

CHAPTER - I

OBSERVATIONS ON EFFECTS OF CASTRATION AND REPLACEMENT WITH TESTOSTERONE PROPIONATE ON LIPID, CHOLESTEROL AND ASCORBIC ACID CONTENTS OF THE SUBMANDIBULAR SALIVARY GLANDS OF MALE RATS

As early as 1940, Lacassagne described sexual dimorphism in the salivary glands of mice and rats and also demonstrated that sex hormones could produce morphological changes in the submandibular salivary gland. Studies by Berkman and Kronman (1970) revealed that testosterone is important in preserving structural integrity of the submandibular gland, and that the sublingual and parotid glands are not sensitive to alterations in available levels of testosterone. They showed that castration of male mice caused reduction of size, diameter and number of granular tubules of the submandibular gland, which was reestablished by testosterone administration. These observations are in conformity with the findings of earlier workers (Lacassagne, 1940; ChaulinServiniere, 1942a & b; Raynaud, 1950; Shafer and Muhler, 1953; Cassano, 1958).

Several reports are available on assessment of various steroid hormones/metabolites in saliva and their close correspondence with respective unbound levels in plasma as means of noninvasive indicators of the gonadal functional states (Seaton and Fahmy, 1979; Smith et al., 1979; Bexendle et al., 1980; Sannikka et al., 1983; Read et al., 1984).

Booth (1972) has shown the occurrence of testosterone and 5 α -dihydrotestosterone in the submaxillary salivary gland of the boar. In 1970, Weiner et al., has further reported that infusion of testosterone 4 - ^{14}C for 10 min into the blood supply of submaxillary gland of male dog and its recovery after 10 min in the submaxillary gland in the form of androstenedione is indicative of the metabolism of testosterone in the submaxillary gland. Katkov et al. (1972) and Booth (1977) have studied the metabolism of androgens in boar submaxillary glands under in vitro condition. Metabolic handling of cortisol by salivary glands of various mammals has also been histochemically demonstrated as evidenced by the presence of 11- β -hydroxysteroid dehydrogenase activity (Ferguson et al., 1970). These observations point to the capacity of mammalian submandibular glands to actively metabolize steroid hormones, apart from the capacity to excrete steroid molecules per se in the mixed saliva.

The present chapter is intended to deal with variations of the contents of total lipids, total cholesterol and total ascorbic acid (AA) of the submandibular glands of male albino rats as influenced by castration and subsequent replacement with testosterone propionate (TP). This has been done with a view to understand in a broad way the influence of androgenic steroids on overall metabolic patterns of submandibular glands.

There have been numerous reports in the literature concerning castration and its effect on accessory sex organs and other peripheral organs. Changes in the lipid profiles of accessory sex organs

after administration of cyproterone, cyproterone acetate and testosterone to adult castrate male rats have been reported (Umapathy and Rai, 1980), wherein it was shown that in caput epididymis, free and esterified cholesterol increased under the influence of antiandrogens and decreased with TP treatment. Further, it was observed by Umapathy and Rai (1980) that antiandrogenic treatment decreased esterified cholesterol content of prostate and increased with TP treatment. Another interesting observation of Patsch et al. (1980) pertains to patterns of general lipid metabolism in rats as influenced by sex steroids and reflected in the apoprotein composition of plasma lipoprotein fractions. It has been shown that the apoproteins of HDL, LDL and VLDL plasma fractions were higher in females and estradiol treated castrated males than in males or TP treated spayed females. However, the plasma levels of triglycerides, cholesterol, few other apoproteins and lipoproteins with lower densities remained unaltered in all the experimental groups (Patsch et al., 1980). According to Lars-Ake et al. (1980) administration of dehydroepiandrosterone enanthate for 30 days led to a depletion in plasma HDL, VLDL, cholesterol ester, free cholesterol, total cholesterol and phospholipids.

Administration of ethynyl estradiol to adult female rats was reported to decrease lipase activity in the liver and to induce increase in blood triglyceride levels (Valette et al., 1978). These authors showed that progesterone alone did not modify blood triglyceride levels but in combination with ethynyl estradiol decreased lipase activity of liver and adipose tissue. Jose and Coimbra (1981)

reported that lipid droplets in male preputial gland were significantly higher than that in female, and androgen administration increased the diameter of lipid droplets while estrogen treatment decreased the same. According to Minetti et al. (1985) murine submandibular glands are influenced by testosterone and its metabolites, and that, replacement with testosterone, TP and other related steroids are able to restore to near control values the glandular weight gain and total protein content. It is known that androgens regulate general patterns of lipid metabolism of mammals (Kritchevsky et al., 1963; Doeg, 1968; Raheja et al., 1971; Ambadkar and Gangaramani, 1976). The present study was carried out to understand the effects of short-term castration and subsequent replacement therapy on the alterations in the content of total lipids and cholesterol in the submandibular glands of male albino rats.

Realization of significance of the metabolic turnover of Ascorbic acid in reproductive and non-reproductive tissues has become apparent on the basis of the repeatedly observed fact that higher content of ascorbic acid in the tissues was almost always associated with greater metabolic activity (Chinoy, 1969, 1970, 1971 & 1973; Chinoy et al., 1974). Deficiency of this vitamin is known to disturb carbohydrate, lipid and iron metabolism (Rusch Kline, 1941; Banerjee and Ghosh, 1947; Mazur et al. 1961). A general relationship between the metabolism of ascorbic acid and the circulating level of testosterone has been suggested (Dieter, 1969; Majmudar and Chatterjee, 1974; Chinoy and Parmar, 1975; Chinoy et al., 1975). Studies by Stubbs et al. (1967) on influence of hypophysectomy and castration have led them to opine that the level of testicular secretion

have led them to opine that the level of testicular secretion significantly influences the maintenance of ascorbic acid levels in the liver of male rats. Decrease in ascorbic acid content of liver, urine and blood of male rats, six weeks after castration, has been reported by Khandwekar et al. (1973). In the light of the literature cited above on varied roles of ascorbic acid in several physiological processes, it was thought desirable to study variations in the AA content of submandibular glands of male rats under the experimental conditions employed in the present study.

M A T E R I A L A N D M E T H O D S

Adult male albino rats (Rattus norvegicus albinus) weighing 120 ± 20 g served as experimental animals. The rats were maintained on a balanced diet and water ad libitum. The animals were divided into two batches:

i) Normal batch of untreated animals, which served as controls.

Experimental batch of animals was subdivided into following two groups:

a) Consisting of male rats, which were bilaterally castrated under mild ether anaesthesia, 48 hr prior to quantitative estimations.

b) Comprising of 48 hr castrates replaced with 100 μ g of testosterone propionate (TP) intramuscularly. A single injection in 0.5 ml tributyrin (vehicle) was administered to each of the animals. The TP administered animals were sacrificed for assays at very short intervals of 1, 2 and 4 hours post administration.

At these selected intervals the rats were weighed and then sacrificed by decapitation. Both submandibular glands were quickly removed, freed of connective tissue and weighed on an electronic balance. One of the glands was utilized for the estimation of total lipids and total cholesterol while total AA was assayed from the other gland. For the estimation of total lipids, weighed gland was ground thoroughly with sand and chloroform-methanol mixture (2:1) for extraction as per the method of Folch *et al.* (1957). Total cholesterol content was determined employing ferric-chloride reaction (Crawford, 1958). The other gland was homogenized in 6% trichloroacetic acid in pre-chilled mortars. Aliquots of these extracts were utilized for the determination of total AA by the method of Roe (1954) using dinitrophenyl hydrazine. The total lipid and total cholesterol contents were calculated in terms of gram percentage of fresh tissue weight. Total AA was calculated in terms of mg percentage of the fresh tissue weight of submandibular gland. Data were analysed using Student's 't' test.

R E S U L T S

The present investigation was an attempt to know the possible relation between the sex hormones and the patterns of metabolism of submandibular gland with respect to lipids, cholesterol and AA. Castrated animals.

A significant decrease was observed in the total lipid content of the submandibular gland 48 hr after castration. Value of total cholesterol was seen to increase significantly after

48 hr of gonadectomy (Table - 1). Concentration of AA in the submandibular gland was also affected by deprivation of androgens; a significant high level was noted.

Castrated and hormone treated animals.

The results (Table - 1) showed an immediate rise in the total lipid content of the submandibular gland within an hr of 100 ug TP administration. Thus, replacement therapy was found to restore lipid content of normal value. By 2 hr of replacement, the total lipid content of submandibular gland was found to be slightly lowered, nevertheless, it remained noticeably higher than the normal value.

Replacement therapy was found to be able to reestablish the cholesterol content of the gland to near normal values within an hour and upto 2 hr remained close to that level. However, these values were comparatively higher than those of untreated rats. Levels registered at 4 hr interval were noticeably higher.

Administration of TP to 48 hr castrates was found to lower the AA content of the submandibular gland within 1 hr as compared to that of castrate level, but these values were significantly ($P < 0.02$) high when compared to normal values. With lapse of time upto 2 hr; it was seen that there was an increase in the AA content, almost corresponding to that of castrate level. By 4 hr the AA content of the gland was slightly reduced when compared to 2 hr interval, though the value was significantly above the normal AA content.

Table 1

Showing the influence of castration and replacement with TP
to male rats on various biochemical parameters

	Experimental regimes				
	Normal	Castrates	48 hr castrates injected with 100 µg TP (i.m.)		
			Post injection intervals 1 hr	2 hr	4 hr
ASCORBIC ACID mg/100 mg tissue	0.022 ±0.001	0.036*** ±0.002	0.028* ±0.002	0.039* ±0.002	0.032** ±0.003
TOTAL LIPIDS g/100 g tissue	4.491 ±0.211	2.847*** ±0.147	4.436 ±0.171	7.7096*** ±0.231	6.093** ±0.402
CHOLESTEROL g/100 g tissue	0.295 ±0.03	0.462*** ±0.015	0.341 ±0.011	0.354 ±0.018	0.439** ±0.033

Values are mean ± SE (not less than 8 animals)
*** - $P < 0.001$; ** - $P < 0.01$; * $P < 0.02$

D I S C U S S I O N

Adequate information regarding the influence of sex hormones on patterns of metabolism in submandibular gland is not available. The present study was, therefore, undertaken to get some idea about this aspect in case of androgen-sensitive submandibular salivary gland of male albino rats subjected to castration and subsequent replacement with TP. Influence of androgen deprivation and its replacement on glandular contents of lipids, cholesterol and AA are discussed.

Overall general significance of lipid metabolites and their metabolism on their functional states of various tissues has been reported (George and Jyoti, 1957; Fredrikson and Gordon, 1958; Ambadkar, 1969; Ambadkar and Gangaramani, 1976; Valette et al., 1978; Patsch et al., 1980; Schuler et al., 1981). De Smet (1953) has shown that castration of male rats led to an increase in the fat content under the skin and around the kidneys but to a decrease in abdominal fat. Watkins et al. (1972), on the basis of their studies on rats, concluded that the hepatic output of triglycerides may be regulated by gonadal or gonadotrophic hormones. Acetyl CoA carboxylase, one of the rate limiting enzymes, is responsible for the synthesis of fatty acids in the extramitochondrial system in liver, kidney, brain, lung and mammary gland. Long chain acyl CoA moiety competitively inhibits acyl CoA carboxylase activity by forming an acyl-S-enzyme complex. So, if acyl CoA moieties get esterified at a correspondingly faster rate, then it would lead to enhancement of lipogenesis (Harper, 1988).

The evident reduction of total lipid content of submandibular gland 48 hr after castration could have possibly led to either reduced lipogenesis or increased lipolysis by slowing the rate of esterification of fatty acids or enhancing lipase activity.

Replacement therapy was conducted to see whether the effects observed with castration could be restored. Many workers have studied the replacement of the hormones in castrates to explain the effects of hormones (Thapliyal et al., 1975; Moore et al., 1977; Patsch et al., 1980; Umapathy and Rai, 1980). TP (100 µg) was found to reestablish the total lipid value to that of normal after 1 hr of its administration and by 2 hr further increase could be seen however, after 4 hr a decreasing trend was evident. This would imply that a single dose of 100 µg of TP reverses the castration effect for initial 2 hr or so but by 4 hr returns towards normal level of lipid content. It could be suggested that continuous monitoring of testicular androgen levels might be necessary for proper maintenance of normal lipid levels in submandibular gland.

Thus, from the present study, it could be said that total glandular lipid content decreased due to deprivation of sex hormones. Such depletion could be due to reduction in some of the lipid components like phospholipids or neutral lipids as the cholesterol content was noticed to be higher after castration. This conclusion finds support in the work of Umapathy et al. (1979) wherein increase in total lipids primarily due to elevation of neutral and phospholipid components was reported in the case of caput epididymis of male rats after administration of testosterone.

Metabolism of cholesterol has received great attention due to its role in steroidogenesis, nutritional value and endocrine functions (Kritchevsky, 1958; Goodman, 1963; Nelson, 1960; Pageaux, 1980; Sultan, 1980; Umapathy and Rai, 1980). It has been shown that HMG-CoA reductase is the key enzyme in cholesterol biosynthesis (Horton et al., 1970; Bortz and Steele, 1973; Bortz et al., 1973; Gregory and Booth, 1975). It is also known that this enzyme is influenced by hormones (Kurt et al., 1981) like hydrocortisone, which stimulates the enzyme activity in cultured skin fibroblasts.

Extract of Malvaviscus konzatti flower, which has an anti-fertility effect, has been shown to increase cholesterol content in the testes, epididymis, vas deferens and seminal vesicles of albino mice (Verma et al., 1980). Increase in the plasma free cholesterol concentration was reported by Barnett et al. (1981) after daily dose of ethynyl estradiol for three weeks. Recently Haug et al. (1986) have reported that orchidectomy in rats led to hypercholesterolemia accompanied by hypotriglycerolemia, as was also observed in the present study. Results obtained here reveal that lack of androgens might possibly lead to enhanced HMG-CoA reductase activity as manifested in higher levels of cholesterol in submandibular gland. This finds support in the present study itself where castrates replaced with TP were found to exhibit a restoring response in case of cholesterol content of the gland. This influence of TP was, however, seemingly washed off by about 4 hr later and evidently the glandular cholesterol content registered a higher level comparable to that of 48 hr castrates. This would logically mean

that the administered single TP dose of 100 μ g was not enough to exert its effect in maintaining normal physiological level of the cholesterol in the gland for longer than a couple of hours. It appeared that the mechanism of maintaining normal levels is similar to that expressed in the case of lipids.

It has been demonstrated that the metabolism of AA is intimately associated with the circulating levels of androgens (Dieter, 1969; Majmudar and Chatterjee, 1974; Chinoy and Seethalakshmi, 1978). Androgen deprivation (castration) has been observed to reduce concentration of ascorbate and its turnover in both liver and adrenal of white rats (Chinoy et al., 1979). Observations of Stubbs and Mckerman (1967), Stubbs et al. (1967), Khandwekar et al. (1973) and Chinoy (1979) have already indicated the interdependence of vitamin C and androgens. Most of these reports deal with steroidogenic organs.

Besides the above mentioned aspects, it has been shown that AA is also involved in redox potential associated with metabolic reactions of various mammalian tissues by forming its free radical. From this point of view it could be surmised that such tissues as are not involved in steroidogenesis may also exhibit, to a certain extent, dependence on AA content as far as general oxidative processes are concerned. This fact is born out by a report by Patro and Patnaik (1979), wherein it has been shown that a short-term cold stress reduces the AA content in the fish muscle. Moreover, other endocrine disturbances were also reported to lead to alter-

ations in tissue AA levels. An example in this direction is the work of Nathani et al. (1979), wherein they have shown that adrenalectomy in rats led to depletion of AA synthesis and simultaneous increase in its degradation in the liver and kidney.

There exists sufficient amount of literature on effects of castration on the AA contents of various tissues but these observations were made a few weeks after castration, wherein reduction in AA content has been reported. In contrast to this, the present observations were made after 48 hr of castration, hence the results need to be interpreted in a new context and with due precaution. During the course of this investigation it was observed that there was an increase in AA content of submandibular gland due to castration. This is in contrast with the earlier literature where AA content has been reported to be reduced in various tissues studied by different authors. Hence, the presently observed AA increase could possibly be an early effect of castration on the submandibular gland. In the recent past it has been increasingly realized that the effects of various steroid hormones are mediated through cyclic AMP levels in responding tissues.

As has been reported elsewhere (Chapter II) in this report, an increase in phosphodiesterase enzyme activity at early intervals, in all probability, indicated a lowering effect on the cyclic AMP levels in the submandibular gland. From this it could be suggested that lower cyclic AMP levels facilitate the rate of AA synthesis in the submandibular gland. An indirect evidence in this context

is obtainable from the work of Earp et al. (1970), who have shown that cyclic AMP content mediates the AA depleting effect of ACTH on rat adrenal glands.

Replacement experiments, during the course of present investigation showed that submandibular gland promptly responded to TP administration. Within 1 hr of 100 μ g TP administration the AA content of the gland was seen to be restored almost to normal level. However, this dose of TP apparently was not sufficient to hold normal condition, as was evident from increase in AA content by 2 hr that continued to be so even at 4 hr interval. It is, therefore, possible that continuous monitoring of the TP level at a certain minimum critical level is essential for regulating the glandular AA content.

The data considered in the present report indicate that the short term effects of castration as well as replacement reveal interesting and paradoxical influences on metabolic patterns. It is well known that androgens are attributed with lipid mobilizing influence (Laron and Kowadlo, 1963 & 1964). However, the presently observed effect of reduction in total lipid content of the gland due to castration and the reversal of this effect by TP administration are contradictory. Hence, it is suggested that early effects may ultimately reveal a new understanding about the hormonal influence. The results in respect of total cholesterol and AA content may be looked at from the same point of view. It is, therefore, desirable to pursue this line of work extensively before any general conclusion can be drawn about the early effects of hormones.