Chapter - 1

EFFECTS OF CASTRATION AND ADRENALECTOMY ON LIPID METABOLISM OF THE PREPUTIAL GLANDS OF MALE RATS

The preputial gland of rat is a modified sebaceous structure, which is active in secreting lipids (Beaver, 1963). Lipids of preputial glands of male mice have been well characterized by Snyder and Blank (1969), who have shown that the major lipid constituents are wax esters, alkyl glycerols amd triglycerides, while phosphotidyl ethanolamine and phosphatidyl choline are found only in traces. Effects of various steroid hormones on the preputial and/or sebaceous glands have been studied by a number of workers (Yip and Freinkel, 1964; Burgess and Wilson, 1963; Ebling et al., 1969, 1970, 1971).

Attempts have also been made to study lipogenesis in the preputial glands. Patterson (1960) has studied biosynthesis of squalene by rat preputial gland from C^{14}-acetate. Burgess and Wilson (1963) have shown that squalene synthesis in the preputial gland and skim is enhanced by administration of testosterone. Sansone et al. (1971) have observed increased

lipogenesis from glucose in mouse preputial gland after stimulation by testosterone.

In the present investigation an attempt has been made to study the effects of androgen deprivation on the total lipid content as well as on the two concerned enzymes - Glucose-6-phosphate dehydrogenase (G-6-PDH) and 'malic' enzyme - involved in lipogenesis in the preputial gland of male rats.

MATERIALS AND METHODS

Adult male albino rats (Haffkine strain) weighing 120-140 gms were used for the present investigation. Rats were divided into four groups : (1) intact males (2) bilaterally castrated rats (3) bilaterally adrenalectomized-castrated rats (4) testosterone propionate treated animals. Rats were castrated through scrotal incision; and adrenalectomized via dorsal approach under light ether anesthesia. 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. Some of the 120 hours' castrated animals were treated with intramuscular injection of 0.1 mg of testosterone propionate dissolved in 0.5 ml of tributyrine and were sacrificed 24 hours after the injection. Castrated and adrenalectomized-castrated

animals were sacrificed 24, 48 and 120 hours postoperatively.

The preputial glands were obtained by excision immediately after decapitation. The glands were dissected free of fat and other tissues. Total lipid content of the gland was estimated by extracting the lipids with chloroform : methanol (2:1 v/v) mixture according to the method of Folch et al. (1957). For the assessment of activities of G-6-PDH and 'malic' enzymes the tissue was homogenized in 0.15 M KCl. The tissue homogenate was centrifuged at 20,000 g in a refrigerated centrifuge and enzyme activities were assayed in the supernatant collected. G-6-PDH (E.C. 1.1.1.49) was assayed employing the method of Kornberg and Horecker (1955) with modifications as described by Marks (1966). NADP-malate dehydrogenase (E.C. 1.1.1.40) was assayed as described by Hsu and Lardy (1969). Protein content of the supernatant was estimated by the biuret method of Layne (1957).

RESULTS

TOTAL LIPIDS : Table-1.

Total lipid content of the gland was very high (27.01%) which showed decrease 120 hrs. after castration (24.96%) and was significantly reduced further in adrenalectomized-castrated animals (18.09%). Testosterone propionate treatment

Table 1

Total lipid content of the preputial gland of normal, castrated and adrenalectomized-castrated rats. Mean ± S.D.

Experimental Group	Total lipid content mg/100 mg wet weight	Significantly different from the normal at the level P
Normal	27.01 ± 2.59	
24 hrs castrated	22.00 ± 1.95	< 0.05
48 hrs castrated	20.56 ± 2.65	< 0.05
120 hrs castrated	24.96 ± 3.22	< 0.05
24 hrs adrenalectomized -castrated	20.07 ± 2.57	< 0.05
48 hrs adrenalectomized -castrated	21.05 ± 1.50	< 0.05
120 hrs adrenalectomized -castrated	18.09 ± 2.85*	< 0.005
Testosterone propionated injected	25.10 ± 1.21	< 0.05

* Significantly different from the 120 hours castrated rats at the level $P < 0.005$

Table 2

Levels of Glucose-6-phosphate dehydrogenase and 'malic' enzyme activities in the preputial glands from normal, castrated, adrenalectomized-castrated and testosterone propionate injected rats.
Expressed as mμ of $NADPH_2$ formed/mg protein/min. Mean value ± SD.

Experimental group	G-6-PDH	'malic' enzyme
Normal	127.07 ± 4.03	26.56 ± 6.07
24 hrs castrated	81.43 ± 9.15*	24.23 ± 8.66
48 hrs castrated	74.22 ± 9.24*	20.18 ± 3.76@
120 hrs castrated	64.95 ± 7.88*	14.28 ± 5.17@@
24 hrs adrenalectomized -castrated	87.88 ± 7.17*	20.95 ± 7.78
48 hrs adrenalectomized -castrated	71.65 ± 9.27*	19.98 ± 3.21@
120 hrs adrenalectomized -castrated	50.63 ± 7.18**	15.32 ± 5.72@@
Testosterone propionate treated rats	111.27 ± 15.36***	25.30 ± 2.74@@@

* Significantly different from the normal at the level $P<0.0005$

** Significantly different from the normal at the level $P<0.0005$ and also from 120 hrs castrated group at the level $P<0.05$

*** Significantly different from 120 hrs castrated group at the level $P<0.0005$

@ Significantly different from the normal at the level $P<0.05$

@@ Significantly different from the normal at the level $P<0.01$

@@@ Significantly different from the 120 hrs castrated group at the level $P<0.001$

did not alter the level of total lipids in the preputial glands of castrated rats.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE :

From Table-2 it becomes evident that the preputial glands from normal males exhibit quite high G-6-PDH activity which is of the value of 127.07 and it decreased significantly due to castration to a level of 64.95 (120 hrs postoperatively) and in the glands from adrenalectomized-castrated animals the enzyme dropped further to 50.63. Testosterone propionate injection to the castrated animals raised it upto 111.27.

MALIC ENZYME :

Comparatively, the malic enzyme activity was less than that of G-6-PDH in this gland (26.56).This was observed to be significantly lowered due to castration (14.28). The glands from adrenalectomized-castrated rats (15.32) failed to exhibit any additional significant change in the activity of this enzyme as compared to castrated rats. Like G-6-PDH, 'malic' enzyme activity also was found to increase in the preputial gland after testosterone propionate administration to the castrated rats.

No significant differences were observed in the total lipid content, G-6-PDH and 'malic' enzyme activities of the

preputial glands between the animals of castrated group and the adrenalectomized-castrated group; sacrificed at the intervals of 24 and 48 hours postoperatively. However, 120 hours postoperatively total lipid content and the G-6-PDH activity in the preputial gland of adrenalectomized-castrated rat were significantly lower than that in the gland of castrated rat.

DISCUSSION

Results obtained indicated that preputial glands of male rats are rich in lipids and that total lipid content of the gland decreased as early as 24 hours after castration; it reduced further when adrenalectomy was performed along with castration suggesting that, both testicular and adrenal steroids are involved in the regulation of lipid content of the rat preputial gland. Similarly, there are reports which indicate that sebum production levels are considerably lower in castrated men than in intact men (Emanuel, 1936; Pochi et al., 1962). Thody and Shuster (1971) have shown that removal of adrenal glands in the rat produces a decrease in sebum secretion, and Pochi et al. (1963) have reported similar fimdings in man.

Considerably high activity of G-6-PDH in these glands is indicative of an active participation of hexose mono-

phosphate shunt in the metabolism of rat preputial gland. G-6-PDH catalyzes the oxidation of G-6-P providing pentose phosphatases and reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$). Through the Embden - Meyerhof pathway two molecules of NADH are formed per mole of glucose, which is sufficient for slow or moderate fatty acid synthesis but cannot sustain a high rate of lipogenesis. To attain higher rate of fatty acid synthesis additional amounts of non-mitochondrial reduced nicotinamide dinucleotide phosphate are required and this can be provided through HMP shunt (Katz and Rognstad, 1966). Layne (1960) proposed that synthesis of fatty acids is regulated by the levels of available $NADPH_2$, generation of which depends on the levels of G-6-P added. McKerns (1967) has also emphasized the importance of HMP shunt in the production of $NADPH_2$. The study on malate dehydrogenase (Chapter 2) and 'malic' enzyme reported here reveal that malate cycle is also active in the rat preputial gland. It becomes evident from the present study that; of the two $NADPH_2$ generating systems in the preputial gland of rat i.e. HMP shunt and malate cycle, the former contributes more to the supply of cytoplasmic $NADPH_2$. Michael and Hoopes (1974) have also reported the active participation of pentose shunt in sebaceous glands of human skin. In this respect both the sebaceous glands and the preputial gland

differ from epidermis, wherein malate cycle is suggested to be a major source of cytoplasmic $NADPH_2$ (Wheatley, et al., 1973). Nevertheless, preponderance of HMP shunt over the malate cycle in the preputial gland is more plausible, as that can provide pentose sugars essential for nucleic acid synthesis which may compliment the gland's high mitotic index. Function of 'malic' enzyme is to provide additional amount of $NADPH_2$ for reductive synthesis. Snyder and Malone (1970) have suggested that $NADPH_2$ is an effective source of hydrogen ion for lipogenesis in the mouse preputial tumors.

After castration, there was a drop in activities of Glucose-6-Phosphate dehydrogenase and 'malic' enzyme and the former was found to be reduced further at 120 hrs. interval in adrenalectomized-castrated rats. Such a decrease in the activities of these enzyme would result in reduced supply of $NADPH_2$. This attenuated supply of $NADPH_2$ may contribute to reduction of total lipid in the gland after experimental alterations. Again it becomes obvious under these experimental conditions, that as compared to 'malic' enzyme, HMP shunt is involved to a greater extent in the physiology of the rat preputial gland. Hershey (1959) has also suggested that androgenic steroids act on enzyme systems which provide $NADPH_2$. Takayasu and Adachi (1972) have shown that endogenous levels

of $NADPH_2$ in the sebaceous gland are reduced to half after castration which supports the present observations.

Effects of testosterone administration on lipid content (Freinkel, 1963) and rate of sebum secretion (Archibald and Shuster, 1967; Ebling and Skinner, 1967; Ebling, 1974) have been well documented in the literature. Moreover, Sansone et al. (1971) have shown that glucose oxidation via HMP shunt in the mouse preputial gland is stimulated by testosterone propionate injection. Increased G-6-PDH and 'malic' enzyme activities after testosterone propionate injection observed herein, suggest that sebum production following repeated testosterone injection reported by above mentioned authors could be mediated through imduction of G-6-PDH and 'malic' enzyme. However, in the present investigation it is shown that though the activities of enzymes increased, the lipid level did not show any change following the androgen administration. On the basis of these observations it could be suggested that a single injection of the given dose of testosterone is not sufficient to cause any change in lipid content of this gland.

Comparatively low values of lipid and G-6-PDH in preputial glands from adrenalectomized-castrated rats than that in glands from castrated rats give some indication

about the role of adrenal gland in the physiology of rat preputial gland, and lower level of the enzyme in the gland of adrenalectomized-castrated rat may be due to lack of androgenic stimulation from the adrenal cortex. There is an evidence that gonadectomy results in increased adrenal steroid production (Howard and Kitay, 1972). Moreover, Pochi et al. (1963); Pochi and Strauss (1969) and Thody and Shuster (1971) have shown that adrenal steroids play an important role in regulation of sebum secretion.

From this study it could be concluded that the lipid synthesis in the preputial gland of rat is under the control of androgenic steroids, both of gonadal and adrenal origin. The hormonal action, in this case, might be mediated through the regulation of enzymes like Glucose-6-phosphate dehydrogenase and 'malic' enzyme affecting overall synthesis of lipids in the gland.