CHAPTER II

EFFECT OF GONADAL HORMONES ON THE HEPATIC GLUCOSE-6-PHOSPHATASE AND GLYCOGEN SYNTHETASE ACTIVITIES IN THE MALE ALBINO RATS (Rattus norvegicus albinus).

Influence of testicular androgens on functional changes in various tissues are vivid, (Brooks,1976; Ambadkar and Gangaramani,1980 and Engel et al., 1980). Bulk of literature available deals with the alterations observed after few weeks of orchidectomy (Konopkova and Nedvidek,1972; Moore et al., 1977; Guraya and Arbans,1981; Takayasu and Saloshi,1981). There are also reports showing that various tissues, apart from accessory sex organs, do respond to sex steroid deprivation and subsequent replacement in males (Jakubovic and Cekan,1967; Gangaramani,1979; Kudsen and Max, 1980; Max and Toop,1983; Chandrashekaran et al., 1985; Hymavathy et al., 1985; Sreedevi et al., 1985). The importance of hepatic tissue in the general economy of the body functions is a well documented fact; and its importance in the regulation of carbohydrate metabolism cannot be over looked. Androgenic control of carbohydrate metabolism in the prostrate and seminal vesicles has been well documented by Singhal in 1974, who has discussed the mechanism of enzyme induction in male accessory sex glands. He has also reported on inhibition of biochemical responses by the anti-metabolite

actinomycin-D. Singhal has opined that testosterone influences carbohydrate metabolism, through modulation of genic sites; thereby governing the synthesis of some of the rate limiting enzymes. Further, Singhal (1974) states that such an influence of androgen induces quantitative alterations in specific proteins or enzymes. This would imply an important means of regulation of metabolic processes in the male secondary sexual tissues.

Androgenic influence on hepatic carbohydrate metabolism in the male rats was observed in our laboratory (Ambadkar and Gangaramani,1982). It was found that early influence of androgen deprivation and subsequent replacement caused a disturbance within hours in the carbohydrate metabolic patterns. Another noteworthy feature that emerged from these previous studies in our laboratory was the crucial post orchidectomy interval of 48-hours, at which maximally altered metabolic sensitivity to subsequent TP administration (Gangaramani, 1979) was noticeable. Androgenic control of glucose metabolism in different tissues within few hours has also been reported (Pirkko,1981; Ambadkar and Gangaramani,1980 and 1982; Max and Toop, 1983). The results obtained earlier in our laboratory indicated a hypoglycaemic condition within first four hours of replacement with TP to 48-hour castrates (Ambadkar and Gangaramani, 1982) in contrast to a clear cut hyperglycaemic condition prior to TP administration. These findings, by necessity, demanded further investigation at enzymic levels in search of a plausible

explanation of the observed early alteration in the metabolic patterns. In order to understand the alterations induced in respect of the pattern of hepatic carbohydrate metabolism after castration (48-hours) and replacement with TP (First, Second and Fourth hour) an evaluation of activity levels of Glucose-6-Phosphatase and glycogen Synthetase has been undertaken.

Material and Methods :

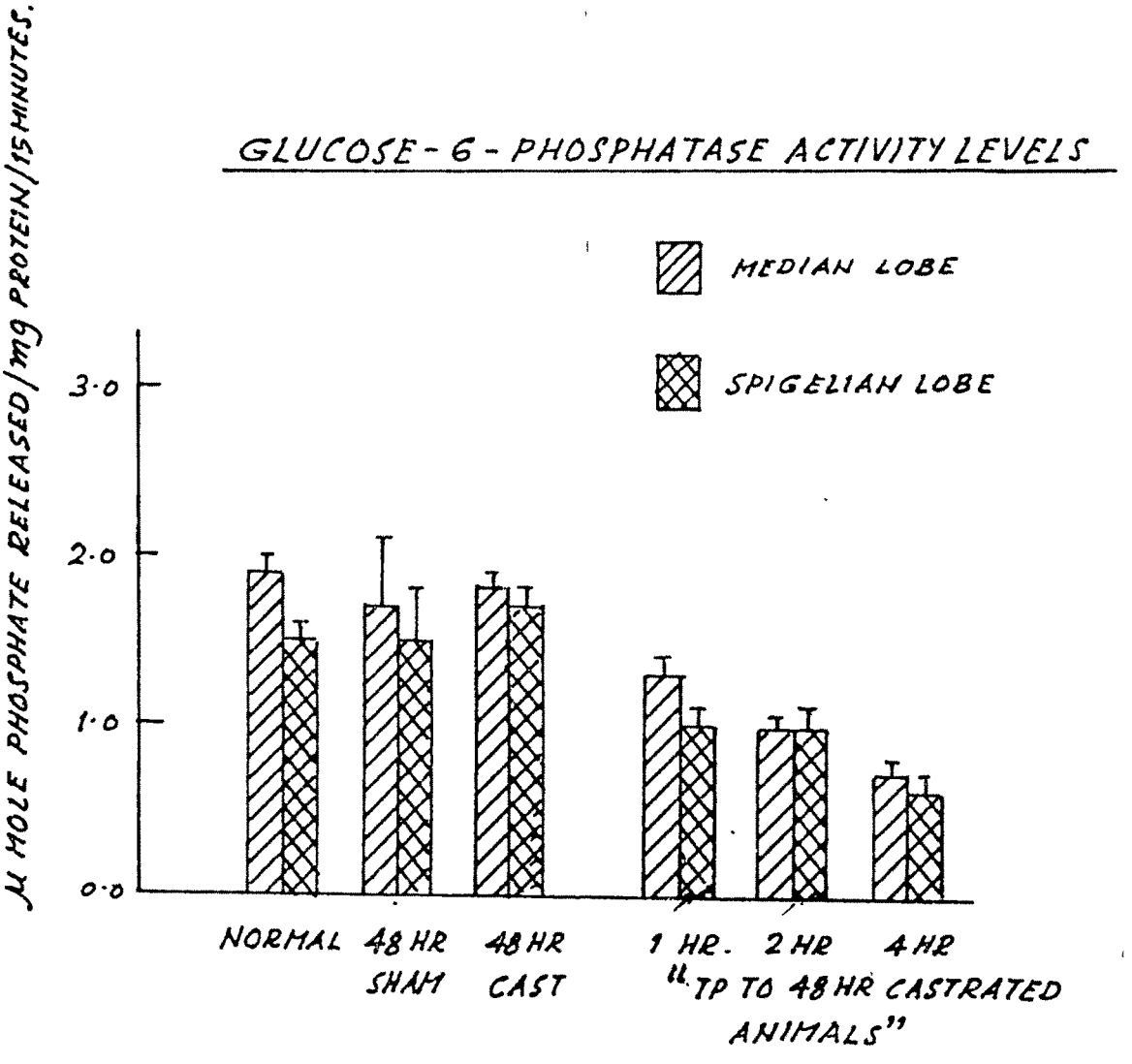
Adult male albino rats (120-160 gms. body weight) maintained in the laboratory on ad libitum food and water, served as experimental animals. Rats were divided into the following experimental categories.

(1) 48-hour orchidectomized animals.

(2) 48-hour Sham-operated animals.

(3) 48-hour orchidectomized animals which were given 0.1 mg of TP and sacrificed after first, second and fourth hour.

After decapitation, the median and Spigelian lobes of the liver were dissected out, blotted free of blood and tissue fluids and were immediately weighed and processed for enzyme estimations. Glucose-6-Phosphatase and glycogen Synthetase activities were assayed according to the methods as described in Chapter-I.


GLUCOSE-6-PHOSPHATASE ACTIVITY LEVELS
MEDIAN LOBE
SPIGELIAN LOBE
μ MOLE PHOSPHATE RELEASED/mg PROTEIN/15 MINUTES.
3·0
2·0
1·0
0·0
NORMAL
48 HR SHAM
48 HR CAST
1 HR.
2 HR
4 HR
"TP TO 48 HR CASTRATED ANIMALS"

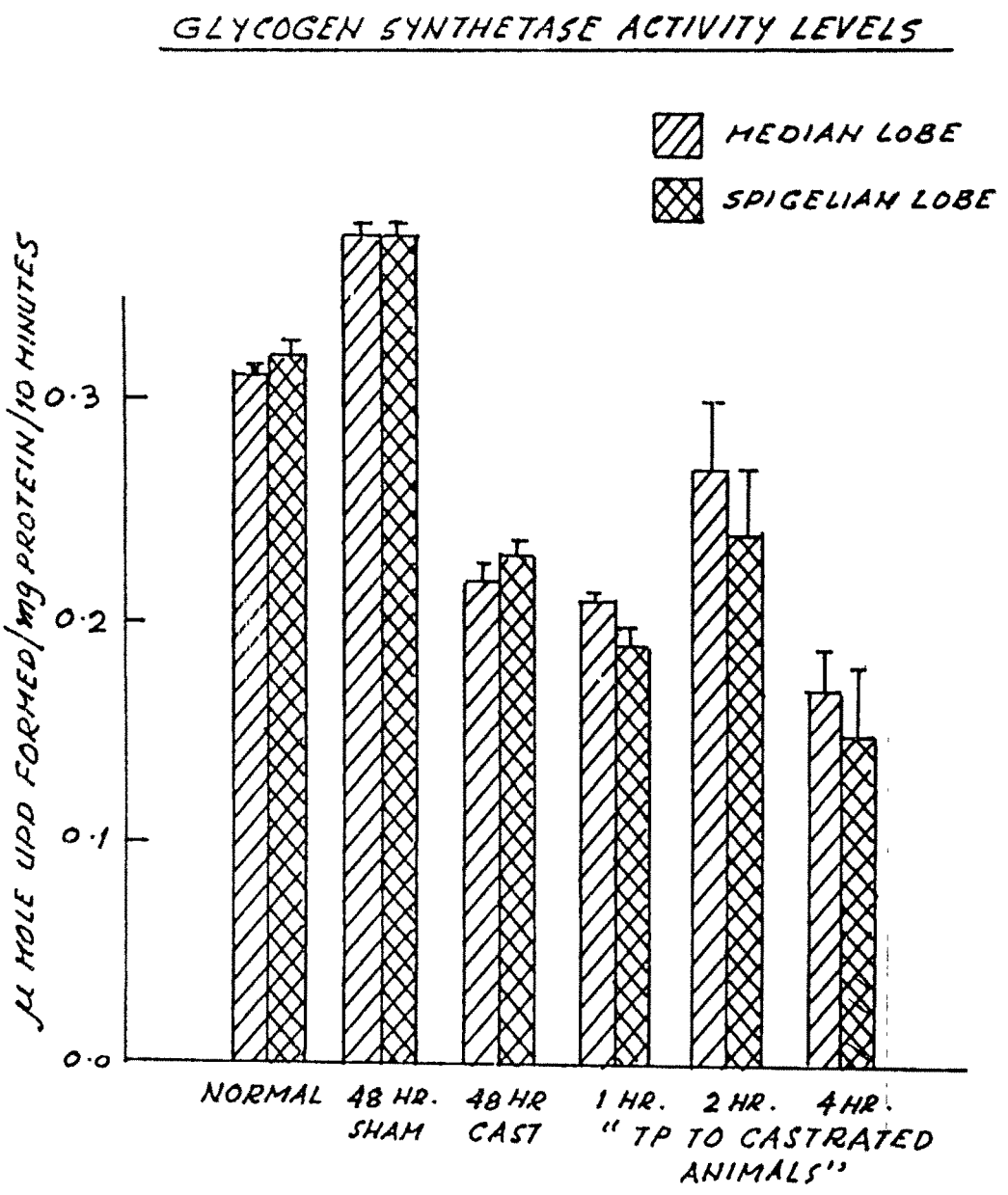

GLYCOGEN SYNTHETASE ACTIVITY LEVELS
MEDIAN LOBE
SPIGELIAN LOBE
μ MOLE UPD FORMED/mg PROTEIN/10 MINUTES
0·0
0·1
0·2
0·3
NORMAL
48 HR. SHAM
48 HR CAST
1 HR.
2 HR.
4 HR.
" TP TO CASTRATED ANIMALS"

Table 2.1 : Early effect of orchidectomy and replacement with TP on hepatic G-6-Pase and glycogen synthetase activity of male albino rats-*Rattus norvegicus albinus*.

	Glucose-6-Phosphatase activity- μ Moles of phosphate released/ mg protein/15 minutes		Glycogen synthetase activity- μ Moles UDP formed/mg protein/ 10 minutes	
	Median lobe	Spigelian lobe	Median lobe	Spigelian lobe
Normal	1.932 ±0.132	1.5699 ±0.101	0.3191 ±0.031	0.3231 ±0.031
48 hrs. Sham operated animals	1.7440^{NS} ±0.44	1.5058^{NS} ±0.31	0.3810^{NS} ±0.044	0.3850^{NS} ±0.053
48 hrs. Orchide-ctomized animals	1.8383^{NS} ±0.092	1.740^{NS} ±0.09	0.2273* ±0.053	0.2332* ±0.034
One Hr.@	**** 1.3399 ±0.180	*** 1.046 ±0.192	** 0.2173 ±0.017	* 0.1945 ±0.044
Two Hrs.@	**** 1.007 ±0.06	**** 1.0285 ±0.102	0.2768^{NS} ±0.044	0.2435^{NS} ±0.032
Four Hrs.@	**** 0.750 ±0.02	**** 0.645 ±0.08	**** 0.1704 ±0.02	*** 0.1585 ±0.036

* P $<$ 0.05 ** P $<$ 0.02 *** P $<$ 0.01 **** P $<$ 0.001

NS - Nonsignificant

± S.E.M. of at least 8 animals

@ 48 hrs-orchidectomized animals injected with 0.1 mg TP and sacrificed after intervals indicated here.

Table : Alterations in the level of plasma glucose and hepatic glycogen content as well as phosphorylase, SDH and G6PDH activities due to castration and hormone replacement in rats. +

	Plasma glucose mg/100ml	Liver Glycogen % of fresh tissue wt		Liver phosphorylase µg/Po4/ MgPr/15 min.		SDH µg/formazon/ mgPr/30min		G6PDH mµ MTPNH/mgPr/ min	
		M	SP	M	SP	M	SP	M	SP
Normal	81.12 ± 8.35	3.83 ±0.39	3.66 ±0.53	78.82 ± 5.41	58.74 ± 4.32	11.29 ± 0.58	7.72 ±0.58	33.11 ± 2.60	28.5 ± 1.49
48-hrs sham operated animals	84.55 ± 5.82	10.51 ± 5.53	9.00 ±0.58	71.81 ± 2.83	51.05 ± 3.18	10.11 ± 0.39	7.99 ±0.18	26.99 ± 2.16	22.12 ± 2.08
48-hrs castrated animals	96.80 ± 6.52	7.77 ± 0.58	8.18 ±0.43	90.00 ± 4.49	59.74 ± 2.58	11.52 ± 0.12	8.11 ±0.32	15.01 ± 2.38	16.29 ± 1.16
48-hrs castrate injected with .1mg/.5ml TP 1HR	39.50 ± 3.15	8.33 ± 0.07	6.51 ±0.06	17.04 ± 2.05	13.36 ± 2.22	4.24 ± 0.97	4.71 ±0.78	16.23 ± 2.83	17.13 ± 1.93
48-hrs castrate injected with .1mg/.5ml TP 2 HR	46.00 ± 2.68	6.61 ± 0.06	5.79 ±0.05	21.38 ± 1.69	22.20 ± 2.29	5.72 ± 0.31	3.81 ±0.61	17.83 ± 2.05	18.29 ± 2.53
48-hrs castrate injected with .1mg/.5ml TP 4 HR	62.58 ± 2.16	6.63 ± 0.14	6.47 ±0.10	17.16 ± 1.75	17.78 ± 1.91	6.29 ± 0.20	4.55 ±0.59	16.33 ± 2.31	18.67 ± 1.90

Each value represents average of ten different samples.

Sp - Spigelian lobe of the liver M - Median lobe of the liver.

* Hours after castration ** Hours after TP injection

\+ P.M.Ambadkar and N.F.Gangaramani (1982).

Results :

The results obtained are presented in the Table 2.1. Fig.2.1 and 2.2. Orchidectomy did not alter G-6-Pase activity when compared with the normal and Sham-operated animals; whereas TP administration to the castrates caused a decline in the enzyme activity (Fig.2.1) with a concomittant hypoglycaemic condition as was observed previously (Ambadkar and Gangaramani, 1982). Glycogen Synthetase activity was found to be marginally lowered by removal of circulating androgen within 48-hours but subsequent TP administration caused a further reduction by fourth hour in the enzyme activity (Fig.2.2), however, a slight transitional improvement in the enzyme activity was apparent by the second hour (Table 2.1).

Discussion :

The balance between the processes involving uptake and release of glucose by hepatic tissue depends on the prevailing hormonal influences at any particular time that govern the various enzyme activities of a tissue. Singhal (1974) has amply proved that the mechanism which controls metabolic processes in the accessory reproductive organs, in response to varying levels of sex steroids, manifests itself through alterations in the activity levels of key enzymes involved in the carbohydrate metabolism.

The data presented here corroborate the results of earlier work (Ambadkar and Gangaramani, 1982), wherein it was

shown that the removal of circulating androgens affected the hepatic glycogenolysis by increased phosphorylase activity and concomittant fall in hepatic glycogen levels. On the other hand, same authors have shown that, there occurs a reduction in the direct oxidative utilization of glucose. Further, it was reported that subsequent replacement with androgen (0.1 mg TP) lead to a drastic decline in blood glucose level, reduced hepatic glycogen content as well as phosphorylase activity and suppression of tricarboxylic acid (TCA) cycle, as evident by reduced succinate dehydrogenase activity. This statement is supported by the present observation that, androgen replacement drastically reduces the G-6-Pase activity, as this would contribute to reduction in the rate of release of glucose into the circulating blood. As a corollary, hexokinase enzyme activity should be affected in an inverse manner. In this context, the work of Singhal (1974) on accessory reproductive organs could be cited as an indirect support, since he has shown that this is so with respect to hexokinase activity.

From the foregoing observations it appears that the mechanism of glycogenolysis and glucose release from liver into blood are influenced differently and independently by TP. The hepatic tissue probably responds to the exogenous androgen by increasing the rate of glycolysis and apparently simultaneously suppressing gluconeogenic pathway as well.

This naturally would lead to reduced hepatic glycogen level, as was noted. Moreover, reduced level of glycogen synthatase activity observed here would also complement this phenomenon. On the other hand, hypoglycaemia observed during the present investigation could possibly be due to increased glucose uptake from blood and its utilization by those peripheral tissues which are influenced by such experimental treatment as castration and TP administration. One such recent report is that of Pirkko (1981). He has studied the androgenic control of glucose metabolism in the ventral prostate of 48-hour castrates and testosterone replaced rats, wherein he has reported on increased glucose utilization by the tissue after 12-hours of testosterone administration independently of pentose cycle activity and pyruvatekinase as well as pyruvate dehydrogenase activities. In the light of these observations it could be surmised that the hepatic tissue responds to variations in circulating androgen levels in an independent manner as regards the glycogenolysis vis-a-vis release of glucose into blood.