CHAPTER V

EARLY INFLUENCE OF TESTOSTERONE PROPIONATE ADMINISTRATION TO NORMAL INTACT MALE ALBINO RATS (Rattus norvegicus alninus) ON CERTAIN ASPECTS OF HEPATIC METABOLIC PATTERNS : II TRANSAMINASES, 5'NUCLEOTIDASE AND cAMP SPECIFIC PHOSPHODIESTRASE ENZYME ACTIVITIES

Observations presented in chapter-3 have clearly shown that androgen deprivation and subsequent replacement influences the hepatic enzymic activity levels of GOT, GPT, and 5'-nucleotidase. Additionally, the cAMP specific phosphodiestrase activity level has also been reported to be influenced by circulating androgen levels (Gangaramani, 1979). Similar information regarding the influence of circulating levels of androgen on different enzymes of various tissues are also available (Van Pilsum et al., 1968; Illsleiy et al., 1980; Conti et al., 1981; Pirkko, 1981; Ambadkar et al., 1985, 1986). Now it is well known that c-AMP is an important intracellular substance through the agency of which many hormones appear to manifest their respective actions. c-AMP is elaborated through the mediation of adenylcyclase system. The adenylcyclase has now been shown to be a complex comprised of three components viz.- a receptor, a catalytic unit and a GTP-binding protein 'G' (Spiegal et al., 1981). This 'G' unit is important for triggering hormone action and any clinical abnormality involving this unit may lead to altered c-AMP concentration. 'G' unit functions only in the presence of GTP nucleotide.

The functional interrelationship between the c-AMP concentration and androgen action is well known (Singhal, 1974). It has also been worked out in our laboratory that androgen (TP) does influence the level of c-AMP, which in turn causes changes in hepatic metabolic patterns. Androgens are known to facilitate activity levels of many enzymes involved in carbohydrate metabolism by elevating c-AMP levels of the seminal vesicles and the ventral prostate gland. (Singhal and Valadares, 1968; Singhal et al., 1968; Santti and Villee 1971; Singhal et al., 1971; Mangan et al., 1973).

During the initial observations on impact of orchidectomy on the enzyme activities of 5'-nucleotidase and transaminases (Chapter-3) were found responsible for changes in the nucleotide as well as amino acid pools of the hepatic gland. Concomitant changes in protein content and nucleic acids also have been already reported (Ambadkar et al., 1987). Thus, such alterations in the levels of GOT, GPT, 5'-Nucleotidase and c-AMP-specific phosphodiesterase enzyme activity in hepatic tissue by necessity, demanded further investigation. Hence, the major interest of the present investigation was to find out the early influence of exogenously administered circulating androgen, on these enzyme activities in the liver of intact albino rats. It is pertinent here to mention that measurement of c-AMP-specific phosphodiesterase activity is an indirect pointer to the level of c-AMP. As the phosphodiesterase instantaneously

hydrolyses intracellularly elaborated c-AMP; any change in this enzyme activity would reflect on c-AMP concentration of the tissue. Since during the course of the present work, due to lack of appropriate facilities it was not possible to directly assess the c-AMP content; the level of c-AMP-specific phosphodiesterase instead was assayed as an indirect indicator of intracellular c-AMP concentrations. It was thought desirable to study both the dose-dependent as well as time-dependent responses of the median and Spigelian lobes of liver.

MATERIALS AND METHODS :

Adult male albino rats (Rattus norvegicus albinus) weighing 120-160 gms were employed as experimental animals. Testosterone propionate (TP) was injected intramuscularly as a single dose per animal before sacrifice. Three different experimental groups were injected with 0.1, 0.25 and 0.5 mg of TP and these animals were sacrificed after 30, 60, 90, and 120 minutes. c-AMP-specific phosphodiesterase, 5'-nucleotidase, GOT and GPT activity levels were investigated in the hepatic tissue (median and Spigelian lobe). The details of experimental methods employed were as described in Chapter-1.

RESULTS :

The results obtained during the course of present investigation are represented in Tables 5.1 - 5.4 and Figs 5.1 - 5.4.

From table 5.4 it is obvious that administration of TP, in general, leads to an increase in the c-AMP-specific phosphodiesterase activity in both the liver lobes. It could, however, be pointed out that with 0.1 mg TP dose the M-lobe showed persistent step-wise increase as the time interval was prolonged. On the other hand, the Sp.lobe registered a slight increase in the enzyme activity up to 60 minutes and thereafter exhibited a tendency towards recovery by 90 minutes. Nevertheless, it did respond, like M-lone, by exhibiting a very significant rise at 120 minutes. Such a tendency towards recovery was noticed in the case of M-lobe with 0.25 mg TP dose as late as 120 minutes. With 0.5 mg TP dose a sharp increase in enzyme activity in case of both the liver lobes was apparant at 30, 60 and 90 minute intervals, but by 120 minutes a discernible reduction of enzyme activity was noticed.

With respect to 5'-nucleotidase enzyme activity levels in both the liver lobes under study (Table 5.1) it could be said that TP administration did not lead to alterations worth noting. It could be pointed out here that though there were fluctuations in the enzyme activity in both the lobes, no definite pattern was apparent. However, a noteworthy point was that the Sp.lobe was influenced by TP administration comparatively at lower dose and early time interval (0.1 mg TP-60 minutes) than the M-lobe (0.5 mg TP-60 minutes).

From the perusal of tables 5.2 and 5.3 it is evident that administration of TP to normal intact animals leads to a vide range of alterations in the activities of transaminases and that the patterns of fluctuations are also very varied.

With reference to GOT enzyme activity it was apparent that 0.1 mg of TP dose induces immediate and drastic reduction within first 30 minutes in the case of M-lobe (10.8% of the normal level) and also Sp.lobe (32.8% of the normal level). In marked contrast to this, by 60 minutes the enzymic levels in both the liver lobes were found to be raised almost close to normal values, thereby exhibiting almost total recovery. However, both the liver lobes, once again exhibited a reduction of enzyme activity by 90 minutes that got futher depressed very significantly to 25% of normal level in M-lobe and 20% in the Sp.lobe. Strangely enough, the two higher doses i.e. 0.25 mg and 0.5 mg induced in both the liver lobes almost 1.5 to 2 fold and more than 2 fold increase in the enzyme activity respectively within first 30 minutes only. Further changes at different intervals, as stated earlier, did not exhibit any pattern worth mentioning, except for the fact that the values were more or less fluctuating around normal levels only.

Almost similar trend could be noticed in respect of the GPT enzyme activity with reference to M-as well as Sp-lobes of liver. A particular notice should be taken

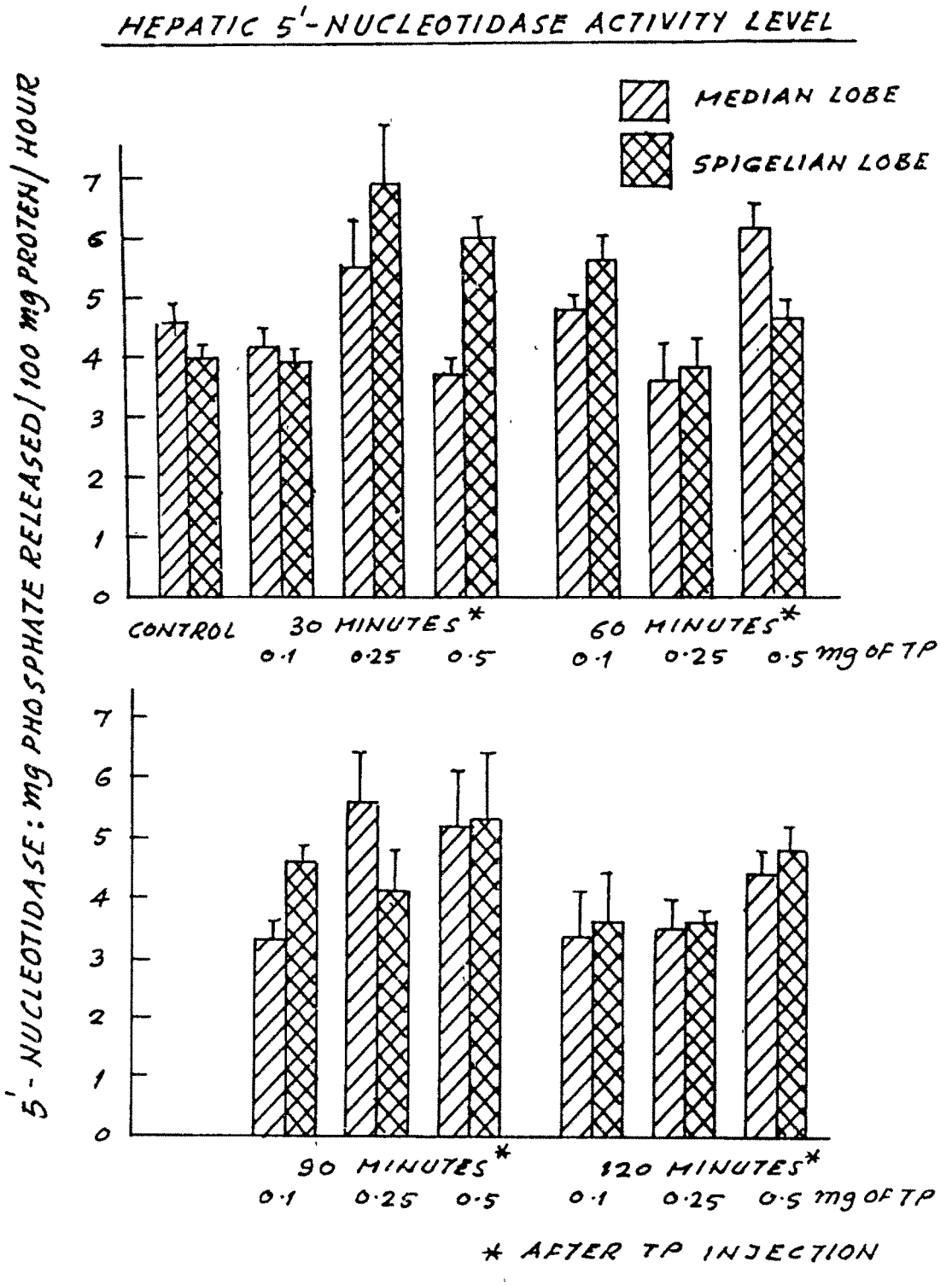
HEPATIC 5'-NUCLEOTIDASE ACTIVITY LEVEL
MEDIAN LOBE
SPIGELIAN LOBE
5'-NUCLEOTIDASE: mg PHOSPHATE RELEASED/100 mg PROTEN/HOUR
0
1
2
3
4
5
6
7
CONTROL
30 MINUTES*
0·1
0·25
0·5
60 MINUTES*
0·1
0·25
0·5 mg of TP
90 MINUTES*
0·1
0·25
0·5
120 MINUTES*
0·1
0·25
0·5 mg of TP
* AFTER TP INJECTION

Table 5.1 : Immediate influence of exogenous TP administration to white rats, with respect to hepatic 5'-nucleotidase activity level

Post operative intervals	Normal animals administered with TP					
	0.1 mg		0.25 mg		0.5 mg	
	M	SP	M	SP	M	SP
30 minutes	4.22*	3.97	5.53****	6.93****	3.79*	6.04*****
	±0.37	±0.25	±0.8	±1.1	±0.34	±0.3
60 minutes	4.84**	5.69****	3.69*	3.86	6.14*****	4.68*
	±0.22	±0.44	±0.3	±0.5	±0.51	±0.39
90 minutes	3.31**	4.60**	5.06	4.1	5.2*	5.3*
	±0.33	±0.32	±0.8	±0.6	±1.0	±1.1
120 minutes	3.42	3.67	3.56*	3.59	4.44*	4.87*
	±0.78	±0.8	±0.5	±0.29	±0.4	±0.43

Normal 5'-nucleotidase activity level of the hepatic tissue mg phosphate released/100 mg protein/hour

M (Median lobe)	Sp (Spigelian lobe)
4.60 ±0.34	4.03 ±0.25

* $P < .05$ ** $P < .02$ *** $P < .01$ **** $P < .001$ ***** $P < .0005$

± S.E.M. of at least eight animals.

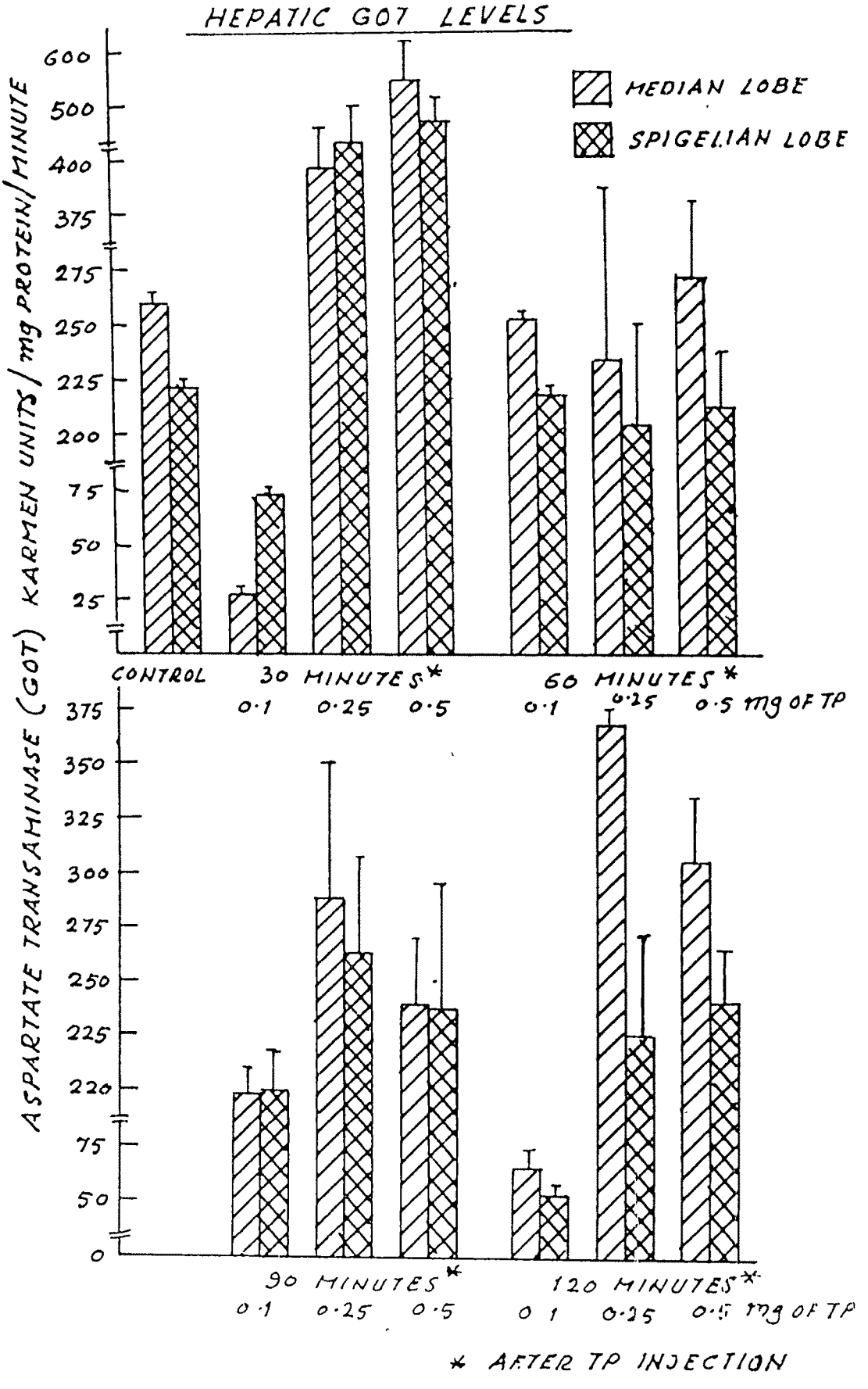
HEPATIC GOT LEVELS
MEDIAN LOBE
SPIGELIAN LOBE
ASPARTATE TRANSAMINASE (GOT) KARMEN UNITS / mg PROTEIN / MINUTE
600
500
400
375
275
250
225
200
75
50
25
CONTROL
30 MINUTES*
0·1
0·25
0·5
60 MINUTES*
0·1
0·25
0·5 mg of TP
375
350
325
300
275
250
225
220
75
50
0
90 MINUTES*
0·1
0·25
0·5
120 MINUTES*
0·1
0·25
0·5 mg of TP
* AFTER TP INJECTION

Table 5.2 : Immediate influence of exogenous TP administration to white rats, with respect to hepatic GOT activity level

Post operative intervals	Normal animals adminstered with TP					
	0.1 mg		0.25 mg		0.5 mg	
	M	SP	M	SP	M	SP
30 minutes	28.44 ± 2.7 *****	73.2 ± 6.3 *****	391.8 ±62.9 ***	433.5 ±60.7 *****	548.5 ±73.3 *****	470.6 ±41.2 *****
60 minutes	253.206 ± 5.7	210.52 ± 4.6	231.5 ±94.5	209.8 ±50.7	270.4 ± 26.8	219.5 ± 18.9
90 minutes	152.46 ± 8.2 ****	157.12 ±14.6 ****	281.3 ±59.9	264.3 ±52.06 **	237.7 ± 26.1	235.63 ± 41.8
120 minutes	65.49 ± 7.7 *****	52.19 ± 4.16 *****	369.1 ± 66.4 *	224.5 ± 43.9	306.7 ± 31.4 *	240.8 ± 25.8

Normal GOT activity level of the hepatic tissue

Kermen units/mg protein/minute

M (Median lobe)	Sp (Spigelian lobe)
262.28 ± 9.9	222.57 ± 5.9

* P < .05 ** P < .02 *** P < .01 **** P < .001 *****P<.0005

± S.E.M. of at least eight animals.

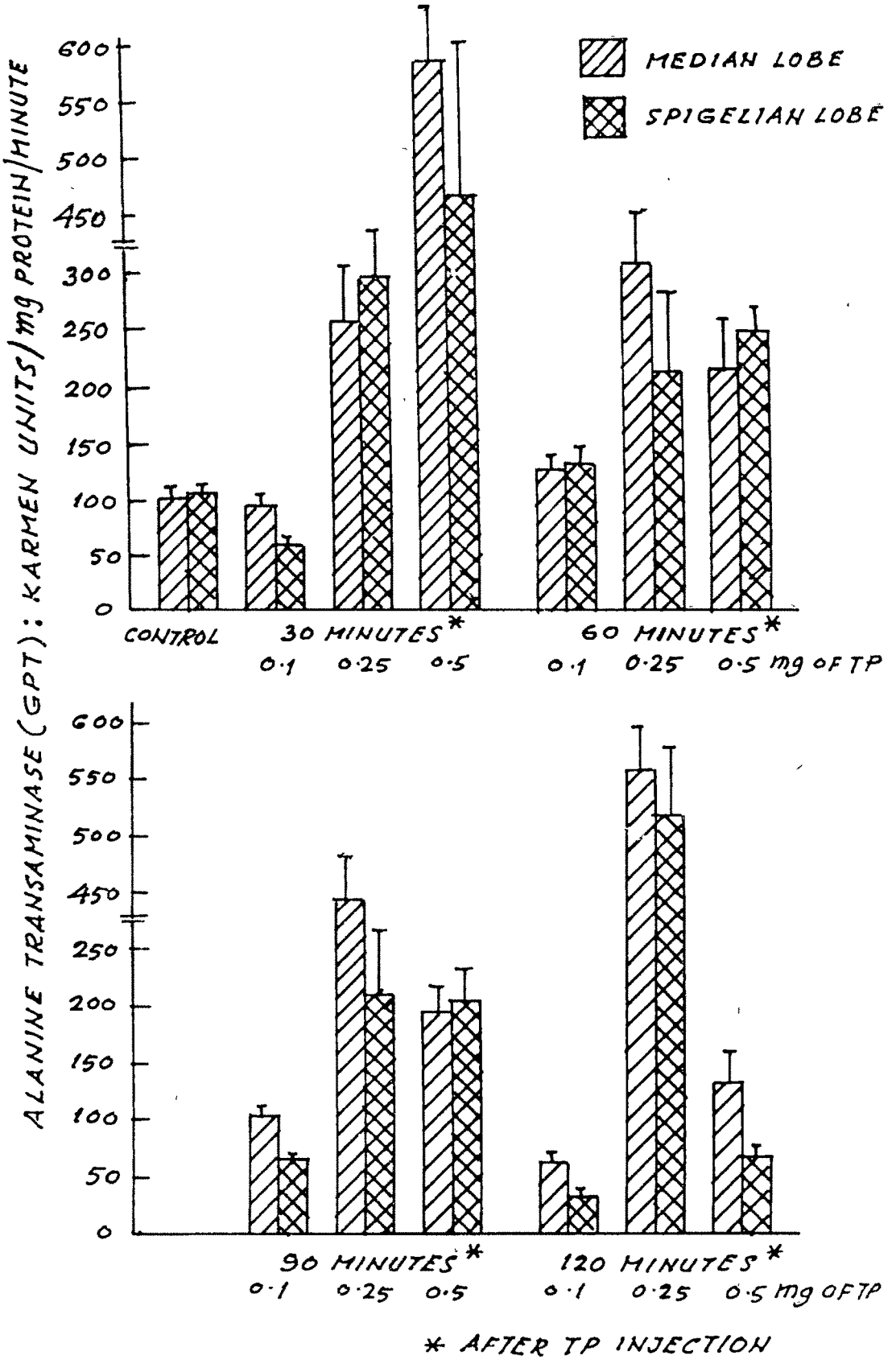
HEPATIC GPT LEVELS
MEDIAN LOBE
SPIGELIAN LOBE
ALANINE TRANSAMINASE (GPT): KARMEN UNITS/mg PROTEIN/MINUTE
600
550
500
450
300
250
200
150
100
50
0
CONTROL
30 MINUTES*
0·1 0·25 0·5
60 MINUTES*
0·1 0·25 0·5 mg OF TP
90 MINUTES*
0·1 0·25 0·5
120 MINUTES*
0·1 0·25 0·5 mg OF TP
* AFTER TP INJECTION

Table 5.3 : Immediate influence of exogenous TP administration to white rats, with respect to hepatic GPT activity level

Post operative intervals	Normal animals administered with TP					
	0.1 mg		0.25 mg		0.5 mg	
	M	SP	M	SP	M	SP
30 minutes	91.51 ± 8.5	57.5 ± 4.4	253.1 ± 51.5 ****	295.8 ± 40.2 *****	587.35 ± 82.8 *****	462.7 ± 153.2 *****
60 minutes	126.5 ± 11.3 **	130.05 ± 12.3 ****	435.3 ± 123.1 ***	137.1 ± 21.7 ***	212.8 ± 48.3 **	145.2 ± 20.4 *
90 minutes	112.37 ± 7.5 *	65.798 ± 3.8	308.6 ± 49.4 ****	211.5 ± 69.3 **	194.1 ± 37.6 *	214.9 ± 36.2 ***
120 minutes	61.30 ± 12.7	31.09 ± 1.9	559.5 ± 96.4 *****	486.5 ± 88.2 *****	137.5 ± 27.2 *	69.5 ± 11.9 *

Normal GPT activity level of the hepatic tissue

Karmen units/mg protein/minute

M (Median lobe) 101.03 ± 7.6

Sp (Spigelian lobe) 107.40 ± 4.4

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* $P < .05$ ** $P < .02$ *** $P < .01$ **** $P < .001$ ***** $P < .0005$

± S.E.M. of at least eight animals.

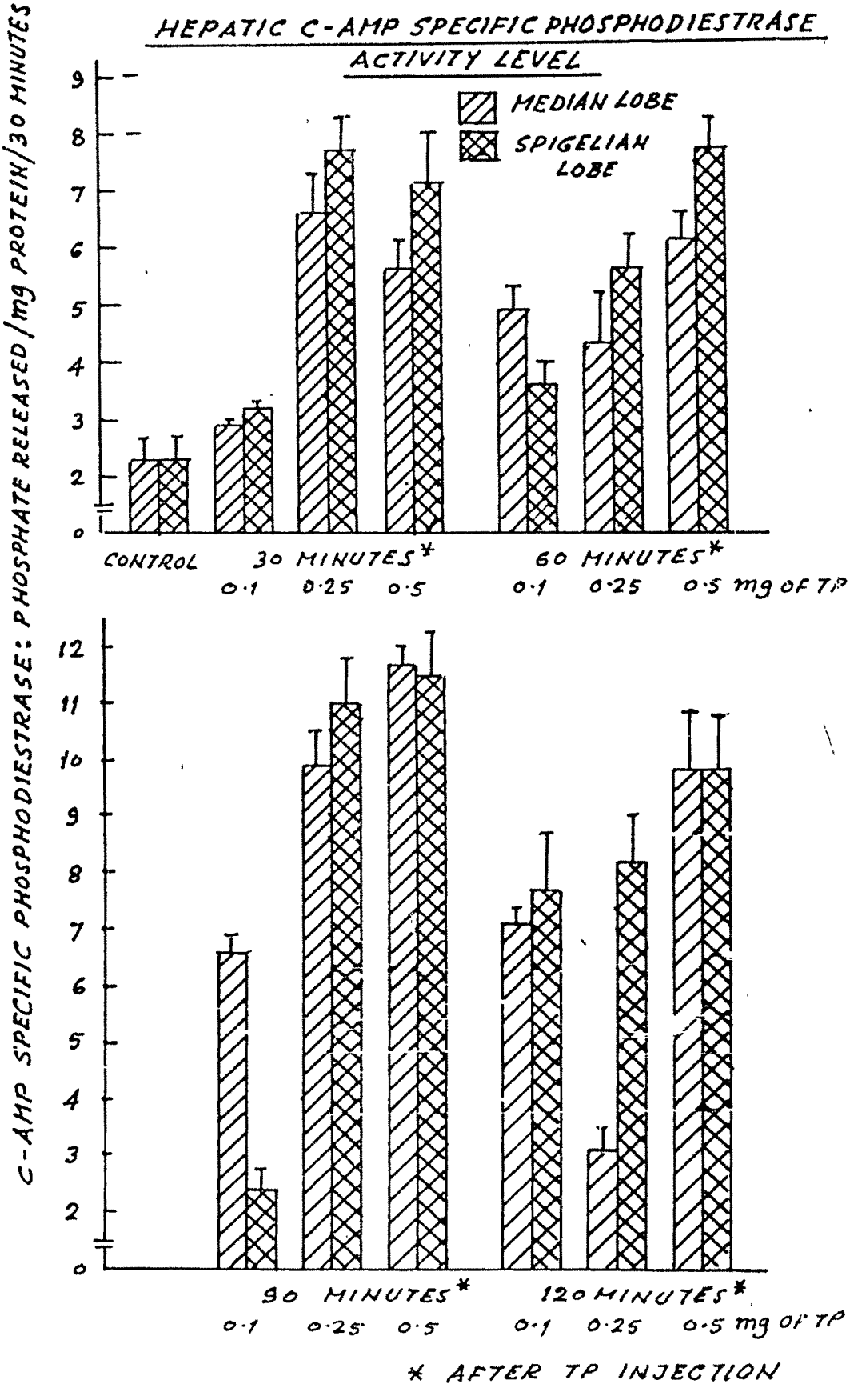
HEPATIC C-AMP SPECIFIC PHOSPHODIESTRASE ACTIVITY LEVEL
MEDIAN LOBE
SPIGELIAN LOBE
C-AMP SPECIFIC PHOSPHODIESTRASE: PHOSPHATE RELEASED /mg PROTEIN/30 MINUTES
CONTROL
30 MINUTES*
60 MINUTES*
0·1 0·25 0·5 0·1 0·25 0·5 mg OF TP
90 MINUTES*
120 MINUTES*
0·1 0·25 0·5 0·1 0·25 0·5 mg of TP
* AFTER TP INJECTION

Table 5.4 : Immediate influence of exogenous TP administration to white rats, with respect to hepatic c-AMP-specific phosphodiesterase activity level

Post operative intervals	Normal animals administered with TF					
	0.1 mg		0.25 mg		0.5 mg	
	M	SP	M	SP	M	SP
30 minutes	2.94* ±0.01	3.28** ±0.01	6.59***** ±0.7	7.71***** ±0.61	5.67***** ±0.5	7.10***** ±0.9
60 minutes	4.93***** ±0.041	3.69*** ±0.041	4.33***** ±0.91	5.66***** ±0.6	6.15***** ±0.51	7.7***** ±0.52
90 minutes	6.64***** ±0.049	2.49 ±0.049	9.92***** ±0.6	11.0***** ±0.83	11.79***** ±0.47	11.51***** ±1.1
120 minutes	7.17***** ±0.31	7.72***** ±1.1	3.11* ±0.4	8.23***** ±0.88	9.82***** ±1.0	9.83***** ±0.9

Normal c-AMP-specific phosphodiesterase activity level of the hepatic tissue phosphate released/ mg protein/30 minutes

M (Median lobe)	Sp (Spigelian lobe)
2.34 ± 0.041	2.32 ± 0.041

* P < .05 ** P < .02 *** P < .01 **** P < .01 ***** P < .0005

± S.E.M. of at least eight animals.

regarding the degree of increase in enzyme activity at 30 minutes interval with 0.25 mg as well as 0.5 mg TP doses being almost double that found in case of GOT. Another noteworthy fact was that the lowest as well as the highest dose of TP led to significant depression of enzyme activity by 120 minutes (except M-lobe with 0.5 mg TP) whereas the median dose of 0.25 mg TP resulted into remarkable 4.5 to 5.5 fold increase in enzyme activity at this interval.

DISCUSSION :

The over all picture that emerged from the data presented in this chapter depicts the following :-

(a) increased c-AMP-specific phosphodiesterase,

(b) marginal rise in 5'-nucleotidase enzyme activity, and

(c) significant rise in the levels of alanine and aspartate transaminase activity.

These results could very aptly be discussed in the light of observations reported in the Chapters 3 and 6.

Alterations in c-AMP levels due to androgen administration to orchidectomized rats has been reported in accessory sex organs (Singhal et al., 1971) and in the hepatic tissue (Gangaramani, 1979). According to Jhon et al. (1973) testosterone had no significant effect on c-AMP in the liver, oviduct, muscle and blood of intact fowl. However, the present investigation clearly points out that there is

an early influence of TP administration on the c-AMP levels of hepatic tissue of rats. Increased levels of c-AMP-specific phosphodiesterase activity in both the liver lobes at almost all the time intervals under consideration and the three arbiterily chosen TP doses indicated that the c-AMP concentration of hepatic tissue is markedly reduced. Further, there are few reports which deny the possibility of adenylcyclase activity being stimulated by androgens (Rosenfeld and O'Mally, 1970; Liao et al., 1971; Mangan et al., 1973). It is known that c-AMP-specific phosphodiesterase activity rapidly hydrolizes the intracellular c-AMP concentrations. Recently hormonal influence on phosphodiesterase activity levels has been documented by Gangaramani (1979) and Conti et al. (1981). c-AMP is known for its intracellular intermidiary action, through the agency of which many hormones are known to act (Barker and Warren, 1966; Hilf et al., 1972; Mangan et al., 1973). Thus, the observed reduction in the c-AMP concentration is suggestive of lowering inhibition of protein synthesis; and indeed a markedly reduced total protein content in hepatic tissue was obtained during the course of the present study (Chapter-6). Further, it is interesting to refer to the work of McManus et al. (1972), wherein the authors clearly state that c-AMP concentration positively increases immediately prior to DNA synthesis of partially hepatectomized or hormone infused rats. According to Short et al. (1975) hepatic DNA synthesis in rats is influenced by c-AMP

concentration. In consonance with these observations the nucleic acid (RNA/DNA) contents were found to be drastically reduced. During the course of present investigation it was observed that though the administration of TP to intact animals influenced the 5'-nucleotidase activity in a marginal way, yet sporadic spurts in hepatic 5'-nucleotidase enzyme activity were noted. These may indirectly contribute to catabolism of nucleic acids and/or may lead to unavailability of specific nucleotides needed for nucleic acid synthesis. In this context it is worthwhile noting the work of Spigel *et al.*, (1981) which has pointed out the importance of GTP-nucleotide in triggering hormone action as far as adenyl-cyclase system is concerned.

Higher enzymic levels of aspartate and alanine transaminases are clinically well known indicators of wasting of tissue proteins (Worblewski *et al.*, 1955, '56; De Rittis *et al.*, 1956; Waldman and Borman, 1959; Sacks and Landantin, 1960; Harper, 1975, '77) and consequently, alterations in the status of general amino acid pool, significantly enhanced transaminase levels, accompanied by lowered hepatic protein levels obtained during the present work corraborate these observations.

Hence, one can suggest that early influence of TP administration to normal intact albino rats induces conditions akin to hepatic cell injury which are known to be accompanied

by enhanced transaminase activity and protein degradation. Further, this response greatly depends on dose levels of TP as well as the lapse of time after exogenous administration. 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 mechanisms of early influence of exogenous androgen on hepatic metabolic response and as to how and why this differs from those reported on the basis of late longterm effects.