EARLY EFFECTS OF ANDROGEN DEPRIVATION AND ADMINISTRATION ON CERTAIN ASPECTS OF HEPATIC METABOLISM IN MALE ALBINO RATS *Rattus norvegicus albinus*

CONCISE SUMMARY

Investigation of the mechanism of action of gonadal steriods constitutes an important aspect of endocrinology. Generally this is carried out by studying the alteration in biochemical patterns induced by the said steroidal hormones in prime target tissues. Androgens are responsible for the growth and maintenance of male reproductive structures in mammals (William, 1961). Effects of androgens on the accessary reproductive organs have been reviewed as early as in 1939 by Moore, and by many others thereafter like Willams-Ashman *et al.* (1967), Coffey (1974), Tuohimaa and Neimi (1974), Engel *et al.* (1980), Guraya and Arbans (1981), Franklin, *et al.* (1982). They are also equally important for their influence on metabolic patterns of sex specific tissues (Farookhi, 1980; Guraya and Arbans, 1982 and Rukmini and Reddy,1983), as well as non sexual tissues *viz.*- skin, muscle, liver and kidney (Drofman,1961; Van Pilsum, 1968; Bergamini, 1972; Dube *et al.*, 1975; Max and Toop,1983). The androgens have also been reported to induce *de novo* enzyme synthesis (Umbreit, 1951; Rioton and Fishman, 1953). Although the androgen action is due to

the steriod molecule *per se* the specific oxygen functions at positions 3 and 17 are chiefly responsible for androgenicity. In recent years it has been increasingly realized that the steriodal as well as non-steriodal androgenic hormones act via the agency of the c-AMP-adenylcyclase system. This has been amply demonstrated by the works of Robinson and Sutherland (1972); Singhal (1974); Spigel *et al.* (1981) on the mechanism of androgen action.

Further, importance of hepatic tissue in the general economy of the bodily functions is a well documented fact. Major bulk of literature deals with alterations observed after few weeks of orchidectomy (Konopkova and Nedvidek 1972; Moore *et al.*, 1977, Engel *et al.*, 1980, Guraya and Arbans,1981) or prolonged/repeated hormone therapy. On the other hand, it is also known that different tissues, including accessary sex organs, respond to hormonal deprivation within a few days (Kochakian,1969; Baulien and Jung,1970; Santti and Ville,1971; Chinoy *et al.*, 1973; Filipenko *et al.*, 1981; Pirkko,1981; Franklin *et al.*,1982) or even within a few hours (Liang and Liao,1975; Ambadkar and Gangaramani,1981; Pirkko,1981; Max and Toop,1983; Aruldhas and Govindrajulu,1985; Sreedevi and Oomen,1985). It has also been reported that the effect of testosterone treatment on prostatic tissue could be seen as early as 30 minutes after the hormone administration (Singhal,1973). However, information available on the hepatic functions leaves much to be desired.

Changes induced by testicular androgens are so vivid that their probable influence on intermediate metabolism of the hepatic tissue certainly warrents further investigation. Even though vast amount of literature regarding the influence of androgens on intermediate metabolism of the hepatic tissue is available, it falls short of a coherent account of the action of testicular androgens on over all physiology of liver. During the last few decades the published literature (Ambadkar and Gangaramani, 1980, 1981; Pirkko, 1981; Max and Toop, 1983; Muddashwar et al., 1984; Din-udom et al., 1985; Ambadkar et al., 1986, 1987) has amply proved that action of steroids become apparent within matter of few hours of administration. Hence it is desirable to know very early effects on mammalian hepatic metabolism. The major issue therefore for the present study was to observe the early effect, in terms of few hours of orchidectomy, replacement therapy as well as administration of androgen to normal intact animals on hepatic metabolism in adult male albino rats.

A previous attempt in this direction was made in the author's laboratory and it was established that maximal alterations in various functional parameters occured by about 48-hours of gonadectomy and that the Spigelian lobe of the liver is more sensitive and intense in its response to alterations in level of androgens. On the basis of these

earlier results obtained in this laboratory, and, in view of the fact that the alterations in certain enzymic activities are manifestations of hormonal regulation, an attempt is made in the present study to observe the hepatic enzymic levels pertaining to the carbohydrate and protein metabolism at 48-hour post orchidectomy interval. Further, during the earlier observations different dose levels of testosterone propionate (TP) were administered to the orchidectomized rats so as to counter act the effects of castration and it was found that the maximally effective dose of TP was 0.1 mg which was able to bring about most of the parameters studied towards normality.

Hence, in the present study only this effective dose, administered as a single intramuscular injection, was employed to evaluate the effects of replacement. A study of few rate limiting enzymes of carbohydrate metabolism would help understand biochemical alterations better and may provide clues regarding causative factors. In order to substaniate this hypothesis experimentally, the present work involving the activities of key enzymes concerning this aspect viz.- Glycogen synthetase and Glucose-6-phosphatase (G-6-Pase) was carried out.

Glucose-6-Phosphastase (G-6-Pase) by its action releases glucose into blood as a ready source of energy. It was observed during the course of the present investigation that G-6-Pase activity was not altered at all by androgen

deprivation, whereas TP administration to orchidectomized animals, caused decline in the enzyme activity leading to a hypoglycaemic condition. Glycogen synthetase activity was marginally reduced by orchidectomy and TP replacement did not lead to recovery in the enzyme activity even by the fourth hour of replacement. These results extend further support to the observations made by the previous investigators (Ambadkar and Gangaramani, 1982). It was opined by them that the removal of circulating androgenic compounds may possibly affect the hepatic glycogenolysis, as evidenced by increased phosphorylase activity, ultimately leading to the release of glucose into blood. These authors had also reported that, on the other hand, TP administration to castrates leads to hypoglycemia. Data obtained here corroborate the suggestion offered by earlier workers to a good extent, as is apparent from the following explanation. Reduction in glycogen synthesis observed presently, could possibly contribute to the previously reported reduced hepatic glycogen content after castration. Similarly previous findings about decline in blood glucose level after replacement therapy is evidently supported by present finding that such a treatment is a manifestation of presently observed reduction in G-6-Pase activity. These observations lead one to suggest that probably the mechanism of hepatic glycogenolysis and that of release of glucose by liver into the blood are differently and independently influenced by androgenic treatment (Chapter-2).

Alterations observed in the nucleic acids and protein content of the hepatic tissue due to orchidectomy and subsequent TP replacement by Gangaramani (1979) prompted the idea of studying the 5'-nucleotidase and transeaminases (GOT/GPT) activity levels under various experimental conditions. 5'-nucleotidase enzyme activity is known to reflect on the flux in nucleotide pool of the cell. It is worth nothing here that this has been shown to be dependent on the androgens in case of accessary sex organs (Chalet et al., 1979). The transaminases (GOT/GPT) too, are reported to be affected by androgens (Khilchevs,1971; Frankline et al., 1982).

During the present study it was observed that there was significant decline in the 5'-nucleotidase enzyme activity after orchidectomy. TP administration within the first 60 minutes was seen not only to just counteract the effect of castration but the enzyme activity registered slightly higher than normal values. The second hour of hormone replacement showed sudden drop in the activity. By the fourth hour the trend towards normalization was again apparent. Hence, one is tempted to assume that 5'-nucleotidase activity is dependent on circulating androgen level, but disturbances in activity level due to castration and replacement therapy are apparently not having any immediate and direct repercussions on nucleic acid or protein metabolic patterns. As far as the transaminase activity

levels are concerned it was observed that there is a reduction in GOT/GPT level after androgen deprivation. TP administration brought back the enzyme activity to normality by 1st hour, however, at 2nd hour of treatment a transient decrease in the activity was observed. Thus this result indicate that probably the reduction in the transaminase activity levels facilitates some how the protein synthesizing mechanism leading to the increase in total protein content of the liver lobes (Chapter-3).

Additionally, androgen administration in three different doses viz.- 0.1, 0.25 and 0.5 mg of TP to normal intact male rats were studied. Hepatic ascorbic acid level was observed to show an early influence of androgen when in excess of normal physiological concentration. It is quite obvious from the work of Ambadkar and Gangaramani (1981, 1987) that not only the contents of protein and carbohydrate moities and levels of some of the concerned enzymic activities are influenced by variations in correlating androgen level, but even such important constituents as DNA and RNA also get affected. This situation naturally demands further extensive as well as intensive investigations in order to understand the issue in a better perspective. However, within the limited scope and time available, it was thought desirable to atleast make an attempt to help solve the issue a bit more. With this view,

a pilot project was undertaken to elucidate the possible early metabolic response of the hepatic tissue of normal intact adult rats to exogenous administration of TP. Three arbitrarily chosen dose levels of the hormone viz.- 0.1 0.25 and 0.5 mg were administered intramuscularly as a single injection per head in 0.5 ml volume of tributyrin (vehicle). Rats were sacrificed at 30, 60, 90 and 120 minutes after the hormone administration. Quantitative biochemical analyses of the two hepatic lobes (Median and Spigelian - Chapter-1) were carried out to evaluate the early androgen affects in the normal, healthy, intact rats. The main objective of taking up this pilot project was to be forwarned of the metabolic disturbances that the hormones may induce, and hence, of the subsequent interference with the over all body welfare. This was specifically thought of in the light of the fact that recent trends in clinical practice tend towards frequent use of natural and synthetic sex-hormones to deal with fertility disturbances, carcinomas and even as anti-fertility drugs.

The data collected during this study were quite confusing and contrary to expectations that would normally be based on already existing knowledge concerning the effects of androgenic hormones. It is a well known fact that the androgens exert an anabolic influence in general, but the present investigation clearly showed that the early response of hepatic tissue was quite contrary, as was evident from biochemical evaluation of the total hepatic

protein content, and the enzymes involved in the protein/nucleic acid metabolism. It seems probable from the present findings, considered along with the previous observations, that normally expected TP-induced generation of c-AMP through activation of adenylcyclase system, especially at the early intervals employed here, is apperently nullified very fast due to observed high levels of c-AMP-specific phosphodiesterase. This in its turn possibly leads to a transient disturbance in the well known 'cascade' effect. Secondly, the raised levels of transaminases might carry such an influence further through accelerated process of deamination leading to formation of more carboxylic acids and there by reducing the normally available pool of amino acids for protein synthesis (Chapters 5 and 6).

On the basis of these observations such tentative suggestions only could be made which need be dealt with further before any thing could be said with confidence. A consistent induction of a hyperglycaemic state after all the three doses of TP at almost all intervals studied here is noteworthy. This is further substantiated by a general rise in the level of glucose-6-phosphatase, which is known to be responsible for release of glucose from the liver into the blood. Further, the observed alterations in the carbohydrate metabolic pattern might tangentially point to as yet unnoticed influence of TP on the regulation

of hepatic carbohydrate metabolism, perhaps of different etiology than that of either insulin or glucagon (Chapter-4).

Further, an interrelationship between the circulating level of testosterone and ascorbic acid (A.A. - Vitamic C) metabolism has been suggested (Stubbs et al., 1967; Chinoy and Parmar, 1975; and Chinoy et al., 1975 a and b). Ascorbic acid is synthesized in the hepatic tissue of mammals and plays an important role in the oxidation-reduction processes of the tissues. It was evident from the report of Ambadkar and Gangaramani (1981) that the A.A. levels in the hepatic tissue of male rat registered a significant rise after orchidectomy and that it showed a time-dependent fall after the initial increase. These workers also reported that in case of 24-hour castrates the effect of replacement therapy was contrary to expected reparative effect of androgen administration. Taking all these points into consideration an investigation was carried out so as to bring out possible reflections on the whole blood and hepatic tissue A.A. levels due to androgen deprivation and replacement (not incorporated in thesis but separately published: Ambadkar et al., 1987).

The results obtained in Chapter-7 revealed that exogenous administration of TP to intact male albino rats, did alter the blood and hepatic A.A. levels. Marked reduction in hepatic A.A.level was noted in both the liver lobes,

irrespective of dose and time-interval under consideration. Results reported were indicative of the fact that even small elavation in circulating androgen level exerts regulatory effect on hepatic A.A. synthesis as well as its concentration. Further, it brings about intrahepatic and peripheral utilization of A.A. It is suggested there, that hepatic A.A. synthesis and its retention are influenced by TP in an independent and different manner.

Finally an attempt was made to compare the influence of hormons in normal and 48-hour orchidectomized animals. This was carried out at different time intervals and with different dosages of TP. This could help in understanding the mechanism of hormone action on hepatic tissue (Chapter-8).

Another noteworthy aspect that was kept in the lime-light during the entire study was to investigate the median and the Spingelian lobes (nomenclature of Green, 1959) of the hepatic gland separately, because the Spigelian lobe of rat liver was found to exhibit decidedly different responses than the remaining lobes to androgen deprivation as well as replacement (Ambadkar and Gangaramani, 1980, 1981, 1982). Such functional differences in metabolic response of the two different lobes of liver has also been reported in respect of some other physiological adjustments but only by few workers like Hems et al. (1972) and Tyagi and Mishra (1977).

The chapters presented in this thesis are prepared as separate entities only for the sake of convenience and clarity of explanations. Special efforts have been made to avoid repetitions. The last chapter "General Considerations" is an attempt to interlink the entire data represented herein so as to have an integrated picture of the same.