Chapter - 2

ACTIVITIES OF CERTAIN DEHYDROGENASES IN THE PREPUTIAL GLAND OF NORMAL, CASTRATED AND ADRENALECTOMIZED-CASTRATED RATS

Of recent, thepreputial glands of male rats and mice are being increasingly employed as a convenient model in the investigation of physiology of sebaceous glands. Studies carried out on these specialized and enlarged sebaceous glands have clearly shown that steroids, mainly androgens, play an important role in regulation of functions of these glands. Androgen dependency of these glands was suggested mainly on the basis of positive correlation between steroid metabolism within the gland itself and the effects of this group of hormones on the rate of sebum production and size of the gland. Sebum, the secretory material of the gland, is rich in lipids. Lipogenesis, is thus, a prominant feature of the metabolism of preputial glands. The synthetic process, such as this, would naturally demand enough energy as well as the required precursors. While it is fairly well known that androgens influence the rate of lipogenesis in preputial glands, practically little is known about metabolic pathways making provisions for energy demand and

supply of precursors necessary for lipogenesis in normal rat preputial glands.The same is true regarding the influence of androgens on these aspects. Enzymatic and biochemical studies on lipogenesis in guinea pig epidermis and sebaceous glands (Wheatley, 1974) and carbohydrate metabolism in human sebaceous glands (Michael and Hoopes, 1974) explain certain features of metabolic pathways in these tissues. However, it is not clear as to what extent these metabolic pathways are influenced by testicular or adrenal steroids. A study of certain dehydrogenases viz., α-glycerophosphate dehydrogenase (α-GPDH), Lactate dehydrogenase (LDH), β-hydroxybutyrate dehydrogenase (BDH), Malate dehydrogenase (MDH), and Succinate dehydrogenase (SDH), was carried out in the preputial glands of normal intact, castrated and adrenalectomized-castrated rats, with a view to know about the metabolic peculiarities of the glands.

MATERIALS AND METHODS

Adult male albino rats (Haffkine strain) weighing 120-160 gms were used for the present investigation. Animals were divided into three groups : (1) normal males (2) bilaterally castrated rats and (3) bilaterally adrenalectomized-

-castrated rats. 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. Rats were castrated through scrotal incision and adrenalectomized via dorsal approach under light ether anaesthesia. The animals were sacrificed 24, 48 and 120 hours post-operatively.

The preputial glands were obtained by excision immediately after decapitation. The glands were made free of fat and other tissues 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 demonstration of α-GPDH, LDH, MDH, BDH and SDH activities employing the method of Ogata and Mori (1964).

The preputial glands from normal, 24, 48, 120 hours castrated and 120 hours adrenalectomized-castrated rats were homogenized in chilled distilled water and LDH activity was assayed employing the method of Wroblewski and La Due (1955). Protein content of the homogenate was estimated by the biuret method of Layne (1957).

RESULTS

Histochemical Study (Figs. 15, 16) : All the five dehydro-

EXPLANATIONS FOR FIGURES

Figs. 1 to 3 Photomicrographs of sections of rat preputial glands showing LDH activity. 65X

Fig.1 LDH activity in the sections of the preputial gland obtained from normal rat.

Fig.2 LDH activity in the section of the preputial gland obtained from the rat - 120 hrs after castration.

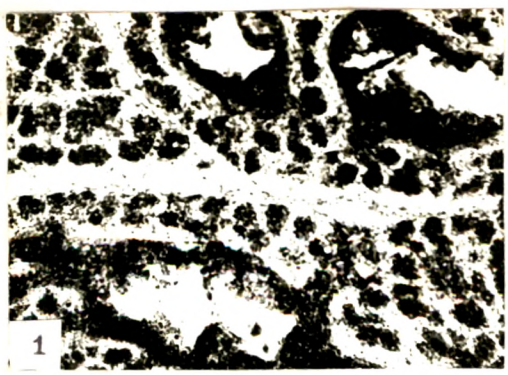
Fig.3 LDH activity in the section of the preputial gland obtained from adrenalectomized-castrated rat - 120 hrs postoperatively.

Figs. 4 to 6 Photomicrographs of the sections of the rat preputial glands showing BDH activity. 65X

Fig.4 BDH activity in the section of the gland obtained from normal rat.

Fig.5 BDH activity in the section of the gland obtained from castrated rat - 120 hrs postoperatively.

Fig.6 BDH activity in the section of the gland obtained from adrenalectomized- castrated rat - 120 hrs postoperatively.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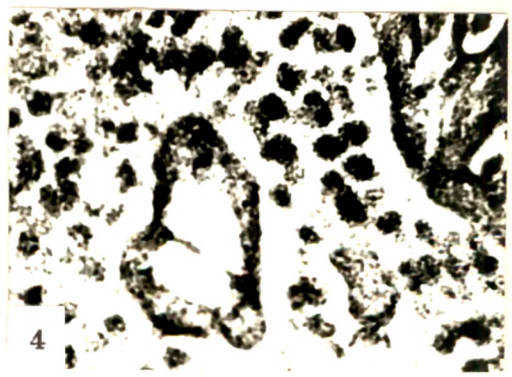
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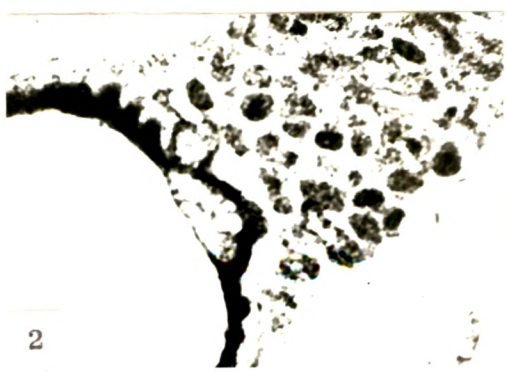
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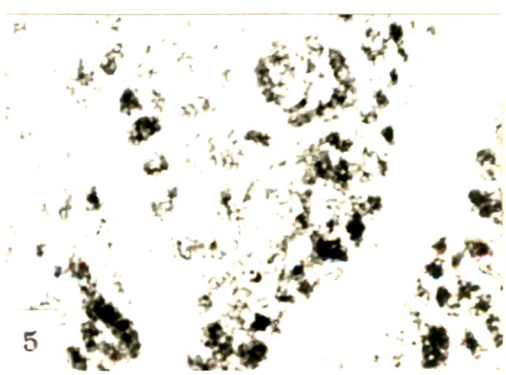
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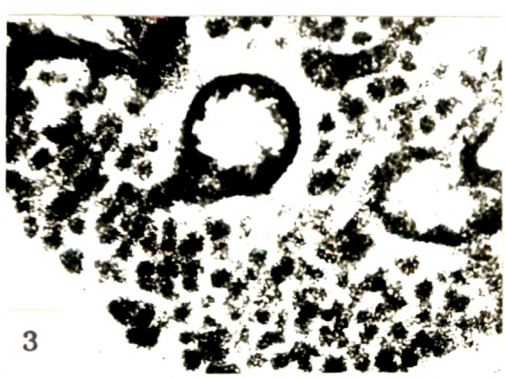
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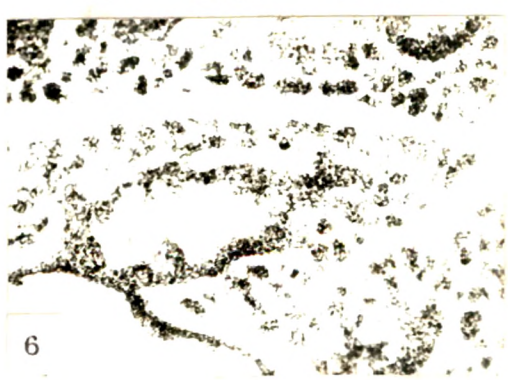
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EXPLANATIONS FOR FIGURES

Figs.7 to 9 Photomicrographs of the sections of rat preputial glands showing MDH activity. 65X

Fig.7 MDH activity in the section of the gland obtained from normal rat.

Fig.8 MDH activity in the section of the gland obtained from the castrated rat 120 hrs postoperatively.

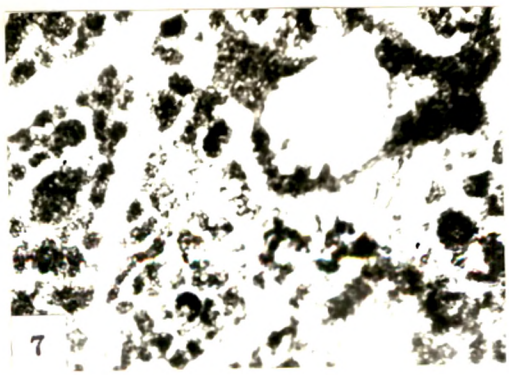
Fig.9 MDH activity in the section of the gland obtained from the adrenalectomized-castrated rat - 120 hrs postoperatively.

Figs.10 to 12 Photomicrographs of the sections of rat preputial glands showing α-GPDH activity. 65X

Fig.10 α-GPDH activity in the section of the gland obtained from normal rat.

Fig.11 α-GPDH activity in the section of the gland obtained from the castrated rat - 120 hrs postoperatively.

Fig.12 α-GPDH activity in the section of the gland obtained from adrenalectomized-castrated rat - 120 hrs postoperatively.



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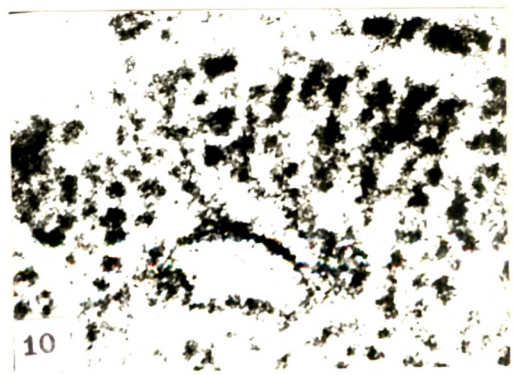

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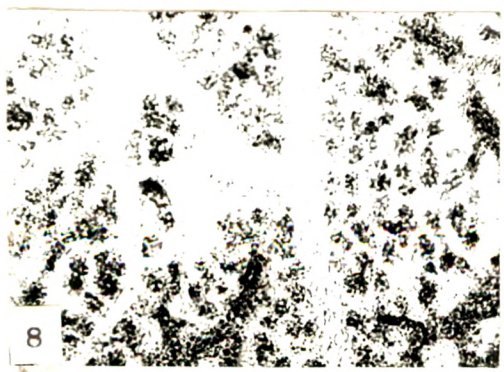

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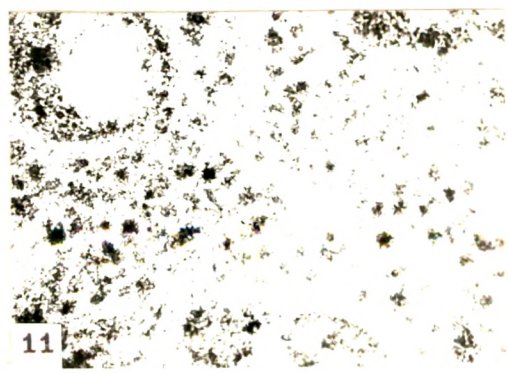

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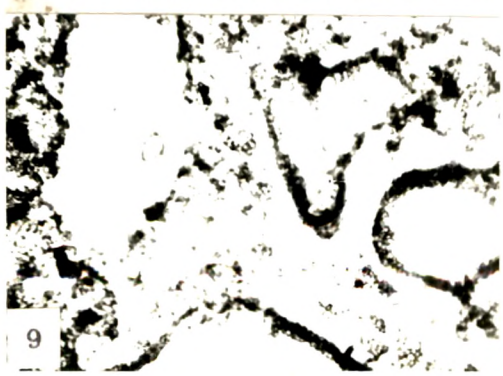

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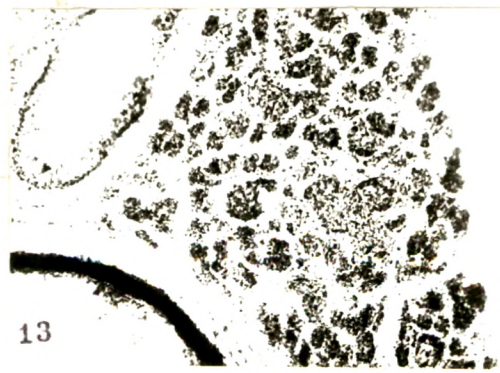
EXPLANATIONS FOR FIGURES

Figs.13 to 15 Photomicrographs of the sections of the rat preputial glands showing histochemical localization of SDH activity. 65X

Fig.13 SDH activity in the section of the gland obtained from normal rat.

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

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



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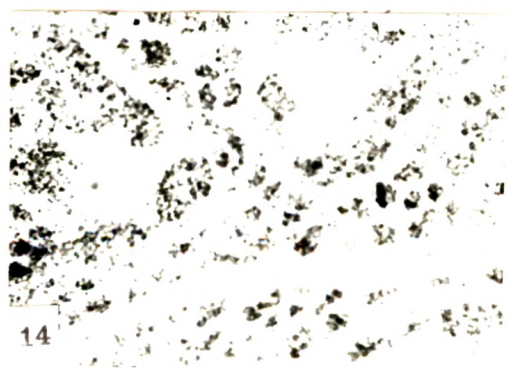

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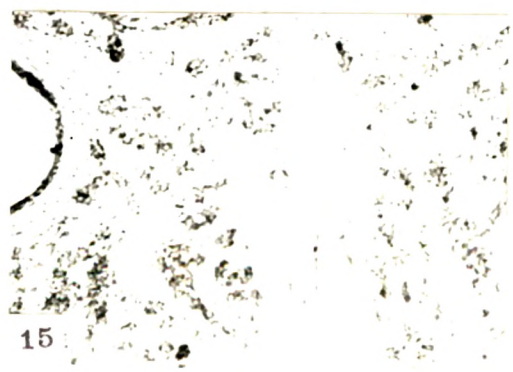

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Table 1

Levels of lactate dehydrogenase in the preputial glands obtained from normal castrated and adrenalectomized-castrated rats. Expressed as mμ NADH oxidized/mg protein/min. Mean value ± S.D.

Experimental Group	LDH activity	Significantly different from the normal at the level
Normal	323.44 ± 20.97	
24 hrs castrated	244.10 ± 36.20	$P < 0.001$
48 hrs castrated	230.50 ± 53.69	$P < 0.005$
120 hrs castrated	210.88 ± 69.42	$P < 0.005$
120 hrs adrenalectomized -castrated	340.83 ± 37.78	NS

NS = Nonsignificant

genases studied i.e., LDH, BDH, MDH, α-GPDH and SDH depicted high reactivity at the periphery and showed decrement towards the centre of the preputial glands of the rats (Figs. 1,4,7,10 and 13). Cells of the duct system of the gland also showed moderate enzyme reactivities. However, SDH activity was found to be higher in the cells of the duct. Castration resulted in a gradual reduction of the activity of the dehydrogenases without any change in the distribution pattern (Figs. 2,5,8,11 and 14). Cells lining the duct system were found to be less affected by castration than the acinar cells. Adrenalectomy of castrated rats caused further reduction in the intensity of all the enzyme activities (Figs. 6,9,12 and 15) except for LDH which showed activity similar to that of normal animals (Fig.3).

Quantitative study : Data presented in Table-1 clearly showed that the activity of LDH in the preputial gland decreased significantly after castration. The values for LDH in the gland of adrenalectomized-castrated rats did not differ significantly from those of normal rats.

DISCUSSION

SDH activity in the preputial gland, though lower than other dehydrogenases studied, is indicative of contribution

of TCA cycle towards energy production. The contention finds support in the work of Montagna and Noback (1947) who have reported high cytochrome oxidase activity in these glands. SDH activity has also been demonstrated in sebaceous glands of human skin (Montagna, 1963) as well as in the uropygeal gland of birds (Bhattacharya and Ghosh, 1971). Michael and Hoopes (1974) have studied carbon flow in sebaceous gland and have shown a high turnover of TCA cycle. Very high LDH activity observed in the preputial gland is an indication of anaerobic energy production through glycolytic pathway in this gland. Among the dehydrogenases studied, the activity of LDH was found to be the highest and it is worth noting that tissues exhibiting a high mitotic index derive energy mainly through anaerobic glycolysis. Richard (1971) has shown that in epidermal slices most of the glucose is converted to lactate. Mier (1969), on the basis of enzymatic study, also suggested that anaerobic glycolysis contributes towards energy production in the skin. However, very high LDH activity in the preputial gland could not be accounted totally for lactate production. It is uneconomical from the point of view of energetics that the activity of this enzyme should lead to production of lactate as the end product of glycolysis, since it would thereby mean wasting metabolic

availability of pyruvate; which could otherwise be utilized for fatty acid synthesis. Since lipid synthesis in the preputial gland occurs at a higher rate and since there is also high LDH activity, a reaction in the direction of pyruvate formation from lactate is very likely to be operative at a higher rate. This may indicate that lactate being utilized in such a manner comes, to a greater extent, from circulation rather than as the end product of glycolytic pathway. Such a statement is based on the observation that a major part of glucose appears to be metabolized through (1) HMP shunt at Glucose-6-phosphate level as revealed by very high G-6-PDH activity (Chapter-1). Such alternative pathway would generate a major part of reductive hydrogen ($NADPH_2$) required for lipogenesis and (2) (at the 3-carbon level) triose sugar is apparently metabolized to some extent to glycerophosphate for glycerogenesis through the reaction catalyzed by α-glycerophosphate dehydrogenase, the activity of which was found to be high in the preputial gland of rat (Fig.10). Freinkel (1960) also figured that major source of carbon atom, which finally appears as CO_2 (via Krebs cycle), is not glucose. It has also been suggested that apart from glucose, lactate can sustain a rapid rate of lipogenesis; comparable with that obtained with glucose (Wheatley, 1974). Moreover, utilization of lactate would generate cytosolic

reductive hydrogen in the form of $NADH_2$, $NADH_2$ in turn can drive glycerogenesis and can also support the malate cycle thereby generating $NADPH_2$.

Thus, glycolysis could contribute pyruvate as a major metabolite rather than lactate. However, Freinkel (1960), Halprin and Ohkawara (1966) and Wheatley (1974) on the basis of their in vitro studies have reported that the chief end product of glucose metabolism in skin is lactate. But, the conditions in vivo may be different. Also, there is a possibility that metabolic features of skin and preputial glands may differ in their metabolic pattern and before arriving at a valid conclusion further investigation to evaluate this aspect is necessary.

Presently observed high MDH and 'Malic' enzyme (Chapter-1) activities are also suggestive of higher turn-over of intermediates through malate cycle.

Activity of α-GPDH was found to be high in the acinar cells of the gland of normal male rats. The α-GPDH catalyzes the interconversion of glycerophosphate and dihydroxyacetone phosphate. Glycerophosphate produced by this reaction can serve as an important precursor for the synthesis of lipids (Kornberg and Pricer, 1952; Kennedy, 1953).

The stimulatory effect of glycerophosphate on fatty acid esterification was reported by Tzur et al. (1964). Howard and Lowenstein (1965) have shown the importance of glycerophosphate in the synthesis of glycerides and phospholipids. It has been suggested by Adachi and Yamasawa (1967) that in sebaceous gland this enzyme may participate in glycerophosphate synthesis as well as in the anaerobic glycolytic pathway. As this enzyme was found to be high in the cells of the normal preputial gland, it could be suggested that the α-GPDH might be aiding the synthesis of neutral fats and phospholipids.

BDH activity being apparently low, in the given order of intensity of enzyme activity (LDH > BDH > MDH = α-GPDH > SDH), indicated its possible role in the channelization of available free fatty acid via acetyl CoA and TCA cycle for energy production rather than utilization for lipogenesis, which would be a wastage as suggested by Wheatley (1974).

Thus, presently observed differential magnitudes of activities of dehydrogenases in the preputial glands of rat are suggestive of possible alternative pathways leading to production of intermediates and cofactors required for synthetic activity of cells.

Another feature, that was noticed in the present investigation, is that the cells of the duct compared to the acinar cells showed relatively less activity of enzymes studied, except that of SDH, which was found to be higher in the cells of the duct. Among the acinar cell, high reactivity for dehydrogenases was observed in the peripheral cells and differentiating cells indicating significant involvement of TCA cycle in the metabolic processes of the gland during active cell proliferation and differentiation. Similar type of distribution of SDH has been reported by Montagna (1963) in human sebaceous glands.

The preputial glands of castrated rats revealed a gradual decrease in the intensities of all the enzymic reactions studied, thereby clearly indicating that depletion of androgens, through castration, adversely affected the energy yielding as well as synthetic machinery of preputial glands of white rats. It could be suggested that the effect of androgens on lipid synthesis in preputial gland is possibly mediated through their effect on the HMP shunt and 'malic' enzyme as suggested in Chapter-1, as well as through the effect of hormones on energy yielding mechanism which is evident from presently observed reduced activities of dehydrogenases following castration. Takayasu and Adachi

(1970) have also reported that castration of male hamster resulted in reduction of rate of glycolysis to one half in its costovertebral glands. It was observed that the activity of these enzymes, except that of LDH, at the end of 120 hrs in the preputial gland of castrated rats were significantly higher than those of adrenalectomized-castrated rats. It could be inferred from these observations that adrenal steroids, to some extent, are implicated in preputial gland physiology through their influence on the metabolism in the gland. Exceptionally, LDH activity showed an unusual pattern by exhibiting a significant increase in the gland of adrenalectomized-castrated rat, which is difficult to explain at this stage.