Chapter - 3

HISTOCHEMICAL OBSERVATIONS ON CERTAIN HYDROXYSTEROID DEHYDROGENASES IN THE RAT PREPUTIAL GLAND

Since last few years steroid metabolism in the sebaceous glands has been the subject of extensive study. It has been shown that the skin is capable of metabolizing several steroids including testosterone and the literature has been reviewed by Hsia (1971). Histochemical studies on hydroxysteroid dehydrogenases (HSDHs) in the skin have provided enough evidence regarding a dynamic state of steroid metabolism in the sebaceous glands of mammalian skin (Baillie et al., 1965; Calman et al., 1970; Muir et al., 1970).Several metabolites of steroids have been identified not only in the sebaceous glands but also in analogous skin glands such as preputial gland of rat (Bardin et al., 1970; Richardson and Axelrod, 1971; Sansone and Reisner, 1974) and constovertebral gland of hamster (Takayasu and Adachi, 1972). These findings clearly

point to the presence of necessary enzyme systems in such locations. Balogh (1966) and Muir et al. (1970) described the histochemical localization of HSDHs in the preputial glands. It is apparent that hydroxysteroid dehydrogenases are important for metabolic interconversions of the steroids in the preputial gland. However, these enzymes have not been studied in the preputial glands of castrated rats. Moreover, it has been suggested that hormonal control of the sebaceous glands in the rat appears to be exercised on at least two levels, those of cell division and intracellular synthesis showing different responses to different steroids (Ebling, 1974; Ebling et al., 1973). Such variations in response may be due to differences in the pattern of steroid metabolism of the cell types engaged in cell division and intracellular synthesis. Since isolation of such cell types is difficult for biochemical study, interpretation on the basis of histochemical study would be helpful. The present study reports possibilities of correlation of HSDHs activity with the metabolism of the steroids in the preputial glands of the rat.

MATERIALS AND METHODS

Laboratory bred male albino rats weighing 120± 40 gms were used for the present investigation. Experimental animals were divided in to three groups *viz*., Group I - untreated animals as controls, Group II - bilaterally castrated animals and Group III - bilaterally castrated-adrenalectomized animals.

Castration was performed by trans-scrotal route and adrenalectomy by abdominal route. Animals were kept under laboratory conditions with food and water provided *ad libitum*. Adrenalectomized-castrated animals were provided with glucose saline instead of plain drinking water. All the animals were sacrificed at intervals of 24, 48, and 120 hours post-operatively.

Preputial glands were removed immediately after cervical decapitation of the animals and made free of the blood and adipose tissue and kept on a chuck of the cryostat microtome maintained at -20°C. 9 to 12 μ thick sections were cut and processed for histochemical study of 17β-HSDH employing testosterone and estradiol-17β

as substrates (Kellogg and Glenner, 1960), 3β-HSDH using dehydroepiandrosterone (DHEA) and pregnenolone (P) as substrates(Wattenberg, 1958) and 3α-HSDH using androsterone as the substrate (Balogh, 1966).

RESULTS

17β-Hydroxysteroid dehydrogenase (17β-HSDH) : In normal rats, when testosterone was the substrate, this enzyme [17β-HSDH (T)] activity was found to be localized in the cells towards the periphery of the preputial gland acini. The cells of the duct system of the gland also showed the enzyme activity (Fig.1). The enzyme activity reduced in the preputial glands of the castrated rats (Fig.2). 17β-HSDH (T) activity in the glands of adrenalectomized-castrated rat (Fig.3) did not differ much from that in the case of the castrated rats.

17β-HSDH activity, when estradiol-17β, [17β HSDH (E)] (Figs. 4,5 and 6) was the substrate, also demonstrated a distribution pattern similar to 17β-HSDH (T). However, it was observed that this enzyme activity remained comparatively lower than that of 17β-HSDH (T).

EXPLANATIONS FOR FIGURES

Figs. 1 to 3 Photomicrographs of sections of the preputial gland of rat showing the activity of 17β-hydrosteroid dehydrogenase using testosterone as substrate. 125X

Fig.1 17β-HSDH (T) activity in the section of the preputial gland obtained from normal rat.

Fig.2 17β-HSDH (T) activity in the section of the preputial gland obtained from castrated rat 120 hrs postoperatively.

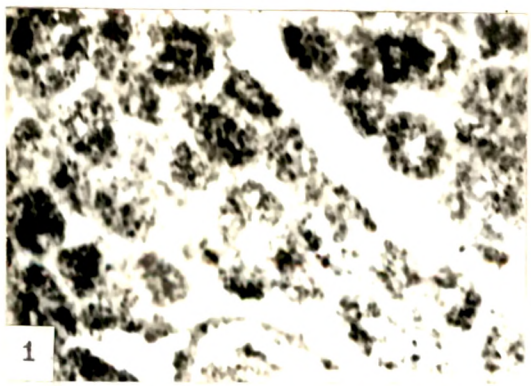
Fig.3 17β-HSDH (T) activity in the section of the gland obtained from adrenalectomized-castrated rat 120 hrs postoperatively.

Figs. 4 to 6 Photomicrographs of the sections of rat preputial gland showing activity 17β-hydroxysteroid dehydrogenase using 17β-Estradiol as substrate. 125X

Fig.4 17β-HSDH (E) activity in the section of the gland obtained from normal rat.

Fig.5 17β-HSDH (E) activity in the section of the gland obtained from castrated rat 120 hrs postoperatively.

Fig.6 17β-HSDH (E) activity in the section of the gland obtained from adrenalectomized-castrated rat 120 hrs postoperatively.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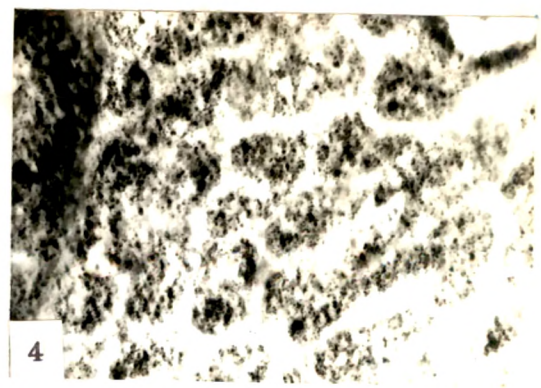
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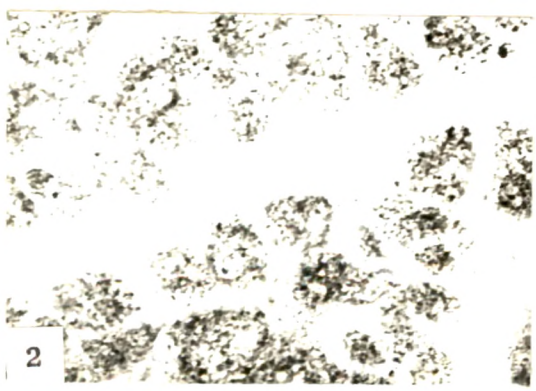
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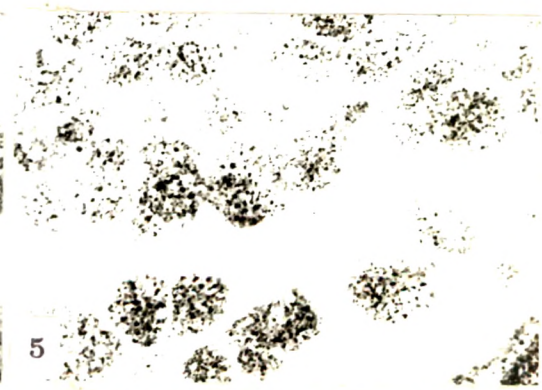
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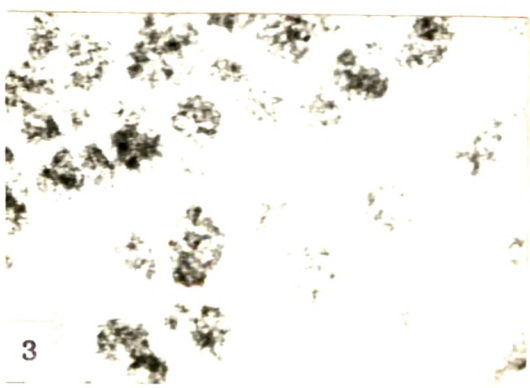
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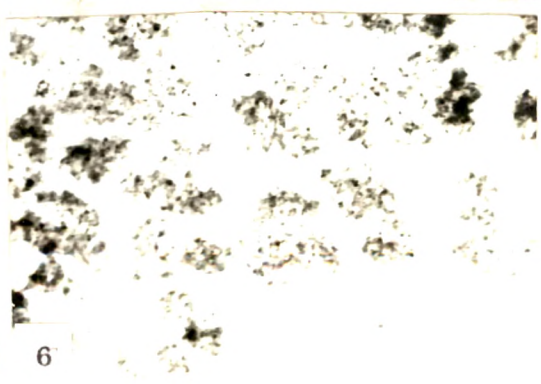
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EXPLANATIONS FOR FIGURES

Figs. 7 to 9 Photomicrographs of the sections of the rat preputial gland showing 3β-hydroxysteroid dehydrogenase activity using DHEA as substrate. 125X

Fig.7 3β-HSDH activity in the section of the gland obtained from normal rat.

Fig.8 3β-HSDH activity in the section of the gland obtained from castrated rat - 120 hrs postoperatively.

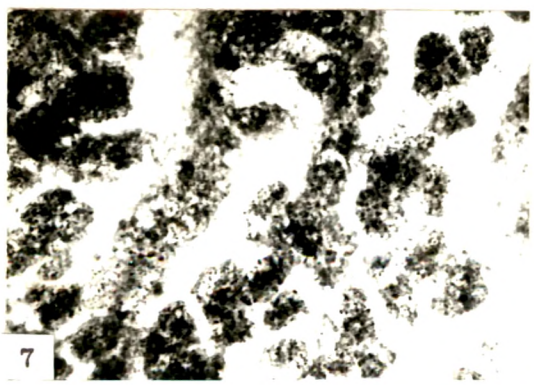
Fig.9 3β-HSDH activity in the section of the gland obtained from adrenalectomized-castrated rat - 120 hrs postoperatively.

Figs.10 to 12 Photomicrographs of the sections of the rat preputial gland showing 3β-hydroxysteroid dehydrogenase activity using pregnenolone as substrate. 125X

Fig.10 3β-HSDH (P) activity in the section of the gland obtained from normal rat.

Fig.11 3β-HSDH (P) activity in the section of the gland obtained from castrated rat - 120 hrs postoperatively.

Fig.12 3β-HSDH (P) activity in the section of the gland obtained from adrenalectomized-castrated rat - 120 hrs postoperatively.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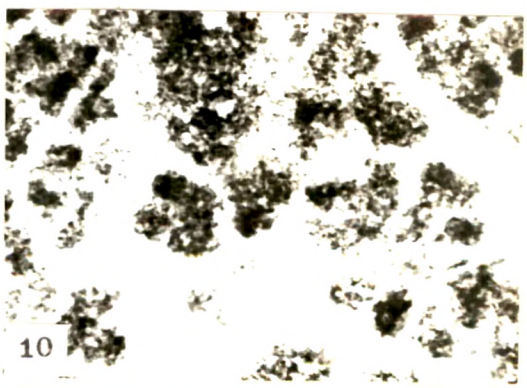
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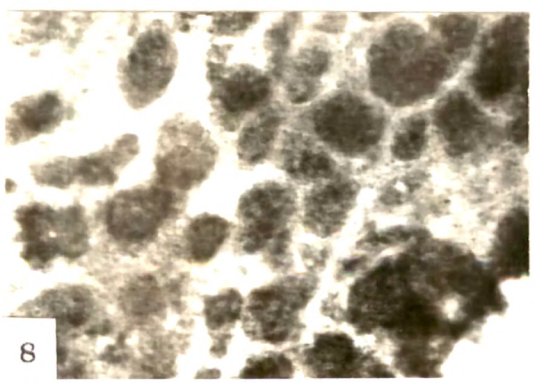
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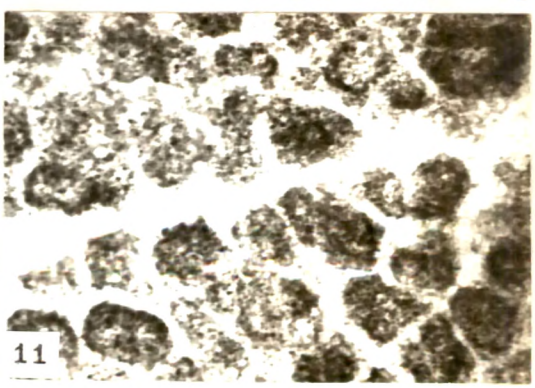
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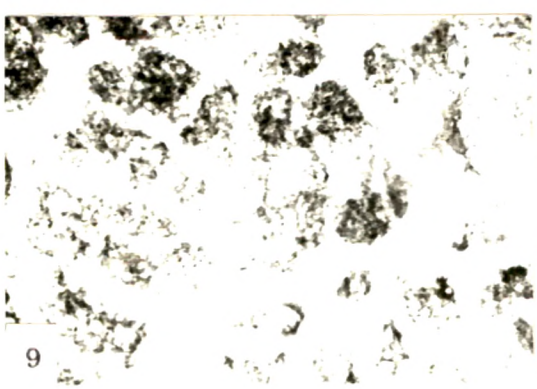
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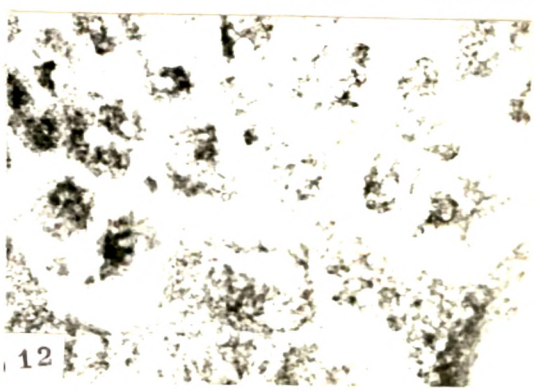
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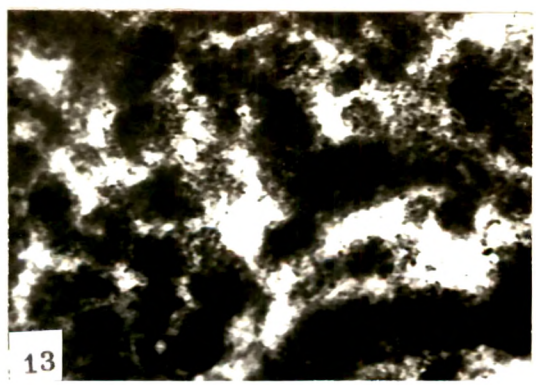
EXPLANATIONS FOR FIGURES

Figs. 13 to 15 Photomicrographs of the sections of rat preputial gland showing 3α-hydroxysteroid dehydrogenase activity. 125X

Fig.13 3α-HSDH activity in the section of the gland obtained from normal rat.

Fig.14 3α-HSDH activity in the section of the gland obtained from castrated rat - 120 hrs postoperatively.

Fig.15 3α-HSDH activity in the section of the gland obtained from adrenalectomized-castrated rat - 120 hrs postoperatively.



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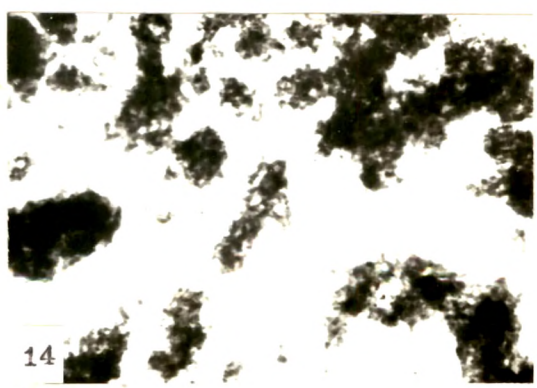

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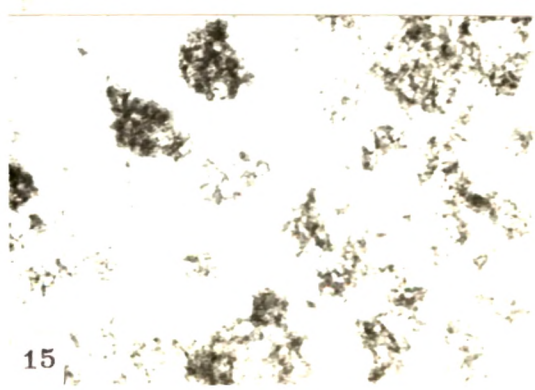

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3β - Hydroxysteroid dehydrogenase (3β HSDH) : When DHEA was employed as the substrate; this enzyme [3β-HSDH (DHEA)] activity was found to be distributed uniformly in the acini of the preputial gland of normal rat (Fig.7) which did not alter after castration (Fig.8), but the intensity of the enzyme reactivity decreased in the glands of the adrenalectomized-castrated rats (Fig.9). The distribution pattern of 3β-HSDH with pregnenolone as the substrate was similar to that of 3β-HSDH (D) (Figs. 10, 11 and 12), but the overall intensity was noticeably lower than that of the preceeding type.

3α - Hydroxysteroid dehydrogenase (3α-HSDH) : Intense 3α-HSDH activity was observed in the preputial glands of normal rats (Fig. 13). Castration seemed not to alter this enzyme activity to any discernible extent. Localization was found to be confined to the central parts of the acini (Fig.14). Nevertheless, adrenalectomy of the castrated rats lead to a reduction in the activity of the enzyme (Fig.15).

Irrespective of distribution pattern the intemsity of reactivities of HSDHs were inorder of 3α-HSDH > 3β

HSDH (DHEA) > 17β-HSDH(T) > 3β-HSDH(P) = 17β-HSDH (E).

DISCUSSION

The preputial glands of male rats exhibited 17β-HSDH activity with testosterone as well as estradiol as substrates and the enzyme activity was higher with testosterone as compared to that with estradiol. This suggests that the 17β-HSDH which is involved in the metabolism of sex steroids of the rat preputial glands, shows greater preference for testosterone than for estrogen. It also ultimately supports the view that the activity of the preputial gland is androgen dependant. The observation that the 17β-HSDH activity was more or less confined towards the periphery of the preputial gland acini, where undifferentiated cells are located, is essentially similar to that reported by Calman (1970) who also observed that the 17β-HSDH was localized in the limited area around the periphery of the sebaceous glands. It has been suggested that the sex steroids increase the mitotic rate of the undifferentiated cells at the periphery of the acini (Ebling

et al., 1957a). Frost et al. (1973), using hamster flank organ, reported that castration affects gland cells present near the periphery of the acini within 48 hours and diminishes cell replication activity. In the light of these findings and the observations reported here on the distribution of 17β-HSDH (T) in the acini of the rat preputial gland it could be suggested that the cells near the periphery of the acini of the gland are predominantly involved in the metabolic responses to the levels of testosterone.

Employing DHEA and pregnenolone as substrates ; 3β-HSDH activity was demonstrated in the preputial gland of rat. In contrast to the distribution patterns of 17β-HSDH, 3β-HSDH depicted almost uniform distribution throughout the acini of the gland. It was observed that 3β-HSDH activity was comparatively higher with DHEA than that obtained with pregnenolone, suggesting that the former is the preferred substrate. It is known that DHEA is rapidly metabolized in the skin also (Faredin et al.,1970). According to Cooper et al. (1976) there is a direct correlation between the rate of lipogenic activity in

acne bearing skin and DHEA metabolism, and the latter in its turn is dependant on the 3β-HSDH activity in the sebaceous glands. Chakraborty et al. (1970) also suggested that 3β-HSDH is an enzyme involved in the condition associated with hyperactivity of the sebaceous glands. Pochi and Strauss (1969) have suggested that androgenic steroid (DHEA) from adrenal can influence the lipogenic activity in the sebaceous gland. Uniform distribution of 3β-HSDH activity observed here and earlier observations (Chapter-1) on lipid metabolism in the preputial glands of the rats indicate that DHEA metabolism of the preputial gland of the rat is closely related to lipogenic activity of the acinar cells.

3α-HSDH was found to be highly active among all the HSDHs studied. The activity of this enzyme was confined more or less to the central region of the acini, where the cells undergo fatty degeneration. Baillie et al. (1966) studied HSDHs in the skin and suggested that 3α-HSDH was involved in degradation and excretion of the steroids. Presence of intense 3α-HSDH

activity in the central part of the acini is clearly indicative of its role in the catabolic reactions preceeding fatty degeneration of the maturing holocrine secretory cells. The cells burst open liberating sebum and the excretory products of steroid hormones in the final steps.

Thus, on the basis of this histochemical study it appears that regional variations in the activities of the HSDHs in the cells of the different regions of the acini are involved in controlling the hormonal micro-environment at different sites in the preputial gland acini; as has been suggested for the skin by Gomez and Hsia (1968).

Observations on the different HSDHs in the preputial glands of the castrated rats gave an evidence that deprivation of potent androgens through castration, leads to marked reduction in the activity of 17β-HSDH 24 hours after castration which remains low throughout the experimental period (i.e. up to 120 hours). It has been suggested that castration results in altered metabolism of androgen (Sansone and Reisner, 1974; Takayasu and

Adachi, 1972). Thus a reduction in the activity of 17β-HSDH in the rat preputial gland could be a change in association with altered androgen metabolism. However, castration did not affect the activities of 3β-HSDH and 3∝-HSDH. This condition presumably is due to the presence of small quantities of androgenic steroids emanating from adrenal cortex which should be metabolized. This contention finds support in the observation reported here that both these enzymes showed considerable reduction in the activities after the removal of adrenals from the castrated rats.