CHAPTER VII

INFLUENCE OF ADMINISTRATION OF 17β-ESTRADIOL TO MALE ALBINO RATS ON SOME METABOLIC PATTERNS OF SUBMANDIBULAR GLAND

That there exists sexual dimorphism in the rodent submandibular glands and administration of sex hormones leads to structural changes is well known (Lacassagne, 1940; Shafer and Muhler, 1953). According to Lacassagne (1940), treatment of male mice with estrone benzoate results in feminization of the gland. Shafer and Muhler (1953) reported that administration of diethylstilbesterol to female rats for 17 weeks caused a decrease in size and number of the granular ducts. According to Cassano (1958) and Houssay and Harfin (1973) prolonged administration of estradiol to gonadectomized mice of both sexes resulted in hypertrophy of the granular ducts. Later, Raynaud (1960) failed to observe any alteration in the histological structure of the submandibular glands of mice after administration of female sex hormones. Implantation of estradiol pellets in female rats caused a change in the cytology of the granular ducts and this change, according to them, was accompanied by alteration in protein synthesis (Flynn et al., 1983). Morvay et al. (1983) showed that it was possible to get adequate idea about the ethynyl estradiol content of the serum from the measurement of its concentration in saliva. While the effect of estrogenic hormones in histological and histochemical alterations have been investigated, its effect on metabolic variations in male rats have not yet been sufficiently

clarified. In the light of literature cited here and on the basis of observations reported in previous chapters it was thought desirable to study the effect of administration of estradiol to normal as well as castrated male albino rats on the metabolic patterns of submandibular glands.

Bulk of literature is available on the effect of estrogen on various animal tissues. Treatment with 17 ∝-ethynyl estradiol was reported to decrease rat plasma lipids, while total cholesterol and cholesterol ester contents of rat liver and cultured hepatocytes were increased (Kazo et al., 1987). Valette et al. (1987) have shown that administration of ethynyl estradiol to rats for 10 days caused an increase in lipoprotein lipase (LPL) activity in fed state and decrease in fasting state. Further, they showed that feeding the animals a diet supplement with 20% lard reversed estrogen dependent LPL activity increase in fed state causing depletion of fat. Prostatic weight was observed to be increased due to 3∝-androstenediol (3 ∝-diol) alone and in combination with 17β-estradiol (Ulf et al., 1981). Lee and Reed (1977) have shown that estradiol potentiates c.AMP response of human lymphocytes to isoproterenol and PGE_1. On the basis of their work on rat liver mitochondria, Kritchevsky et al. (1963) have shown that circulating androgen levels rather than those of estrogen determine the efficiency of cholesterol oxidation. Subcutaneous implantation of estradiol-17β pellets for 14 days in the heifer calves increased sweat and sebaceous gland volumes in the perineal region than in the neck region (Blazquez et al., 1987). Alves et al. (1986) have shown that estrogen is

able to stimulate preputial lipogenesis in female rats. Administration of estradiol dipropionate to ovariectomized monkeys inhibited MDH activity but stimulted SDH and ATPase in all genital tissues except the cervix and vagina (Kushwah et al., 1987). 17β-estradiol was reported to have more potent antiandrogenic effect on the rat epididymis than cyproterone acetate (Tindall et al., 1981). Long term treatment with low doses of estradiol benzoate was observed to decrease the weight of testes and accessory sex organs (Raychoudhury and Chowdhury, 1987). All the above cited literature show how estrogenic hormones affect various tissues in addition to accessory sex organs. Submandibular glands known to be androgen-sensitive. The present work was carried out to know the possible influence of estrogenic hormone on this gland. In this light, variations in various biochemical metabolites viz. - glycogoen, total lipids, total cholesterol, total ascorbic acid alongwith a few concerned enzymes viz. - glycogen synthetase, total phosphorylase, c.AMP-specific phosphodiesterase, total as well as Na^{+}-K^{+}-ATPase, aldolase and SDH activity levels were studied in submandibular gland of rats after exogenous administration as well as hormone replacement to 48 hr castrates.

MATERIAL AND METHODS

1. A total of 166 male albino rats (120 ±20 g) were maintained in the laboratory on food and water ad libitum.
2. 24 rats were treated as normal while the remaining 142 were grouped into two batches:

a) 72 rats were castrated under mild ether anaesthesia. These rats were administered 100 µg/100 g b.w. of 17β-estradiol (Sigma) dissolved in 0.5 ml tributyrin i.m., 48 hr after castration. Each of 24 hormone replaced rats were sacrificed at 1, 2 and 4 hr time interval.

b) The remaining 72 intact rats were injected with 17β-estradiol (100 µg/100 g b.w.) and sacrificed at similar intervals stated above.

3. The submandibular glands were removed and were freed of connective tissue and following parameters were assayed:- a) metabolites - glycogen, total lipids, total cholesterol, total ascorbic acid (AA) contents and (b) enzyme activities:- glycogen synthetase, total phosphorylase, c.AMP-specific phosphodiesterase (PDE), total and Na^+-K^+-ATPase, aldolase and SDH.

4. Methods employed for determining these parameters were same as given in the earlier chapters (Chapter I & II).

Statistical significance of the data was determined using Student's 't' test.

R E S U L T S

Exogenous administration

1. The results obtained (Table - 1) showed a significant increase in the total lipid, cholesterol and glycogen content of the submandibular gland.
2. AA content did not show any significant alterations.
3. The c.AMP activity was observed to register an increase

(near 3 fold) by an hr and further time interval revealed a trend towards recovery.

4. Total ATPase as well as Na^{+}-K^{+}-ATPase activity was not affected by an hr of estradiol administration but by 2 hr the total ATPase activity was markedly suppressed while Na^{+}-K^{+}-ATPase was significantly ($P < 0.001$) increased.

5. Estradiol administration caused a noticeable suppression in the activity levels of SDH and aldolase enzymes.

Hormone replacement

1. Administration of estradiol led to a significant increase in the glycogen, lipids, cholesterol and AA contents of the submandibular gland as compared values obtainable in case of normal intact rats.

2. A significant ($P < 0.001$) increase was noticed in the glycogen synthetase activity while phosphorylase registered a significant decrease.

3. By 2 hr the PDE was found to increase 2 fold which, with further lapse of time was seen to get reduced.

4. The total ATPase activity was observed to be depleted significantly at 2 hr. Similar was the case with Na^{+}-K^{+}-ATPase enzyme.

5. The Na^{+}-K^{+}-ATPase activity not only recovered by 4 hr but it exceeded the normal value significantly.

6. An overall suppression of the SDH activity was apparent from 1 hr onwards which led to marked reduction by 4 hr. Aldolase activity registered a significant ($P < 0.001$) elevation by an hr while by 2 hr time interval, about 50% reduction over normal value was evident. By 4 hr the activity exhibited recovery.

Table 1

Showing the influence of estradiol-17β administration by way of replacement and exogenous dose to intact male rats on various biochemical parameters

D I S C U S S I O N

The results are discussed here on their own merits and also with reference to the observations on 48 hr castrates as stated in Chapter I & II. From the results obtained (Table - 1) it can be said that administration of estradiol-17β to 48 hr castrate rats led to an increase in the glandular glycogen content. There is an increase in the glycogen synthetase activity alongwith a depletion in the phosphorylase activity. However, the increase in the glycogen content was much lower than that observed in 48 hr castrates. As compared to castrate level, the glycogen synthetase activity is depleted but the phosphorylase activity is not modified due to hormone replacement, rather a further depletion is observed. It could be said that aadministration of estradiol tries to bring the glycogen level back to normal but by 4 hr the effect of estrogen wanes off. The c.AMP-PDE activity which had increased significantly due to castration was noted to get reduced to normal due to estradiol by an hr but was seen to increase again above normal at next time interval. Reduction of PDE activity 1 hr after estradiol administration should lead to an increase in intracellular c.AMP levels, thereby to a corresponding increase in phosphorylase activity and decrease in glycogen synthetase activity. However, during the course of present study a reduction in the glycogen synthetase activity is noticed but that of phosphorylase does not seem to be influenced by estradiol treatment. This would logically lead to lowering of glycogen content of the gland and that was borne by the results. The increase in PDE obtained at 2 and 4 hourly intervals led to

a depletion of phosphorylase activity, which gets reflected in the observed rise in glycogen conent.

The Na^{+}-K^{+}-ATPase activity, that was shown to be increased due to castration, gets reduced to normal level an hr after estradiol administration. This suppression continued to be so by 2 hr leading to below normal level; there is no uptake of glucose molecules during that period. A tremendous increase in its activity by 4 hr indicate its role in taking up glucose molecules which get incorporated into glycogen as evidenced by raised glandular glycogen content. A decrease in the SDH activity reveals that the Krebs' cycle was functioning at low tune. Decrease obtained in the total ATPase activity could possibly be due to depletion of Mg^{++}-ATPase involved in intramitochondrial energy transfer process. Aldolase activity is found to increase above the normal level by an hour. By 2 hr it reduces below the normal level. However, concomitant reduction in SDH activity could possibly mean that glucose molecules broken down by heightened aldolase were not oxidized at a commensurate rate. Hence, it may be suggested that the products of aldolase action in all probability, diverted towards lipid synthesis. This is borne out clearly by the elevated total lipid content at this interval. Similar increase in the hepatic total lipid and cholesterol content in rats (Kozo et al., 1987) and esterified cholesterol (Fewster et al., 1967) has been reported due to administration of ethynyl estradiol and estradiol benzoate respectively.

The results obtained at 2 and 4 hr interval of estradiol-17β administration to castrates reveal an increase in the glycogen content. Estradiol-17β injected to normal intact rats showed an increase in the glycogen content by an hour which marginally decreases with further lapse of time interval. Such inverse relationships in the enzyme activity could have led to the observed increase in the glandular glycogen content. Comparatively, the effects are more obvious in case of hormone replaced animals. In support of this, is the escalated level of PDE activity thereby leaving very less intracellular c.AMP which causes depletion of phosphorylase and elevation of glycogen synthetase activity as is evident from the values reported here. Overall suppression of phosphorylase, SDH and aldolase activities are suggestive of negligible utilization of glucose molecules through the usual glycolytic pathway during the period of experimentation. Over the period of 2-4 hr, the general trend in Na^{+}-K^{+}ATPase activity was seen to be higher which may enhance the uptake of glucose which is utilized for the glycogen synthesis. All the above mentioned results were in favour of increased glycogen content, which was evident at 4 hr after estradiol administration to intact as well as 48 hr castrates. The total ATPase activity was seen to decrease significantly. Mg^{++}-ATPase enzyme, one of the components of total ATPase spectrum is mitochondrial where TCA cycle is involved. As evident from the observed reduction in the SDH activity, it could be said that the Krebs' cycle was functioning at low tune. Hence it would be inferred that low Mg^{++}-ATPase activity must have led to the observed decrement in the total ATPase activity due to estradiol administration.

Strangely enough, no further significant alterations were noticed under the present experimental regime (estradiol administration to castrates) at 2 and 4 hourly interval in the total lipids as well as cholesterol contents. Such enigmatic response, contrary to normal expectation, would certainly need more work to explain this phenomenon. Exogenous administration of estradiol-17β to intact rats led to a further increase in the lipids as well as cholesterol contents. Similar increase in hepatic cholesterol due to administration of estradiol benzoate to rats has been reported by Fewster et al. (1967).

Administration of estradiol to normal male rats did not lead to any noticeable variation in the AA content of the submandibular gland. As against this, similar treatment given to 48 hr castrates led to a gradual rise of glandular AA content, which was well above normal level throughout the period of observation. This was essentially similar to that reported in Chapter I after administration of testosterone propionate to 48 hr castrates, however, the values obtained in present chapter were slightly higher. It could be suggested that slightly higher levels of the circulating sex steroids, due to exogenous administration, do not induce alterations in tissue AA levels. In contrast to this, administration of either estrogens or androgens to the castrates, result in an overt response by the submandibular gland leading to more than normal AA level. From this, it could be inferred that in case of castrate rats administration of steroids of either sex induce a sparing effect on AA content of the submandibular gland.

Houssay and Harfin (1954) observed that administration of estradiol benzoate to castrated male mice produced hypertrophy of the submandibular gland similar to that of androgenic type. Looking at the overall effects of administration of estradiol-17β it could be surmized that the effect is in the same direction as that obtained after administration of TP. Here, the author is tempted to suggest that at least the submandibular gland of the male rats is capable of converting TP and/or estradiol-17β to some metabolite which affects common pathways of metabolic changes, as has been shown in the case of several integumentary glands (Moger and Anakwe, 1983 and Moger and Murphy, 1983). More studies need to be conducted to unravel the possible mechanisms involved.