CHAPTER II

A STUDY OF ALTERATIONS IN GLYCOGEN CONTENT AND ENZYMES OF CARBOHYDRATE METABOLISM OF SUBMANDIBULAR GLAND INDUCED BY CASTRATION AND REPLACEMENT WITH TP

Carbohydrate metabolism in various tissues is known to be influenced by the circulating hormones (Matute and Kalkhoff, 1973; Froberg et al., 1975; Whitton and Hems, 1976; Martirosyan, 1980; Meshchishen et al., 1980; Balasubramanian et al., 1981; Hiroyuk et al., 1981; Panse, et al., 1981; Srivastava et al., 1981; Malbon and Camphell, 1982). One of the major functions of carbohydrates is to provide immediate source of energy in the form of glucose for metabolic processes, from the glycogen store. Degradation of glycogen is catalysed by an initial rate limiting enzyme glycogen phosphorylase. A similar rate limiting step in the synthesis of glycogen, is the incorporation of glucose molecule through the agency of uridine diphosphoglucose (UDPG) into glycogen that is catalysed by the enzyme - glycogen synthetase. Martirosyan (1980) has reported that castration of male rats led to hypoglycemic conditions after 14-60 days, which was correlated by the author with the disturbed uptake of glucose but at later interval of 90 days it was seen to intensify glucogenesis. According to Meshchishen et al. (1980) ovariectomy in prepubertal female rats led to reduction of glucose, hepatic glycogen, glucokinase, G-6-P-glucosephosphate isomerase and aldolase activities whereas G-6-Pase and fructose-1,6-diphospha-tase activities were elevated. Administration of thyroid hormone

in vivo was observed to decrease basal glycogen phosphorylase activity and increased phosphorylase a phosphatase activity of rat hepatocytes (Malbon and Camphell, 1982). Levels of glycogen and maltase activity were observed to get elevated above that of control in Cowper's glands of rats on TP administration while removal of glands led to a decrease in these parameters (Srivastava et al., 1981). Daily administration of progestagens for 7 days to infertile human females showed an increase in endometrial glycogen content and glycogen synthetase activity (Hiroyuk et al., 1981). Panse et al. (1981) showed on the basis of experiments with mice, that administration of cyproternone acetate and flutamide (antiandrogen) resulted in a significant activation of glycolysis, which was manifested in increased glycogen phosphorylase and depleted fructose-1,6-diphosphatase activities. Administration of ACTH to starved South American Caimans has been shown to elevate hepatic glycogen (Daniel, 1972). It has been reported that hypophysectomy leads to a fall in the blood sugar level and hepatic glycogen, while administration of prolactin and corticosterone to hypophysectomized lizards was effective in restoring both the parameters to normal levels (Callard and Chan, 1972). Maity et al. (1981) concluded that oral administration of ethynyl estradiol and dl-norgesterol in rats inhibited spermatogenesis which was accompanied by enhanced glycolytic activity. A striking increase in the uptake of 2-deoxyglucose in the bulbocavernosus, levator ani and extensor digitorium longus muscles was observed in response to TP injections to male rats (Stephen and Toop, 1983). Extract of Malvaviscus conzatti flower extract (antifertility effect) was observed to elevate glycogen

content of the rat testes (Verma et al.,1980). Spayed female rats showed no effect in the activity levels of the enzymes of Krebs' cycle and those of glycolysis in the uterus except the G-6-PDH (glucose-6-phosphate dehydrogenase) which had depleted and that estradiol replacement therapy also was seen not to stimulate the enzymes of Krebs' cycle (Eckstein and Villee, 1966). Taking to account such varied reports it is apparent that there occour several alterations in carbohydrate metabolism of different organs and tissues in wide variety of animals.

However, in the case of salivary glands such reports are not easily available. Smith and Frommer (1975) have shown that steroid hormones lead to an increse in carboxylated mucosubstance within the acini as well as granular tubules of mouse submandibular gland. The possible influence of sex hormones on overall carbohydrate metabolism of submandibular gland of male rats is not yet properly understood, hence, the present study was undertaken to know the effect of deprivation and replacement of androgens on some of the aspects of carbohydrate metabolism. The following paragraphs describe the background on the basis of which the parameters for assessment were chosen to probe into the aspects of carbohydrate metabolis of the submandibular gland of male albino rats.

It is now recognized that androgens do influence the carbohydrate metabolism of various tissues of rats. As a basic indicator such an influence on the glycogen content of submandibular gland was estimated 48 hr after castration and after few hours of TP

replacement therapy to castrated rats. Various enzymes involved in carbohydrate metabolism are known to be influenced by steroid hormones in case of different tissues of laboratory rats (Dahm, 1971; Martirosyan, 1980; Balasubramanian et al., 1981; Maity et al., 1981). In order to know the status of carbohydrate store in the submandibular gland the two key enzyme activities viz.- glycogen synthetase and phosphorylase were quantitatively assayed.

Succinate dehydrogenase (SDH) is one of the key enzymes which is an index of oxidative metabolism of a tissue/organ. It is also reported that SDH activity in levator ani muscle of guinea pig is androgen dependent (Chinoy et al., 1973). Hence, SDH activity in the submandibular gland was determined to know the possible influence of variation in androgen levels on the oxidative metabolism.

Sodium-potassium dependent Adenosine triphosphatase (Na^+-K^+-ATPase) is widely accepted to be intimately associated with the mechanism of ions and water across glandular epithelial tissues (Tanaka, et al., 1987). It is known that Na^+-K^+-ATPase activity contributes to the hypotonic saliva in the parotid glands of rats by facilitating the transport of sodium ions both intracellularly and paracellularly (Tadashi et al., 1987). Owing to its importance in regulating the content of saliva, this enzyme activity was studied alongwith total ATPase enzyme activity.

By now it is well recognized that cyclic AMP (c.AMP), the second messenger, is involved in bringing about cellular responses

to several hormones which are known to activate the membrane-bound enzyme adenylate cyclase that catalyses the formation of c.AMP from ATP. Increased c.AMP levels within the cells help in facilitating manifestation of action of hormones through specific activation of certain enzymes or enzyme systems underlying the response. Androgens have been shown to activate many enzymes of the seminal vesicles and ventral prostate glands involved in carbohydrate metabolism mainly by increasing c.AMP levels (Singhal et al., 1971; Mangan et al., 1973). Inactivation of c.AMP to 5'-AMP within cells is normally brought about by the action of the enzyme - c.AMP-specific phosphodiesterase (PDE). Thus, such a specific PDE activity represents a controlling step in the manifestation of hormonal regulation of cellular functions. Hence, the activity of PDE was assayed to establish information regarding its influence on levels of c.AMP vis a vis submandibular gland metabolic patterns.

MATERIAL AND METHODS

Submandibular glands from adult male albino rats (140-160g b.w.) were obtained from 48 hours castrates and from those subsequently administered 100 µg of TP by way of replacement therapy after 1, 2 and 4 hr. A single intramuscular injection of TP dissolved in 0.5 ml of tributyryn was given to each of the 48 hr castrated rats. In course of this work twelve 48 hr castrates, thirty-six 48 hr castrates each treated with 100 µg of TP and twelve normal intact male albino rats were utilized. For the purpose of study of influence of TP administration at different intervals in each

case 12 animals were utilized. Average fresh weight of the right and left submandibular gland together in case of normal intact male rats was 200 mg. Glycogen content was estimated by the method of Seifter et al. (1950). Total phosphorylase activity was assayed by the method of Cahill et al. (1957). Glycogen synthetase (GS) activity was determined by the method of Leloir and Goldemberg, (1962). SDH activity was quantitatively assayed by the method of Kun and Abood (1949) using INT (indole-nitrophenyl-triphenyl-tetrazolium salt) as the electron acceptor. c.AMP specific PDE activity was estimated employing the method of Butcher and Sutherland (1962). Na^{+}-K^{+}-ATPase and total ATPase activities were measured following the method of Umbreit et al. (1957). Ouabain was used to specifically inhibit Na^{+}-K^{+}-ATPase activity. The total protein content was determined employing the method of Lowry et al. (1950). Aldolase enzyme activity was assayed by the method of Umbreit, K., 1957.

R E S U L T S

Effects of castration

It was observed (Table -1) that 48 hr after castration glycogen content of the submandibular gland increased significantly ($P < 0.001$); the increase being nearly 2.5 fold. Correspondingly, the activity of GS too, after castration registered a significant ($P < 0.001$) increment. However, the total phosphorylase activity was not found to be significantly affected by castration, though a negligible decrease could be observed.

Membrane-bound Na^{+}-K^{+}-ATPase enzyme activity was found to increase significantly ($P < 0.02$) due to castration. Contradictory

to this, the total ATPase activity in the submandibular gland was noted to be lowered.

SDH activity did not register alterations 48 hr after gonadectomy. On the other hand, aldolase activity exhibited a reduction.

c.AMP-specific PDE levels were observed to rise with the removal of gonads.

Total protein content in the submandibular gland was found to be reduced significantly ($P < 0.001$) 48 hr after castration.

Effect of hormone replacement

Administration of 100 µg TP to 48 hr castrated rats did not revert glycogen content to pre-castrate levels. However, a tendency towards slight depletion could be observed within an hour of hormone replacement. By 2 hr it showed a fluctuating response; wherein the glycogen content was found to increase slightly. However, by 4 hr glycogen content was seen to be lowered to a good extent, nevertheless, it was significantly ($P < 0.001$) higher than the normal glycogen values. GS activity was found to get reduced by 1 hr to about 50% of the values obtained in the case of 48 hr castrates, but it was significantly ($P < 0.001$) higher than those of normal intact animals. 2 hr after replacement a decrement in the synthetase activity was apparent, however, by 4 hr it was noted to increase again ($P < 0.001$). Initially, the acitivity showed a slight increase above the castrate values and also above the normal values. After

Table 1

Showing alterations in the glycogen content and enzymes of carbohydrate metabolism induced by castration and replacement with TP to male rats.

2 hr the activity reduced to the values close to that of castrates. Contrastingly, at 4 hr interval the phosphorylase activity registered and increase going above the values obtained in case of 48 hr castrates. It should be noted that this rise was, however, only marginally above the values obtained in case of normal intact animals. Administration of TP did not induce any alterations in Na^{+}-K^{+}-ATPase activity at 1 and 4 hr interval, but at the 2 hr interval a significant ($P < 0.001$) depletion on administration of TP to 48 hr castrated rats. The decreased activity was maintained at low level upto 2 hr of replacement but the same increased by 4 hr to the levels obtained in case of normal untreated rats. Replacement therapy was seen to reverse the reduction in aldolase activity induced by castration. The activity returned to normal by an hour of TP administration. With further lapse of time after TP injection upto 2 and 4 hr a significant ($P<0.001$) reduction almost to the castrate level was observed. Replacement therapy was not initially observed to effect any significant variation in the c.AMP specific PDE activity from the comparatively high levels obtained in 48 hr castrates. However, a marginal depletion was observable by 2 and 4 hr. Total protein content was restored to normalcy by TP replacement at 1 and 2 hr, however, at 4 hr interval it was noted to show further increment ($P<0.01$).

D I S C U S S I O N

Regulation of glycogen metabolism in tissues is effected by the activities of two very important enzymes among others viz.

phosphorylase and synthetase. The glycogen phosphorylase activity and metabolism of glycogen have already been well correlated ever since 1943 (Shapiro and Wertheimier, 1943) and further corroborated later in 1960 by Stetten and Stetten. These enzyme activities, in their turn are hormonally as well as non-hormonally influenced (neuroactive drugs) (Sutherland and Rall, 1960; Krebs and Fisher, 1962; Larner, 1966). Glycogen synthetase catalyses the synthesis of 1,4 linkages of glycogen molecule. According to Hers and Wulf (1968) there is a direct and positive correlation under in vitro conditions between the degree of stimulation of glycogen synthesis and the activity of this enzyme. During the present study an increase in the glycogen synthetase activity was observed 48 hr after castration. This increase was reflected in an increase in the glycogen content of the submandibular gland. Similar rise in glycogen content due to castration was also observed in the case of hepatic tissue of albino rats and garden lizards respectively by Ambadkar and Gangaramani (1982) and by Sreedeviamma and Oommen-(1987). As has been observed during the course of this work, there was an insignificant reduction in the activity of total phosphorylase after castration it is obvious that the increase in the glycogen content was mainly due to increased rate of synthesis. Further, a decrement in the aldolase activity implies that the glycolytic pathway was also not active. Additionally, the SDH activity was also noted to show marginal reduction, hence it could be surmized that oxidative breakdown of carbohydrate was also on low key.

Now it has been amply realized that administration of androgenic steroids stimulates adenylate cyclase system (Singhal et al., 1968; Santi and Villee, 1971; Mangan et al., 1973) and thereby leads to formation of c.AMP content intracellularly. This in its turn has been shown to enhance phosphorylase activity and to reduce the glycogen synthetase system (Lerner et al., 1968; Drummond et al., 1969; Hers et al., 1970; Rindi, 1971). However, during the course of present investigation it was clearly observed that not only castration but subsequent androgen administration also led to some degree of reduction in phosphorylase activity. Such a paradox gets resolved in the observed significant rise in the c.AMP specific PDE activity under the experimental regimen employed here. This observed enhancement of PDE activity is apparently responsible for quick conversion of whatever little c.AMP, that might be formed, into 5'-AMP, and thereby, leading to a hitherto unexpected result; wherein there was actually a rise in synthetase activity and a marginal decrease in phosphorylase activity. The situation led to the observed increase in intracellular glycogen content of the submandibular gland. On this basis, it could be suggested that in case of 48 hr castrated rats, the early response of the submandibular gland was a significant deviation from the normally expected results.

Increased Na^+-K^+-ATPase activity observed here 48 hr after gonadectomy could possibly mean active uptake of glucose by acinar cells and its incorporation into glycogen, providing further support to preceeding observations.

As has been mentioned earlier, 48 hr castrated rats were subjected to a single dose of 100 µg of TP in each case with a view to assess immediate influence on parameters under study at intervals of 1, 2 and 4 hr. Results obtained showed a very different response of enzymic machinery concerned with carbohydrate metabolism in submandibular gland of male albino rats at such early intervals. Injection of 100 µg TP to 48 hr castrates was found to induce a mild degree of depletion in the synthetase activity and a concomitant but a marginal elevation in the phosphorylase activity. This probably indicated a beginning of glycogen mobilizing effect on submandibular gland accompanied by a fall in the synthesizing activity. Provided, as stated here, if glycogen is mobilized under the influence of TP by 1 hr, the glucose thus released should logically be metabolized glycolytically. This is borne out by restoration of aldolase activity at this interval. Not withstanding this reparative influence of 100 µg TP within first 60 min, it was apparent that the same waned as quickly as 120 min and this was evident from the corresponding fluctuations in concerned enzyme activities. If this be the case, then there should be adequate proof emanating from observations on oxidative capacity too, as was borne out by reduction in the SDH level. Nevertheless, this suppressing influence of TP too, was seen to get nullified by 4 hr.

It would not be out of context here to mention that increase in oxidative metabolism in case of orchidectomized rats has been reported by Chandola et al. (1974). and by Brooks et al. (1979). Reversal of observed influence of castration at 48 hr interval only

for the fleeting first 60 min after administration is enigmatic one. What could be surmised is (a) 100 µg of TP dose is not enough and (b) to understand such early responses intensive further work is necessary to arrive at any reasonable inference.

The PDE activity was not found to be altered after an hour of administration of TP but a marginal reduction could be noticed at 2 and 4 hr intervals. Apparently, whatever slight decrease was noticed in PDE activity that was perhaps sufficient enough to permit rise in intracellular c.AMP content. This presumption finds adequate experimental support in the enhancement of phosphorylase activity evident at the interval of 4 hr. Thus, the influence of TP administration by 4 hr interval in bringing down the glycogen content is concerned it could have rather been more due to enhanced rate of glycogen breakdown, as synthetase system was apparently not suppressed but was active simultaneously. From this it is logical to state that the rate of glycogenesis outweighed by the enhanced rate of glycogenolysis at the interval of 4 hours.

One of the basic mechanisms underlying the influence of steroid hormones on their respective target organs involves an alteration in the rate of transport of substances across the plasma membrane (Metcalf and Gross, 1960; Novikoff et al., 1962; Mills and Spaziani, 1968; Klein and Boyer, 1972; Simoni and Shallenberger, 1972). Na^{+}-K^{+}-ATPase has been shown to facilitate the rate of transport of molecules across the plasma membrane against the concentration gradient (Judah and Ahmed, 1964; Skou, 1965; Fransworth,

1972). Thus, alterations in the Na^{+}-K^{+}-ATPase activity would reflect on the flux of various substances across the cellular membrane in the submandibular gland also. No alteration was observed in this activity by an hour of replacement. However, by 2 hr interval a highly significant enhancement of Na^{+}-K^{+}-ATPase activity was observable but again at 4 hr interval the same was reduced to almost 48 hr castrate level. As against this the total ATPase activity registered noticeably higher levels at all the three intervals. It is thus apparent that Na^{+}-K^{+}-ATPase was less sensitive to TP administration and it took 2 hr to respond and that too, in a fleeting manner only. By 4 hr it was once again reduced. Hence, it was total ATPase complex that responded better to the experimental regime. However, this complex comprises of Mg^{++} as well as Ca^{++}-ATPase activities. The Mg^{++}-ATPase enzyme being mitochondrially bound and since the oxidative metabolic index - SDH activity - also being suppressed, it could not have possibly contributed to the observed rise in total ATPase activity. Logically then it might have been Ca^{++}-ATPase activity that was more sensitive and got changed. If that is the case, then it could be said that 100 µg of TP administration to 48 hr castrate rats led to augmentation of Ca^{++}-ATPase activity preferentially. This would mean that such a treatment resulted in alterations in intracellular electrolyte fluxes leading to localized pH changes at these early intervals, the significance which demands further intensive work for a better understanding of this situation. The problem is receiving attention at present.

The total protein content of the submandibular gland increased significantly ($P < 0.001$) 48 hr after castration. This result was in contrast with the work reported by other workers, wherein a depletion in the total protein content was observed after several weeks of castration (Kochakian et al., 1955; Brooks and Higgins, 1980). However, a similar rise in protein content has been previously obtained after 48 hr of castration in the hepatic tissue of rats. (Gangaramani, 1979). The protein content reduced to the normal values within an hour of TP replacement. This was maintained by 2 hr but by 4 hr again a rise was noted in the submandibular protein content. These observations clearly point to the fact that the unexpected rise in protein content of submandibular gland was not an aberrant finding, since it was decidedly of such a nature as could be reversed by administration of TP, a proof enough that it was due to variations in androgen levels at the early intervals. However, it should be added here that 100 µg of TP was just not sufficient to introduce normalcy, as was evident from its waning influence by 4 hr interval. The present author is of the oipinion that these early changes in protein content need reinvestigation so as to understand the implications in a proper light.