under investigation.

Prolactin

Rat leydig cells contain prolactin receptor and the binding of hormone to its receptor increases the testosterone synthesis. The possible mechanism for the testosterone biosynthesis could be the enhancement of lipoprotein transport in side the cells and increasing the availability of cholesterol for steroidogenesis (Bartke, 1976). Also the regulation of testosterone involves mechanisms in the testis through testicular peptides (inhibin, activin) and growth factors (transforming growth factors and, fibroblast growth factor, insulin-like growth factor I [IGF-I]).

1.12 Prostate

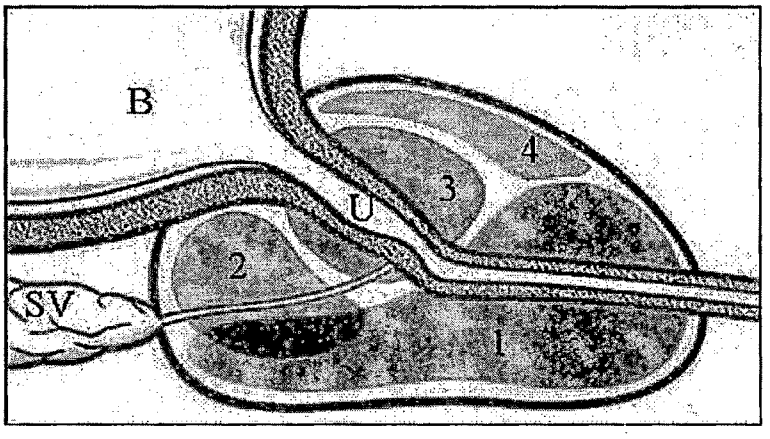
The term 'prostate', originally derived from the Greek word prohistani, which means 'to stand in front of', has been attributed to Herophilus of Alexandria who used the term earlier to describe the small organ located in front of the bladder. The prostate gland is a small firm organ, about the size of a chestnut, located below the bladder and in front of the rectum. The urethra, the channel through which urine is voided, passes from the bladder and through the prostate and penis. The primary function of the prostate gland, which contracts with ejaculation, is to provide enzymes to maintain the fluid nature of seminal fluid and to nourish sperm as they pass through the prostatic and penile urethra to outside the body.

The prostate is linked inextricably with the endocrine system. During the development of the prostate, epithelium and mesenchyme are under the control of testicular androgens, and interact to form an organized secretory organ. Furthermore, many of the disease processes are attributed to, and therapies aimed at the manipulation of the endocrine system. The gland resides in the true anatomical pelvis and forms the most proximal aspect of the urethra. It has been stated that the prostate gland is the male organ most commonly afflicted with either benign or malignant neoplasms (Presti, 2000). Benign prostatic

hyperplasia (BPH) is the most prevalent of benign disorders affecting the prostate.

According to McNeal's model of the prostate (McNeal, 1981), four different anatomical zones may be distinguished that have anatomo-clinical correlation (Figure 6).

Figure 6. The prostate structure.



1= Peripheral Zone, 2= Central Zone, 3= Transitional Zone, 4= Anterior Fibro muscular Zone. B= Bladder, U= Urethra, SV= Seminal Vesicle (adapted from Algaba).

- **The peripheral zone:** is the area forming the postero-inferior aspect of the gland and represents 70% of the prostatic volume. It is the zone where the majority (60-70%) of prostate cancers form.
- **The central zone:** represents 25% of the prostate volume and contains the ejaculatory ducts. It is the zone, which usually gives rise to inflammatory processes (eg. prostatitis).
- **The transitional zone:** this represents only 5% of the total prostatic volume. This is the zone where benign prostatic hypertrophy occurs and consists of two lateral lobes together with periurethral glands. Approximately 25% of prostatic adenocarcinomas also occur it this zone.
- **The Anterior Zone:** Predominantly Fibromuscular with no Glandular Structures.

Histologically prostate consists of stromal and epithelial elements. Smooth muscle cells, fibroblasts and endothelial cells are in the stroma and the

epithelial cells are secretory cells, basal cells and neuroendocrine cells. The columnar secretory cells are tall with pale to clear cytoplasm. These cells stain positively with prostate specific antigen (Epstein, 1997). Basal cells are less differentiated than secretory cells and so are devoid of secretory products such as prostate-specific antigen (PSA) (Warhol and Longtine, 1985). Finally, neuroendocrine cells are irregularly distributed throughout the ducts and acini; with a greater proportion in the ducts .The prostate has the greatest number of neuroendocrine cells of any of the genitourinary organs (Di Sant, 1992). Most cells contain serotonin but other peptides present include somatostatin, calcitonin gene-related peptides and katacalcin (Epstein, 1997). The cells co-express PSA and prostatic acid phosphatase. Their function is unclear but it is speculated that these cells are involved with local regulation by paracrine release of peptides (Epstein, 1997). The prostate becomes more complex with ducts and branching glands arranged in lobules and surrounded by stroma with advancing age (Figure 7).

Figure 7. Structural relationship between ducts, glandular cells of the prostate.

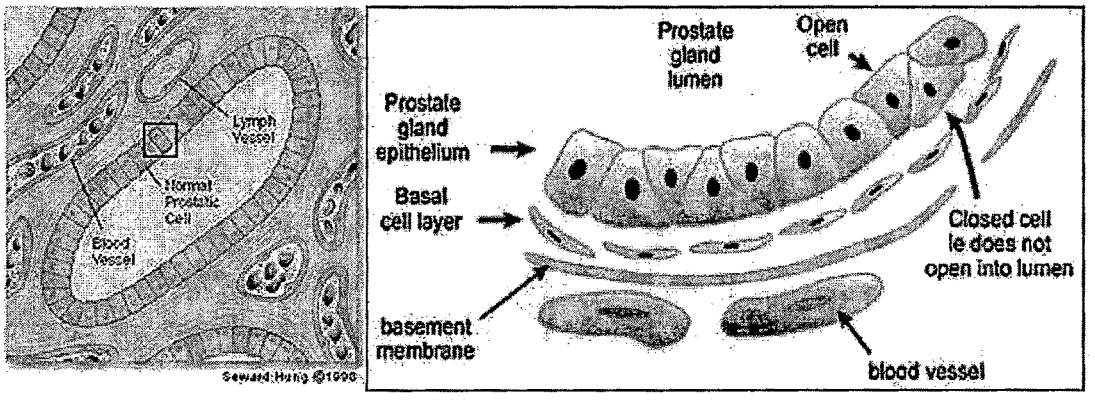


Diagram outlining the structure of the prostate gland with regard to ducts, glandular cells and their relationship to blood vessels.

1.13 Physiology

The main role of the prostate as a male reproductive organ is to produce prostatic fluid, which accounts for up to 30 per cent of the semen volume. Sperm motility and nourishment are aided by the prostatic fluid constituents. Prostatic

fluid is a thin, milky alkaline fluid containing citric acid, calcium, zinc, acid phosphatase and fibrinolysin among its many constituents (Donkervoort et al., 1977). Prostate specific antigen (PSA) is also a constituent found in prostatic secretions. During ejaculation, α-adrenergic stimulation results in transport of the seminal fluid containing sperm from the ampulla of the vas deferens into the posterior urethra (Brawer and Kirby, 1999). Interestingly, humans and dogs only experience abnormal growth of the prostate while other mammals are spared (Partin and Rodriguez, 2002).

1.14 Prostate endocrinology

It is becoming clear that intraprostatic signaling systems are important for the regulation of cell proliferation and extracellular matrix production in prostatic stroma.

Testosterone

Prostatic epithelial cells express the androgen receptor (Marengo and Chung, 1994). From the beginning of embryonic differentiation to pubertal maturation and beyond, androgens are a prerequisite for the normal development and physiological control of the prostate (Cooke et al., 1991) and help to maintain the normal metabolic and secretory functions of the prostate. They are also implicated in the development of benign prostatic hyperplasia (BPH) and prostatatic cancer. Androgens do not act in isolation but role of other hormones and growth factors in manifestation of prostatic cancer are being investigated (Gao et al., 2001).

Androgens also interact with prostate stromal cells that release soluble paracrine factors that are important in the growth and development (Cuntha et al., 1992). These paracrine pathways may be critical in regulation of the balance between proliferation and apoptosis of prostate epithelial cells in the adult (Gao et al., 2001).

The metabolism of testosterone to dihydrotestosterone (DHT) and its aromatisation to estradiol are recognised as the key events in prostatic steroid response. Testosterone, to be maximally active in the prostate, must be converted

to dihydrotestosterone (DHT) by the enzyme 5α-reductase (Bartsch et al., 2002). DHT has a much greater affinity for the androgen receptor than testosterone which allows it to accumulate in the prostate and demonstrate twice as potent effect as testosterone (Wright et al., 1999; Grino et al., 1990). A DHT concentration remains similar to those in young men as in the prostate of elderly men, despite the fact that serum testosterone levels may decline with age (Bartsch et al., 2002). In the prostate, the total level of testosterone is 0.4 ng/g and the total of DHT is 4.5 ng/g (Forti et al., 1989). The total concentration of testosterone in the blood (18.2nnmol/lL) is approximately 10 times higher than that of DHT (Ganong et al., 1997). Circulating DHT, by virtue of its low serum plasma concentration and tight binding to plasma proteins, is of diminished importance as circulating androgen affecting prostate growth (Partin and Rodriguez, 2002).

Estrogen

Role for estrogens in the prostate pathology of the ageing male appears likely with accumulating evidence that estrogens, alone or in combination with androgens, are involved in inducing aberrant growth and/or malignant change. Animal models have supported this hypothesis, where estrogens "sensitize" the ageing dog prostate, to the effects of androgen (Barrack and Berry, 1987). The evidence is less clear in humans. Estrogens in the male are predominantly the products of peripheral aromatization of testicular and adrenal androgens (Gooren and Toorians, 2003). While the testicular and adrenal production of androgens declines with ageing, levels of total plasma estradiol do not decline. This has been ascribed to the increase in fat mass with ageing (the primary site of peripheral aromatization) and to an increased aromatase activity with ageing. However, free or bioavailable estrogens may decline due to an increase in sex hormone binding globulin (SHBG), which could translate to lower intraprostatic levels of the hormone. The potentially adverse effects of estrogens on the prostate may be due to a shift in the intra-prostatic estrogen: androgen ratio with ageing.

1.15 Prostate disorders

The prostate disorders are of three types:

1. **Benign Prostatic Hyperplasia (BPH)**: BPH is an age related & progressive neoplastic condition of the prostate gland.
2. **Prostatitis**: Prostatitis is an acute/chronic bacterial infection of the prostate gland, occasionally progressing to debilitating illness.
3. **Prostate Cancer (CaP)**: Proliferation of the prostatic cells in an uncontrolled manner.

1.15.1 Benign prostatic hyperplasia

Benign Prostatic Hyperplasia (BPH) is an age-related and progressive neoplastic condition of the prostate gland (Shibata K. et al, 1996) (Shibata et al., 1996). BPH may only be defined histologically. BPH in the clinical setting is characterised by lower urinary tract symptoms (LUTS).

Table 1. Lower Urinary Tract Symptoms

Voiding or Obstructive Symptoms	Storage or Irritative Symptoms
Hesitancy	Urinary frequency
Poor stream	Urgency
Intermittent stream	Urge incontinence
Straining to pass urine	Nocturia
Prolonged micturition	
Sense of incomplete bladder emptying	
Terminal dribbling	

There is no causal relationship between benign and malignant prostatic hypertrophy (Ekman et al., 2000). Clinically apparent BPH represents a considerable health problem for older men, due to the negative effects it has on quality of life (QOL). A recent study has demonstrated an overall prevalence of 10.3%, with an overall incidence rate of 15 per 1000 man-years, increasing with age (3 per 1000 at age 45-49 years, to 38 per 1000 at 75-79 years). For a symptom free man at age 46, the risk of clinical BPH over the coming 30 years, if he

survives, is 45% (Verhamme et al., 2002). The true prevalence and incidence of clinical BPH will vary according to the criteria used to describe the condition. It is crucial to acknowledge that LUTS can exist without signs of BPH - as the symptoms can be caused by variations in the sympathetic nervous stimulation of prostatic smooth muscle, variability of prostatic anatomy (viz., enlarged median lobe of the prostate), and the variable effects of bladder physiology from the obstruction and aging.

Risk factors

The only clearly defined risk factors for BPH are age and the presence of circulating androgens. BPH does not develop in men castrated before the age of forty (Moore, 1994). But other factors may influence the prevalence of clinical disease, which include:

Hereditary

Clinical BPH appears to run in families. If one or more first degree relatives are affected, an individual is at greater risk of being afflicted by the disorder (Geller et al., 1998). The incidence of BPH is highest and starts earlier in blacks than Caucasians and is lowest in Asians. (Ekman, 2000).

Diet

Diet has been reported as a risk factor for the development of BPH. Large amounts of vegetables and soy products in the diet may explain the lower rate of BPH in the orient when compared to westernized countries. In particular, certain vegetables and soy are said to be high in phyto-estrogens, such as genestin, that have anti-androgenic effects as determined (Geller et al., 1998).

Other risk factors

It has not been possible to delineate any other risk factors for BPH such as coronary artery disease, liver cirrhosis or diabetes mellitus. There is also no causal relationship between malignant and benign prostatic hypertrophy (Ekman, 2000).

Pathophysiology

Estrogen, which acts through estrogen receptors (ER) alpha and beta, has been implicated in the pathogenesis of benign and malignant human prostatic tumors (Tsurusaki et al., 2003). As stated above, benign prostatic hyperplasia is thought to originate in the transitional zone (TZ) while prostate cancer originates in the peripheral zone (PZ) of the prostate. Receptor studies have indicated the precence of ER-α and ER-β sub-types of ER, distributed in human with normal and hyperplastic prostate tissues, using in situ hybridization and immunohistochemistry. ER-α expression was restricted to stromal cells of the PZ. In contrast, ER-β was expressed in the stromal and epithelial cells of PZ as well as TZ. These findings suggest that estrogen may play a crucial role in the pathogenesis of benign prostatic hyperplasia through ER-β (Tsurusaki et al., 2003). Investigations are ongoing and could result in a new range of therapies directed against BPH and prostate cancer. Dietary phytoestrogens (in soya and other vegetables) or selective estrogen receptor modulators are currently being investigated, with regard to their role in the development of BPH and prostate cancer (Gooren and Toorians, 2003). Such ER modifiers may oppose some of the effects of natural estrogen by modulating ER receptors, thus reducing the local impact of androgens that need active ER receptors, effectively making them anti-androgenic compounds (Jenkins et al., 2003).

The pathological or first phase of BPH is asymptomatic and involves a progression from microscopic to macroscopic BPH. Microscopic BPH will develop in almost all men if they live long enough but in only about half it will progress to macroscopic BPH (Isaacs and Coffey, 1978). This would suggest that additional factors are necessary to cause microscopic BPH to progress to macroscopic BPH. The pathological phase involves development of hyperplastic changes in the transitional zone of the prostate (Caine, 1986). While there is wide variability in prostate growth rates on an individual level, prostate volume appears to increase steadily at about 1.6% per year in randomly selected community men (Rhodes et al., 1999).

The clinical or second phase of BPH involves the progression from pathological to 'clinical BPH', which is synonymous with the development of LUTS. Only about one half of the patients with macroscopic BPH progress to develop clinical BPH (Isaacs and offey, 1978). BPH consists of mechanical and dynamic components and it is these components that are responsible for the progression from pathological to clinical BPH (Caine, 1986). In clinical BPH, the ratio of stroma to epithelium is 5:1 whereas in the case of asymptomatic hyperplasia the ratio is 2:7:1. A significant contribution is therefore made by stroma to the infravesical obstruction of BPH (Akduman and Crawford, 2001).

Common complications associated with BPH are:

- Urinary retention
- Recurrent Urinary Tract Infections
- Bladder Calculi
- Haematuria
- Secondary bladder instability
- Renal Impairment

Treatment

Transurethral resection of prostate (TURP)

TURP remains the most common surgical treatment for BPH (Lu-Yao et al., 1994). TURP involves either regional or general anaesthesia, with most patients spending a minimum of one night in hospital. TURP involves surgically debulking the periurethral and transitional zones of the prostate to relieve obstruction. Debulking is done by electrocautery in the standard TURP through endoscopic instruments introduced into the urethra and bladder. Tissue is resected in small pieces until the adenoma is removed and a new channel for passage in the prostatic urethra created, much like fashioning a pumpkin for Halloween with the capsule left behind.

Open prostactectomy

This is the oldest, most invasive therapy for BPH (Jepsen and Bruskewitz, 1998). It is commonly done through a transvesical approach, but may be done

retropubically. Early complications of this operation include haemorrhage, blood transfusion, sepsis and urinary retention with the most common late complication being bladder neck stricture (2-3%) (Han et al., 2002). TURP has lower perioperative morbidity but open prostatectomy produces equivalent, if not superior improvement with a similar or lower re-operation rate (Serretta et al., 2002). Sexual dysfunction is not likely to be altered by surgery (Gacci et al., 2003).

Laser therapy (HoLRP)

There are several evolving therapies for BPH involving lasers including Holmium laser resection of the prostate (HoLRP) and Holmium laser enucleation of the prostate (HoLEP) as well as more minimally invasive laser therapies. Laser as an energy source has an advantage of standard electrocautery by being relatively bloodless and does not carry the risk of hyponatraemia, which may rarely occur via absorption of irrigation fluid in a standard TURP (Aho and Gilling, 2006). HoLRP is an operation involving laser resection of the prostate tissue via an endoscope, similar to a standard TURP using electrocautery as outlined above. The fragments of prostate tissue are made small enough to irrigate out prior to detachment from the prostate (Gilling et al., 1996). HoLEP again uses a holmium laser but the laser acts like a finger would at an open prostatectomy, shelling out tissue until it floats in the bladder. The tissue is then morcellated and extracted. This technique may be safely used in large prostate glands (those weighing >100g) as an alternative to open prostatectomy (Aho and Gilling, 2006).

1.15.2 Prostatitis

Prostatitis is a common condition that must be excluded from other causes of LUTS and is a common cause of visits to primary care physicians and urologists. It may be present as an acute bacterial infection or may be chronic, occasionally progressing to a debilitating illness. In practice, the clinical diagnosis of prostatitis depends on the history and physical examination, but there is no characteristic physical finding or diagnostic laboratory test. Patients

with prostatitis experience considerable morbidity and may remain symptomatic for many years.

1.15.3 Prostate cancer

Traditionally, Prostate Cancer (CaP) was considered a disease of "older men." As such, it was generally accepted that 'men never died from prostate cancer, they died of other conditions associated with prostate cancer'. Prostate cancer is an uncontrolled (malignant) growth of cells in the prostate gland. Prostate cancer, unlike many other forms of cancer, tends to be slow growing. Eventually it can spread to other organs and tissues, including bones.

1.16 Environmental pollutants

Many chemicals in common use enter the bodies of human, domestic animals, and wildlife, chiefly through contaminated food and water. Environmetal pollutants are produced mainly from industries and extensive use of new products. These pollutants mainly affect human physiology as they tend to accumulate to larger time span due to reduced half life. Heavy metals are the chief representative of these classes of chemicals, which hinder the body process.

1.17 Heavy metals

Metals are indispensable for life. Some metals are micro-nutrient that are essential for normal metabolic function and called as trace elements. Of these, some are rarely toxic, even at relatively high levels of exposure. Beside sodium, potassium, calcium and magnesium, which sustain the internal physiological balance, there are approximately several other trace elements, which have proven essential. Some are less benign at high levels of exposure; manganese, although essential in trace amounts, can cause Parkinson's like syndrome at high levels and selenium has been linked to an increased risk of cancer. There are metals such as lead and cadmium, which are called heavy metals, and essentially they have no known functions in cells, but have established toxic effects. Heavy metal belongs to class of transition elements and are present in the environment in higher concentration due to extensive application of modern technology and

technical advances. The functional, morphological and biochemical effects of these elements manifest themselves at different levels; the organism, organs and tissues, the cell and even at the subcellular level due to their high solubility in water. The biological properties of heavy metals are discussed in terms of three important characteristics: the ability to form irreversible complexes and chelates with organic ligands, which influence greatly the dynamics of transport, distribution and excretion of several important metal cations; the properties to form organic-metallic bonds and the potential to undergo oxidation-reduction reactions. Field and laboratory studies indicated that bioaccumulation of heavy metals, occurs in primary and secondary consumers of the food web. Among them lead and cadmium have been shown to accumulate in various tissues such as kidney and the liver. Also their accumulation in other organs as the hypothalamus, pituitary or gonads has been reported (Lafuente and Esquifino, 1999; Lorenson et al., 1983; Ronis et al., 1998; Paksy et al., 1990).

1.18 Lead exposure

Lead is a naturally occurring, bluish grey metal that is found in small quantities in earth's crust. It is a divalent heavy metal with atomic weight 207.19 and vapor pressure - 1.0 mm Hg at 980° C. Lead in the atmosphere comes from various natural, anthropogenic sources. All human beings have lead in their body, primarily as a result of exposure to manmade sources. The most important pathways are ingestion of chips from lead painted surfaces, inhalation of lead from automobile emissions, food from lead soldered cans, drinking water from lead soldered plumbing and medications in the form of folk remedies. Although inhalation of lead from gasoline is no longer considered as a public problem, the lead from dusts in automobile emissions has been deposited in the soil.

Toxicokinetics (Absorption/Distribution/Metabolism/Excretion)

In the body, inorganic lead is not metabolized but is directly absorbed, distributed and excreted. The rate at which lead is absorbed depends upon its chemical and physical form and on the physiological status of the exposed person (e.g. nutritional status and age). Inhaled lead deposited in the lower

respiratory tract is completely absorbed. The amount of lead absorbed from GIT of adults is around 10-15% and the rest 85%-90% is excreted in faeces. In pregnant women and children, the amount of lead absorbed can increase to as much as 50%. The quantity absorbed increases significantly under fasting condition and with iron or calcium deficiency. GIT absorption in children may be only 30% for lead present in dust and dirt and 17% for lead in paint chips, compared with 50% for lead in food and beverages.

The rate of absorption of different lead compounds may vary considerably. A study in rats showed that relative to lead acetate (100%), lead carbonate was absorbed 164%; lead thalliate 121%; lead sulfite, lead napthenate and lead octanate 62-67%; lead chromate 44% and metallic lead 14% (Barlthrop and Meek, 1975). The limited data available indicate that laboratory animals absorb lead from the respiratory tract as efficiently as humans and the absorption rate is not affected by any chemical form or concentration of lead in air (EPA, 1986).

Once in the blood, lead is distributed primarily among three compartments-blood, soft tissue (kidney, bone marrow, liver and brain) and mineralizing tissues- bones and teeth. Mineralizing tissue contains about 95% of the total body burden of lead in adults. In bone, there is both labile component, which readily exchanges lead with the blood and an inert pool (ASTDR, 1999). The lead in the inert pool poses a special risk because of a potential endogenous source of lead. When the body is under physiological stress such as pregnancy, lactation or chronic disease, this inert pool can show mobilization, thus increasing blood lead level (ASTDR, 1999). It has been shown that lead may be released from the bone after menopause (Silbergeld, 1991) and clearly higher blood lead levels are seen in postmenopausal than in premenopausal women (Silbergeld et al., 1989).

Of the blood lead, 99 % is associated with RBC; the remaining 1 % is available for transport to tissues (Desilva, 1981; EPA 1986). The blood lead is not retained as it is and is either excreted by the kidneys or through biliary clearance

into the GIT. In single exposure study with adults, lead has a half-life of 25 days, 40 days in blood soft tissues and 25 years in bone.

The blood distributes lead to various organs. Animal studies have shown that liver, lungs, kidneys have greater accumulation of lead concentrations after acute exposure (inhalation, oral, dermal, intravenous routes) (ASTDR, 1999). Selective accumulation of lead occurs in hippocampus region of the brain. This accumulation is more in children than in adults (EPA, 1986).

Approximately 75% of inorganic lead absorbed into the body is excreted in the urine and less than 25 % in faeces. Lead is also excreted in breast milk and therefore, available for intake by infants (Jensen, 1991; EPA, 1986).

Signs and symptoms of lead toxicity

Symptoms of lead intoxication and their onset vary due to differences in the susceptibility and duration of exposure. In symptomatic lead intoxication, blood lead levels generally ranges from 35 to 50μ g/dl in children and 40- 60 μg/dl for adults. Severe toxicity is frequently associated with blood lead levels of 70 μg/dl or more in children and 100 μg/dl in adults.

Table 2. Signs and symptoms associated with lead toxicity

Mild Toxicity	Moderate Toxicity	Severe Toxicity
Myalgia or paresthesia, Mild fatigue, Irritability Lethargy, Occasional abdominal discomfort	Arthralgia, General Fatigue, Difficulty in concentrating, muscle exhaustibility, tremor Headache,diffuse abdominalpain, vomiting, weight loss, constipation	Paralysis, Encephalopathy leading to seizures, changes in consciousness, coma and death, gingival tissue, intermittent or severe cramps

Reproductive effects

Lead is known to affect all systems of the body. The toxicity of lead may be largely explained by its interference with different enzyme systems: lead inactivates several enzymes by binding to -SH, amine, phosphate, carboxyl groups of its proteins or by displacing other essential metal ions (ASTDR, 1999).

For this reason, effect of lead on several organs and organ systems has been well documented.

A study of 2,111 Finnish workers occupationally exposed to inorganic lead showed a significant reduction in fertility relative to 681 unexposed men (Sállmen et al., 2000a). Studies have shown that sperm quality is affected by occupational exposure to lead. Although there is some variation in the results, most of the available studies suggest that reductions in sperm concentration, indicate of adverse effects on sperm chromatin, and evidence of sperm abnormalities may occur in men with mean blood Pb > 40 µg/dL but not in men with lower PbBs. Other effects reported in recent studies in rats following oral dosing with lead include disorganization and disruption of spermatogenesis and reduction in the activities of the enzymes alkaline phosphatase and Na+-K+-ATPase (Batra et al. 2001), and an increase in the percentage of seminiferous tubules showing apoptotic germ cells (Adhikari et al. 2001).

1.19 Cadmium exposure

Cadmium (Cd) is an element that occurs naturally in the earth's crust. Pure cadmium is a soft silver-white metal. Cadmium is not present in the environment as a pure metal, but as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide). Cadmium is most often present in nature as complex oxides, sulfides and carbonates in zinc, lead and copper ores. The chlorides, sulfides are easily soluble in water to varying degrees. Cadmium is used extensively for electroplating and galvanization processes, in the production of pigments, in batteries, as a chemical agent and in various industrial processes (ATSDR, 1989). Cadmium compounds have varying degrees of solubility which affects their absorption and toxicity.

Cadmium is present in ambient air in the form of particles in which cadmium oxide is probably an important constituent. Cigarette smoking increases cadmium concentrations inside houses. The average daily exposure from cigarette smoking (20 cigarettes a day) is 2–4 µg of cadmium (Ros and

Slooff, 1987). Cadmium concentrations in unpolluted natural waters are usually below 1 µg/litre. Contamination of drinking-water may occur as a result of the presence of cadmium as an impurity in the zinc of galvanized pipes or cadmium-containing solders in fittings, water heaters, water coolers, and taps. Levels of cadmium could be higher in areas supplied with soft water of low pH, as this would tend to be more corrosive in plumbing systems containing cadmium.

Food is the main source of cadmium intake for nonoccupationally exposed people. Crops grown in polluted soil or irrigated with polluted water may contain increased concentrations and meat from animals grazing on contaminated pastures (IARC, 1976). Animal kidneys and livers concentrate cadmium. Levels in fruit, meat, and vegetables are usually below 10 µg/kg, in liver 10–100 µg/kg, and in kidney 100–1000 µg/kg. In cereals, levels are about 25 µg/kg wet weight.

Toxicokinetics (Absorption/Distribution/Metabolism/Excretion)

Cadmium is more efficiently absorbed from the lungs than the GIT (ATSDR, 1999). Inhalation and absorption usually involves cadmium in a particulate matter form where absorption being a function of deposition, which in turn is dependent upon the particulate size (particles >= 10µm diameter) tend to be deposited in the upper respiratory tract and particles <=0.1µm diameter are deposited in the alveolar region. Alveolar deposition efficiency in animal models ranges from 5% to 20% (Boisset et al., 1978) and in humans, it is estimated to be up to 50% for small particles (Nordberg et al., 1985). Actual cadmium absorption via inhalation exposure has been estimated to be 30% to 60% in humans (Elinder et al., 1976).

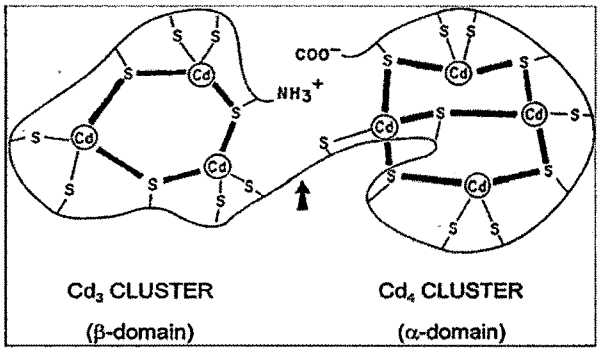
Absorption through gastrointestinal tract appears to be a saturable process with the fraction absorbed decreasing at high doses (Nordberg et al., 1985). The absorption of cadmium through GIT has modified many physiological factors such as high fat or protein content in the diet. Shaikh and Smith (1980) reported a mean retention time of 2.8% (1.1% to 7 %) for 12 human

subjects given at a single oral dose of radiolabelled cadmium chloride. Cadmium absorption is decreased by coabsorption of divalent and trivalent cations like Zinc, Chromium, and Magnesium and increased by iron and calcium deficiencies (Goyer. 1991). Dermal absorption of cadmium is generally low (0.2-0.8%).

Absorbed cadmium is transported in the blood by RBC and albumin (Goyer, 1991). Acceptable blood cadmium levels in adults are 1 μg/dl. Although cadmium is widely distributed throughout the body, most of it (50% to70% of the body burden) gets accumulated in the kidneys and liver (Goyer, 1991). Cadmium burden in the kidneys tends to increase in a linear fashion with age up to 50 to 60 years of age after which it remains somewhat constant or slightly declines (Goyer, 1991). During pregnancy, cadmium present in maternal body is almost impermeable through the placenta so that fetus is exposed to only small amounts of maternal cadmium (ATSDR, 1999).

Cadmium is not transformed into any other form but rather binds to various biological components, such as protein and non-protein sulfahydryl groups and anionic groups of various macromolecules. Major binding protein of cadmium is metallothionein. Metallothionein is very effective in binding with cadmium and some other metals and is instrumental in determining the disposition of cadmium in the body. It is a family of proteins with a molecular weight of 6.5 Kd, which is rich in cysteine residues. It contains 20 cysteine residues that remain invariant along the amino acid sequence. All cysteines are known to participate in the coordination of 7 mol of Cd or zinc (Zn) per mol of MT (Kagi and Vallee, 1960). Coordination of these cysteine residues results in a high binding affinity for Zn (10^{-18}) and Cd (10^{-22}) (Kagi and Vallee, 1960). The seven atoms of bound Cd are arranged in two separate polynuclear metal clusters, one containing three and the other four metal ions (Figure 8).

Figure 8. Metal clusters of metallothionein.



There are tremendous differences in the half-life of MT synthesized as a result of chemical induction of the MT gene. For example, the half-life of Zn-MT is approximately 18–20 h, whereas that of Cd-MT is about 3 days (Feldman et al., 1978). Concurrent titration studies indicated that at lysosomal pH, most of the Zn is released from MT whereas most of the Cd is not (McKim et al., 1992). This could be the reason Cd MT has a higher half-life in vivo than does Zn MT. Studies showed that the half-life for constitutive MT in adult rats is about 4 h (Kershaw and Klaassen, 1992). There are four isoforms of metallothionein-MT-1, MT-2 that are expressed in almost all tissues, MT-3 is present in the brain and MT-4 specifically expressed in keratinocytes. Several studies have shown MT protects the renal tubule cells from toxicity of cadmium. Cd not bound to MT induces denovo synthesis of MT in liver cells. In long term Cd exposure, there occurs a slow release of CdMT from the liver to the blood. Cadmium–MT is the form that is readily taken up into the kidney and transported into the lysosomes, where they are catabolised. However, the rate of influx of Cd-MT into renal tubular cells and rate of de novo synthesis of MT in the kidney regulates the pool of free intracellular cadmium ions that can interact with renal tubular cells.

After transportation, one of the mechanism by which Cd2+ affects cell function and gene expression were recently reviewed by Bhttacharyya et al. (Bhattacharyya et al., 2000). Cd2+ can easily enter into the cells through the L-type voltage Ca2+ channels (Hinkle and Osbourne, 1994) and receptor-mediated Ca2+ channels (Blazka and Shaikh, 1991) because both cations have similar radii size and charge (Ca2+ = 0.97 A°, Cd2+ = 0.99 A°).

The principal route of excretion is via urine, with a daily average excretion of 2 to 3 μg for human beings (ATSDR, 1999). Daily excretion represents only a small percentage of the total body burden, which accounts for 17 to >30 years half-life of cadmium in the body (Friberg et al., 1950). Unabsorbed cadmium is removed from GIT by fecal excretion. Typical daily excretion has been reported to be about 0.01% of the total body burden (ATSDR, 1999).

Table 3. Signs and symptoms of cadmium toxicity

Acute Intoxication	*Chronic Intoxication*
Fever, Pulmonary effects, Headache, Chills, Muscle aches, Nausea, Vomiting, Diarrhea	Renal effects- proximal tubular dysfunction, with excretion of low molecular weight proteins; Fanconi syndrome (amino aciduria, glycosuria, phosphaturia, renal tubular acidosis); Pulmonary effects; Bone effects – osteomalacia

Reproductive effects

In male rats and mice, acute oral exposure to near-lethal (60-100 mg/kg) doses can cause testicular atrophy and necrosis (Andersen et al. 1988; Borzelleca et al. 1989) and concomitant decreased fertility (Kotsonis and Klaassen 1978). Lower-dose acute exposures of 25-50 mg/kg did not result in reproductive toxicity in male animals (Andersen et al. 1988). Reproductive effects on both male and female rats orally exposed to 2.5 mg/kg/day via drinking water for 180 days may have resulted in the observed decrease in litter size and increased interval between litters. Male rats were exposed to 0-14 mg Cd/kg/day via food for 77 weeks. The incidence of prostatic hyperplasias was increased above controls (1.8%) from the 3.5 mg Cd/kg/day dose. Xu et al. (1993) measured trace elements in blood and seminal plasma and their relationship to sperm quality in 221 Singapore men (age range 24-54; mean 34.8). Parameters monitored included semen volume and sperm density, motility, morphology, and viability.

1.20 Aims and objectives

The constant increase in environmental levels of heavy metals due to urbanization and industrialization has stimulated interest in the study of toxic substances and its consequences to biological systems. Environmental pollution by toxic metals is a global problem, resulting in an increase in heavy metals including cadmium and lead in air, water and food, above the recommended safety levels causing a deleterious effect on human health. Lead (Pb) and Cadmium (Cd) are two non-essential metals, which are not required by the body for any physiological function. Their solubility in water makes them partially harmful, allowing easy entry into the cells where they inhibit and interact with several vital functions. Lead and cadmium have been shown to accumulate in reproductive tissues such as testis and prostate (Benoff et al., 2000). Our lab has also reported their accumulation in various organs including hypothalamus, pituitary, ovary and liver, which has led to disturbances in function of Hypothalamic-Pituitary-Gonadal (HPG) axis in female rats (Pillai et al., 2002; Laxmipriya et al., 2006; Pillai et al., 2005).

In recent decades, the increasing incidence of infertility has been associated with exposure to endocrine disruptors like pesticides and industrial residues (Eertmans et al., 2003; Carman, 2005). Many external causes of infertility are exposure to substances related to occupation such as pesticides, polychlorinated biphenyls (PCBs), dioxins , furans, ethanol, phenols and phthalates, and metals like cadmium, lead, mercury etc. (Eertmans et al., 2003; Santamarta, 2001). Industrial cadmium exposure is extremely relevant, affecting more than 1,500,000 workers a year in the United States alone (Ragan HA, Mast, 1990). Reproductive dysfunction has been described in men exposed to lead and cadmium at the workplace, including oligozoospermia and dose-dependent astenozoospermia (Rom, 1976). Blood and semen samples were analyzed from battery factory workers, showing an inverse association between plasma lead levels and sperm volume and concentration. Significant correlations were observed between lead, dehydratase, and protoporphyrin levels and reproductive parameters, indicating a decrease in sperm count, density, motility,

viability and an increase in abnormal sperm head morphology (Telisman et al., 2000). Studies on workers in lead foundry workers showed hypogonadism and decreased serum testosterone, with a reproductive and endocrine impact, especially in the hypothalamic-pituitary-testicular axis, associated with occupational exposure to inorganic lead (Cullen et al., 2000).

In a study by the World Health Organization (WHO) on the effects of lead and cadmium in the blood of adult men, the overall results indicate that even low-level exposure to lead and cadmium can significantly reduce the quality of semen, although the study did not show conclusive evidence of male endocrine reproductive alterations (Telisman et al., 2000).

Since reproductive physiology is under the regulation of endocrines, it is important to study their effects as endocrine disruptors. One major aspect of heavy metal associated endocrine disruption is reproductive dysfunction. Various reports have suggested that these heavy metals act at all the level across the HPG axis (Klein et al., 1994; Lafuente et al., 1999). At hypothalamus, it affects the levels of various neurotransmitters, involved in the release of hormone (GnRH) required for the secretion of the pituitary hormone that is involved in the various reproductive activities at gonadal level. Since sex steroids control many reproductive functions, a possibility ,that changes in the synthesis or breakdown of these hormones may alter reproductive capacity in man and other animals exposed to these heavy metals cannot be excluded.

Other reproductive disorder associated with environmental pollutants (lead and cadmium) is benign prostatic hyperplasia as indicated by epidemiologic and laboratory animal studies (Waalkes et al., 2000). The most convincing evidence for the involvement of environmental factors in increasing the hyperplasia risk comes from migration studies which show that the incidence in immigrants moving from low-risk to high-risk countries, increases with successive generations (Dunn, 1976; Angwafo, 1998). Pro-oxidant properties of these heavy metals are also known to be the mediators of phenotypic and genotypic changes that lead from mutation to neoplasia.

Studies in literature have proven lead and cadmium as reproductive terotogens. Data from literature also has suggested that Pb and Cd in isolation are known physiological disrupters. But in environmet there is constant presence of one or more metals to which humans are exposed to, leading to synergestic, additive or antagonistic effects. This proves that there is a need to study the effects that are caused by such multiple exposures that too at low level. Earlier work from our lab has shown that simultaneous exposure of Pb and Cd affect the hypothalamic-pituitary-ovarian function at each organ level in praganant and non praganant rats (Laxmipriya et al., 2007; Laxmipriya et al., 2006; Pillai et al., 2005; Pillai and Gupta, 2005).

It is clear from literature that incidence of male infertility increases due to Pb and Cd exposure. Hence, it was of great interest to study the effects caused by co-exposure of Pb and Cd on male reproductive system mainly on hypothalamic-pituitary-gonadal-hepatic axis and on accesary organ-prostate. Thus, to understand biochemical, cellular and molecular mechanism of lead and cadmium coexposure, current study was performed. Demographic study has also been carried out to understand the association of environmental pollutants (cadmium and lead) with the incidence of BPH in patients of western India.

Objectives of the present study:

I. Effects of lead and cadmium Co-exposure on hypothalamic, pituitary, testicular and hepatic steroid metabolism in adult rats.

II. Effects of lead and cadmium Co-exposure on hypothalamic-pituitary-testicular axis function in rats.

III. Biochemical, cellular and molecular mechanism of lead and cadmium co-exposure on male reproductive system.

IV. Association of environmental pollutants like lead and cadmium with incidence of benign prostatic hyperplasia in patients of western India.

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