CHAPTER 3

EFFECTS OF CASTRATION AND HORMONE REPLACEMENT ON SOME ASPECTS OF HEPATIC CARBOHYDRATE METABOLISM

Carbohydrates are important substances in the general metabolism of the body. Glucose is an important immediate source of energy that is essential for several metabolic processes and also for maintenance of normal regulation of intracellular and extracellular electrolytes. Excess of glucose is normally converted into lipids or glycogen. Glycogen is widely distributed in the animal body but the largest bulk occurs in the muscles and the liver. Liver glycogen may be considered as a ready source of glucose. Defects in the enzymes involved in the synthesis or degradation of glycogen may lead to disturbances of glycogen metabolism.

The balance between processes involving uptake of glucose by tissues and supply of glucose by the liver depends on the prevailing hormonal influences at any particular time that govern the various enzyme activities of a tissue. Hormones act at different levels of biological systems and on the regulatory mechanisms involved in homeostasis. Studies on the mechanisms that

control metabolic processes in accessory reproductive organs have demonstrated that sex-hormones are capable of inducing synthesis of several important enzymes involved in carbohydrate metabolism (Singhal et al., 1967; 1969; Vijayvargiya et al., 1969; Singhal and Valadares, 1970). Since only scanty information is available on the action of sex-hormones in terms of their effects on hepatic carbohydrate metabolism, attention was directed towards a study of possible influence of sex-hormones on certain enzymes that are rate-limiting in carbohydrate metabolism. An attempt has, therefore, been made to study alterations in the glycogen content and total phosphorylase activity (a catabolic enzyme) in the liver of normal, castrated and hormone replaced rats. In view of the fact that alterations in certain enzyme activities are manifestations of hormonal regulation, it would be desirable to carry out a study of dehydrogenases which are important enzymes catalysing various metabolic pathways. Succinate dehydrogenase (SDH) is one such enzyme of the Tricarboxylic acid cycle (TCA cycle). According to Putilina and Eshchenko (1969), amongst

the Krebs cycle dehydrogenases, SDH is more active than any of the other enzymes. It is therefore, obvious that assessment of SDH activity would provide a very good index of the state of oxidative metabolism, hence it was thought desirable to study this enzyme activity under the experimental conditions employed in the present investigation on liver functions.

Glucose-6-phosphate dehydrogenase (G-6-PDH) is that enzyme which brings about direct oxidation of the glucose moiety, liberating reduced NADP (Nicotinamide adenine dinucleotide phosphate) and pentose phosphates. Thyroxine, 17-β estradiol, dehydroepiandrosterone, cortisone and insulin (Glock and McLean, 1955) have been shown to alter the level of G-6-PDH activity, therefore, this enzyme serves as a model indicator for the study of interrelationships between hormones and the energy metabolism in liver. The present report deals with alterations in the concentration of glycogen and levels of phosphorylase, SDH and G-6-PDH activities in the liver of white rats under castration and replacement therapy.

MATERIALS AND METHODS

The adult male albino rats maintained in the laboratory on an ad libitum food and water served as the experimental animals. Castration, sham-operation and the hormone treatment were performed as outlined in Chapter-1. After decapitation, the liver lobes were dissected out, blotted free of blood and tissue fluids, and were weighed immediately. Estimation of hepatic glycogen was performed by the method of Seifter et al. (1950). Total liver phosphorylase activity was assessed according to the method of Cahill et al. (1957). SDH activity was measured as described by Kun and Abood (1949) using Triphenyl tetrazolium chloride (TTC) as the electron acceptor. Activity of G-6-PDH was assayed spectrophotometrically employing the method of Kornberg and Horecker (1955) with modification as described by Marks (1966).

RESULTS

In the normal intact animals no lobe-wise differences in the concentration of hepatic glycogen were discernible. Liver glycogen content of normal animals was found to be

3.5 gms/100 gms (average value) in all the different lobes. Subsequent to castration there was a sudden increase in glycogen content but later a lower average value of 4.5% was recorded on the fifth day (120 hrs.) of castration. In sham-operated animals also glycogen content exhibited a sharp rise and in fact the levels were higher than those obtained in 24 hr. castrates. After 48 and 120 hrs. of castration, depletion in glycogen content was apparent leading to nearly the normal values (Table I, Fig. 1).

Regarding the phosphorylase enzyme activity a significant difference between the median and Spigelian liver lobes was recorded in the normal intact condition. It was found to be higher in the median lobe. Sham-operation induced a non-significant decrease in the two lobes. The enzyme activity was found to increase in both of these lobes after 24 hrs. of castration. After 48 hrs. of gonadectomy, though a slight depression in enzyme activity was noticeable, it still remained nearer to the level obtained in 24 hr. castrates than to the normal ones. There was a highly significant rise in the phosphorylase activity at 120 hrs. post-operatively. The two dehydrogenases (SDH and G-6-PDH) were also more active

in the median lobe than in the Spigelian. Their activity was found to decline after 24 hrs. of castration. Under sham-operation also a decrease of similar magnitude in the enzyme activity of SDH as well as G-6-PDH was obtained. Variation in SDH activity of the median lobe was almost negligible whereas it was significant in case of the Spigelian lobe. Fluctuations in G-6-PDH activity were more obvious for both the lobes. In comparison to sham-operated animals, the 24 hr. castrates showed a greater depletion in G-6-PDH activity. By 48 hrs. of operation, the two dehydrogenases started showing increase in their respective activities, going more towards the normal levels by 120 hrs. of gonadectomy (Table I, Fig.2).

Effects of replacement therapy:- Testosterone propionate (TP) was injected in three different doses viz., 0.05, 0.1 and 0.5 mg intramuscularly as single shots to the rats after 24 hrs. of castration. After having found that 0.1 mg dose of TP was capable of bringing about maximum restoring effect this dose was administered to animals after 120 hrs. of castration to compare changes brought about that late. The results obtained are represented in Table II.

Glycogen concentration was observed to register values higher than normal but lower than sham-operated ones after 24 hrs. of castration. Later the levels decreased upto 120 hrs. (Table I, Fig. 1). Administration of TP to 24 hr. castrates seemed to lower the glycogen levels, nevertheless, maximum possible influence was observed with 0.1 mg dose which was effective on both lobes. When this same dose was given to 120 hr. castrates a very obvious effect was seen in case of the median lobe (Table II, Fig. 3). Spigelian lobe responded less evidently. There was a clear difference in sensitivity to TP injection between the Spigelian and median lobe of the liver of white rats.

Phosphorylase activity was slightly lower in sham-operated animals than in normal but castration decidedly led to a considerable rise within 24 hrs. These values registered negligible lowering at 48 hrs. post-operatively but by 120 hrs. a very significant raising was evident. Administration of 0.05 mg of TP to 24 hr. castrates led to markedly highest levels recorded during all the experimental conditions employed (Table II, Fig. 3). This was certainly a very problematic observation. Nevertheless, 0.1 mg of TP did bring down

the values close to normal levels which was more so in the case of median lobe. 0.5 mg dose did not differ much from the 0.1 mg dose. When 0.1 mg TP was injected into 120 hr. castrates the phosphorylase activity was very close to normal in case of both of the lobes (Table II, Fig. 3).

SDH activity was not observed to exhibit any significant variation from the normal values in both of the liver lobes with any of the experimental treatments except for the value obtainable in the case of Spigelian lobe in 24 hr. castrates, which showed a significant depletion.

G-6-PDH activity was lowered significantly due to sham-operation itself but castration brought it down further after 24 hrs. At 48 hrs. of castration it was not very different yet signs of restoration were apparent and by 120 hrs. the values were closer to normal ones, particularly in case of the Spigelian lobe. Administration of TP was observed to effect only a partial restoration to normality. Obviously, 0.1 mg dose was found to be most satisfactory. 120 hr. castrates responded comparatively poorly to hormone administration.

Table I : Glycogen content and activity levels of phosphorylase, SDH and G-6-PDH of rat liver (median and Spigelian lobe) in normal, sham-operated and castrated rats.

	Normal Animals		Sham-operated Animals		Castrated Animals					
			24 H*		24 H*		48 H*		120 H*	
	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp
Glycogen % on fresh tissue wt.	3.83 ±0.395	3.66 ±0.531	12.93 ±0.783	8.28 ±0.442	9.13 ±0.907	6.93 ±0.517	7.77 ±0.500	8.17 ±0.439	4.99 ±0.672	4.30 ±0.326
Phosphorylase /µg PO_4/ mg PR/15 min.	78.82 ±5.41	58.74 ±4.32	71.30 ±4.03	53.09 ±3.12	94.45 ±1.18	63.52 ±4.30	90.00 ±4.49	59.74 ±2.58	124.37 ±3.04	88.74 ±5.57
SDH /µg Formazan/ mg PR/30 min.	11.29 ±0.584	7.72 ±0.581	10.21 ±0.601	7.031 ±0.391	10.80 ±0.994	4.29 ±0.414	11.52 ±0.120	8.11 ±0.321	11.27 ±0.458	7.03 ±0.510
G-6-PDH mµM TPNH/ mg PR/min.	33.11 ±2.60	28.50 ±1.49	19.28 ±1.58	21.68 ±2.48	13.88 ±1.77	15.91 ±2.03	15.01 ±2.38	16.29 ±1.61	21.48 ±2.14	26.29 ±2.40

Each value is the mean of at least fifteen different samples.
M - Median lobe
Sp - Spigelian lobe
*Hours after operation.

Table II : Glycogen content and activity levels of phosphorylase, SDH and G-6-PDH of rat liver (median and Spigelian lobe) in normal and hormone treated castrated rats.

	Normal Animal		24 H Castrates injected with TP						120 H Castrates injected with TP	
			0.05 mg*		0.1 mg*		0.5 mg*		0.1 mg*	
	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp
Glycogen % on fresh tissue wt.	3.83 ±0.395	3.66 ±0.531	5.05 ±0.600	6.31 ±0.348	4.09 ±0.403	4.26 ±0.488	5.04 ±0.411	3.99 ±0.154	3.85 ±0.553	5.35 ±0.406
Phosphorylase µg PO_4/ mg PR/15 min.	78.82 ±5.41	58.74 ±4.92	147.4 ±4.10	97.50 ±4.28	81.66 ±4.88	80.37 ±6.12	83.60 ±3.65	86.10 ±3.60	68.24 ±8.86	69.69 ±5.89
SDH µg Formazan/ mg PR/30 min.	11.29 ±0.584	7.72 ±0.581	12.86 ±0.476	10.03 ±0.806	10.80 ±0.381	10.90 ±0.538	11.87 ±0.532	9.88 ±0.880	11.95 ±0.470	9.96 ±0.269
G-6-PDH mµM TPNH/ mg PR/min.	33.11 ±2.60	28.50 ±1.49	18.40 ±2.52	19.81 ±2.38	28.32 ±2.81	19.27 ±1.67	15.52 ±3.10	14.22 ±1.81	16.23 ±1.77	18.81 ±2.29

Each value is the mean of at least fifteen different samples.
M - Median lobe
Sp - Spigelian lobe
*Dosages of TP administered.

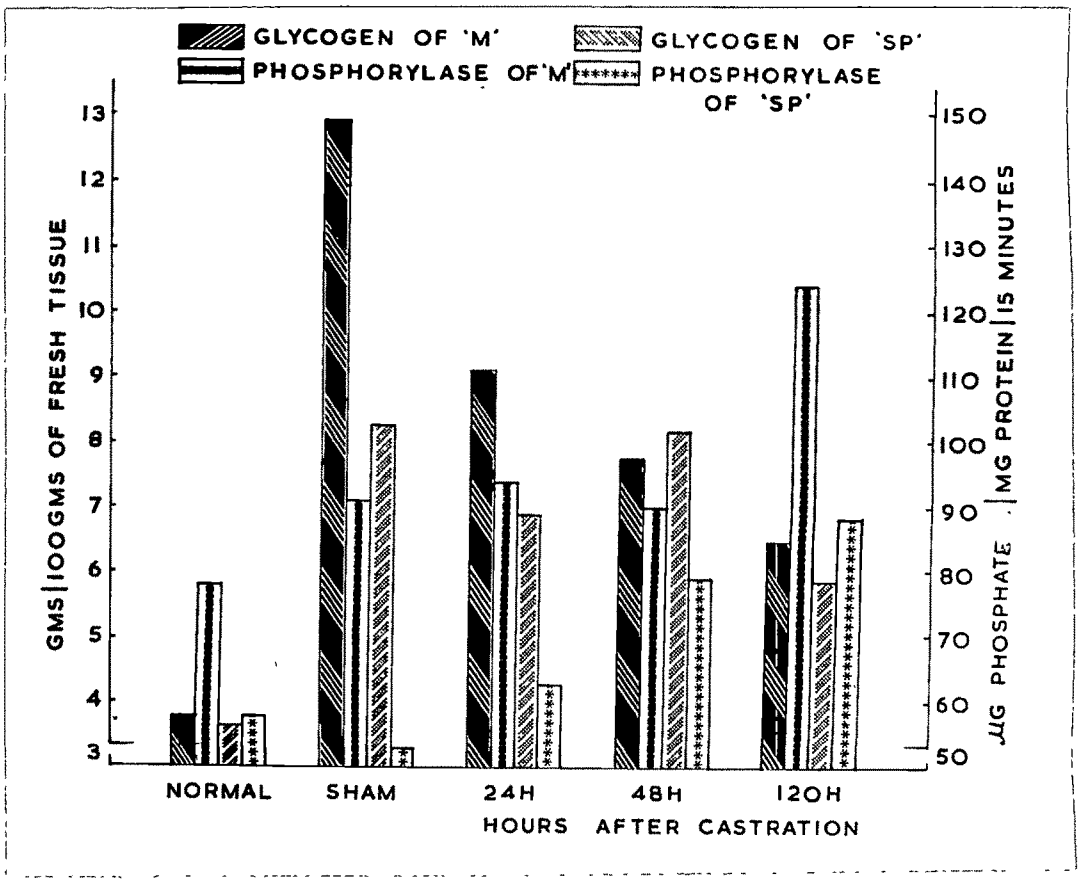

Fig. 1. Depicts the effect of castration on glycogen concentration and phosphorylase enzyme activity in the median and Spigelian lobes of liver.

M- Median lobe of the liver.

Sp- Spigelian lobe of the liver.

Glycogen values expressed as gms/100 gms of fresh tissue

Phosphorylase activity expressed as $\mu g\ PO_4$ / mg Pr / 15 min.

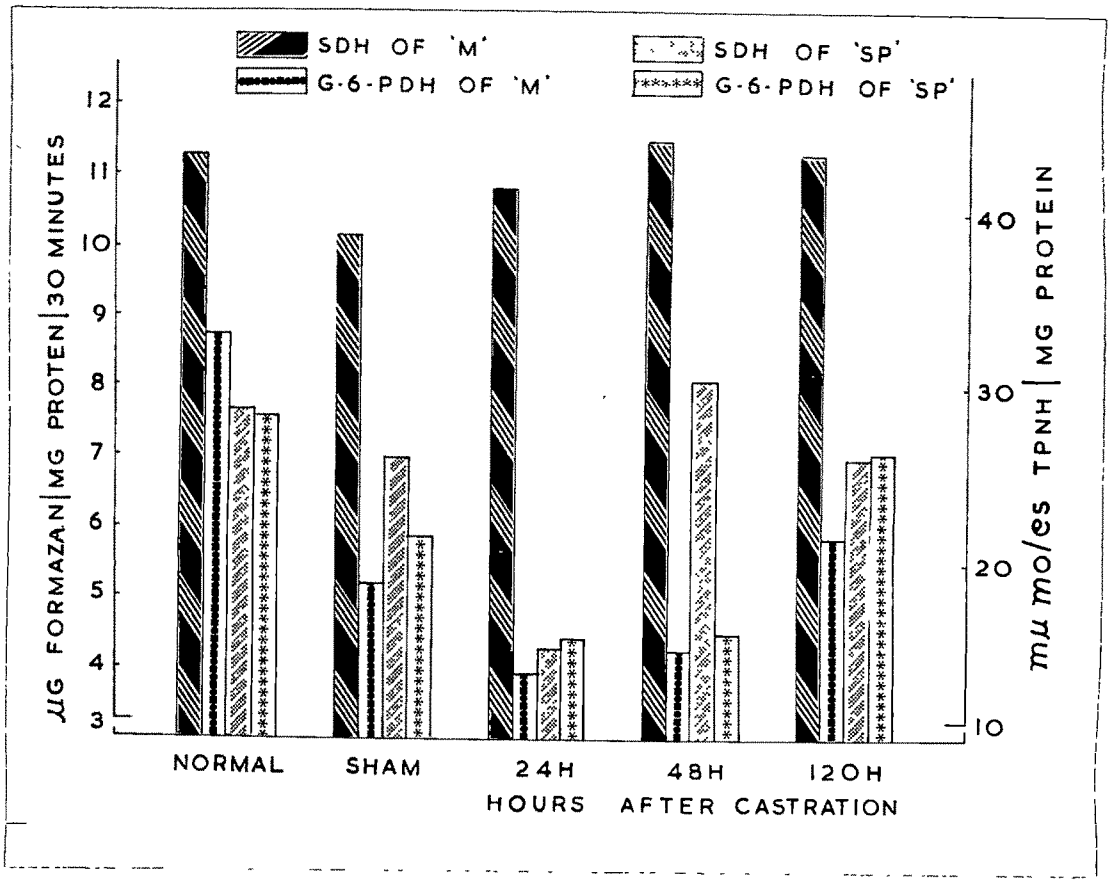

Fig. 2. Variations in the activity of Succinate dehydrogenase and Glucose-6-phosphate dehydrogenase in two different liver lobes (median and Spigelian) of normal, sham-operated and castrated rats.

M – Median lobe of the liver.

Sp – Spigelian lobe of the liver.

SDH activity expressed as µg formazan/mg Pr/30min.

G-6-PDH activity expressed as mµM TPNH/mg Pr/min.

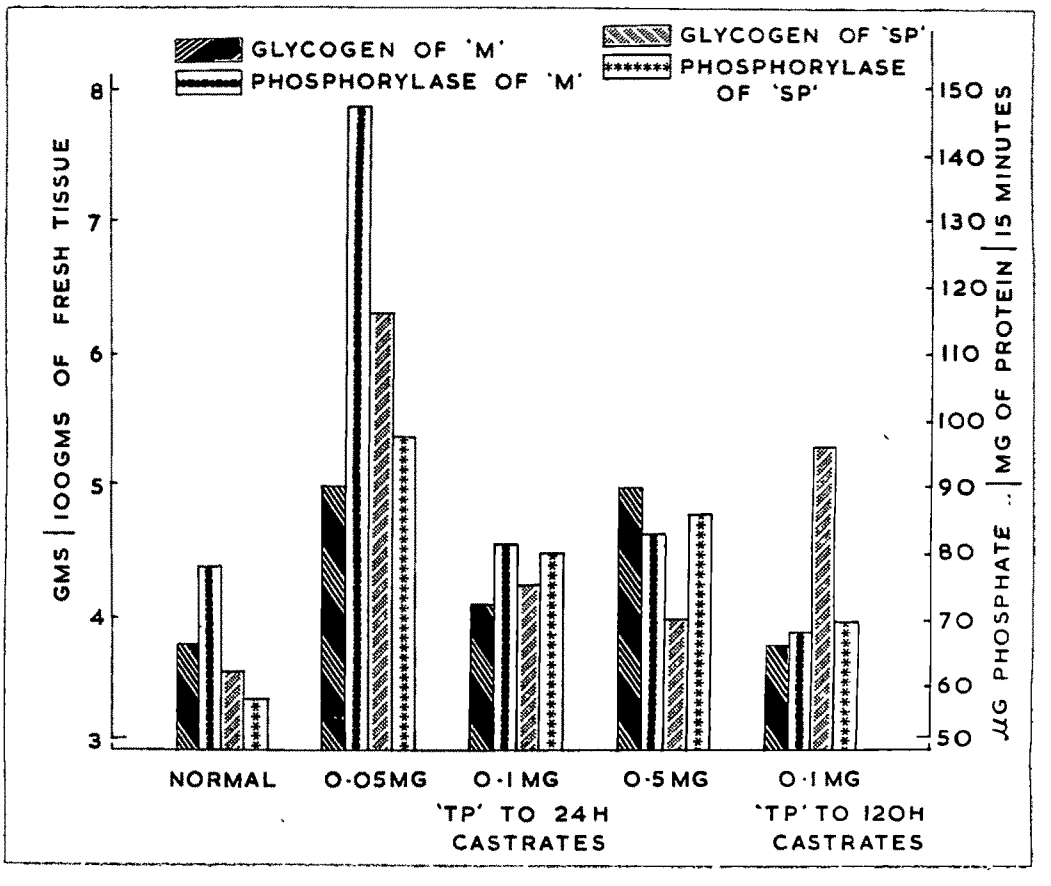

Fig. 3. Represents the influence of TP administration on glycogen content and phosphorylase activity levels of the two liver lobes.

M - Median lobe of the liver.

Sp - Spigelian lobe of the liver.

Glycogen values expressed as gms/100 gms of fresh tissue

Phosphorylase activity expressed as $\mu g\ PO_4$ / mg Pr / 15 min.

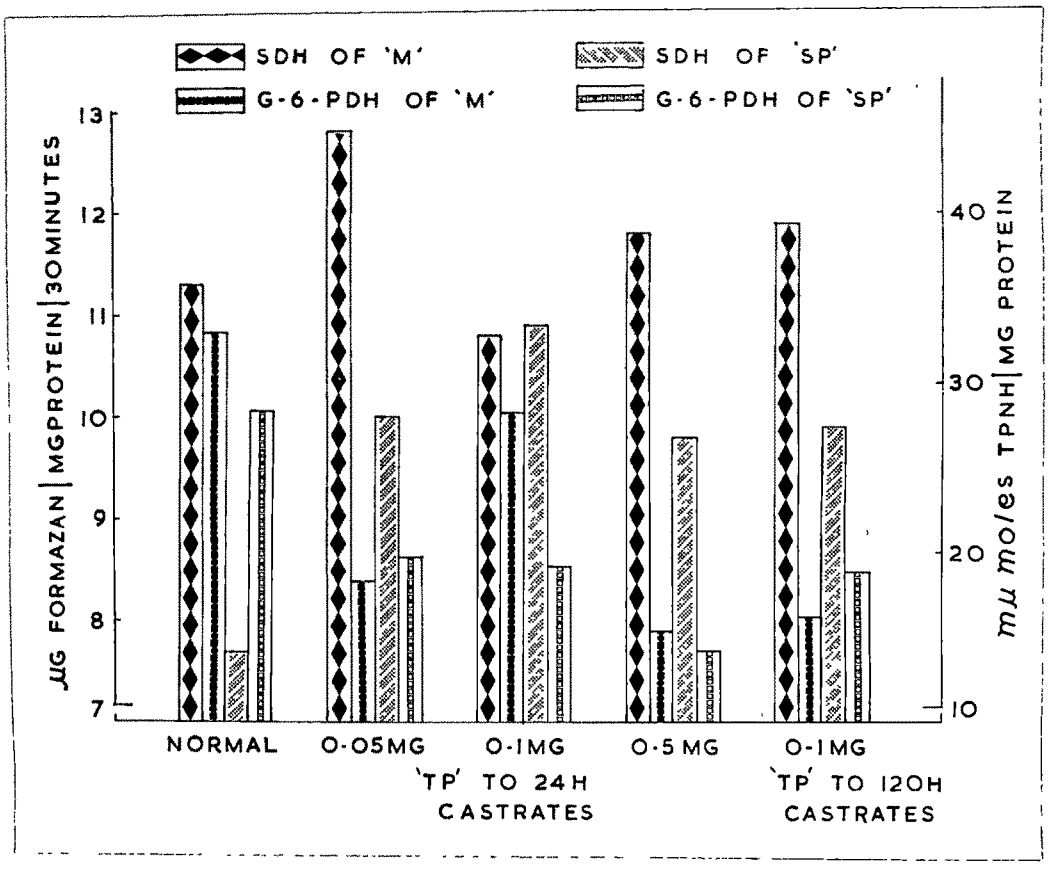

Fig. 4. Representation of the influence of replacement therapy on the levels of SDH and G-6-PDH activities.

M - Median lobe of the liver.

Sp - Spigelian lobe of the liver.

SDH activity expressed as μg Formazan / mg P_2 / 30min

G-6-PDH activity expressed as mμ M TPNH / mg P_2 / min.

DISCUSSION

Phosphorylase activity and glycogen metabolism of a tissue have been positively correlated as early as 1943 by Shapiro and Wertheimer. Phosphorylase occupies a predominent position in the metabolism of carbohydrates, since it is the initial catalytic force in the chain of chemical events that lead to the phosphorylative degradation and utilization of glycogen (Stetten and Stetten, 1960). In the present study an increase in phosphorylase activity was obtained after 24 hrs. of castration and simultaneously the glycogen level was also found to be increased. When these results were considered with regard to the values obtained for sham-operated rats (Table I, Fig. 1), it became obvious that the glycogen content was actually depleted. Similar agreement between the high activity of phosphorylase and low glycogen content have been observed by Cahill et al. (1957) and Palasi and Larner (1960). The phosphorylase activity also increased after 120 hrs. of operation in both the liver lobes. The markedly steep rise observed in teh glycogen content of the liver 24 hrs. after sham-operation is not easy to explain. However, considering a minor reduction in the phosphorylase and SDH activities and a

highly significant decrease in the G-6-PDH activity, it could be suggested that there is a considerable reduction in glycólytic as well as direct oxidative utilization of glucose that is obtainable from hepatic glycogen. This sparing does not account fairly for the striking rise in the concentration of glycogen in liver. It appears that alternative glycogenic/ glucogenic substances may be available and/or glyco-neogenetic stimuli may prevail under these conditions leading to fast glyconeogenesis. However that rise in hepatic concentration of glycogen may be brought about; it is sufficiently clear from the data on 24 hr. and 48 hr. castrates that castration does counteract such an influence. This becomes more apparent when one looks at the values obtained at 120 hrs. after gonadectomy. By this stage almost normal levels of liver glycogen are restored, but rate of its breakdown is stepped up. Alongwith the rise in phosphorylase activity there is a corresponding, though less significant, rise in the activity of G-6-PDH indicative of direct oxidation of glucose.

Injection of 0.05 mg of TP to 24 hr. castrates shows reduction in hepatic glycogen (when compared with

24 hr. castrate levels) with an unduly high rise in phosphorylase activity and marginal elevation of SDH as well as G-6-PDH activity. This indicates a mobilizing effect on liver glycogen with a rise of marginal magnitude in glycolytic and TCA cycle activity and a good rise in direct oxidation of glucose. Elevation in the rate of oxidative metabolism with male hormone administration to orchidectomized calotes has also been observed by Chandola et al. (1974). With higher dose (0.1 mg) of TP though the phosphorylase activity per se exhibits a significant lowering, the rate of glucose utilization, particularly direct oxidation, is improvised. 0.5 mg dose does not show any improvement on that of immediate lower one. It is quite clear that administration of 0.1 mg of TP to 120 hr. castrates influences all the parameters considered here in a better way except in the case of G-6-PDH, which remains sufficiently low (Table I, Fig. 4). This may mean that the effective dose of TP (0.1 mg), that works well on 24 hr. castrates, is incapable of restoring direct oxidation of glucose by the hepatic tissue. From the foregoing account it

could be suggested that castration induces disturbances in the hepatic carbohydrate metabolism within 24 hrs. by lowering proper utilization of glucose through HMP (hexose monophosphate) shunt and to lesser extent through TCA cycle. These fluctuations smoothen as the post-operative interval is increased upto 120 hrs. save for that involving HMP shunt. A single injection of 0.1 mg TP 24 hrs. after castration is capable of counteracting these adverse effects to significant level. It would be much desirable to expect that results from wider and more intense enzymological investigation on very early effects of castration and hormonal replacement only can resolve the problem further about influence of male sex hormones on metabolic patterns in non-target tissue such as the liver of laboratory rats.