GENERAL CONSIDERATIONS

Although there is a wealth of information on the androgenic regulation of sebum secretion, there is a dearth of substantive literature on neural control of the functions of sebaceous glands. As for hormonal control, the problem has a history of about four decades only. However, the comparatively poor progress in this field leaves the question of neuronal regulation of sebum secretion at an equivocal stage.

The work reported in the present thesis mainly comprises of two components. The first one deals with possible neural influence on the functions of the preputial glands of male rats and the second part deals with the hormonal influences by employing the methods of surgical ablation of gonads and the adrenals.

Neural Control :

Three decades ago, a feed-back mechanism was generally accepted as the controlling force for sebaceous glands. The assumption was for the first time challenged by Kligman and Shelley (1958) who, after careful experimental work, proposed that the sebaceous glands secrete continuously. The theory of feed-back mechanism of control of sebaceous gland secretion was rejected on the ground that neurohistologic studies yielded no evidence of secretory innervation for these glands. Even Montagna (1963), after studying acetylcholinesterase histochemically, showed that no positive fibres were present in the gland itself and the innervation of the sebaceous gland was considered to be fortuitous. Thus, the very question of

innervation itself is disputable. However, later it was convincingly shown that rat preputial gland is supplied by both adrenergic as well as cholinergic nerve fibres (Ambadkar and Vyas, 1981a), despite the previously reported conflicting views on the subject (Boecke, 1934; Hurley et al., 1953; Rothman, 1954; Hellmann, 1955; Thies, W and Galente, 1957; Winklemann, 1960; Montagna, 1963).

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 α-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, while 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. Rates of sebaceous lipogenesis were shown to be reduced after L-Dopa therapy (Burton and Shuster, 1973; Burton et al., 1973; Wheatley and Brind, 1981). Moreover, Wheatley and Brind (1981)

opined on the basis of a report from our laboratory (Ambadkar and Vyas, 1980) that adrenal neurohormones may play a role in stimulating sebaceous gland to replenish its secretion. Conversly, adrenal neurohormones have been reported to inhibit sebaceous lipogenesis (Wheatley et al., 1971). It is, thus, apparent that practically only a hazy image seems to emerge out of the literature relevant to neuronal regulation of sebaceous gland functioning. 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 neurohormones. The gland was shown to exhibit metabolic alterations in response to administration of one of such neurodynamic neurohumoral agonists viz.- isoproterenol- which are usually known to follow androgen deprivation in male rats. Precisely these observations from this laboratory prompted the idea of making use of isoproterenol for further understanding about inhibition of sebum synthesis, which could prove to be clinically beneficial for acne and related disorders of elevated sebaceous lipogenesis. However, it is not known as to what extent such neurodynamic compounds 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. The salient features from the results of these investigations are highlighted as under.

An experimental animal model - viz. albino rat - has been employed to investigate the influence of one of the most potent β-adrenergic receptor agonist, isoproterenol, on steroid

dehydrogenase profile of the preputial gland. The drug administration brought about changes in the steroid dehydrogenases' profile which have been reported to follow androgen deprivation on the pattern of steroid metabolism of the preputial gland of male rats (Ambadkar and Vyas, 1982). Since the steroid metabolism in the target tissue depends on the plasma levels of testosterone, possibly decreased circulating testosterone levels due to the drug therapy might have caused the observed reduction in the steroid metabolism of the gland. At this juncture, two possibilities had been put forward to explain such altered steroid metabolism of the gland in response to chronic IPR therapy. Firstly, it was presumed that IPR might have some direct effect on the endogenous steroid metabolism of the gland. Alternatively, it was thought that chronic IPR administration might bring about enhancement of steroid catabolism in liver and may lead to the observed influence on the steroid dehydrogénases profile of the gland. Such enhancement of steroid catabolism could be accomplished with a number of mechanisms and the oxidoreduction reaction of a steroid molecule is one such metabolic process.

In the beginning, the investigation was directed at alterations in the histochemical localization and intensity of 3α -HSDH and 17β -HSDH enzyme activities in the hepatic and renal tissues of male rats in response to chronic IPR therapy which has been presented in Chapter : 11. Histoenzymological profile revealed a spurt in 3α -HSDH activity in the areas which reacted positively in both liver and kidney after the drug therapy. Thus, testosterone and 5α -DHT could be expected to be converted to 3α-diols in liver

and kidney at a faster rate. Moreover, elevated 3α-HSDH activity in both the organs also could be considered to be involved in the interconversions between Δ^4-ketosteroids and 3α-hydroxysteroids. Obviously, the drug therapy would have hampered plasma testosterone levels. Similarly, the drug administration intensified 17β-HSDH activity in both the organ to different degrees. Thus, it sounds appropriate to note the increased conversion to biologically inactive 17-ketosteroids. Elevated HSDH reactivity levels in tissues like liver and kidney could collectively lead to decrease circulating testosterone levels. This assumption, to a certain extent, could explain the alterations evident in case of certain other biochemical and histochemical parameters of male rat preputial glands, in response to chronic IPR therapy, that have been dealt with in details in the succeeding chapters.

Certain histological (Vyas, 1978), histochemical (Ambadkar and Vyas, 1982) and enzyme quantitation (Ambadkar and Vyas, 1981b) studies have provided clues that the preputial lipogenesis could be significantly influenced by chronic isoproterenol administration to male rats. This gland being eminently of holocrine type; the rate of production of sebum directly reflects the overall biological activity of the sebaceous cells. Sebum secretion is a synthetic potential and is determined by the rates of both maturation and subsequent disintegration of lipid-laden mature cells, and replacement by undifferentiated cells. Undifferentiated cells at the periphary of acini are engaged in lipid production. Measurement of glandular total lipid content after IPR therapy indicated a significant decline

as compared to normal values (Chapter : 2). The observations clearly indicate that preputial lipogenesis is inhibited by IPR. Hence, adrenal neurohormones could be considered to inhibit sebaceous lipogenesis. Indeed, such a role assigned to adrenal neurohormones has been reported in guinea pig ear skin model (Wheatley et al., 1971).

A unique biosynthetic potentital acquired by preputial sebaceous acini would naturally demand cofactors and precursors. This could be brought about through anaerobic and/or aerobic reactions and could either be stepped up or toned down according to the hormonal microenvironment at the cellular level. A histoenzymological study in this context revealed drastic reduction in the activities of all three TPN-linked dehydrogenases viz., ICDH, ME and G-6PDH, which are known to provide reductive hydrogen for lipogenesis (Chapter : 2). This finds further support in the observation that drug therapy reduced the production of lipid precursors like α-glycerophosphate and acetyl Co-A (Chapter : 3) as evinced from reduced α-GPDH activity as well as that of BDH. Further, as reported in Chapter : 4, IPR therapy was seen to reduce another enzyme activity viz., MDH. This enzyme activity is known to facilitate lipogenesis through its stimulatory effect on malate cycle. Reduction in its activity as observed here would logically reduce preputial lipogenesis. This is in corroboration with the statements made in connection with TPN-linked dehydrogenases on one hand and BDH-α-GPDH on the other. It is, thus, appropriate to note that the entire process of lipid synthesis in the preputial gland is retarded in response to chronic IPR therapy (Chapter : 2, 3 & 4).

Holocrine mode of secretion essentially underlies a programmed autolysis of lipid-laden mature preputial acinar cells. This obviously needs a very high rate of mitosis so as to compensate the orderly loss of lipid-laden mature cells. IPR therapy in male rats was seen to lead to deceleration of cAMP specific PDE activity in the gland (Chapter : 7). This can be considered as an indication of elevated intracellular cAMP levels. Chronic IPR therapy has been reported to result in retarding the rate of mitosis in the gland of male rats (Vyas, 1978). The present observation about elevated cAMP level is indicative of a possible mechanism of inhibition of mitosis as has been reported (Harris and Mackenzie, 1981). Another inference that can be drawn pertain to the role of cAMP in stabilizing the lysosomal membrane system. The latter would be a manifestation of retardation of normal process of programmed maturation of acinar cells. Hence, it can be suggested that chronic IPR therapy has a two-pronged influence on the programmed holocrine secretory mode of preputial gland. On one hand, it retards the rate of mitosis and thereby influencing the process of replacement with potentially undifferentiated cells and on the other hand it also hampers the process of autolytic disintegration by stabilizing cellular membranes in general and the lysosomal membranes in particular. The observations, thus, suggest a possible inhibitory role for β-adrenergic neurotransmitters in the entire array of preputial acinar functions considered here. The catecholamine emanating from adrenal medulla (via circulation) may possibly have a significant role to play in the processes under consideration, since denervation of the gland was reported to be

without any significant effect on the rat preputial gland (Vyas, 1978).

From the present observations it also becomes evident that stimulation of β-adrenergic function mimics certain changes that are known to follow androgen deprivation. The study on steroid metabolism of the gland (Ambadkar and Vyas, 1982) indicated that IPR administration interferes with the metabolism of steroids in this gland. It is also apparent that IPR, by increasing the oxidoreductior rates of steroids in liver and kidney (Chapter : 1), decreases the local availability of steroids to the gland. Thus β-adrenergic receptor functions are probably implicated either through their influence on overall steroid metabolism in the body as a whole, as has been hypothetised in the first chapter or by virtue of their direct action on the gland itself. However, it is not possible, at this stage, to comment on definite mechanisms involved and to assess the extent of involvement of β-adrenergic receptor functions on the glandular metabolism. Neverthless, it can be assumed, on the basis of present investigation, that any condition leading to increase in levels of circulating catecholamines would consequently counteract the stimulating androgenic influence on sebaceous analogues. The present observation may have significant pharmaceutical implications in the pathology of the sebaceous glands. With further studies in this field, it may become possible to formulate therapeutic measures. Further, the finding of Bullough and Laurence (1970) that a water soluble substance from the skin, which is activated by adrenaline, inhibits mitotic activity in the sebaceous gland, substantiates the above mentioned contention. It is also supported

by the observation that IPR inhibits rates of mitosis, glycolysis and amino acid incorporation in the epidermal components (Harris and Mackenzie, 1981). Moreover, yet another observation that adrenal neurohormones inhibit sebaceous lipogenesis (Wheatley et al., 1971) confirm the present findings reported in Chapter 2, 3, 4, 6 and 7). Though the precise mechanism of adrenergic influence on the physiology of sebaceous gland is not known at this stage, findings presented (Chapter 1, 2, 3, 4, 6 and 7) do open newer avenues for further studies on factors controlling the physiology of sebaceous glands. Such studies need be made not only to vet the academic appetite of researchers in basic sciences but also due to possible far-reaching pathological and pharmacological implications.

During the course of this investigation it was observed that chronic IPR treatment leads to reduction (1/3 or more) in case of ATPases, SDH and FDP-aldolase enzyme activities. This treatment, therefore, seems to reduce the tone of oxidative phosphorylation as well as the process of active transport (Chapter : 5). Reduction of aldolase activity is indicative of slowing down glycolytic pathway as was also reported by Harris and Mackenzie (1981). Chronic IPR therapy to male rats elevated the transaminase levels in the gland with a corresponding decrement in the total protein content. The observation is in good agreement with inhibitory role assigned to IPR therapy towards protein synthetic capabilities (Jefferson et al., 1972; Harris and Mackenzie, 1981; Chao and Walkenbach, 1986).

If one tries to summerise the results obtained, it could be seen that chronic IPR to male rats leads to following alterations :

Enhancing the rate of steroid degradation by the liver and the kidney (Chapter : 1) and in the case of preputial gland, reduction of lipogenesis (Chapter : 2, 3, 4), glycolysis (Chapter : 3, 5), oxidative phosphorylation (Chapter : 2, 3, 4, 5), reduction of total protein content and increase in transaminase activity (Chapter : 8) and in an increase in cAMP-PDE levels (Chapter : 7).

From the above changes, it can be surmised that the administration of IPR induces overall suppression of normal activities of the preputial gland. However, one could easily note that this β-adrenergic agonist apparently has a direct influence on various enzymic systems in addition to its distant reductive influence on circulating androgenic levels. The latter may also tell upon the involutionary tendencies as a consequence. Hence, it is apparent that IPR therapy acts on preputial gland proximately and also involving the liver and kidney in an indirect manner.

Androgenic Regulation :

As the results discussed so far were accuring, it became increasingly apparent that the adrenal catecholamines, in conjunction with gonadal steroids, are responsible for the normal functional state of the preputial glands of male rats. Keeping this view into consideration, it was thought desirable to look into the possible involvement of adreno-medullary compounds, an investigation was carried out on the effects of ablation of gonads alone and along with that of adrenals on the various functional aspects of the preputial glands of the male rats. The results obtained indicated very clearly

that gonadal androgenic influence exerts regulatory action on some important processes like glycolysis (Chapter : 5), oxidative metabolism (Chapter : 5), transport phenomena (Chapter : 5) and cellular proliferation (Chapter : 6 and 7). When C was accompanied by Adx, the alterations were seemingly additive. From this, one can tentatively conclude that the adrenal glands perform a facilitatory role for the action of gonadal steroids on the preputial glands. However, from the results at hand, it is not known whether the adrenal steroids on their own could have any direct role in the physiology of these glands. It is, therefore, suggested that further studies in this context may provide an insight of this issue.

In contrast to the preceding additive results, the influence of C and C + Adx was vis a vis at variance as far as the total protein content of the gland is concerned. As is generally expected, C leads to decrease in protein content but if it is accompanied by Adx, strangely enough the protein content of the preputial gland registered a significant increase. Probable implications of these observations have already been discussed (Chapter : 8). However, at this juncture, it could be said that there is a pressing need of further intensive investigation so as to resolve this enigmatic and apparent increase in the protein content of the gland.

It is, thus, apparent that the adrenal glands may have two-pronged action on the biological activities of the preputial gland. The adrenals might play an additive role in the maintenance of the physiological functions of the preputial glands via the agency of cortical steroids among castrates. However, the adrenals might also

exert an inhibitory role in the regulation of structural and functional integrity of the preputial gland via the agency of medullary catecholamines. Obviously, both the cortical and the medullary hormonal factors of the adrenals exert direct and opposable influences on the physiology of androgen-dependent preputial glands. Hence, it is envisaged that responses exhibited by the preputial gland separately towards cortical manipulations alone should be assessed in order to deal with such a complex issue. Additionally, in-vitro responses of the preputial gland towards IPR also should be assessed. Later on, the combined influence could be analysed for a better understanding.