CHAPTER-4

INFLUENCE OF ADMINISTRATION OF 17β-ESTRADIOL TO OVARIECTOMIZED RATS ON SOME ASPECTS OF CARBOHYDRATE METABOLISM 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, 1940a; Shafer and Muhler, 1953; Cassano, 1958). According to Lacassagne (1940b) treatment of male mice with estrone benzoate results in feminization of the gland. Long term administration of diethylstilbesterol and estradiol benzoate to normal female rats has been reported to cause significant decrease in number of granular tubules and their diameter in submandibular gland (Shafer and Muhler, 1953; Liu *et al.*, 1969). On the other hand, prolonged administration of estradiol to gonadectomized mice of both sexes was reported to result in hypertrophy of granular ducts and increase in weight of submandibular gland (Cassano, 1958; Houssay and Harfin, 1973; Curbelo *et al.*, 1974a). However, Raynaud (1950) failed to observe any alterations in histological structure of submandibular glands of mice after administration of female sex hormones. Implantation of estradiol pellets in female rats was reported to cause changes in cytology of granular ducts and that this change was found to be accompanied by alterations in protein synthesis (Flynn *et al.*, 1983). According to Rybakova (1975) presence of estrogenic compounds in physiological doses in the tissue culture media not only improves chances of survival of salivary gland explants but also leads to epithelial differentiation of gland along with formation of specific secretory products. It is also known that the rat salivary glands exhibit an estrogen-induced increase in peroxidase activity (Laine and Tenovuo, 1983) analogous to that observed in the estrogen treated uterus (Lyttle and Jellink, 1973; Lyttle and Desombre, 1977). 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 on histological and histochemical alterations in the salivary glands have already been investigated; their influence on metabolic aspects of these glands of female rats have not yet been studied to a desirable extent. In the light of literature cited above and on the basis of observations reported in previous chapters; it was thought desirable to study the effects of administration of estradiol to spayed female albino rats on the metabolic patterns of submandibular glands.

It would not be impertinent here to scan through the varied literature on multiparious effects of estrogens on a variety of animal tissues. Treatment with 17α-ethynyl estradiol was reported to decrease rat plasma lipids, while content of total cholesterol and cholesterol esters of rat liver and cultured hepatocytes were increased (Kozo *et al.*, 1987). Valette *et al.*(1987) have shown that administration of ethynyl estradiol to rats for 10 days causes an increase in plasma lipoprotein lipase (LPL) activity in the fed state but the same brought about a decrease under fasting condition. Further, they showed that feeding the animals a diet supplemented with 20% lard repressed estrogen dependent LPL increase in the fed state. Prostatic weight of dog was observed to be increased due to 3α-androstenedione (3α-dione) 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 isoproterinol and PGE_1. Subcutaneous implantation of estradiol-17β pellets for 14 days in the heifer calves increased sweat and sebaceous gland volumes more 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 stimulated SDH and ATPase in genital tissues except the cervix and vagina (Kushwah *et al.*, 1987a). 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 (Raychoudhary and Chowdhury, 1987). Administration of estradiol dipropionate for 7 days to ovariectomized mice produced

about a 3-fold increase in uterine glycogen content and approximately 4-fold decrease in vaginal glycogen (Tripathi, 1983). According to Zanca *et al.* (1983) physiological concentration of estradiol can approximately double the activity of the endometrial Na^+-K^+ ATPase in immature mammals. Subcutaneous implantation of estradiol pellets in gonadectomized rats of both sexes resulted in enhanced hepatic G-6-PDH activity (Ibim, 1989). The dehydrogenases of the pentose cycle have been reported to increase after estradiol administration in rat uterus (Eckstein and Villee, 1966). Intravaginal instillation of a very small amount of estrogen elicited marked increase in glycogen concentration of the rat vagina (Randau, 1968). It is known that estrogen administration in immature (21 day old) female rats brings about a significant increase in uterine glycogen deposition (Demers and Jacobs, 1973). According to Rinard (1970) estrogen treatment increased the uterine phosphorylase-a activity. All the above cited literature showed how estrogenic hormones influence various tissues in different ways. As the submandibular salivary glands of rodents are known to be estrogen-sensitive (Laine and Tenovuo, 1983) , it was thought desirable to know the possible influences of estrogenic hormones on the metabolic aspects of this gland. Variations in some enzyme activities concerned with carbohydrate metabolism *viz*- glycogen phosphorylase, c.AMP-PDE, total ATPase as well as Na^+-K^+ ATPase and SDH were studied in submandibular gland of rats after estrogen administration to 48 H spayed females. Plasma glucose levels also were assayed simultaneously.

MATERIAL AND METHODS

To study the effects of 17β-estradiol replacement, the experimental animals were divided into 4 groups of ten animals each as follows:-

1) 48 H post operated females.

2) 48 H sham operated females.

3) 48 H ovariectomized controls injected intramuscularly with 0.1 ml of vehicle (propylene glycol)

4) 48 H ovariectomized females injected intramuscularly with 0.1 ml of 17β-estradiol (E2) solution. Three different doses of E2 (in 0.1 ml of propyene glycol) were administered individually viz.- 5 (D-1), 10 (D-2) and 15 (D-3) μg / animal intramuscularly as a single injection at 09:00 am. The animals were sacrificed at the end of 1, 2 and 4 H post injection intervals. The parameters in the present study were estimated as per methods given in chapter-1.

RESULTS

17β-estradiol administration to 48 H spayed females rats was found to suppress glycogen levels slightly after 1 H in case of all the three doses. However, after 2 H the first two doses (D-1 and D-2) led to non-significant increase in glycogen concentration, but D-3 led to a significant increase. At 4 hourly interval; D-1 resulted in suppression of glycogen concentration, D-2 was found to bring it very close to normal level and the highest dose maintained the level noted at the previous interval. On the other hand, total phosphorylase activity was increased significantly within 1 H of all the three doses of 17β-estradiol administration However, with all the doses at the 2 H post-injection intervals this enzyme activity was normalized. By 4 H post-injection interval the normalizing influence was apparent with D-2 and D-3, whereas only in case of D-1 an increase in enzyme activity was registered.

TABLE - 4.1

Showing the effects of replacement with 5 µg (D-1) dose of 17β - estradiol (E_2) administration to 48 H ovariectomized rats on various biochemical parameters of submandibular gland.

Parameters	Diestrous female normal	48 H OvX female	Post-injection interval		
			1H	2 H	4 H
Glycogen mg/ 100 mg tissue	0.006 ± 0.006	0.121[a] ± 0.007	0.059 ± 0.001	0.078[c] ± 0.002	0.042[b] ± 0.001
Phosphorylase µmoles PO_4 released/ mg protein/ H	73.538 ± 02.026	71.304 ± 01.370	115.027[a] ± 03.543	71.717 ± 03.631	94 599[a] ± 02.220
c AMP-PDE µmoles PO_4 released/ mg protein/ H	4.844 ± 0.515	5.464 ± 0.203	4.887 ± 0.208	10.203[a] ± 0.142	6.134 ± 0.223
Total ATPase µmoles PO_4 released/ mg protein/ H	200.328 ± 012.000	225.619 ± 009.394	202.514 ± 012.600	257.899[b] ± 008.296	276.589[a] ± 010.308
Na^+- K^+ ATPase µmoles PO_4 released/ mg protein/ H	41.824 ± 03.175	14.859[a] ± 01.023	15.436[a] ± 01.429	30.081 ± 03.256	61.291[a] ± 04.404
SDH µg Formazan formed/ mg protein/ H	30.304 ± 01.726	47.784[a] ± 01.705	56.485[a] ± 02.414	55.278[a] ± 01.516	24.320[b] ± 01.138
Protein mg/ 100 mg tissue	24.467 ± 01.446	21.448[c] ± 00.894	25.365 ± 0.963	22.726 ± 01.336	28.079[b] ± 00.759
Plasma glucose mg/ 100 ml plasma	112.500 ± 002.012	100.500[a] ± 000.948	135.000[a] ± 003.286	71.617[a] ± 03 184	49.362[a] ± 01.592

Values are mean ± SE (n = 8)
a - P<0.0005; b - P<0.005; c - P<0.05
Levels of significance have been calculated with reference to diestrous values

TABLE - 4.2

Showing the effects of replacement with 10 µg (D-2) dose of 17β-estradiol (E_2) administration to 48 H ovariectomized rats on various biochemical parameters of submandibular gland.

Parameters	Diestrous female normal	48 H OvX female	Post-injection intervals		
			1 H	2 H	4 H
Glycogen mg/ 100 mg tissue	0.066 ± 0.006	0.121[a] ± 0.007	0.050[b] ± 0.001	0.067 ± 0.002	0.063 ± 0.001
Phosphorylase µmoles PO_4 released/ mg protein/ H	73.538 ± 02.026	71.304 ± 01.370	100.781[a] ± 002.284	63.686[b] ± 02.072	73.607 ± 02.171
c.AMP-PDE µmoles PO_4 released/ mg protein/ H	4.844 ± 0.515	5.464 ± 0.203	5.051 ± 0.255	8.665[a] ± 0.398	7.092[b] ± 0.331
Total ATPase µmoles PO_4 released/ mg protein/ H	200.328 ± 012.00	225.619 ± 009.394	273.652[a] ± 012.440	326.620[a] ± 009.988	324.86[a] ± 008.872
Na^+-K^+ ATPase µmoles PO_4 released/ protein/ H	41.824 ± 03.175	14.859[a] ± 01.023	69.183[a] ± 02.664	80.836[a] ± 04.572	73.855[a] ± 02.619
SDH µg Formazan formed/ mg protein/ H	30.304 ± 01.726	47.784[a] ± 01.705	54.821[a] ± 01.808	50.348[a] ± 01.628	22.623[b] ± 01.714
Protein mg/ 100 mg tissue	24.467 ± 01.446	21.448[c] ± 00.894	22.073 ± 00.494	18.728[b] ± 00.647	26.039 ± 00.880
Plasma glucose mg/ 100 ml plasma	112.500 ± 02.012	100.500[a] ± 00.948	87.897[a] ± 03.574	97.451[a] ± 01.909	86.669[a] ± 03 366

Values are mean ± SE (n = 8)
a - P<0.0005; b - P<0.005; c - P<0.05
Levels of significance have been calculated with reference to diestrous values.

TABLE - 4.3

Showing the effects of replacement with 15 µg (D-3) dose of 17β-estradiol (E_2) administration to 48 H ovariectomized rats on various biochemical parameters of submandibular gland.

Parameters	Diestrous female normal	48 H OvX female	Post-injection intervals		
			1 H	2 H	4H
Glycogen mg/ 100 mg tissue	0.066 ± 0.006	0.121[a] ± 0.007	0.063 ± 0.002	0.123[a] ± 0.010	0.123[a] ± 0.001
Phosphorylase µmoles PO_4 released/ mg protein/ H	73.538 ± 02.026	71.304 ± 01.370	93 964[a] ± 01.699	72.459 ± 02.975	74.016 ± 01.851
c.AMP-PDE µmoles PO_4 released/ mg protein/ H	4.844 ± 0.515	5.464 ± 0.203	7.134[a] ± 0.224	9.014[a] ± 0.191	5.589 ± 0.121
Total ATPase µmoles PO_4 released/ mg protein/ H	200.328 ± 012.00	225.619 ± 009.394	369 432[a] ± 015.342	353.852[a] ± 005.956	271.44[a] ± 011.741
Na^+-K^+ ATPase µmoles PO_4 released/ mg protein/ H	41.824 ± 03.175	14.859[a] ± 01.023	102.640[a] ± 003.379	48.565 ± 02.332	48.329 ± 02.278
SDH µg Formazan formed/ mg protein/ H	30.304 ± 01.726	47.784[a] ± 01.705	42.339[a] ± 00.822	38.510[b] ± 01.812	35.619[c] ± 00.724
Protein mg/ 100 mg tissue	24.467 ± 01.446	21.448[c] ± 00.894	22.533 ± 00 661	18.322[a] ± 00.499	24.901 ± 02 136
Plasma glucose mg/ 100 ml plasma	112.500 ± 02.012	100.500[a] ± 00.948	80.987[a] ± 02 036	101.924[c] ± 03.296	90.764[a] ± 02.136

Values are mean ± SE (n = 8)
a - P<0.0005; b - P<0.005; c - P<0.05
Levels of significance have been calculated with reference to diestrous values.

cAMP-PDE activity was significantly enhanced at 2 H interval with all the three doses of 17β-estradiol administered. Such an influence of 17β-estradiol was not apparent at 1 H interval in case of the first two doses but D-3 did increase the enzyme activity. Another fact was that by 4 H the enzyme activity exhibited almost normal level; more so in case of the D-3. By the end of very first hour of post-injection interval after administration of D-2 and D-3 the total as well as Na^+-K^+ ATPase activity levels were significantly raised. On the other hand, both of these enzyme activities enhanced only after 2 H of D-1 administration with further increase at 4 H interval. In the case of D-2 and D-3 after 2 and 4 H post-injection interval the trend of recovery in the behaviour of the two enzymic activities differed significantly between the two doses. From the results presented here it is apparent that E_2 administration enhances general ATPase activity but less so than that of Na^+-K^+ ATPase. The former was found to remain significantly above normal level with all the three doses upto 4 H of administration. Only the D-3 dose did enhance Na^+-K^+ ATPase markedly upto 60 minute of hormone administration but thereafter the same reached almost normal level at 2 and 4 H intervals.

In case of SDH activity a very different kind of influence of 17β-estradiol administration to 48 H ovariectomized females became apparent. All the three doses enhanced the enzyme activity at the first two intervals, but by 4 H interval the same was decreased, going below normal level in case of D-1 and D-2. Contrastingly, D-3 brought the level of SDH enzyme activity more or less within the normal range at 4 H post-injection interval.

In general, the glandular protein concentration exhibited slight variations. A very exceptionally noticeable fact was that at 2 hourly interval only after any of the three estradiol doses reduction in the protein levels was quite apparent.

Ovariectomy was found to have a slightly hypoglycemic effect by 48 H. Administration of D-1 was noted to induce a noteworthy hyperglycemic influence

within 60 minutes, but thereafter this dose induced a remarkable hypoglycemia at later two intervals. On the other hand, D-2 as well as D-3 were not found to alter glycemic level in a significant way. However, it can be seen that D-3 induced least possible variation from 48 H ovariectomized level.

DISCUSSION

From the values given in Table - 4.1 to 4.3 it can be seen that, irrespective of different numerical values recorded with all the three doses of 17β-estradiol, only at 2 hourly intervals, in each case, the glycogen level was comparatively higher, phosphorylase enzyme activity was lower and that of c.AMP-PDE was significantly higher. This situation was almost the converse of that at 1 and 4 H intervals with the same three doses of 17β-estradiol. According to Bitman *et al.* (1965) maximum uterine glycogen synthesis occurs between 2-10 H after a single injection of estradiol to rat. Two points emerge from these observations. Firstly, higher c.AMP-PDE activity seems to be favourable for glycogen build up, than reducing the rate of glycogen break down. Secondly, D-3 administration leads to a better reparative influence on glycogen level and phosphorylase enzyme activity of submandibular salivary glands of ovariectomized females rats, but at different time intervals. If ovariectomy led to enhancement of SDH activity; then administration of 17β-estradiol would be expected to counter the effect of ovariectomy. However, this enzyme activity was noted to be further elevated with D-1 and D-2 at 2 H but later at 4 H interval it was lowered even below normal level. As against this D-3 did not alter SDH activity as significantly as with D-1 and D-2. Hence, it could be suggested that D-3 is a better dose level in countering the effect of OvX as far as this enzyme activity is concerned. Variations in glandular protein concentration were obvious; particularly at 2 H intervals after all the three doses. It has been shown by Butcher (1978) that supraphysiological blood titres are attainable 2 H after infusion of 17β-estradiol in rat. Aschkenasy-Lelu and Aschkenasy (1959) have proved that low level of estradiol is slightly anabolic and the same at higher level is catabolic in its action on hepatic protein levels. Considering these

observation; it can be explained that the higher estradiol level at 2 H interval led to catabolic influence on protein concentration of the submandibular salivary glands in rat. This was clearly seen to be so in the present observation. Maximum influence of different doses of 17β-estradiol administration to 48 H OvX females was noticeable only at 2 H post-injection interval. It may be added here that D-3 appears to exhibit better influence in reversing the effects of spaying, of course at 4 H time interval.

Considering the influence of OvX and subsequent E_2 administration on total and Na^+-K^+ ATPase activities of the submandibular salivary gland of female rats in relation with variations in other parameters studied it can be seen that in this class of enzymic activities, that is those of Ca^{++} and Mg^{++} activated ones, are more susceptible to hormonal manipulations rather than that of Na^+-K^+ ATPase enzyme activity. It could, therefore, be suggested that E_2 administration generally favours glandular catabolic tendency, which was more obvious with respect to glandular protein concentration.

Regarding the plasma glucose levels it can be said on the basis of present findings that though estrogen influences this parameter it is perhaps not directly related to glandular glycogen concentration. This may be an organ-specific response to fluctuations of ovarian hormones, as far as glycogen level of submandibular gland is concerned. Similar differential organ specificities in respect to ovarian hormones have been noted between uterus and vagina by Tripathi (1983) and Kushwah *et al.* (1987a & b).

It can be suggested that a wider range of doses as well as time, and inclusion of a few more concerned parameters need to be studied before any tenable inference could be drawn.