

## Chapter - 7

EFFECTS OF ADRENERGIC AGENTS ON THE PREPUTIAL  
GLAND : A HISTOMORPHOMETRIC STUDY

Sebaceous glands of man and other mammals are known to be stimulated by androgens. Androgens have been reported to enlarge the sebaceous glands in rats (Ebling, 1948; Haskin, et al., 1953), rabbits (Montagna and Kenyon, 1949), hamsters (Hamilton and Montagna, 1950), mice (Lapiere, 1953) and the analogues such as preputial glands of rats (Beaver, 1960) and supracaudal glands of guinea pigs (Montagna, 1962; Martan and Price, 1967).

It is interesting to know the relationships between different facets of sebaceous activity. The rate of sebum production depends upon two factors : the synthetic capacity of sebocytes and the rate of proliferation of undifferentiated cells. Under the influence of testosterone sebaceous gland shows high incidence of cell division. Castration reduces thymidine incorporation and mitotic index in sebaceous glands (Frost et al., 1973). Androgen dependency of the gland for its secretory function has been repeatedly reported by many workers.

Though it is known that the preputial gland, a sebaceous analogue of rats, is innervated by cholinergic and adrenergic nerves (Chapter-4), yet, it is not known if either adrenergic or cholinergic agents have any influence on sebaceous glands or on its analogues. Adrenergic agents and cyclic nucleotides are known to alter the rate of cellular proliferation in the epidermis (Birnbaum et al., 1976; Voorhees et al., 1972). In the light of these observations, it was deemed worthwhile to study the effects of certain pharmacodynamic agents (viz., Isoproterenol and phenylephrine) on histological characteristics of the preputial glands with a view to comprehend possible implications of these reports.

#### MATERIALS AND METHODS

Male albino rats (120-140 gms) were used for the present investigation. Rats were divided into three groups : Group I - consisted of untreated normal animals. Group II - phenylephrine treated animals and Group III - isoproterenol treated animals.

The pharmacodynamic agents were administered intraperitoneally. Each of the animals of group III received 25 mg of isoproterenol/kg body weight and those of the Group II 6 mg of phenylephrine/kg body weight. These agents were

administered twice a day, 12 hours apart, for 10 days. Twelve hours after the last injection of the drugs, rats were sacrificed and preputial glands were removed and fixed in Bouin's fluid for routine histology. Paraffin sections of 6  $\mu$  thickness were cut and stained with Haematoxylin-Eosin for observations. A large number of sections were observed at the magnification of 500 x. The number of acini in an area projected on the screen (area under observation was kept constant) were counted. The area of the screen remaining constant; differences in the number of acini per unit area were compared. Acini with central acinar cells showing disintegration (broken cell membranes) were also counted, and the percentage of such disintegrating acini was calculated (Table-1).

## RESULTS

In the normal untreated animals about 41.5% acini were found to be in a stage of disintegration, while in isoproterenol treated animals 26.25% acini showed signs of disintegration. Number of acini per unit area showed an increase in the isoproterenol treated animals, but the size of the acini was comparatively smaller in isoproterenol treated animals (Table-1 and Figs. 1 and 2). Phenylephrine was found to have no demonstrable effects on histological characteristics of the gland (Table-1).

Table 1

Number of acini and percentage of disintegrating acini in preputial glands of normal, isoproterenol and phenylephrine treated rats per a given area of  $283.64 \text{ cm}^2$  observed at a magnification of 500. Mean value  $\pm$  S.D.

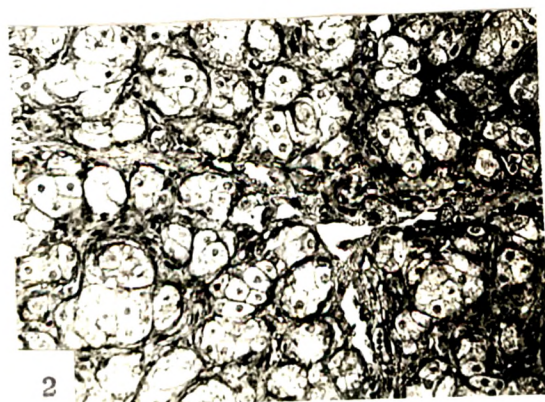
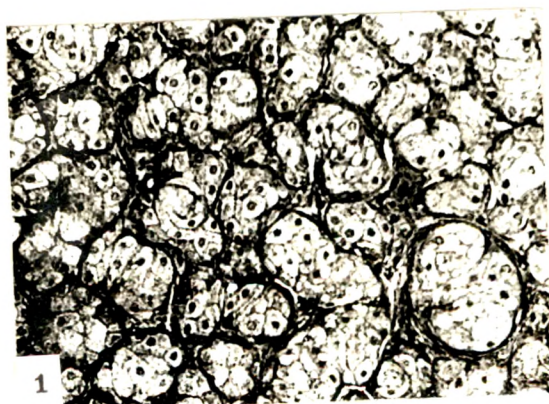
Treatments	Number of acini	Percentage of disintegrating acini
Normal	$27.6 \pm 5.2$	$41.5 \pm 10.2$
Isoproterenol	$46.5 \pm 7.5^*$	$26.2 \pm 4.6^{**}$
Phenylephrine	$26.2 \pm 6.5$	$42.5 \pm 8.3$

\* Significantly different from the normal at the level  $P < 0.0025$ .

\*\* Significantly different from the level at the level  $P < 0.01$ .

## EXPLANATIONS FOR FIGURES

- Fig.1      Photomicrograph of the section of the preputial gland of normal rat stained with Haematoxylin-eosin. 125X
- Fig.2      Photomicrograph of the section of the preputial gland of isoproterenol treated rat stained with Haematoxylin-eosin. Note the smaller size of acini and reduction in number of disintegrating acini as compared to that shown in Fig.1. 125X



## DISCUSSION

Preputial gland, a sebaceous analogue, in rats is a holocrine secretory gland. Secretion is formed by complete breakdown of lipid laden mature cells of the acini. For the normal functioning of the gland, number of disintegrating cells should bear a certain proportion to the number of cells produced. If number of disintegrating cells goes down, the rate of cell production remaining the same; then the acini ought to show hypertrophy. However, here it was observed that after isoproterenol administration the number of disintegrating acini and acinar size both were reduced considerably as compared to normal ones, which suggested that the rate of cell proliferation as well as the process of cell disintegration also underwent retardation leading to comparatively greater number of acini per unit area under observation with fewer disintegrating cells in the acini and smaller size of the acini themselves.

Isoproterenol is known to elevate intracellular concentration of c-AMP in the epidermis (Powell et al., 1971). Further, the cyclic nucleotides are known to be involved in the regulation of cell proliferation and differentiation in various types of cells (Goldberg et al., 1976). Presently observed decrease in cell proliferation, therefore could well

be due to an increase in c-AMP concentration induced by the administration of the drug.

Voorhees et al. (1971, 1975) have shown the existence of a c-AMP system in the mammalian epidermis which is sensitive to stimulation by  $\beta$ -adrenergic agonist. They have further reported that elevation of epidermal levels of c-AMP can inhibit epidermal cell division in vitro. Chopra (1977) has also reported that theophylline and c-AMP depress mitosis in the epidermis. In the present investigation also it has been observed that isoproterenol ( $\beta$ -adrenergic agonist) has a pronounced depressing effect on the rate of proliferation of acinar cells. On the other hand, phenylephrine ( $\alpha$ -adrenergic agent) did not influence significantly the mitotic activity of the gland. From these observations it can safely be suggested that  $\beta$ -receptor agonists influence the process of cell division as well as that of cell disintegration in the preputial glands of rats.

Taylor and Shuster (1977) have reported decreased mitotic activity in the sebaceous glands under stress and suggested that it may be due to increased release of adreno-medullary hormones, which are known to affect both  $\alpha$ -as well as  $\beta$ -adrenergic receptors. Observed retardation of disintegration in the acinar cells of the gland and possible inhibition of

mitotic activity may result in decreased 'sebum' production. It was also observed that in most of the acini all the cells are in a similar state of maturation after isoproterenol administration, unlike the normal situation, where the central cells in almost all the acini are fat-laden and are on the way towards disintegration. In an earlier report on the activity of acid phosphatase, which is directly associated with programmed disintegration of holocrine secretory cells of the rat preputial glands (Chapter-6), was observed to be decreased on administration of isoproterenol. Thus, the present histological observation that isoproterenol administration decreases cellular disintegration, leading consequently to reduction of sebum production in the gland, finds good support in the biochemical finding reported in Chapter-6.

These results suggest a possible role for adrenergic neurotransmitters in the mechanism of cell replacement in the acini of the preputial glands of white rats. The neurohormones may come from adrenal medulla via circulation. It appears quite possible that circulating adrenergic hormones may be of greater importance, since denervation of the gland was found to be without any apparent significant effect on the rat preputial glands (Chapter-5).

From the present report it also becomes clearly evident that  $\beta$ -adrenergic stimulation mimics, to a certain extent, the changes that are known to follow androgen deprivation. The latter condition has been reported to cause decrease in mitotic index of the sebaceous glands of hamsters (Frost et al., 1973). The study on steroid metabolism of the gland (Chapter-8) indicated that isoproterenol administration interferes with the metabolism of steroids in this gland. It was also suggested that isoproterenol, by increasing the rate of steroid hydroxylation in the liver, decreases the availability of steroids to the gland. Thus,  $\beta$ -adrenergic system may be implicated either through its influence on steroid hydroxylation in non-target organ like liver or by virtue of its direct action on the gland. However, it is not possible, at this stage, to comment on definite mechanisms involved and to decide the magnitude of  $\beta$ -adrenergic influence directly on the gland.