SUMMARY AND GENERAL CONSIDERATIONS

Many facets of androgen functions and their interplay in the general metabolism are now being widely accepted in the scientific world. The field of research as far as reproductive hormones are concerned is not restricted to the target tissue as such but also includes non-target tissues and at times tends to amend the definition of a target organ. The hepatic tissue is one such organ which is no more considered a non-target tissue as far as the steroid actions are concerned. Further, in more recent years, it has been reported that lack or excess of steroid hormones leads to significant alterations in the normal hepatic metabolic patterns (Konopkova and Nedvidek, 1972; Khandwekar *et al.*, 1973; Pirkko *et al.*, 1975; Pirkko, 1981; Muddeshwar *et al.*, 1984; Ambadkar *et al.*, 1987).

It is rather difficult to incorporate herein the vast literature available on the effects of androgen deprivation and its subsequent replacement therapy. Nevertheless, it is important to mention that majority of the earlier studies deal with long-term effects of androgens. The literature available on short-term early influences of androgens somehow fall short of a coherent account of the action of testicular androgens on overall physiology of

liver. Hence, early addrogenic influence on the hepatic functions leaves much to be explored.

Overall alterations induced by testicular androgens are so vivid that their probable influence on intermediate metabolism of the hepatic tissue certainly warrants further investigation. The literature published during the last few decades (Pirkko, 1981; Max and Toop, 1983; Muddeshwar et al., 1984; Din-udom et al., 1985) has amply proved that the action of steroids becomes apparent within a matter of few hours/minutes of its administration. Furthermore, recent trends in clinical practice of frequent administration of natural as well as various synthetic sex hormones to deal with problems of fertility disturbances, carcinomas, and sometimes as antifertility drugs, do provide enough cause for calling special attention today to take a more than cursory view of the situation; particularly, with reference to overall body welfare. It is highly necessary to be forewarned of the undesirable metabolic influences such hormones may have as side effects.

Thus, the major goal of the present investigation was to gain better insight into the mechanism of androgen actions on the response of such a vital organ as the liver.

On the basis of earlier observations reported from this laboratory (Gangaramani, 1979) where the present study was carried out and in view of the fact that the alterations in enzymic activities are manifestations of hormonal regulation, to begin with, an attempt was made in the present study to observe the hepatic enzymic levels pertaining to carbohydrate and protein metabolism of the hepatic tissue.

Quantitative evaluation of different metabolic parameters of hepatic tissue constitutes the basic blocks of methodology reported herein; after trying out several histochemical and histological techniques. A detailed outline of the entire methodology has been systematically represented in the very first chapter of this thesis.

Spigelian lobe (nomenclature of Green, 1959) of rat liver has been reported to exhibit decidedly different responses than the remaining lobes to androgen deprivation as well as replacement (Ambadkar and Gangaramani, 1980, '81 and '82). Consequently, the primary focus of the entire present study was on separate investigation of the median (representative of rest of the liver lobes) and the Spegelian lobes of liver. This aspect of the study is noteworthy as such regional functional differences in metabolic responses

of different lobes of liver has been reported in respect of other physiological adjustments (Hems et al., 1972; Tyagi and Mishra, 1977).

Before going further into the details of the data, it is necessary to mention here that employing the only maximally effective dose of TP administration viz.- 0.1 mg of TP to 48-hour castrated rats was made a fixed locus of the present investigation. This important assumption was justifiably made as it had already been reported that 48-hours of castration induced maximum alterations in the liver and that 0.1 mg TP administration could bring back most of the altered parameters towards normality (Gangaramani, 1979).

In the second phase of the present study an investigation of dose-dependent hepatic response of normal intact rats was taken up employing three different doses viz.-0.1,0.25 and 0.5 mg of TP administration. The main objective of taking up this pilot project was to be forewarned of the metabolic disturbances that the hormones may induce, and hence, of the subsequent interference with the overall body welfare. The data were collected as early as 30 minutes after TP administration and thereafter at 60, 90 and 120 minutes. Quantitative biochemical analysis of the two liver lobes - the median and the Spigelian -

were carried out separately to evaluate the early androgen effects. The data obtained during this experimental set-up were quite contrary to expectations that would normally be based on already existing knowledge concerning the effects of androgenic hormones. Once again it became clear that of all the three arbitrarily chosen doses of TP, 0.1 mg was found to be the physiologically least damaging and most compatible.

The pattern of hepatic carbohydrate metabolism immediately after castration (48-hours) and TP replacement (0.1 mg) was investigated. The enzymic activities under investigation were G-6-Pase and Glycogen Synthetase. It was revealed that G-6-Pase activity showed no significant variation after castration but the same registered reduction after TP replacement. Glycogen synthetase activity was marginally decreased after orchidectomy and TP administration also decreased it further. The results also indicate differential response of the two hepatic lobes to varying levels of circulating androgens. It is, therefore, possible to assume that hepatic tissue responds in different ways as far as glycogenolysis and release of glucose into blood are concerned (Chapter 2). In hormone infused intact male albino rats a consistent induction of hyperglycaemic state after the three doses of TP at almost all intervals studied

is note-worthy (Chapter 4). This is further substantiated by a general rise in the level of G-6-Pase known to be responsible for release of glucose from liver into the blood. The alterations noted in the other parameters of carbohydrate metabolism studied here tangentially point to as yet unnoticed influence of TP on the regulation of hepatic carbohydrate metabolism, perhaps of different etiology than that concerning either insulin or glucagon. Further, from the perusal of data obtained regarding changes in carbohydrate metabolism after various experimental regimes it was thought important to compare the results of 0.1 mg TP administration to 48-hours castrates and to intact animals after 60 and 120 minutes (Chapter 8). This study revealed that as far as early hepatic response of the rats to TP administration is concerned the sensitivity of liver to androgenic hormone is basically altered due to orchidectomy. This can easily be noted from the fact that TP administration to castrates led to hypoglycaemic state where as exogenous administration of the same hormone to intact animals led to diametrically opposite influence. It is, therefore, possible that the circulating androgenic levels might somehow be influencing the sensitivity of the hepatic metabolic patterns. This is further borne out by the data obtained in case of glycogen content as well as the enzymic activities of glycogen synthetase, phosphorylase,

G-6-Pase and c.AMP-specific phosphodiesterase. Hence, it is clear that the hepatic gland responds differently when the cells are starved of circulating androgen levels and when exposed to an excess of androgen level under in vivo condition. The observed hypoglycaemic condition after TP injection to 48-hour castrates is possibly due to the increase in glucose utilization by peripheral tissues rather than due to glycogenolysis, while the hyperglycaemia in normal animals after TP administration can be attributed to increased release of glucose(as G-6-Pase level is enhanced) into blood from the liver; despite the fact that phosphorylase activity exhibits sub-normal levels (Chapters 2, 4 and 8).

Alterations observed in the total protein content and that in the nucleic acids of the hepatic tissue due to orchidectomy and subsequent replacement with TP (Gangaramani, 1979) prompted the idea of assaying enzyme activities of 5'-nucleotidase, aspartate transaminase and alanine transaminase. From the results obtained one is tempted to suggest that 5'-nucleotidase enzyme activity is dependent on circulating androgen level. However, alterations noted in the enzyme activity level due to castration and replacement therapy did not show any tangible relation, at least during the period of observations employed here, with the changes

in either nucleic acid or protein contents. Both castration as well as TP administration to 48-hours castrates were noted to exhibit significant rise in hepatic protein contents. It was noted that transaminase activity levels were reduced. This in its turn could facilitate the protein synthesizing mechanism leading to observed increased total protein content of the liver lobes (Chapter 3). In sharp contrast to these observations it was seen that exogenous administrations of TP to normal intact rats was found to lead to significant reduction of hepatic protein content. Such a catabolic response was apparent as early as 30 minutes. Simultaneously it was observed that c-AMP-specific phosphodiesterase was also elevated. It seems probable that the normally expected TP induced generation of c.AMP through activation of adenyl cyclase system, especially in the case of very early intervals employed in this study, is apparently, possibly nullified very fast. This in its turn possibly leads to a transient disturbance in the well known 'cascade' effect. As far as alterations of transaminases are concerned (Chapter 6) it can be said that this spectrum of enzymic activity showed a delayed elevation and further that this became more obvious with increased dose levels as well as time intervals. Such a pattern of response was found to parallel total hepatic protein content in general (Chapter 5).

This kind of an influence may ultimately support the accelerated process of deamination leading to reduction in normally available pool of amino acids for protein synthesis (Chapters 5 and 6). Hence, one is temoted to suggest that the very early influence of TP administration to normal intact albino rats may induce conditions akin to hepatic cell injury. It should however, be mentioned that more intensive as well as extensive investigations, particularly at molecular level, are necessary to confirm this hypothesis and to arrive at some definite understanding about the possible mechanism of very early influence of exogenous androgen on hepatic metabolic response and as to how and why this differs from that reported on the basis of late-/long-term effects.

The comparative studies reported in Chapter 8 concerning the enzymic activities viz.- 5'-nucleotidase, GOT and GPT do not permit any difinite conclusions regarding the changes in total protein content, however, one could still maintain that they do indicate altered sensitivity of the liver towards levels of androgenic hormones.

Finally, keeping in view the interrelationship of circulating levels of testosterone and AA metabolism (Stubbs et al., 1967; Chinoy and Parmar, 1975), an

investigation of influence of circulating androgen level on hepatic as well as blood AA levels under different experimental conditions was carried out. The results obtained (Chapter 7) revealed marked reduction in the hepatic AA content in both the liver lobes irrespective of dose and time intervals under consideration. Blood AA level was also reduced. This is suggestive of the fact that even little elevation in circulating androgen level may influence AA synthesis as well as its tissue concentration by enhancing the intrahepatic and peripheral utilization of AA. On the basis of comparative study of the effect of alterations in androgen level (Chapter 8) on blood and hepatic AA contents, it can be suggested that the mechanism of AA synthesis and its retention are seemingly influenced in different ways and probably independent of each other.

As was referred to in the beginning of this chapter, during the course of present investigation, it was observed that the Spigelian lobe responded in noticeably different ways to the experimental manipulations as compared to the Median lobe (representative of the rest of the hepatic tissue) as far as several of the parameters studied here were concerned. Hence, this corroborates the previous contention.

Notwithstanding the blame of repetition, the present author would like to emphasize that the present study points to a desirability of administering physiologically smaller doses of androgenic compounds over an adequately longer period of time than injecting heavy doses over a shorter period of time, if at all desirable for clinical reasons, to circumvent early undesirable influences.

EXPLANATIONS TO FLOW CHARTS

(1) Part 1 : Experiments and results based on castration and replacement therapy.

(2) Part 2 : Experiments and results based on TP administration to normal intact animals.

a. 30 minutes after TP administration

b. 60 minutes after TP administration

c. 90 minutes after TP administration

d. 120 minutes after TP administration

Abreviations used :

Metab.	-	Metabolism
AA	-	Ascorbic acid
PL Glucose	-	Plasma glucose
Gly.Syn.	-	Glycogen synthetose
Phrylase	-	Phosphorylase
PDE	-	Phosphodiesterase
G-6-Pase	-	Glucose-6-Phosphatase
5'-Nu	-	5'-Nucleotidase
DNA	-	Deoxyribosenucleotide
RNA	-	Ribose nucleotide
Pr.	-	Protein.

Median lobe - 

Spigelian lobe - 

Blood - 

Plasma -

Increase - ↑

Decrease - ↓

No change - —