# INTRODUCTION

Vertebrate skin forms the largest organ system exhibiting potentiality of keratinization. Epidermal derivatives of the skin also have the ability to secrete mucins or lipids. While mucin secretion is more common among aquatic vertebrates, lipid secretion is the characteristic of the terrestrial forms. Reptiles and birds have fewer epidermal glands and, some of the most notable ones are the uropygeal glands of birds (Maiti, 1971) and amongst reptiles the chin glands in tortoises (Rose, 1970), cloacal glands of crocodiles, pre-anal/femoral glands of lizards (Maderson, 1967; Athavale, 1980). Mammalian skin has a comparatively larger number of glands and is characterised by a variety of epidermal glands including sebaceous gland, sweat gland, meibomian gland and mammary gland.

Sebaceous gland is a universal attribute of mammals. Although basically similar, sebaceous glands of mammals show many differences in form and distribution. Lemurs have numerous sebaceous glands while cetaceans have virtually none; except in the external auditory meatus and over the genetilia. The glands may be small and simple as in mouse or may be large and complex as in bats and lemurs (Montagna, 1963). Sebaceous glands are wide-spread over the human body, though not normally found on the palms or soles, interspaces of toes and side faces of feet halfway upto the ankles (Johnsen and Kirk, 1952). They are largest and most numerous in the middle of the back, on the

fore-head and face, in the external auditory meatus and on the anogenital surfaces in the human body (Ebling, 1977). In addition, the presence of sebaceous glands has been reported in parotid and submandibular glands (Hamperl, 1931; Hartz, 1946; Meza Chavez, 1949), salivary glands (Bain et al., 1956; Linhartova, 1974) and in thymus (Wolf et al., 1984).

Sebaceous glands generally develop in association with hair follicles and open into piliary canals, except in the lemurs where they open directly onto the skin surface (Montagna and Yun, 1962) and in the meibomian glands in the palpibrae. Large rosettes of sebaceous glands in oral and buccal mucosa, the nipples and areolae (Perkins and Miller, 1926; Montagna, 1970), the prepuce and the labia minora (Machado, 1931) in a human body contain sebaceous glands which do not open into the piliary canals. In addition to their general distribution pattern in varying densities all over the body, most mammals have some specialized glands which are not associated with hair follicles and have been shown to be concerned with production of scent or pheromones. Marmosets, tamarians and shrews have a large field of abdominal sebaceous glands and tarsiers have huge labial glands. In Felidae and Canidae anal sacs contain many large sebaceous glands. Several species of bats, pigs and others have large single or multiple intermandibular sebaceous glands in the gular region. Most primates, including men, have extensive fields of free sebaceous glands around the anogenital surface (Montagna, 1963).

Some of the modified sebaceous glands viz., preputial glands of rodents, the supracaudal gland of the guinea pig, inguinal glands of rabbits and costovertebral glands of hamsters, are easily amenable to surgical isolation for experimentation by virtue of their larger size.

Preputial glands of rodents are a pair of flask shaped specialized glands of sebaceous type, rather gigantic in size for their category, which open by respective single ducts alongside the urethral orifice. These glands are mainly lipid secreting organs. Preputial secretion of rodents is known to contain pheromonic substance(s) working as sex attractant drawing the conspecific together, whereupon various kinds of social interactions occur; including courtship and mating. Such possibilities have attracted the attention of ethologists (Bronson and Caroom, 1971; Orsulak and Gawienowski, 1972). Unusual 4-ethyl fatty acids secreted by sebaceous glands of the neck region of mature male goat during breeding season are responsible for the release of specific odour having pheromonal activity attracting the oestrous female (Sugiyama et al., 1981). Farnysol, occuring in the human skin can be likened to a perfume presumably having some value in sexual attraction (Nicolaides, 1965). Lactones from the horse have a cyclic structure, a methyl branch near the oxygen function and a location of the ethylenic bond (equolides) inviting comparison with the large ringed ketones, muscone and civetone, which perform pheromonal functions in the musk deer and the civet cats, respectively. If the

equolides have such a function for the horse, it presumably is 'nonsexual' since the compounds are found in similar quantities in both sexes (Downing and Colton, 1980).

Apart from such specilized functions accredited to sebaceous secretions, several generalized functions have been ascribed to sebum. Waxes help the furred mammals in preventing their fur from becoming wet. Mono/diacyl glycerol help to retain moisture in the skin by forming monomolecular films over microdrops of sweat, thereby delaying evaporation. Diacyl glycerol and glycerol are released during hydrolysis of triacylglycerol and then help retain moisture (Nicolaides, 1974). Furred mammals tend to secrete only such types of esters that are not susceptible to bacterial hydrolysis (Lindholm et al., 1981). Fatty acids and waxes can be inhibitory to many types of keratinophytic and saprophytic fungi (Baxter and Trotter, 1969; Pugh and Evans, 1970a and b). A fungicidal activity of $\beta$-hydroxy fatty acids of wood pigeon has been reported (Schildknecht and Koob, 1971). Therefore, the role of sebum fatty acids in the broad spectrum of the skin hygiene may not be ruled out. Excess of free fatty acids may be harmful to skin because of their comedogenic and irritant properties (Kligman, 1974), that may be involved in the pathogenesis of acne.

The sebaceous glands in general possess a pool of undifferentiated stem cells (Plewig et al., 1971 a, b) which are then transformed into transition cells and ultimately into a pool of differentiated mature cells (Epstein and Epstein, 1966)

that are destined to be extruded with disruption/disintegration alongwith secretion. During the process of differentiation, the volume of sebaceous cell increases by a factor of 100-150 (Tosti, 1974). Sebaceous differentiation is a complex and serial process of orderly synthesis, segregation and accumulation of lipid droplets culminating into enlarged, mis-shapen cells that fragment to form the sebum and the glands are, thus, holocrine. Sebum is extruded out after a special stimulus (Downing and Strauss, 1982).

More than two decades of investigations have established beyond doubt that the development and secretory activity of the sebaceous glands of man, and those of other mammals are strikingly influenced by hormonal factors (Ebling, 1974; Shuster and Thody, 1974; Pochi and Strauss, 1974). Most investigators have taken the size of glands and the incidence of mitosis as criteria of activity. Numerous studies have established the dependence of sebaceous gland development and functioning on androgens. Ebling (1963) has reviewed the widespread reports on the effect of castration leading to atrophy of the sebaceous glands. Thody and Shuster (1970a, b) and Strauss and Pochi (1963) have also shown that sebum production is decreased following castration. The responsiveness of sebaceous glands in human beings to exogenous androgens depends upon the age of the individuals and circulatory profile of testosterone. In adult man, sebaceous glands are maximally stimulated by endogenous androgens. They do not show further response even to a moderate dose of testosterone administration. Contrary to this, in children,

castrated men and in postmenopausal women where the sebaceous gland activity is perceptibly low and in cases where the activity is suppressed by estrogen treatment, the glands show prompt response to administration of testosterone propionate or methyl testosterone (Pochi et al., 1962; Strauss et al., 1962).

Apart from testosterone, various other androgens and testosterone metabolities have been found to exert their stimulatory effects upon sebaceous glands to different degrees (Archibald and Shuster, 1967, 1969; Nikkari and Valvaara, 1969; Ebling et al., 1971). It appears that the most active androgens for rat sebaceous gland are testosterone, 5$\alpha$-DHT and 5$\alpha$-androstane, 3$\beta$-17$\beta$ diol. On the other hand, dehydroepiandrosterone (DHEA), its sulphate and $\Delta^4$-androstane-3, 17 dione are found to be relatively less potent (Shuster and Thody, 1974). In the ventral ear sebaceous gland model of female hamsters, however, lipogenesis was found to be augmented in an identical dose-dependent pattern after treatment with all androgen metabolites quoted in this paragraph (Hall et al., 1983).

It appears, therefore, from above paragraph that the growth and secretory activity of sebaceous glands in humanbeings and laboratory animals do not entirely depend upon gonadal androgens only, but adrenal and ovarian androgens may also make significant contribution in this respect. Indeed, acne-an androgen dependent disorder of elevated sebaceous lipogenesis-has been shown usually to develop around puberty and is shown to coincide with the

appearance of adrenal zona reticularis and with increased adrenal androgen secretion (Dhom, 1973; Cutler and Loriaux, 1980). Acne is shown to flare up in conditions accompanied by hyperandrogenism such as congential adrenal hyperplasia (Brooks et al., 1960), ovarian tumours, Cushing's syndrome, idiopathic hirsutism and polycystic ovarian conditions of different origin (Plotz et al., 1952; Rose et al., 1976; Rose and Birnbaum, 1979; Smith et al., 1979; Steinberger et al., 1981; Lucky et al., 1986; Moltz and Schwarts, 1986). In females and in children, particularly, DHEA and androstenedione secreted by ovary and adrenal glands are the physiologic sources for the maintenance of sebaceous gland size and sebum production (Pochi and Strauss, 1969, 1974). Barring some contradictory findings (Ebling, 1955) adrenalectomy of intact and castrated men and rats has been found to result in substantial atrophy of sebaceous glands (Lorincz, 1963) and a decrease in the rate of sebum production (Pochi et al., 1963; Ebling et al., 1970a; Thody and Shuster, 1970a, 1971a; Pochi and Strauss, 1974). Several studies indicate that in normal adult humanbeings and in the intact and gonadectomized rats glucocorticoids, even at pharmacological doses, perhaps, do not take any part in the sebaceous gland activity (Pochi et al., 1963; Pochi and Strauss, 1974; Shuster and Thody, 1974). Stimulatory influence of the adrenal in the sebaceous gland growth and sebum production in mammals has, therefore, been attributed to many androgenic steroids emanating from the adrenals. Both DHEA and its sulfate can be metabolized by skin to testosterone and androstenedione (Kim and Herrmann, 1968; Chakraborty et al., 1970; Faredin et al.,

1970; Hodgins, 1971). Additionally, a positive influence of many adrenal steroids on growth and sebum production of mammalian sebaceous glands has been reported (Ramasastry et al., 1970; Hay and Hodgins, 1973; Ebling, 1974; Shuster and Thody, 1974; Cooper et al., 1976; Agache and Blanc, 1982; Hsia et al., 1983; Marynick et al., 1983; Sawaya et al., 1984).

It is apprent from above points that castration and/or adrenalectomy lead to a decrease in lipogenic index of the sebaceous glands, and, replacement therapy with androgenic steroids to reversal of such effects. Obviously, circulating androgenic steroids could be expected to be responsible for the biosynthetic capability of the sebaceous glands. Contrary to this, no differences have been reported in the plasma levels of androgens in normal and acne patients (Chrousos et al., 1982; Schiavone et al., 1983; Lookingbill et al., 1984; Cunliffe et al., 1986). However, invoking the idea of 'end organ hypersensitivity' provides another plausible explanation to such controversial phenomenon. Further, elevated levels of 5 $\alpha$-reductase have been reported for the skin of acne patients (Sansone and Reisner, 1971; Hay and Hodgins, 1974; Vermorken et al., 1986) and also in the skin of hirsute women subjects (Mowszowics et al., 1983; Serafini et al., 1985; Serafini and Lobo, 1985). On the other hand, high plasma levels of a dihydrotestosterone metabolite viz.- androstanediol glucuronide, in acne patients promotes the hypothesis that target organs form excessive dihydrotestosterone (Horton et al., 1984; Lookingbill et al., 1984). Androgens have been shown to be produced in skin from precursor hormones (Baird

et al., 1969; Kirschner et al., 1973; Givens, 1978). Thus, skin is considered as an 'endocrine' organ involved in the production of androgenic substances (Lucky, 1987; Reingold and Rosenfield, 1987).

In men and women ethynyl oestradiol and stilbesterol administered systemically or topically, bring about reduction in the size of sebaceous glands and decrease in sebum secretion (Jarrett, 1955; Strauss et al., 1962; Strauss and Pochi, 1964; Bonelli et al., 1967, 1970; Pochi and Strauss, 1974). Similar effect of estradiol administration on the sebaceous glands of laboratory animals has been reported (Ebling, 1963; Ebling and Skinner, 1967; Sansone et al., 1972). On the contrary, estriol is able to cause an elevation of mitotic rate and the sebum production rate (Bhattacharya et al., 1986). Progesterone metabolites have been shown to inhibit sebum production by skin components (Mauvais-Jarvis et al., 1974; Taam et al., 1982; Foreman et al., 1984).

Thyroidectomy has been shown to decrease lipogenic index of sebaceous gland in men and rats (Jurenka, 1935; Thody and Shuster, 1972 b). Thyroxine treatment has been shown to elevate the levels of lipids in the sebaceous elements of rats (Ebling et al., 1970 b; Toh, 1981). Thyroid hormones, thus, have a specific stimulatory influence on sebaceous lipogenesis.

Pituitary gland has been reported to maintain structural and functional integrity of sebaceous gland (Ebling, 1974; Shuster and Thody, 1974). Hypophysectomy in rats leads to decreased

sebaceous gland volume and rate of sebum excretion (Haskin et al., 1953; Lasher et al., 1954; Lorincz, 1963; Ebling et al., 1969a; Nikkari and Valavaara, 1969; Thody and Shuster, 1970a, b). However, the way in which the pituitary gland exerts its effects upon the sebaceous gland is neither simple nor the opinions generally agree upon a unified theme. Sebum production in hypophysectomized rats was found to be elevated after administration of either TSH (Thody and Shuster, 1972b) or ACTH (Ebling et al., 1970a; Thody and Shuster, 1971b). The existence of a separate 'sebotrophic principle' from the hypophysis (Lasher et al., 1955, Lorincz, 1963; Woodbury et al., 1965 a, b) has been proved to be a non-entity (Strauss and Pochi, 1963; Ebling et al., 1969a; Shuster and Thody, 1974). Similarly, stimulatory influence of prolactin and GH on sebaceous lipid levels (Ebling et al., 1973) has been challenged (Nikkari and Valavaara, 1969; Shuster and Thody, 1974). $\alpha$-MSH treatment has been shown to stimulate sebaceous gland in hypophysectomized rats (Thody and Shuster, 1971c, 1973a, b; Shuster and Thody, 1974; Ebling et al., 1975; Thody and Shuster, 1975). Oxytocin and Vasopressin on the other hand, have been shown to exert negative sebotrophic activity in female rats (Thody and Shuster, 1973 a, b). The sebotrophic activity of $\alpha$-MSH, $\beta$-MSH, $\beta$-LPH and ACTH has been attributed to their structural relationships (Gorbman et al., 1982).

Lastly, antiandrogenic treatments with spironolactone (Goodfellow et al., 1984; Weissmann et al., 1985) and cyproterone acetate (Greenwood et al., 1983; Kaszynski, 1983; Marsden et al.,

1984) have been employed to cure acne. Varied hormonal influences of sebaceous gland have been reviewed recently (Strauss et al., 1983).

Eventhough abundant literature exists in respect of the role of androgenic compounds in maintaining the structural and functional integrity of sebaceous glands, practically only a hazy image emerges from the literature regarding neuronal regulation of functions of sebaceous glands. Problems of sebaceous gland innervation and its possible significance were full of disputes, confusions and contradictions. However, it was convincingly shown that rat preputial gland, an anologue of sebaceous type, is supplied by both adrenergic as well as cholinergic nerve fibres (Ambadkar and Vyas, 1981a), despite the previously reported conflicting view on the subject by Rothman (1954), Boecke (1934) and Montagna (1963), the latter has cited the work of Hurley et al., (1953), Hellmann (1955), Thies and Galente (1957) and Winklemann (1960).

The literature pertaining to secretory innervation of sebaceous glands was full of discrepancies. Contradictory observations had been reported by workers on the basis of their work on patients with seborrhoea and various neuronal disorders (Starling, 1936; Serrati, 1938; Nexmand, 1944; Savill, 1944; Hodgson-Jones et al., 1952; Kligman and Shelley, 1958). It was conclusively shown that extrusion of pre-formed sebum in the preputial gland is under the control of $\alpha$-adrenergic system (Ambadkar and Vyas, 1980).

The literature relevant to effects of drugs influencing sebaceous gland secretion is scanty (Cerutti, 1934). Melczer and Deme (1942) reported an increase in secretion of sebaceous glands after pilocarpine injections, but others could not find such effects (Rothman and Herrmann, 1953). Miescher and Schonberg (1944) found no change in lipid levels of sebaceous glands after atropine, pilocarpine and acetylcholine administration. Harville (1971) reported striking reduction in sebum secretion with L-Dopa therapy. Burton et al., (1973), Burton and Shuster (1973) and Wheatley and Brind (1981) reported that L-Dopa reduces rates of lipogenesis by sebaceous elements. Additionally, Wheatley and Brind (1981) opined on the basis of a report by Ambadkar and Vyas (1980) that norepinephrine may play a role in stimulating the sebaceous gland to replenish its secretion. Conversly, adrenal neurohormones have been reported to inhibit sebaceous lipogenesis (Wheatley et al., 1971). Combined histological (Vyas, 1978), histochemical (Ambadkar and Vyas, 1982) quantitative (Ambadkar and Vyas, 1981b) as well as in-vitro studies (Ambadkar and Vyas, 1980) have provided clues to the effect that preputial overall metabolism is significantly influenced by neuronal compounds. The gland was shown to exhibit metabolic alterations in response to administration of one of such neuronal compounds, viz.- isoproterenol - that are known to follow androgen deprivation. Precisely these observations from our laboratory prompted the idea of making use of one of such neurohormones for further understanding about inhibition of sebum synthesis, which could prove to be clinically beneficial

for acne and related disorders of increased sebaceous lipogenesis. However, it is not known as to what extent such elements are involved in the regulation of overall metabolic patterns of preputial gland of male rats. Hence, a study was undertaken to deal with this issue.

Since the steroid metabolism in the target tissue depends on the circulating levels of testosterone, possible decreased circulating level of testosterone due to the administration of isoproterenol, a $\beta$-adrenergic agonist, might have caused reduction in the steroid metabolism of the gland (Ambadkar and Vyas, 1982). Here a concept of induction of steroid catabolism in a non-target organ like liver was emerging. It is well known that liver and kidney are the organs involved in steroid catabolism.

The histochemical profile of steroid dehydrogenases was elevated in such non-target organs in response to chronic isoproterenol administration (Chapter : 1). It is conceivable from these observations that isoproterenol administration could have diminished circulating testosterone levels.

Possible decreased levels of plasma testosterone due to chronic IPR administration could alter lipid metabolism in target organs like the preputial gland. Such an influence has not so far been studied. Therefore, examination of total lipid content of the gland and the histochemical patterns of certain lipogenic enzymes were taken up in the gland (Chapter : 2).

Having thus obtained an insight into the lipid metabolism of the gland in response to chronic IPR administration, it was deemed worthwhile to focus attention on some unexplored biochemical pathways associated with production of lipid precursors like ∝-glycerophosphate and acetyl coenzyme A in the context of IPR therapy. The results indicated an inhibitory influence of such pathways in response to IPR administration in the preputial glands (Chapter : 3).

Preputial gland cells have an adequate blood supply, and hence, supply of glucose is not a limiting factor as far as the energy demands of the gland are considered. It was therefore thought desirable to know little more about the IPR-induced alterations in lipogenic capacity as far as the histoenzymological variations in preputial oxidative enzymes like LDH and MDH are responsible for regulation of energy balance of the gland. Observations on these aspects are reported in Chapter : 4.

A plethora of literature is available regarding biochemistry and steroidal regulation of sebaceous secretion. The histological and histochemical characteristics of sebaceous glands and influences of androgens on them have been well documented in the literature. Most of these reports dealt with steroidal influences over either the growth of sebaceous gland and/or sebum excretion rate (SER) of the glands. However, quantitation, nature and regulation of metabolic pathways making provisions for overall energy demands by sebaceous glands have not been attempted to such a great extent as has been done in the case of sebum excretion

rate (SER). Hence, a study was undertaken to deal with this issue. Accordingly two key enzymes concerned with energy metabolism, succinic dehydrogenase and $Mg^{++}$ATPase were studied in the preputial glands. Concomitantly, the activity levels of $Na^{+}$-$K^{+}$ ATPase and fructose 1,6 diphosphate aldolase were also assessed in the glands after different endocrine regimes such as castration, adrenalectomy coupled with castration, testosterone propionate injection to castrates and isoproternol-treatment in normal animals. The results obtained are discussed in the light of available literature (Chapter : 5).

The preputial glands are having a unique mode of holocrine secretion and obviously depict physiological autolysis. Previous studies from this laboratory have shed some light on the contribution and neuroendocrine regulation of acid phosphatase activity as related to typical secretory mode of preputial glands of male rats (Ambadkar and Vyas, 1981b). Although acid phosphatse activity is considered as the hallmark of lysosomal hydrolases, a large number of other hydrolases have also been reported in lysosomes (De Duve and Wattiaux, 1966; Tappel, 1968a; Barrett and Dingle, 1971; Barrett, 1972). In addition, it has been reported that the activity levels of certain lysosomal hydrolases do not run in parallel fashion with alterations in the levels of certain lysosomal enzymes in response to different neuroendocrine manipulations in the rat ventral prostate (Helminen et al., 1972), parotid glands (Robinovitch, 1973; Johnson and Sreebny, 1977) and epididymis (Weiss and Gossrau, 1981). In the light of such

reports, it was thought desirable to investigate the action of certain neuroendocrine manipulations on the activity levels of lysosomal hydrolases other than acid phosphatase. The implications of these observations are discussed (Chapter : 6).

The investigation initiated in this laboratory concerning response of the preputial gland to some neurodynamic agents left certain doubts unresolved. One of such unanswered questions relates to probability of elevation of cAMP levels of the gland in response to IPR therapy (Vyas, 1978; Ambadkar and Vyas, 1981 b, 1982). Intracellular levels of cAMP are dependent upon the intricate balance between the activity of adenylate cyclase system, which synthesizes cAMP and the degradative enzyme cAMP-specific phosphodiestrase (cAMP-PDE). At any given time the prevailing concentration of intracellular cAMP from any tissue will depend on the activity of cAMP-PDE (Butcher and Sutherland, 1962). Taking this fact into consideration, an assay of alterations in cAMP-PDE activity in the preputial gland in response to various endocrine manipulations was taken up. The results indicated elevation of cAMP-PDE levels in response to steroidal and neuronal manipulations and thereby help explain reduction of mitotic index, deceleration of cellular disintegration and stabilization of lysosomal membranes in preputial cells (Chapter:7).

Lipogenesis, a unique biosynthetic potential acquired by mammalian sebaceous glands or its analogues, has turned out to be a major theme of scientific curiosity for skin biologists. A voluminous body of literature concerning sebaceous lipogenesis is available (Chapter : 2, 3). Protein, yet another important

constituent of sebaceous secretion, has unfortunately been so far overlooked by the workers. Only some reports are available on the skin and/or sebaceous gland protein metabolism (Beaver, 1960; Dangelo and Munger, 1964; Giegel et al., 1971; Sherins and Bardin, 1971; Wheatley et al., 1979; Bladon et al., 1984, 1985). Barring few reports, hardly any attempts appear to have been made in correlating the role of amino acids concerning alterations of protein, lipid and carbohydrate metabolism in skin components (Borghi, 1940; Velick and English, 1945; Wheatley et al., 1961; Lipkin et al., 1965; Wheatley et al., 1967; Grigor et al., 1970; Im and Hoopes, 1974; Gumenyuk et al., 1979; Kealey et al., 1986). Additionally, what holds true for scarcity of literature pertaining to protein metabolism in sebaceous analogues is equally applicable to transaminase activity levels (Velick and English, 1945; Tickner et al., 1961; Adachi et al., 1967; Im and Hoopes, 1974; Peters and White, 1976; Raab and Gmeiner, 1976). Similarly, hormonal influences on transaminase activity levels in skin components have never been dealt with in desirable details earlier. Hence, an attempt has been made here in this direction by evaluating the responses exhibited by transaminases and total protein content of the preputial glands under the influence of different neuroendocrine manipulations. Implications of these aspects are discussed (Chapter : 8).

At the end, a summary and a general consideration of the data and information collected on various aspects of the investigations on the preputial glands of male rats are presented to highlight the salient features of the present studies and their probable implications.

# CHAPTER : 1

## INFLUENCE OF ISOPROTERENOL ADMINISTRATION ON PATTERNS OF STEROID DEHYDROGENASES IN LIVER AND KIDNEY OF MALE RAT

In course of an investigation initiated in this laboratory a decade ago on endocrine regulation of metabolic patterns of preputial gland - a sebaceous analogue of male albino rats, it was conclusively shown that development and secretory activity of this gland are strikingly influenced by steroids emanating from both testis and adrenal gland (Ambadkar and Vyas, 1975, 1979). Involvement of neuronal elements in maintenance of structural and functional integrity of the preputial gland, too, had been exploited (Ambadkar and Vyas, 1980, 1981a). In the wake of investigating the influence of neurotransmitters on physiology of the preputial gland, they used different experimental models administered with sympathomimetic and parasympathomimetic agonists (Ambadkar and Vyas, 1981b). In this study, $\beta$-adrenergic agonist receptors emerged as possible control measures for lysosomal proliferation. In another report, the $\beta$-adrenergic agonist receptors had been shown to be implicated in regulation of population dynamics of the preputial acini (Vyas, 1978). Later on an experimental animal model had been devised to investigate the influence of one of the most potent $\beta$-adrenergic receptor agonist, isoproterenol, (IPR), on steroid dehydrogenase profile of the preputial gland. The drug administration brought

about changes that are known to follow androgen deprivation in the steroid metabolism of the preputial gland of male rats (Ambadkar and Vyas, 1982). Since the steroid metabolism in the target tissue depends on the circulating level of testosterone, possibly decreased circulating level of testosterone due to the drug administration might cause observed reduction in the steroid metabolism of the gland. At this juncture, two possibilities had been put forward to explain the influence of the drug therapy on observed decrements in the steroid metabolism of the gland in such an experimental animal model. Firstly, it was presumed that IPR might have some direct effect on the endogenous steroid metabolism of the gland. Alternatively, a concept was shown to emerge so as to induce steroid catabolism in liver and as a consequence of which steroid metabolism in the gland was shown to be altered.

Steroid catabolism could be accomplished with a number of mechanisms and the oxidoreduction of a steroid molecule is a representative of one of such metabolic artifacts. 3$\alpha$ hydroxysteroid dehydrogenase (3$\alpha$-HSDH) is involved in the interconversion between $\Delta^4$-ketosteroids and 3$\alpha$-hydroxysteroids which could be excreted out in urine directly (Bird et al., 1974; Kinouchi and Horton, 1974; Kuttenn et al., 1977) or after being conjugated (Tomkins and Isselbacher, 1954; Isselbacher and Axelrod, 1955; McGuire and Tomkins, 1959, Vande Wiele et al., 1963). Formation of 5$\alpha$-androstane-3$\alpha$, 17$\beta$-diols has been reported from

testosterone and 5α-DHT through mediation of 3α-HSDH (Tomkins, 1956; Hilgar and Hummel, 1964; Moor et al., 1975) and a steroid catabolic function has been assigned to such a reaction (Tomkins and Isselbacher, 1954; Isselbacher and Axelrod, 1955; VandeWiele et al., 1963 and Hiroshi et al., 1977). Liver and kidney, the organs involved in detoxification and elimination of steroids show appreciable 3α-HSDH reactivity (Balough, 1966).

17β-hydroxysteroid dehydrogenase (17β-HSDH) function may regulate concentration of androgens and oestrogens through interconversions of biologically potent 17β-hydroxy steroids, testosterone and oestrogen with less active 17-oxosteroids, androstenedione and estrone, respectively (Baillie et al., 1966; Hawkins and Taylor, 1967; Hodgins et al., 1982) which could be excreted out in urine (furman et al., 1958; Hamilton et al., 1959; VandeWiele and Lieberman, 1960). A clear-cut picture crops out of the literature cited above accentuating on the roles executed by both liver and kidney in detoxification and elimination of steroids through mediation of 3α-HSDH and 17β-HSDH. Moreover, it is reasonable to assume, from the aforementioned aspects of the action of IPR therapy on the steroid metabolism of the preputial gland of male rats, that the drug administration must have evoked prompt diminutions in the circulating testosterone levels by altering either the rate of steroidogenesis or the metabolic clearance rate (MCR) of steroids which itself is a

multifactorial phenomenon. One of these factors is a prompt induction of steroid catabolism accomplished with either hydroxylation, A-ring reduction, oxidoreduction or direct conjugation of a steroid molecule. Hence, a study was initiated to evaluate the role executed by IPR therapy on the steroid catabolism in liver and kidney of male rats. At the onset, it was thought worthwhile to study the patterns of 3$\alpha$-HSDH and 17$\beta$-HSDH histochemical reactivity as influenced by IPR administration in both liver and kidney of male rats.

## MATERIALS AND METHODS

Male albino rats weighing 120-140 gms were used for the present investigation. Rats were divided into two groups. One group consisted of untreated rats as controls and second groups consisted of IPR treated animals. IPR (25 mg/kg body wt, i.p.) was injected twice a day for 10 days and the animals were sacrificed after 12 hours of last injection.

Left liver lobe and kidney were taken out, blotted and fixed on a chuck maintained at -20°C and 9 to 12 $\mu$ thick sections were cut. They were processed for histochemical localization of 17$\beta$-HSDH activity with testosterone (T) and oestradiol (E) as substrates and NAD as a co-factor (Kellogg and Glenner, 1960). 3$\alpha$-HSDH was demonstrated as per the method described by Balough (1966) employing androsterone as substrate and NAD as a co-factor. The sections mounted on coverslips were incubated for 20 minutes

at 37°C in case of 17 β -HSDH demonstration and for a period of 60 minutes at 37°C for the demonstration of 3α-HSDH. Control sections were incubated in absence of substrates.

## OBSERVATIONS

3α-HSDH in liver :

Hepatocytes of normal male rats show a very high reactivity of 3α-HSDH in the area surrounding the portal region. The hepatic cords show moderate reactivity while the area around the central vein depicts the least reactivity. Apparently, a periportal distribution pattern is discernible (Fig : 1).

IPR administration increases the enzyme reactivity considerably in all the regions of liver lobules. The hepatocytes in the area surrounding the portal region depict maximal reactivity for the enzyme in both the groups (Fig : 2).

17 β-HSDH in liver :

In general, the distribution pattern of 17 β-HSDH using testosterone (Fig : 3) and oestradiol (Fig : 5) as substrates remains essentially the same as 3α-HSDH, but the least enzyme activity is discernible in normal rat liver.

The drug therapy increases the enzyme reactivity in the hepatocytes of all the regions (Fig : 4 & 6).

3∝-HSDH in kidney :

In normal male rat kidney, the zona glomerulosa shows weaker enzyme activity. The distal and proximal convoluted tubules show intense reactivity. The medullary substance show very weak reactivity (Fig : 7).

IPR administration intensifies the enzyme reactivity in all the regions mentioned above (Fig : 8). In general, 3∝-HSDH in kidney follows the hepatocyte reactivity.

17 β-HSDH in kidney :

In normal male rats, the 17 β-HSDH reactivity remains the same in kidney with both testosterone (Fig : 9) and oestradiol (Fig : 10) as substrates. The zona glomerulosa shows weaker enzyme reactivity. Both the distal and proximal convoluted tubules show intense localization. The medullary substance, too, depicts positive reactions. The loop of Henle shows intense reactivity while the collecting tubules remain non-reactive

IPR administration intensifies the enzyme activity in all the regions as mentioned earlier (Fig : 10 & 12). In general, liver 3∝-HSDH ~ kidney 3∝-HSDH > kidney (T, E) 17β-HSDH > liver (T, E) > 17 β-HSDH is the pattern of enzyme reactivity observable in a descending fashion in both groups of animals.

## EXPLANATIONS FOR FIGURES

Figs. 1 to 6 Photomicrographs of sections of rat liver showing HSDH activity. 150 X.

Fig. 1 3$\alpha$ -HSDH activity in the section of the liver of normal rat.

Fig. 2 3$\alpha$ -HSDH activity in the section of the liver of isoproterenol treated rat.

Fig. 3 17$\beta$ -HSDH (T) activity in the section of the liver of normal rat.

Fig. 4 17$\beta$ -HSDH (T) activity in the section of the liver of isoproterenol treated rat.

Fig. 5 17$\beta$ -HSDH (E) activity in the section of the liver of normal rat.

Fig. 6 17$\beta$ -HSDH (E) activity in the section of the liver of isoproterenol treated rat.

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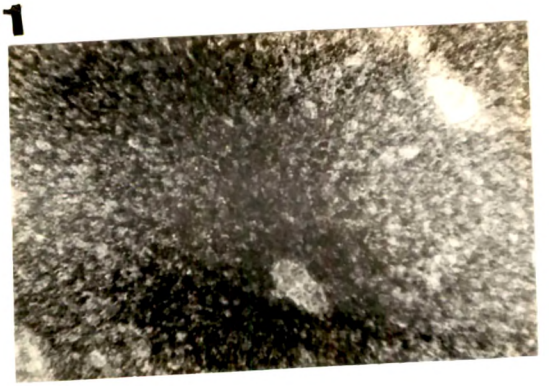

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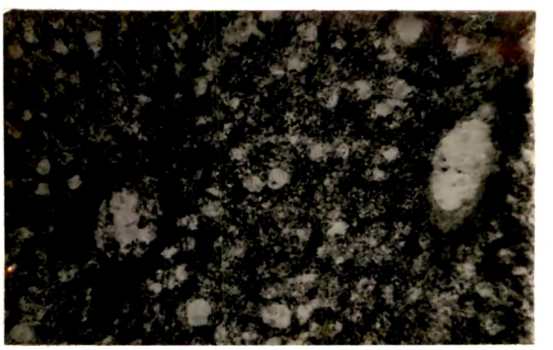

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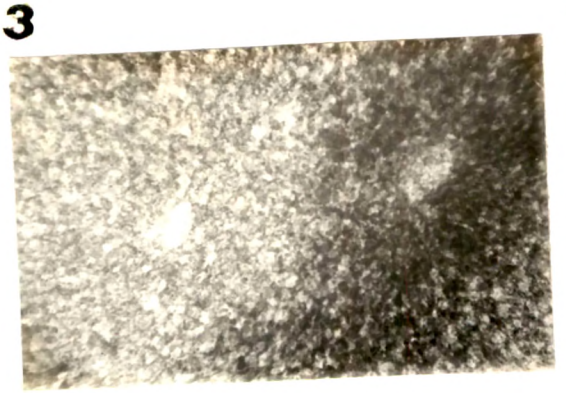

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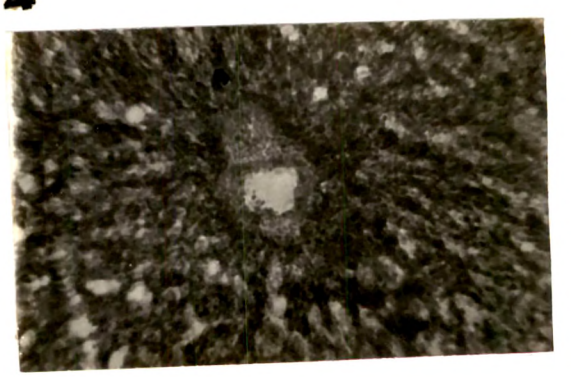

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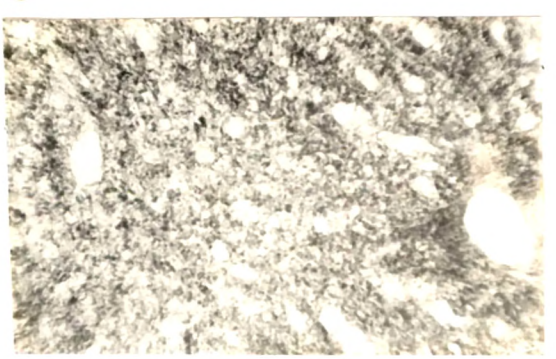

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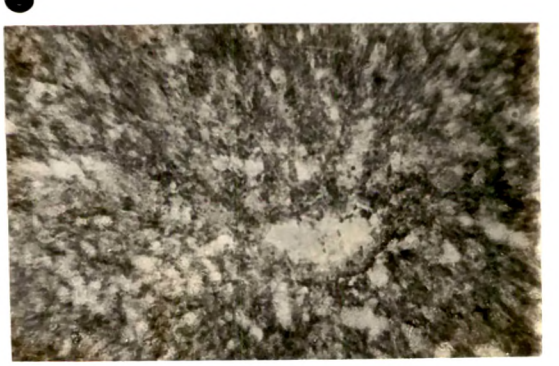

EXPLANATIONS FOR FIGURES

Fig. 7 to 12 Photomicrographs of sections of rat. kidney showing HSDH activity. 150 X.

Fig. 7 3$\alpha$ -HSDH activity in the section of the kidney of normal rat.

Fig. 8 3$\alpha$ -HSDH activity in the section of the kidney of isoproterenol treated rat.

Fig. 9 17$\beta$ -HSDH (T) activity in the section of the kidney of normal rat.

Fig. 10 17$\beta$ -HSDH (T) activity in the section of the kidney of isoproterenol treated rat.

Fig. 11 17$\beta$ -HSDH (E) activity in the section of the kidney of normal rat.

Fig. 12 17$\beta$ -HSDH (E) activity in the section of the kidney of isoproterenol treated rat.

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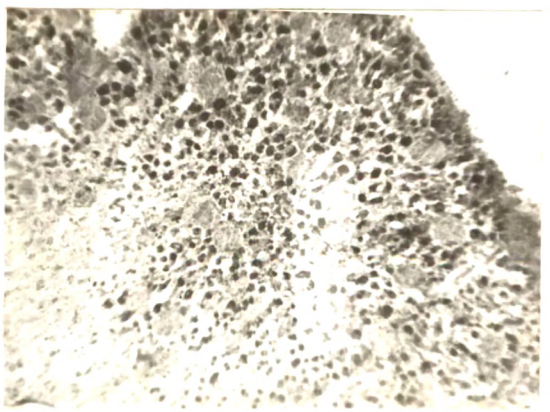

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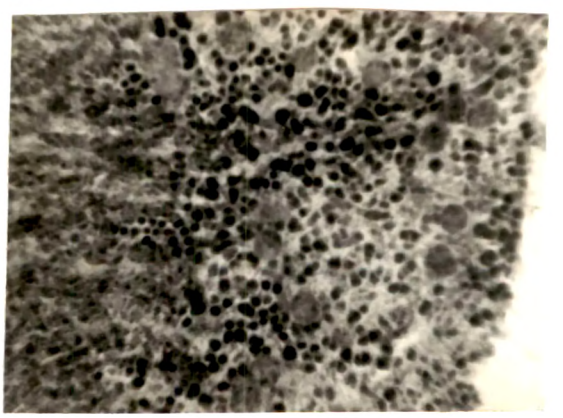

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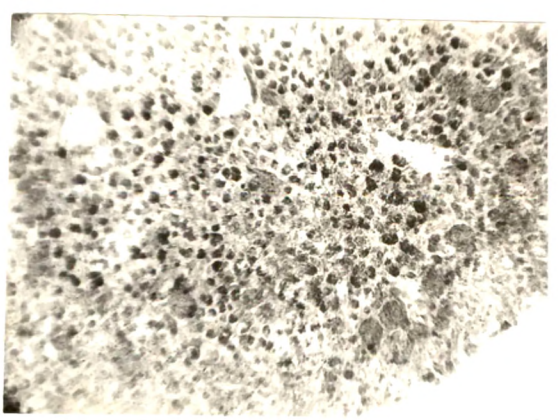

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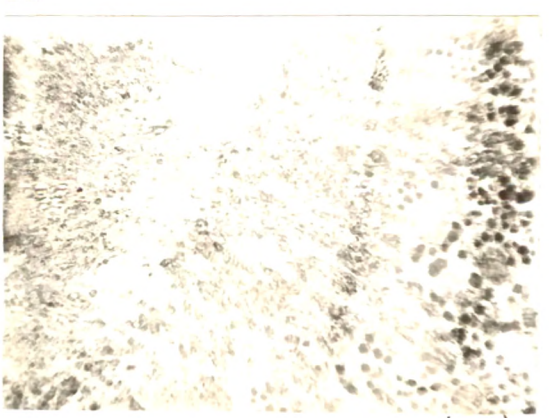

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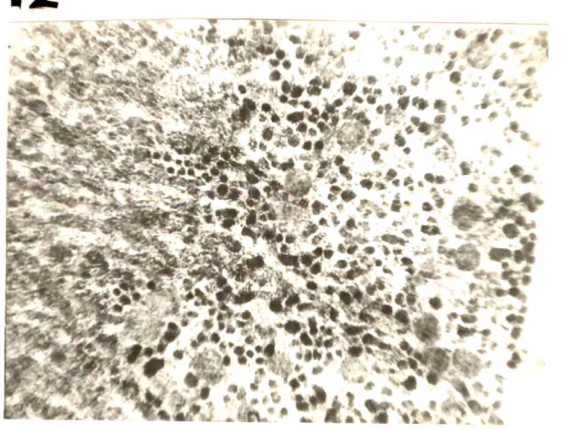

## DISCUSSION

In normal male rat kidney, the zona glomerulosa shows weaker 3∝-HSDH activity. The distal and proximal convoluted tubules in zona fasciculata show intense reactivity. 3∝-HSDH isolated (Asohima et al., 1964) and demonstrated histochemically (Baillie, 1966; Baillie et al., 1966b; Balough, 1966) in kidney has been ascribed a 3∝-hydroxysteroid excretion function (Baillie, 1966). The hepatocytes of normal male rats show a very high reactivity of 3∝-HSDH in the area surrounding the portal region. The hepatic cords show moderate activity while the area around the central vein depicts the least reactivity. Apparently, a periportal distribution pattern is discernible. An identical distribution pattern is observable with 17 /3-HSDH, too. We are at odds with the homogeneous distribution patterns of both 17 /3-HSDH and 3∝-HSDH within the hepatic lobule of adult cockerel and human liver (Baillie, 1966) and the pigeon liver (Kotak; 1979).

Histochemically demonstrable 3∝-HSDH in liver (Pearson and Grouse, 1959) is involved in the interconversions between $\Delta^4$-ketosteroids and 3∝-hydroxysteroids. The enzymic inactivation of $\Delta^4$-3 ketosteroids involves three sequential reactions including saturation of the 4-5 double bond (Tomkins and Isselbacher, 1954) followed by reduction of the carbonyl group at 3 position to an alcohol (Tomkins, 1956) and conversion of water insoluble tetrahydrosteroid to a water soluble glucosiduronic acid (Isselbacher and Axelrod, 1955) which could

be excreted out along with urine or bile. The carbonyl group could be reduced to an alcohol group through mediation of 3 $\alpha$-HSDH in rat liver. Thus, conversions of both dihydrosteroid and 3-ketosteroid to tetrahydrosteroid and 3$\alpha$-hydroxysteroid, respectively are catalyzed by 3 $\alpha$-HSDH (Isselbacher and Axelord, 1955).

Enzymatic conversion of 5 $\alpha$-androstane, 3$\alpha$, 17 $\beta$ diols has been reported from testosterone and 5$\alpha$-DHT in a variety of tissues including liver and kidney (Tomkins, 1956; Hilgar and Hummel, 1964; Voigt et al., 1970; and Verhoeven et al., 1977) through mediation of 3 $\beta$-HSDH. Moor and colleagues (1975) concluded that cytoplasmic activity of 3$\alpha$-HSDH plays a major role in conversion of 5$\alpha$-DHT to 3$\alpha$-diols which compete only weakly with DHT for binding to cytoplasmic androgen receptors isolated from various target organs (Verhoeven et al., 1975; Liao, 1977 and Mainwaring, 1977). This conversion of DHT to 3$\alpha$-diols may be regarded as inactivation of highly potent androgen molecules (Tomkins and Isselbacher, 1954; Isselbacher and Axelrod, 1955; VandelWiele et al., 1963; Hiroshi et al., 1977 and Verhoeven et al., 1977). In fact, 5$\alpha$-androstane, 3$\alpha$, 17 $\beta$ diol is shown to be the major urinary metabolite of DHT (Mauvais-Jarvis et al., 1968 b; Mauvais-Jarvis et al., 1973; Bird et al., 1974 and Kinouchi and Horton, 1974). 5 $\alpha$-DHT formation in liver and kidney is a transient step as it is rapidly converted to tetrahydro-testosterone and other inactive androgens like 5 $\alpha$-diols which are then eliminated through urine (McGuire and Tomkins, 1959).

Taking into consideration the above mentioned views on 3∝-HSDH, positive intense reactions encountered in the hepatocytes of periportal region and the proximal and distal convoluted tubules of kidney in normal male rats indicate that these are the possible sites of sex steroid interconversions and their ultimate excretion through either bile or urine.

Administration of IPR to male rats elicited a spurt in 3∝-HSDH activity in the areas which reacted positively in both liver and kidney. Obviously, the drug administration would have affected 3∝-HSDH function in different ways. Possible IPR therapy would have induced 5∝-reductase activity and hence enzymic conversion of testosterone (T) to 5∝-DHT. Since 5 DHT formation in liver and kidney is a transient step, it would have been converted to 5 ∝-diols through induction of 3 ∝-HSDH, and subsequently would have been eliminated out of circulation. It is pertinent to note here that castration induced 5 ∝-reductase activity in liver (Patterson et al., 1974) and inhibited the same in sex-accessory organs (Gustafsson and Ake, 1974). In liver, possible induction of 5∝-reductase and resultant 5∝-DHT cannot be excluded because of observed least reactivity of 17β-HSDH. The reverse could be true for kidney, i.e. as 17β-HSDH function all along the length of nephrons is evident, 5∝-reduction of T may not be operative to the same extent as in liver and hence, 3 ∝-HSDH function in nephrons stands next to hepatocytes. Thus IPR administration is shown here to induce 3 ∝-HSDH function predominantly in liver and to a lesser extent in kidney cortex of male rats.

In normal male rats, 17β-HSDH activity in hepatocytes is at its lowest ebb with either testosterone or oestradiol as substrates, although the general distribution pattern of the enzyme remaining the same as 3α-HSDH. It sounds probable that the pathway is not operative to the same extent as that of 3α-HSDH. Thus, steroid catabolism is favoured through 3α-HSDH route in liver. The presence of 17β-HSDH has been assigned a steroid catabolic function in a tissue (Samuels et al., 1950; Hay and Hodgins, 1973 and Hodgins et al., 1982).

The drug administration enhanced the histochemically demonstrable 17β-HSDH activity in liver. So the drug therapy could be speculated to enhance conversion of oxosteroids which could be eliminated along with bile or urine.

Normal male rat kidney showed intense reactivity for 17β-HSDH using both T and E as substrates. Positive reactions were encountered all along the length of a nephron, including loop of Henle in the medula, the collecting tubules remaining non-reactive. The observation is in confirmity with Baillie (1966). The positive 17β-HSDH reactions encountered (Akaishi et al., 1974a ; Kotak and Padmanabhan, 1980) in kidney (Baillie et al., 1966b, Liu and Kochakian, 1972; Bhujle and Nadkarni, 1974 and Amanova and Balbich, 1976) are considered to catalyze the interconversions of oestradiol, testosterone and 17β-hydroxysteroids to biologically less active oestrone, androstenedione and 17 -oxosteroids, respectively (Baillie et al., 1966; Hodgins et al., 1982). Such interconversions in kidney

are relevant to excretion of 17-ketosteroids along with urine (Furman et al., 1958; Hamilton et al., 1959 and VandeWiele and Lieberman, 1960).

Chronic IPR administration to male rats intensified 17β-HSDH reactivity all along the length of a nephron including loops of Henle. Thus, it sounds appropriate to note the increased conversion of biologically inactive 17-ketosteroids from more active steroids and hence, decreased plasma testosterone level could be predicted.

Circulating level of T in an animal embodies a conglomerate of two distinctively synchronized processes, namely, (i) steroidogenesis and (ii) metabolic clearance rate (MCR) of steroids from the circulation. The MCR of steroids could result from a variety of factors such as alterations in (a) hepatic and renal blood flow, (b) plasma binding of steroids and (c) activities of steroid metabolizing enzymes. In a normal animal, a precise delicacy is maintained among all these factors and any alteration such as the one inflicted herein by IPR therapy in any one of these factors would bring about disruptive changes in the hormonal status of the animal in question. The male rats challenged herein with IPR would naturally be subjected to register changes in either the process of steroidogenesis or the MCR of steroids from the circulation. At this juncture, it is pertinent to survey the literature pertaining to the effects of IPR administration on both the processes of steroidogenesis and the metabolic clearance rate (MCR) of steroids.

Recently, cultured mouse testicular interstitial cells have been shown to induce androgen production when challenged with IPR, only after 24 hours of culture (Cooke et al., 1982; Moger et al., 1982; Moger and Anakwe 1983; Moger and Murphy, 1983; and Anakwe and Moger, 1984a). This response was not entertained by either freshly isolated cells or whole decapsulated testis (Moger et al., 1982). Ovine LH in vivo induces testosterone production (Damber and Janson, 1978). In vitro induction of steroidogenesis by Leydig cells (Moger and Anakwe, 1983, Anakwe and Moger, 1984a, b) is prone to register a check by a variety of indigenous factors in vivo and in fact, Huang and Mccann (1983) have shown that intraventricular injection of IPR in rats significantly reduced plasma LH concentration which ultimately could be speculated to reduce steroidogenesis by Leydig cells. $\beta$-adrenergic stimulation is shown to inhibit LH release by pituitary (Kar and Ghosh, 1951; Armstrong and Hansel, 1958; Caceres and Taleisnik, 1980; Caceres and Taleisnik, 1982; Dotti and Taleisnik, 1982 and Leungs et al., 1982a, 1982b). Seemingly, these facts lend support to the assumption that IPR would have induced diminutions in the circulating level of testosterone, probably through suppression of LH release.

Apart from the responsive Leydig cells, yet another component of the testis, viz., sertoli cell enriched culture from 19-day old rats has been shown to induce aromatization of testosterone to oestradiol 17 $\beta$ in response to IPR and FSH administration (Dorrington et al., 1976; Rommerts et al., 1978; Verhoeven et al.,

1979 and Verhoeven, 1980). Since estradiol is known to exert a direct inhibitory effect on T production by Leydig cells (Moger, 1980; Keel and Abnoy, 1982), it is possible that chronic IPR administration exploited in the present study would have exerted atleast part of its effect, i.e., probable reductions in the circulating T, through such a step. Once again recalling the *in vivo* work by Huang and Mccann (1983), it is obvious that third ventricular injection of IPR failed to alter plasma FSH levels, contrary to its inhibitory effects on plasma LH concentration. This observation has attained some relevance in the present context. Chronic IPR therapy could be expected to diminish the plasma T levels by reducing the rates of steroidogenesis in the Leydig cells and by enhancing testosterone aromatization to oestradiol in the sertoli cells through the reductions of plasma LH concentration and consistency in FSH levels, respectively.

So far as *in vivo* effects are concerned, only one report exists in the literature pertaining to IPR administration and testosterone production. Kristen (1969) infused non-physiologic dose of IPR at a constant rate via the spermatic artery of dog, the levels of T increased in spermatic venous blood for 30 minutes and declined by 60 minutes, although the response was still evident with HCG infusion. The report is self-explanatory in the present context. The assumption that chronic IPR therapy evokes reductions in the circulatory T levels finds a convincing resolution in this report, since T production was curtailed after 60 minutes.

Circulating catecholamine levels rise sharply in men during physical and mental stress (Barnes et al., 1982; Gustafson and Kalkhoff, 1982; Williams et al., 1982) and in rats during immobilization stress (Buhler et al., 1979 and Kvetnansky et al., 1979). Infusions of catecholamines to men and rats have been reported to diminish the production rate of testosterone (Levin et al., 1967; Damber and Janson, 1978). Physical and psychological stress induce in humans (Kreuz et al., 1972; Aono et al., 1976; Morville et al., 1979) and in animals (Bliss et al., 1972; Gray et al., 1978; Goncharov et al., 1979; Charpenet et al., 1981, 1982), a rapid and drastic fall of plasma testosterone levels. Immobilization stress, a form of psychological stress, induces very rapidly a state of leydig cell hyposensitivity to gonadotropins and a blockade of testosterone biosynthesis (Charpenet et al., 1981 and 1982; Collu et al., 1984). Some of the inhibitory effects of stress on the testicular hyposensitivity to gonadotropins would have been exerted by catecholamines through β-receptors (Collu et al., 1984). IPR, a potent β-adrenergic agonist, when administered chronically in vivo could thus, be expected to commit its inhibitory action on steroidogenesis by leydig cells and hence, plasma testosterone levels could be shown to be diminished as a consequence to such a therapy.

Adrenal gland is yet another important alternate source of steroids. The stimulatory effects of IPR in 2 day culture of bovine adrenocortical cells on steroidogenesis have been reported (Kawamura et al., 1984). IPR is shown to elicit stimulation of

$^{3}$H-corticosterone. Serum corticosterone and serum protein-binding of corticosterone were shown to be decreased as a result of the drug therapy. They showed an increased MCR of corticosterone in the experimental group, despite an equal production rate of corticosterone in both normal as well as experimental animals. In the view of the decreased serum protein binding of corticosterone and increased accumulation of $^{3}$H-corticosterone in the liver of experimental group, steroid catabolism in liver could be anticipated (Slaunwhite et al., 1962). Obviously, IPR therapy in the present study would have increased the MCR of steroids by decreasing serum-protein binding and increasing accumulation of steroids in liver and hence, accumulated steroids could be anticipated to undergo metabolic conversions into biologically inactive steroids in liver.

The MCR of steroids could also be altered by yet another factor, namely, hepatic and renal blood flow. Increased hepatic and renal blood flow at rest in trained versus untrained individuals may be associated with increased MCR of steroids (Keizer et al., 1980). Intravenous IPR therapy has been shown to increase hepatic (Geumei and Mahfouz, 1968; Greenway and Lawson, 1968) and renal (McNay and Goldberg, 1966) blood flow. Thus, more blood is made available to liver and kidney by such a mechanism and the circulating steroid pool is, thus, exposed to the steroid catabolic machinary of respective organs at a faster rate. Hence, steroid level of serum is prone to receive a major setback. Recently, IPR infusion for 4 hours during the midluteal phase of the menstrual cycle in normal women has been shown to reduce serum progesterone and oestrogen levels significantly, by

increasing MCR of steroids (Casper et al., 1984). Similar findings of decreased serum estrogen and progesterone levels during β-adrenergic agonist infusion have been reported in the third trimester of pregnancy (Bibby et al., 1978 and Schreyer et al., 1981).

The MCR of steroids is once again prone to register changes through alterations in steroid metabolizing enzymes. Only 10% of the circulating testosterone is metabolized to active metabolites through mediation of 5α-reductase, while rest of the circulating testosterone is prone to undergo metabolic conversions into inactive metabolites which are ultimately conjugated and excreted out in urine and bile (Smith et al., 1985). The enzymatic conversion of T to biologically inactive metabolites is a multifactorial phenomenon occurring centrally in liver which is a major detoxification center. The reactions include (i) A-ring reduction, (ii) hydroxylation, (iii) oxidoreduction and (iv) direct conjugation of steroid molecules (Vida, 1969).

In an elegant series of experiments, Conney and his colleagues and others have shown that several structurally unrelated drugs and insecticides that stimulate drug metabolizing enzymes also stimulate steroid hydroxylation and thereby decrease plasma steroid levels ( Garren et al., 1961; Conney et al., 1965, 1966; Kuntzman et al., 1964 and 1966; Conney, 1967; Gielen and Nebert, 1972; Conney et al., 1973; Thomas et al., 1973; Gurtoo et al., 1979; Chung and Chao, 1980; Cicero et al., 1980). TPNH-dependent

enzymes in liver microsomes metabolize not only drugs but also a variety of normal body substrates including steroids (Mueller and Rumney, 1957; Forchielli et al., 1958; Conney and Klutch, 1963; Kuntzman et al., 1964). The similarities between drug and steroid hydroxylases in liver microsomes suggested that drugs and steroids are substrates for the same hydroxylating enzymes (Kuntzman et al., 1964 and 1966). IPR, in particular, has been reported to induce aryl hydrocarbon hydroxylase in human lymphocytes (Gurtoo et al., 1979) and in mammalian liver cell culture (Gielen and Nebert, 1972; Conney et al., 1973), and therefore, IPR could be safely presumed to exert potent steroid hydroxylating effects (Kuntzman et al., 1964, 1966). Obviously, a steroid catabolizing function could be assigned to such an agent.

In essence, it could be inferred from the discussion that chronic IPR therapy in male rats could be expected to exert its influence over both the rate of steroidogenesis ( i to iv) as well as the MCR of steroids from the circulation (v to viii). Chronic IPR administration could be expected :

(i) to suppress LH release from pituitary as a result of which the steroidogenic response of leydig cells could be seized,

(ii) to induce very rapidly a state of Leydig cell hyposensitivity to gonadotropins if at all synthesized and hence, reduced rate of steroid synthesis by leydig cells,

(iii) to sustain FSH release from pituitary, FSH in turn could be speculated to induce aromatization of testosterone to oestradiol by sertoli cells. Oestradiol, thus produced by sertoli cells, could be speculated to curtail steroidogenesis by interstitial cells of Leydig which are in the direct vicinity of sertoli cells.

(iv) to suppress corticosteroid synthesis by adrenal cortex probably as a consequence to lost structural integrity.

(v) to induce the MCR of steroids by reducing serum protein binding of steroids and by increasing accumulation of steroids in liver and hence, plasma steroid pool could be anticipated to be decreased,

(vi) to enhance hepatic and renal blood flow and thereby the circulating steroid pool is exposed to steroid catabolic machinary of both the organs at a faster rate and hence, circulating steroid pool could be diminished,

(vii) to stimulate aryl hydrocarbon hydroxylase in liver, the enzyme in its turn could be expected to metabolize both IPR and steroids as a result of which serum testosterone levels could be diminished and above all,

(viii) to induce MCR of steroids through observed acceleration of steroid catabolizing enzymes such as 3$\alpha$-HSDH and 17$\beta$-HSDH in both liver and kidney of male rats.

It is conceivable from the views ( i to vii) and the observations (viii) as well that catecholamines could be speculated to diminish the circulating testosterone levels. This entire array of speculations could explain convincingly the observed reduction in the metabolic patterns of the preputial gland (Ambadkar and Vyas, 1981b, 1982; Vyas, 1978) of male rats as a sequel to chronic exposure to β-adrenergic agonist, IPR and could also be foreseen as possible cause of the alterations in the biochemical parameters of the male rat preputial gland that will be dealt with in details in the succeeding chapters.