CHAPTER-3

A STUDY OF ALTERATIONS INDUCED BY SPAYING IN GLYCOGEN CONCENTRATION AND SOME ENZYMES CONCERNED WITH CARBOHYDRATE METABOLISM OF SUBMANDIBULAR GLAND

Gonadectomy is known to induce alterations in patterns of carbohydrate metabolism in various tissues of mammals as reviewed in the following account. Martirosayan (1980) has reported that castration in male rats leads to a hypoglycemic condition from 14-60 days. This was correlated by this author with disturbed uptake of glucose, but at a later interval of 90 days it was seen to intensify glucogenesis. An increase in plasma glucose level after 24 H of castration was noted in male rats by Ambadkar and Gangaramani (1982). Krotkiewski *et al.*(1980) showed decreased phosphorylase and lactate dehydrogenase activities in broad white fibres of gastrocnemius muscle of the castrated rats. Balasubramnian *et al.* (1981) reported reduced total LDH activity in the vas deferens after castration. The enzymes of glycolytic as well as HMP-shunt pathways studied in epididymis and vas deferens of rhesus monkey showed significant reduction after castration (Gupta *et al.*, 1993). According to Meshchishen *et al.* (1980) ovariectomy in prepubertal female rats led to reduction in hepatic glucose, glycogen, glucokinase, G-6-P-glucosephosphate-isomerase and aldolase activities; whereas glucose-6-phosphatase and fructose-1-6-diphosphatase activities were elevated. Ovariectomy in immature rhesus monkeys was shown to result in depressed MDH activity but without any effect on SDH and ATPase in oviduct, uterus, cervix and vagina (Kushwah *et al*, 1987a). Dixit and Arya (1975) observed depletion of glycogen content of uterus in ovariectomized gerbil. Spaying of female rats was shown to have no effect on activities of enzymes of Krebs' cycle and those of glycolysis in the uterus, except that of G-6-PDH which got depleted, and that, estradiol replacement therapy also was seen not to stimulate the enzymes of Krebs' cycle (Eckstein and Villee, 1966). Ovariectomy was shown to significantly decrease the UDP-galactose

pyrophosphatase enzyme activity in the endometrium Jato-Rodrigez *et al.*, 1976). Ovariectomy in rabbit was reported to reduce acid mucopolysaccharide levels of fallopian tube, uterus and cervix (Kushwah *et al.*, 1980).

However, in the case of salivary glands, reports of such nature, as cited in the foregoing account, are not easily available. According to Berkman and Kronman (1970) castration of mice causes reduction in size, diameter and number of granular tubules of the submandibular glands, and this effect could be reversed by testosterone administration. These observations are in conformity with the findings of earlier workers (Laccassagne, 1940b; Chaulin-Serviniere, 1942a and b; Raynaud, 1950; Shafer and Muhler, 1953; Cassano, 1958). Zebrowski (1973) and Curbelo *et al.* (1974b) observed that castration in rats decreases sialic acid concentration in submandibular gland. On the other hand, spaying was shown not to alter significantly sialic acid concentration in rat submandibular gland (Curbelo *et al.*, 1974a). Iida (1983) reported suppression of G-6-PDH activity in submandibular gland of mice after either prepubertal or postpubertal castration. Short term effects of castration on carbohydrate metabolism of submandibular gland were studied by Desai (1989). As the possible influence of short-term effect of ovariectomy on overall carbohydrate metabolism of submandibular gland in female rats is not yet properly understood, it was thought desirable to investigate the effect of deprivation of ovarian hormones from 24 to 72 H of ovariectomy on some aspects of carbohydrate metabolism. The reasons on the basis of which the parameters for assessment were chosen in order to probe certain aspects of carbohydrate metabolism of submandibular gland of female albino rats are delineated in following paragraphs :-

By now it is recognized that ovarian hormones do influence carbohydrate metabolism, in general (Eckstein and Villee, 1966; Dixit and Arya, 1975; Jato-Rodrigez *et al.*, 1976; Meshchishen *et al.*, 1980; Kushwah *et al.*, 1980). As a basic step, glycogen concentration of submandibular gland was estimated at intervals of 24, 48 and 72 H after ovariectomy. In order to guage the the status of

carbohydrate reserves in the submandibular gland the phosphorylase enzyme activity was quantitatively assayed. Reports are available on increase in salivary glucose level during pre-ovulatory period of menstrual cycles (Davis and Balin, 1973 and Prosser and Hartmann, 1983). These findings do indicate influence of circulating ovarian hormones on salivary glands, therefore, as a complementary parameter, plasma glucose levels were also assyed. As succinate dehydrogenase (SDH) is one of the key enzymes of oxidative metabolism of glucose, its activity in the submandibular gland was determined. It is widely accepted that sodium-potassium dependent adenosine triphosphatase (Na^+-K^+ ATPase) is intimately associated with the mechanism of movement of ions and glucose across cellular membranes (Juadh and Ahmed, 1964; Skou, 1965; Fransworth, 1972). Na^+-K^+ ATPase activity is known to contribute to the production of hypotonic saliva of the parotid glands of rats by facilitating the transport of sodium ions both intracellularly and paracellularly (Tadashi, 1987). Thus, owing to its importance in the regulation of the composition of saliva, this enzyme activity was also studied along with that of the total ATPase.

Of recent, it has become obvious that cyclic AMP, the second messenger, is involved in mediating cellular responses to several hormones through activation of membrane-bound adenylate cyclase system that catalyses the formation of c.AMP from ATP. Increase in c.AMP levels within the cells facilitates manifestation of hormone action through activation of specific protein kinases. Steroid hormones have been shown to activate many enzymes involved in carbohydrate metabolism in seminal vesicle and ventral prostate glands; mainly by increasing intracellular c.AMP levels (Singhal *et al.*, 1971 and Mangan *et al*, 1973). Inactivation of c.AMP to 5' AMP within cells is normally brought about by the action of an enzyme namely c.AMP-specific phosphodiesterase (PDE). This specific c.AMP-PDE activty represents an important controlling step in the regulation of cellular functions. Hence, the activity of c.AMP-PDE was assayed to establish its influence on levels of c.AMP *v/s a v/s* metabolic patterns of submandibular gland.

MATERIAL AND METHODS

Most of the details of methods employed are given in chapter-1. Sham-operation was perfomed on diestrous females only. It was noted that sham-operated females showed normal estrous cyclicity. Hence, the results obtained with sham-operated females were not taken into consideration. The results obtained were therefore compared with the values obtained in case of intact diestrous females.

RESULTS

The result obtained during the course of present investigation revealed an increase in glycogen level of the submandibular gland from 24 to 48 H post-ovariectomy (OvX), but by 72 H tendency toward recovery was noticeable. The total phosphorylase activity remained almost unaltered after 0vX. An abrupt shooting up of Na^+-K^+ ATPase enzyme activity 24 H post-OvX was obvious. Remarkably enough, an equally sudden lowering of the same was apparent by 48 H, going even below the normal level. However, the enzyme was found to be raised above normal level again at 72 H interval. Total ATPase enzyme activity also showed a similar trend of variation, but the magnitude was comparatively much less. c.AMP-PDE enzyme activity showed a transient rise due to OvX upto 48 H but returned to normal level by 72 H post-OvX. Immediately after removal of gonads (24 H) the SDH activity was significantly lowered ($P<0.005$) but it exhibited highly significant rise ($P<0.005$) by 48 H post-operatively. Thereafter, a trend of recovery was apparent. Total protein concentration did not exhibit significant variation at any of the post operative interval. Within first 24 H of OvX a distinct hypoglycemic influence was seen but thereafter, return to normoglycemic condition was apparent.

TABLE - 3.1

Showing effects of ovariectomy on some metabolites and enzyme activities of submandibular gland of rat at 24, 48 and 72 H of post-operative intervals.

Parameters	Diestrous female normal	Post-ovariectomy intervals		
		24 H	48 H	72 H
Glycogen mg/100 mg tissue	0.066 ± 0.006	0.107[a] ± 0.006	0.121[a] ± 0.007	0.073 ± 0.004
Phosphorylase µmoles PO_4 released/ mg protein/ H	73.538 ± 02.026	71.658 ± 02.297	71.304 ± 01.370	70.077 ± 02.455
c.AMP-PDE µmoles PO_4 released/ mg protein/ H	4.844 ± 0 515	5.589 ± 0.293	5.464 ± 0.203	4.657 ± 0 333
Total ATPase µmoles PO_4 released/ mg protein/ H	200.328 ± 012.000	280.972[a] ± 010.403	225.619 ± 009.394	334.833[a] ± 015.754
Na^+-K^+ATPase µmoles PO_4 released/ mg protein/ H	41.824 ± 03.174	113.521[a] ± 005.791	14.859[a] ± 01.023	59.921[a] ± 01.529
SDH µg Formazan formed/ mg protein/ H	30.304 ± 01.726	22.190[b] ± 01.276	47.784[a] ± 01.705	37.778[c] ± 01.916
Protein mg/ 100 mg tissue	24.467 ± 01.446	21.725 ± 01.067	21.448[c] ± 00.894	24.757 ± 00.567
Plasma glucose mg/ 100 ml plasma	112.500 ± 002.012	84.000[a] ± 01.897	100.500[a] ± 000.948	120.000[c] ± 001.887

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.

DISCUSSION

A well defined correlation between glycogen phosphorylase enzyme activity and glycogen metabolism is known since long (Shapiro and Werthemier, 1943 and Stetten and Stetten, 1960). It has been shown that gonadectomy in rats leads to increase in glycogen concentration of submandibular gland (Desai, 1989; Ambadkar and Raval, 1993) as well as that of the liver (Ambadkar and Gangaramani, 1980; Ambadkar and Wagh, 1993). The presently observed increase in glycogen concentration in submandibular gland due to ovariectomy apparently corroborates the findings of the authors mentioned, as far as influence of deprivation of gonadal hormones is concerned.

Taking into consideration the values obtained at 24 H OvX in case of glycogen phosphorylase and Na^+-K^+ ATPase activity it could be said that the increase in glandular glycogen concentration might have been due to enhanced glucose uptake from the blood and its incorporation into glycogen. Strangely enough, by 48 H, despite highly significant drop in Na^+-K^+ ATPase activity; the glandular glycogen concentration continued to increase to a certain extent, which was perhaps due to delayed inhibition of the process of glycogen synthesis as evidenced from lower c.AMP-PDE by 72 H, which by virtue of its property that permits rise in intracellular c.AMP level, which is known to suppress glycogen synthesis (Lamer, 1966; Drummond *et al.*, 1969 ; Hers *et al.*, 1970; Rindi, 1971). On the basis of the data on hand it is possible to explain reduction in the glandular glycogen concentration observed at 72 H despite a rise in Na^+-K^+ ATPase activity and negligible variation in phosphorylase activity. By assuming that glycogen breakdown is enhanced by sustained level of phosphorylase, though slightly low, under the influence of rising intracellular c.AMP level. Here, it may be added, that at this interval the reduced c.AMP-PDE activity has been responsible for this

change, and it could have possibly also led to enhancement of ATPase and maintainance of higher SDH activity. These circumstances probably indicate acceleration of overall general cellular metabolic activities leading to re-establishment of glandular protein concentration.