CHAPTER III

EFFECT OF GONADAL HORMONES ON THE HEPATIC TRANS AMINASES AND 5'-NUCLEOTIDASE IN THE MALE ALBINO RAT (RATTUS NORVEGICUS ALBINUS)

The transaminase levels were observed to be altered with disturbances in the reproductive functions such as rate of sperm production (azospermia and oligospermia) Povoa, H.Jr (1964). Awapara (1952) has reported on variations in transaminase activity levels in accessory sex organs of male rats after castration. Zuchthygjene (1974) has reported on decrease in GOT levels measurable in the ejeculate of stallions as a result of androgen deprivation. Ikegwuonu et al. (1979) have studied the significance of activity levels of transaminases (GOT/GPT) and 5'-nucleotidase (5'-ribonucleotide phosphohydrolase) in the development of fowl testis and functional maturity. Presence of transaminases and their metabolic importance has been reported by Gregoire et al. (1973) in the case of human vas deferens; and in case of reproductive tissues of the common mouse, by Sreeram Chandramurthy (1979), as well as in various tissues of the albino rats by Haqqi et al. (1979), and Palau et al. (1981). From the work of Harding et al. (1961), it is obvious that various hormonal alterations as well as the process of ageing have noticeable influences on rat hepatic transaminase activity. Roussel et al. (1967) pointed out that GOT activity

exhibits comparatively higher titers than that of GPT in various reproductive organs of male Holstein Friesian bulls. Sex-dependent differences have been noted in the GOT/GPT enzymic levels of blood plasma of giant rabbit (Dragnev et al., 1978). Van Pilsum et al. (1968) have also reported on similar sex-dependent variations in transaminases of rat kidney.

Transaminases are known to bring about transfer of amino groups from specific amino acids and thereby affect the overall flux in amino acid pool. Consequently, this could reflect in the rate and patterns of protein metabolism. That impairment of protein metabolism is a reflection of alteration in nucleic acid metabolism and availability of amino acids is a known fact. Sex hormones are known to influence the protein metabolism and their anabolic effect is well documented (Kochakian, 1964; Leathem, 1970; Mainwaring and Wilce, 1972; Brandes, 1974; Liang and Liao, 1975; Rajalakshmi and Prasad, 1976).

Changes in the protein content of tissues have been correlated with the activity levels of transaminases; especially, in the context of few specific clinical conditions, (like damage to liver and heart tissues), registering higher titers of serum GOT/GPT. These are usually indicative of protein catabolism (De Rittis et al., 1956; Worblewski et al., 1956; Waldman and Borman, 1959; Sacks and Landantin, 1960). On the other hand, it is clear that serum transaminases do

not necessarily reflect hepatic changes as was shown to be the case by El-Hefnawi et al. (1963), who studied the serum transaminases of normal human beings and those afflicted with xeroderma pigmentosm.

Though enough literature is available on various aspects of variations in transaminases and status of protein metabolism in a variety of tissues under different experimentations; it is not possible to offer any definite physiological correlation between these two under all conditions.

Another important enzyme that influences the nucleic acids and consequently protein metabolism, is the 5'-nucleotidase. Alterations in this enzyme activity level get reflected in the status of the nucleotide pool of seminal vesicles of mice (Takuma et al., 1977). Hepatic tissue, skin and accessory reproductive tissues of rat have been shown to possess substantial 5'-nucleotidase activity, (Krolikowska et al., 1966, Fukui et al., 1969; Kim Dae Sung, 1982). Probable relationship of this enzyme activity to development, prepubertal changes, adulthood status as well as aging process has been well documented in recent years (Fukui et al., 1969; Takuma et al., 1977; Chalet et al., 1979; Ikegwuonu et al., 1979).

Since earlier work carried out in this laboratory has shown quantitative alterations in respect of concentrations of protein as well as the nucleic acids of the rat hepatic tissue as a result of immediate influence of the variations

in the male sex hormone (Gangaramani, 1979); it was considered necessary to investigate the possible implications of androgen deprivation and replacement on transaminases and 5'-nucleotidase enzyme activity. It is quite likely that changes in GOT/GPT and 5'-nucleotidase enzyme activity levels would have some bearings on alterations noticeable in the contents of tissue proteins and nucleic acids. Thus, in the present investigation the activity of the hepatic 5'-nucleotidase, GOT and GPT are quantitatively studied in the orchidectomized and subsequently hormone replaced male rats.

MATERIALS AND METHODS

Adult male albino rats (Rattus norvegicus albinus) were used as experimental animals. They were divided into following experimental groups -

(1) Normal

(2) 48-hrs.castrates

(3) 48-hrs. sham operated (reference control), and

(4) 48-hrs.castrates injected with 0.1mg testosterone propionate (TP) and sacrificed after one, two and four hrs.

Enzyme activities assayed were GOT, GPT and 5'-nucleotidase. Total protein content of the tissue was also studied. Other details regarding methods employed are described in Chapter I.

RESULTS :

The results obtained in the present study are shown in Table 3.1 and Figs. 3.1, 3.2, 3.3.

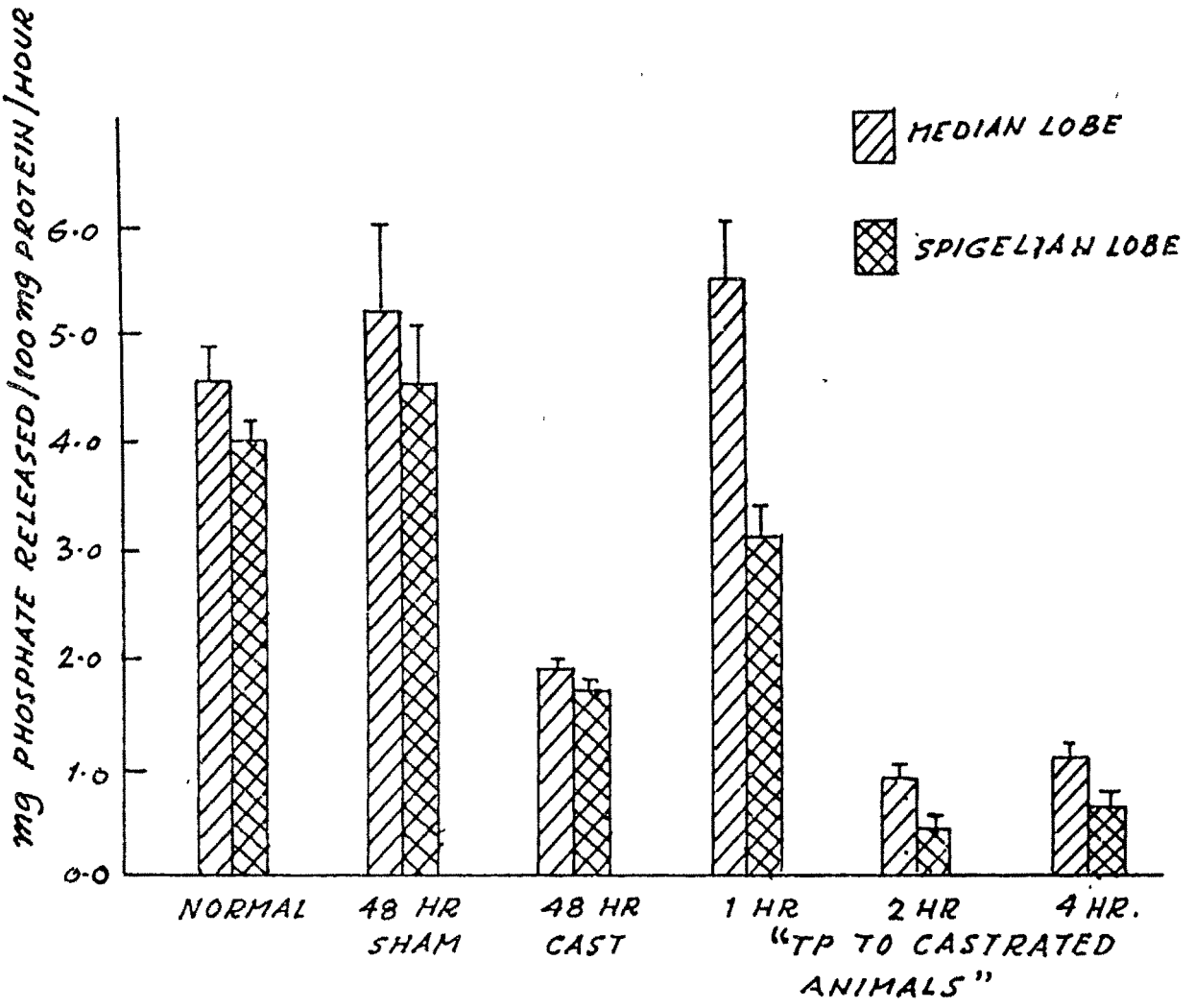
In the normal intact animals, the 5'-nucleotidase activity was found to be quantitatively almost same in both the hepatic lobes (M. and Sp.) studied here. Sham operation did not affect the enzyme activity significantly, while orchidectomy led to marked reduction of 5'-nucleotidase activity level in both the hepatic lobes. Replacement with 0.1mg TP showed an increase in the enzyme activity within the first hour of its administration, compared to 48-hrs castrate levels. A differential response in case of the two liver lobes was quite obvious. The enzyme activity increased to supranormal level in the case of M lobe whereas a less intense recovery was observed in the case of Sp.lobe. The second hour of hormone replacement revealed a drastic reduction, which remained almost at the same level even up to the fourth hour. (Fig:3.3, Table: 3.1).

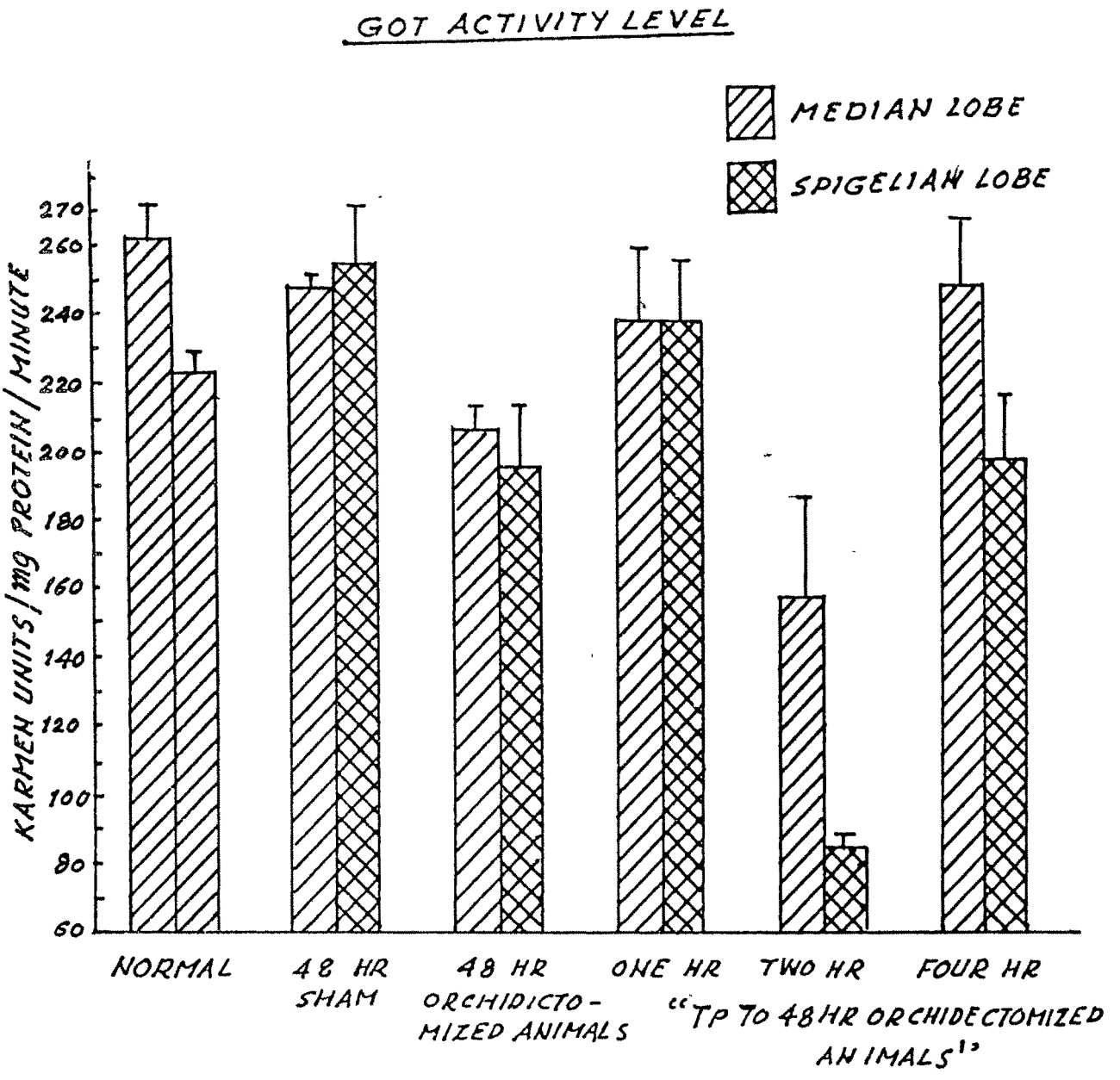
Normally GOT activity levels in both the liver lobes were found to be higher than the GPT activity levels. (Figs: 3.1, 3.2, Table: 3.1). Sham operation did not cause any significant variation in the enzyme activity levels, except for a noticeable decrease in the level of GPT (Fig.3.2) in case of M lobe. 48-hrs orchidectomy led to reduction of GPT level in Sp.lobe, while that of the M lobe was only marginally altered. Contrary to this GOT (Fig.3.1) activity of both the lobes was found to be reduced.

Replacement with 0.1mg TP improved the GOT activity within one hour of its administration in case of both the liver lobes. The readings obtained after 2-hrs of replacement showed significant reduction in case of both the liver lobes but more vividly so, with the Sp.lobe. These levels of GOT activity were lower than even those obtained in castrated rats. Four hrs after TP injection marked recovery was apparent. GPT activity was found to remain mostly unaltered after one hr of TP administration. Thereafter, at two hrs interval the enzyme was significantly reduced in both the liver lobes. At four hrs interval there was significant improvement in both the liver lobes. Both liver lobes (M and SP) exhibited a similar trend of suppression by two hrs and improvement by four hrs of hormone injection.

In case of the M liver lobe the total tissue protein level was found to rise above normal value with sham-operation as well as castration, whereas the Sp.lobe exhibited no alteration with sham operation but did register an increase after castration. With 0.1mg of TP administration, instead of showing reversal of castration effect, a further rise, though slight, but significant was observed at 1 and 2 hrs intervals whereas by four hrs the rise in protein content of both the lobes was remarkably high.

5' NUCLEOTIDASE ACTIVITY LEVELS




GOT ACTIVITY LEVEL
MEDIAN LOBE
SPIGELIAN LOBE
KARMEN UNITS / mg PROTEIN / MINUTE
270
260
240
220
200
180
160
140
120
100
80
60
NORMAL
48 HR SHAM
48 HR ORCHIDICTO-MIZED ANIMALS
ONE HR
TWO HR
FOUR HR
"TP TO 48 HR ORCHIDECTOMIZED ANIMALS"

GPT ACTIVITY LEVEL

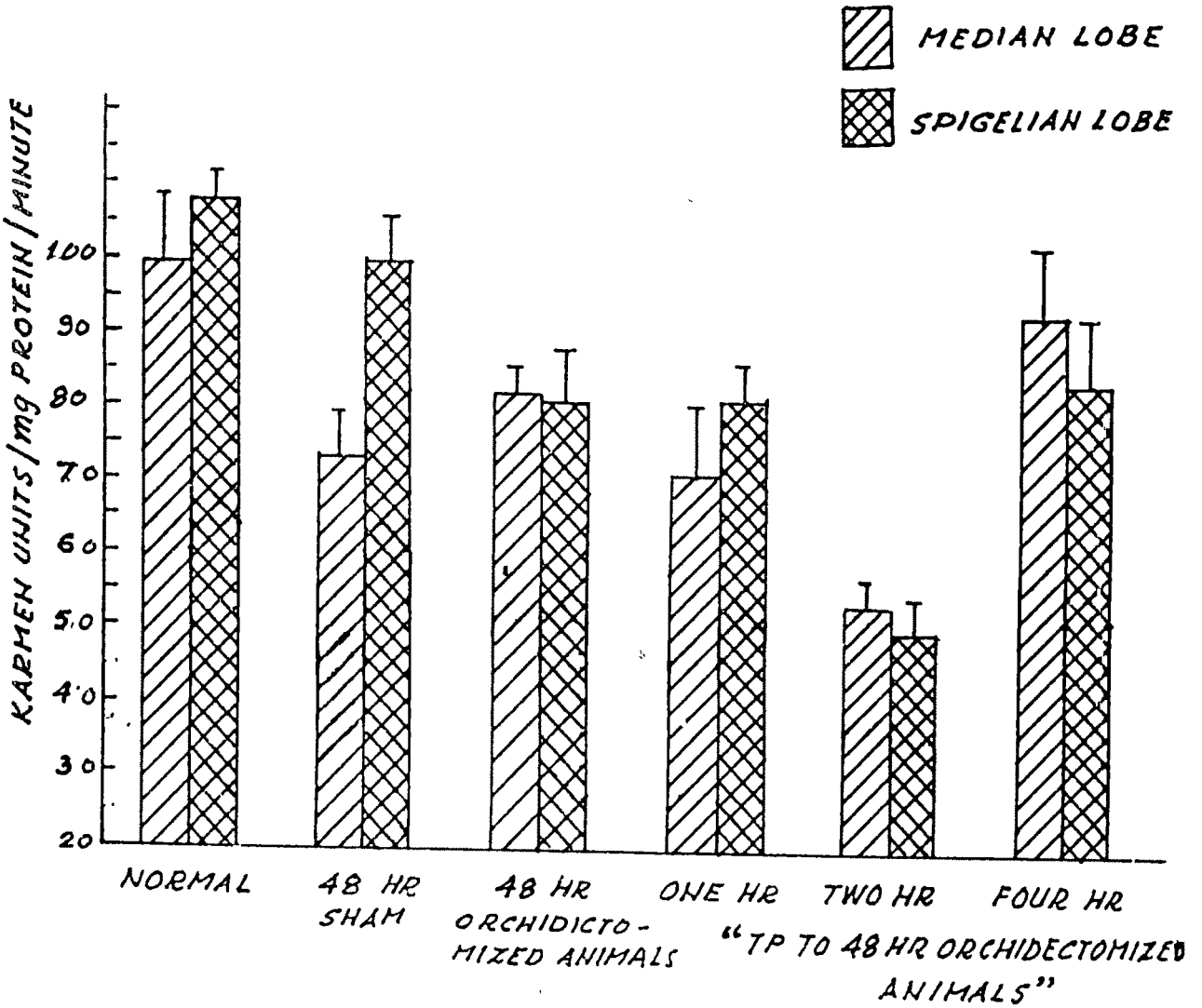


Table 3.1 : Early influence of 48-hrs orchidectomy and replacement with .1 mg TP on hepatic transaminases, 5'-nucleotidase and protein content of male albino rats Rattus norvegicus albinus.

	5'-nucleotidase activity mg phosphate released /100 mg protein/hour		Aspartate transaminase (GOT) activity Karmen units/mg protein/minute		Alanine transaminase (GPT) activity Karmen units/mg protein/minute		Protein content % of fresh tissue weight	
	Median lobe	Spigelian lobe	Median lobe	Spigelian lobe	Median lobe	Spigelian lobe	Median lobe	Spigelian lobe
Normal animals	4.6039 ±0.34	4.0312 ±0.20	262.28 ± 9.9	222.57 ± 5.9	101.03 ± 7.6	107.40 ± 4.4	14.47 ± 0.5	17.55 ± 0.8
48 hrs Sham reference controls	5.2155^{NS} ±1.09	4.5510^{NS} ±0.15	246.82^{NS} ± 4.7	253.14^{NS} ± 16.4	74.7^{NS} ± 6.4	101.15^{NS} ± 7.5	18.44 ± 1.0	16.69 ± 0.98
48 hrs Orchidectomized animals	1.9048**** ±0.19	1.756**** ±0.16	205.28**** ± 6.4	193.47^{NS} ± 18.4	82.51^{NS} ± 3.9	82.756** ± 7.0	18.24 ± 0.2	19.11 ± 0.18
One Hr@	5.5122^{NS} ±0.66	3.1035* ±0.37	236.34^{NS} ± 20.1	236.48^{NS} ± 20.1	71.44^{NS} ± 10.2	82.93** ± 4.6	20.93** ± 0.60	21.01 ± 0.45
Two Hrs@	0.9828**** ±0.05	0.4757**** ±0.01	156.18**** ± 29.2	84.334**** ± 9.1	53.087**** ± 2.6	50.166**** ± 5.0	20.44 ± 0.1	21.36 ± 0.23
Four Hrs@	1.0983**** ±0.13	0.6631**** ±0.04	256.25^{NS} ± 19.4	195.98^{NS} ± 18.41	93.741^{NS} ± 8.9	84.931^{NS} ± 9.9	24.16 ± 0.13	24.06 ± 0.19

* P<.05 ** P<.02 *** P<.01 **** P<.001 NS - Nonsignificant ± S.E.M. of at least eight animals.

@ 48-hrs Orchidectomized animals injected with .1 mg TP and sacrificed after intervals indicated.

DISCUSSION :

That the M as well as Sp lobes of the liver of castrated white rats respond to TP administration earliest by two hrs in a noticeable manner by registering obvious suppression of GOT/GPT levels is apparent from the present results. However, this response seems to be transient as far as transaminases are concerned; since by four hrs, there is a definite trend towards recovery. Kochakian (1959) has also noticed similar reduction in the levels of alanine and gluatamic transaminase activities of rat liver after castration and its restoration by androgen administration. Nevertheless, suppression of 5'-nucleotidase activity evidently persists even upto four hrs. This indicates a transitory variation in the amino acid pool but a comparatively persistent suppressing influence in the case of nucleic acids pool.

Results of the present investigation-considered along with those of Ambadkar and Gangaramani (1980), and also those reported on alterations in DNA/RNA concentrations (Ambadkar et al., 1987) reveal that increase in tissue protein is accompanied, on one hand, by an increase in DNA and maintenance of RNA level near normality, but on the other hand, by decreased GOT/GPT activity and drastic reduction of 5'-nucleotidase activity. This situation is difficult to explain. Giegel (1971) reported increased protein content in rat liver after 48-hrs (two days) of castration. He has also

reported an increase in 3H-uridine uptake in rat liver after castration. Significantly decreased 5'-nucleotidase activity apparently does not have any immediate and direct role in this picture. It is not easy to account for observed rise in tissue protein level unless one is permitted to assume that decreased transaminase activity somehow facilitates enhancement of protein synthesising system. This so-far-unknown mechanism, probably, later gets stimulated by TP administration, despite the observed reduction in RNA, acting on some pre-existing protein synthesising mechanism that is not soluabile. If the effect of castration leads to rise in the tissue protein and DNA, which in itself is not easily understandable, then replacement with TP should lead to restorative influence. Instead, both castration as well as TP administration lead to increased tissue protein levels at such early intervals as employed in the present study.

At this stage, it would be pertinent to recall the now well recognised fact that cyclic AMP acts as a second messenger (Greengard and Kuo, 1970; Beviz et al., 1971; Singhal et al., 1971, Singhal et al., 1974; Jain, 1978; Greengard, 1978). Furthermore, the need for GTP nucleotide in trigerring hormone action via the adenylcyclase system is also reported by Spigel et al., (1981). Elevation of cAMP levels within the target tissue helps in bringing about the specific effect of hormone by activating certain enzymes/ enzyme systems concerned with the response. On basis of these

reports one can assume that the androgenic influence observed in the present study, can be expected to be mediated through induction of alterations in intracellular concentration of cAMP. Possibility regarding occurrence of such changes in the cAMP concentration in hepatic tissue of white rats due to androgen deprivation and subsequent replacement has been suggested by Ambadkar et al. (1986). Additionally, it is known that not only the carbohydrate metabolism but even DNA synthesis is influenced by cAMP concentrations in the rat liver (Short et al., 1975) and RNA and protein synthesis in rat uterri (Hechter et al., 1967; Sharma and Talwar, 1970).

The present investigator, therefore, is compelled to suggest that the early hepatic response to androgen deprivation as well as to administration of TP differs basically from those known so far from reports based on observations made several days after such treatments. In effect, this investigation has raised more querries and has pointed to directions in which further investigations can be carried out. Obviously the parameters studied here do not permit any definite conclusions. Essentially, studies involving assays of enzyme activities concerning DNA replication, RNA and protein synthesis deploying isotopically labelled compounds only, would enable one to throw needed light on the possible differences in very early hepatic response from ones already known.