## CHAPTER IV

## EARLY INFLUENCE OF TESTOSTERONE PROPIONATE ADMINISTRATION TO NORMAL INTACT MALE ALBINO RATS (*Rattus norvegicus albinus*) ON CERTAIN ASPECTS OF HEPATIC METABOLIC PATTERNS

### I CARBOHYDRATE METABOLISM

Recent trends in clinical practice of frequent administration of natural as well as various synthetic sex hormones to deal with fertility disturbances, carcinomas and some times as antifertility drugs (Bloom *et al.*, 1963; Bloom, 1968,'73; Hogarth, 1978) do provide enough cause for calling special attention today to take a more than casual view of the situation; particularly with reference to overall body welfare. It is highly necessary to be forewarned of the metabolic influences such hormones may have as side-effects. It has been established that androgen deprivation and subsequent replacement causes disturbance within a short time in hepatic metabolic sensitivity to androgen by 48-hour post-orchidectomy interval (Gangaramani, 1979). Further, it was also observed in this laboratory that hypoglycemia sets in within the first four hours of TP administration to 48-hour orchidectomized animals in contrast to hyperglycaemic condition obtainable prior to TP injection (Ambadkar and Gangaramani 1982; Ambadkar *et al.*, 1985). The early effect

of androgens on glucose metabolism is evident from the earlier results and the present investigation.

Keeping in view the literature available on the influence of male sex steroids (Bergamini, 1969; Bergamini, 1972; Legrele and Sutter, 1972; Demer and Jacobs, 1973; Matute and Kalkhoff, 1973; Bergamini, 1975; Pirkko et al., 1975; Froberg et al., 1975; Karl and Tisell, 1976; Winitton and Hems, 1976; and Pirkko, 1981) and the results of our earlier investigations, the present study was conducted to assess immediate influence of a single intramuscular injection (i.m.) of TP to normal intact male white rats - Rattus norvegicus albinus, on patterns of hepatic carbohydrate metabolism. Further more hormones are also known to induce rapid influences on various metabolic pathways of different tissue/systems (Geigle, 1971; Teng and Hamilton, 1970a; Koths et al., 1972; Spooner and Goroski, 1972; Chowdhury et al., 1980; Engel et al., 1980; Peek and Watkins, 1980; Summerville and Schwartz, 1981; Vingent et al., 1981; Knudsen et al., 1980; Illsley et al., 1980). Since steroids are known to have qualitatively differential effects with higher dosages - (McPherson and Mahesh, 1969; Gray et al., 1979; Lax et al., 1983), it was also thought necessary to carry out a dose-dependent influence of androgen on hepatic tissue. Taking this into consideration three different arbitrarily chosen doses of TP viz., - 0.1 mg, 0.25 mg, and 0.5 mg were administered in the course of this study.

Material and Methods :

Normal intact white rats (120 - 160 gm body weight) were employed for experimental purpose. They were given a single i.m. injection of 0.1 mg, 0.25 mg and 0.5 mg of TP per animal and sacrificed after 30, 60, 90 and 120 minutes. Blood samples were collected from jugular vein section and plasma samples obtained by centrifugtion were utilized for glucose estimation.

The plasma glucose level, hepatic glycogen content as well as hepatic G-6-Pase, phosphorylase and glycogen synthetase activities were quantitatively assayed in both the liver lobes (M and Sp), according to the methods described in Chapter-I.

RESULTS :

The results obtained in the present study are represented in Tables 4.1-4.5 and Figs.4.1-4.5.

The hepatic glycogen content was found to be reduced within 30 minutes of 0.1 mg hormone injection in case of both the liver lobes. Decreased glycogen levels were found to persist right upto 120 minutes (Table 4.2). Similarly, 0.5 mg TP administration also brought about significant reduction in liver glycogen at 30 and 60 minutes intervals. Later, at 90 minutes, the glycogen content was seen to rise remarkably above normal level and to remain so upto 120 minutes in case of the median lobe only. Whereas, in the

Spigelian lobe by 90 minutes the level was found to recover to near normality (Table 4.2) and to register a fall by 120 minutes. In contrast to these two dosages, that of 0.25 mg TP dose lead to no significant alterations in both the liver lobes at all intervals upto 120 minutes of TP injection except for slight transitional reduction at 60 minutes in the Spigelian lobe and at 90 minutes in the median lobe.

Glucose-6 Phosphatase (G-6-Pase) activity level was seen to register a trend towards increase of varying degrees with all three dosages of TP at different intervals; except for the Spigelian lobe with 0.25 mg dose at 30 and 60 minutes. Maximum increase at 30 minutes was noted in the median lobe with 0.1 mg dose and with 0.5 mg dose in the Spigelian lobe. Similar increase, but of greater magnitude, was apparent in the case of both the liver lobes at 90 and 120 minutes with 0.5 mg TP. A point worthy of mention was overall general recovery towards normal levels that was discernible in both the lobes with 0.1 and 0.25 mg dosages of TP administration by 120 minutes. As against this, 0.5 mg TP injection increased the levels of G-6-Pase activity at 90 minutes interval and continued to elicit further remarkable increase in the enzyme activity by 120 minutes instead of recovery, as was noted with other dose levels (Table 4.3).

Glycogen synthetase activity could be said, in general way, to get suppressed by TP administration in normal intact animals. The overall suppression of this enzyme activity showed a certain pattern. There was an immediate reduction in enzyme activity initially, which later on exhibited recovery to varying degrees depending on lapse of time as well as on dosage levels. Considering the temporal response, it could be seen that by 60 minutes interval suppression was maximally obvious with all the three dosages of TP injection, which continued to be so incase of both the liver lobes even at 90 minutes but with respect to only 0.1 mg dose. Recovery towards normality was clearly discernible in both the liver lobes with all the three dosages of TP administration.

As regards the response to gradually increasing doses it could be said that it was dose-dependent. Maximum suppression being noted with respect to 0.1 mg dose that was lesser with 0.25 mg and the least so with 0.5 mg injection. Temporarily, the suppression of glycogen synthetase enzyme activity was noted to be maximum at 60 minutes interval in an overall way. Recovery too, was maximally observable in case of 0.1 mg TP dose and least so with 0.25 mg TP dose. (Table 4.4).

It could, therefore, be said that 0.1 mg TP dose apparently elicits the maximum response from both the liver lobes. This dose appears to be physiologically most

significant as far as the problem under consideration is concerned.

Phosphorylase activity was found to be stimulated by 0.1 as well as 0.25 mg of TP resulting into higher enzyme activity levels. In a marked contrast, 0.5 mg TP dose induced a drastic reduction of hepatic phosphorylase activity which was also obvious at 60 minutes interval. Reduction in enzyme activity was registered at 60 minutes interval with 0.1 mg and 0.25 mg TP dose levels. Maximum suppression of enzyme activity in case of Spigelian lobe was recorded at 60 minutes with 0.1 mg TP dose and in case of median lobe at the same interval but with 0.25 mg dose level of TP administration. Maximum enhancement of phosphorylase enzyme activity was observable with 0.25 mg TP dose as early as 30 minutes after hormone administration in both the liver lobes. As compared to normal phosphorylase activity levels the increase was of higher degree in case of Spigelian lobe.

Trends in recovery of enzyme activity from suppression apparent between 60-90 minutes, depending on dose level of TP administered, exhibited a pattern of increasingly earlier influence as the dose levels increased and was obvious by 90 minutes, in the case of highest dose administered (0.5 mg). On the other hand, with 0.1 mg of TP, the lowest dose employed, exhibited a comparatively steady pattern with time lapse. However, it should be pointed out here that, the response of Spigelian lobe exhibited greater sensitivity

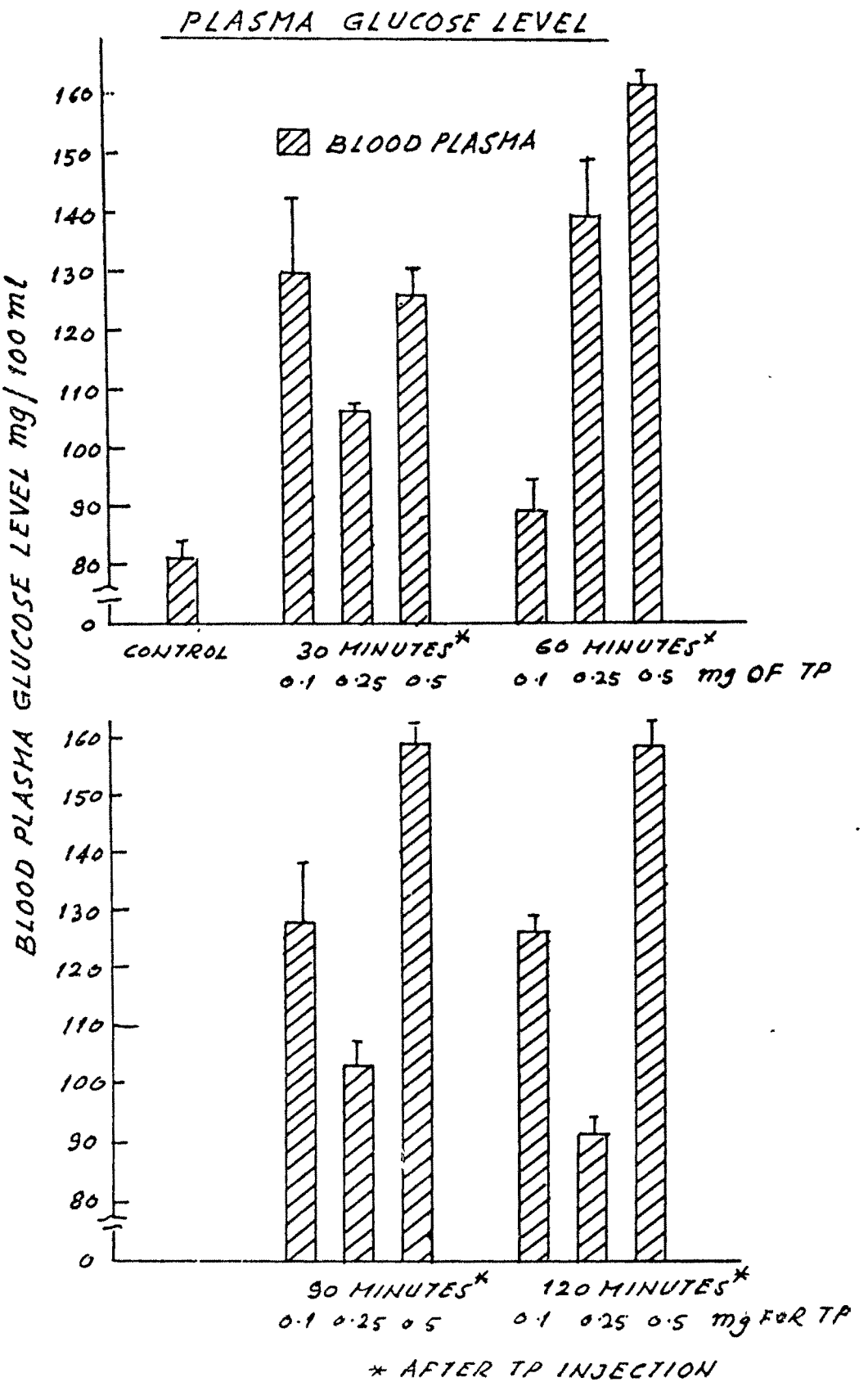

PLASMA GLUCOSE LEVEL
BLOOD PLASMA
BLOOD PLASMA GLUCOSE LEVEL mg/100 ml
160
150
140
130
120
110
100
90
80
0
CONTROL
30 MINUTES*
0·1 0·25 0·5
60 MINUTES*
0·1 0·25 0·5 mg OF TP
90 MINUTES*
0·1 0·25 0·5
120 MINUTES*
0·1 0·25 0·5 mg FOR TP
* AFTER TP INJECTION

Table 4.1 : Immediate influence of exogenous TP administration to white rats, with respect to plasma glucose levels

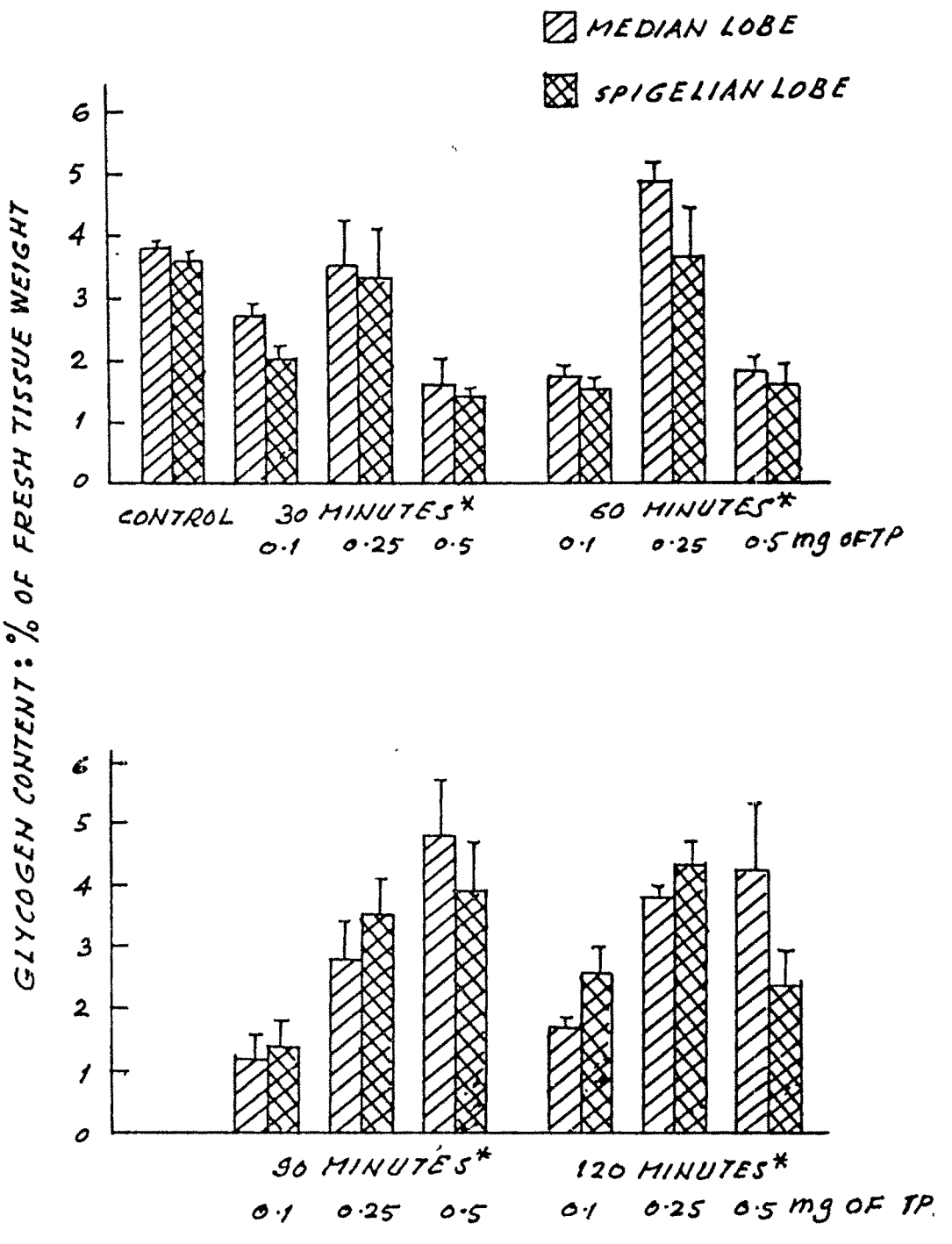
| Post-injection intervals | Normal animals injected with TP | | |
|---|---|---|---|
| | 0.1 mg | 0.25 mg | 0.5 mg |
| 30 minutes | 130.1***** ±12.5 | 106.6***** ± 0.8 | 126.40***** ± 4.1 |
| 60 minutes | 89.83 ± 9.3 | 139.8***** ± 9.5 | 161.00***** ± 3.4 |
| 90 minutes | 129.94***** ±15.6 | 103.8***** ± 3.3 | 160.7***** ± 3.2 |
| 120 minutes | 126.33***** ± 3.2 | 90.25* ± 3.3 | 158.5***** ± 6.3 |

Normal Plasma Glucose level 81.12 ± 2.6 mg/100 ml.

* P < .05 ** P < .01 *** P < .005 **** P < .001

***** P < .0005 ± S.E.M. of at least eight animals.

## HEPATIC GLYCOGEN CONTENT




* AFTER TP INJECTION

Table 4.2 : Immediate influence of exogenous TP administration to white rats, with respect to hepatic glycogen content

| Post operative intervals | Normal animals injected with TP | | | | | |
|---|---|---|---|---|---|---|
| | 0.1 mg | | 0.25 mg | | 0.5 mg | |
| | M | SP | M | SP | M | SP |
| 30 minutes | 2.28 ***** ±0.2 | 2.09 ***** ±0.2 | 3.52 ** ±0.77 | 3.34 ±0.76 | 1.66 ***** ±0.4 | 1.43 ***** ±0.1 |
| 60 minutes | 1.75 ***** ±0.2 | 1.55 ***** ±0.2 | 4.60 ***** ±0.32 | 2.93 * ±0.41 | 1.84 ***** ±0.24 | 1.58 ***** ±0.32 |
| 90 minutes | 1.29 ***** ±0.4 | 1.38 ***** ±0.4 | 2.87 * ±0.6 | 3.44 ±0.62 | 4.82 **** ±0.94 | 3.97 ±0.89 |
| 120 minutes | 1.73 ***** ±0.13 | 2.67 ***** ±0.4 | 3.85 ±0.23 | 4.34 ±0.48 | 4.27 ± 1.1 | 2.33 * ±0.6 |

Normal hepatic glycogen content % of fresh tissue wt.

| M (Median lobe) | Sp (Spigelian lobe) |
|---|---|
| 3.83 ±0.12 | 3.66 ±0.16 |

* P < .05 ** P < .01 *** P < .005 **** P < .001

***** P < .0005 ± S.E.M. of at least eight animals.

## HEPATIC GLUCOSE-6 PHOSPHATASE ACTIVITIES LEVELS

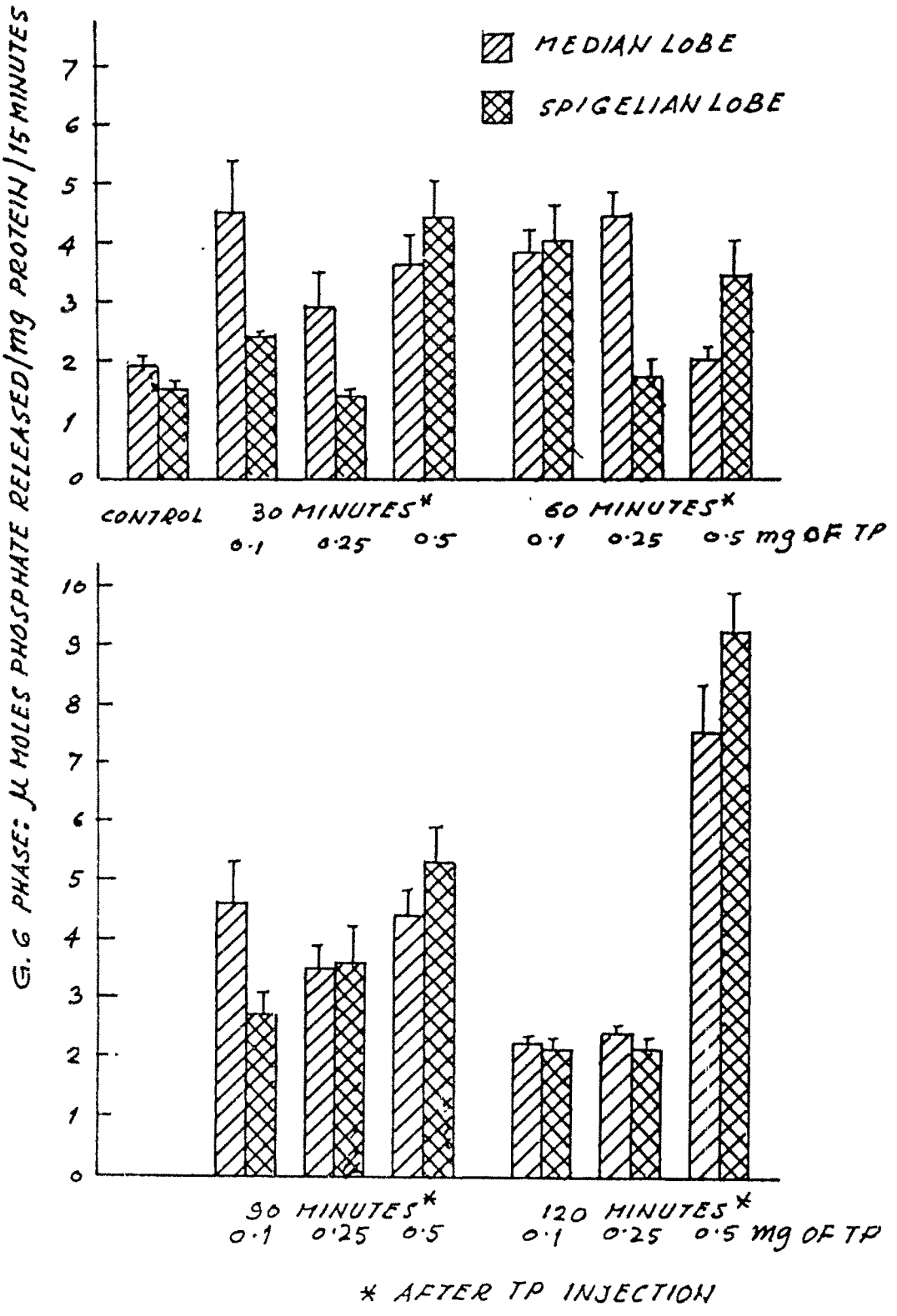


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Table 4.3 : Immediate influence of exogenous TP administration to white rats, with respect to hepatic G-6-Pase activity level.

| Post operative intervals | Normal animals injected with TP | | | | | |
|---|---|---|---|---|---|---|
| | 0.1 mg | | 0.25 mg | | 0.5 mg | |
| | M | SP | M | SP | M | SP |
| 30 minutes | 4.54*** ±0.93 | 2.39** ±0.17 | 2.91** ±0.60 | 1.40 ±0.9 | 3.67*** ±0.56 | 4.43***** ±1.2 |
| 60 minutes | 3.87***** ±0.45 | 4.07***** ±0.61 | 4.45***** ±0.40 | 1.76 ±0.30 | 2.04* ±0.27 | 3.43*** ±0.51 |
| 90 minutes | 4.64***** ±0.71 | 2.74* ±0.44 | 3.56***** ±0.40 | 3.64**** ±0.60 | 4.43***** ±0.42 | 5.32***** ±0.65 |
| 120 minutes | 2.21* ±0.16 | 2.19* ±0.21 | 2.47** ±0.03 | 2.13* ±0.2 | 7.54***** ±0.82 | 9.24***** ±1.1 |

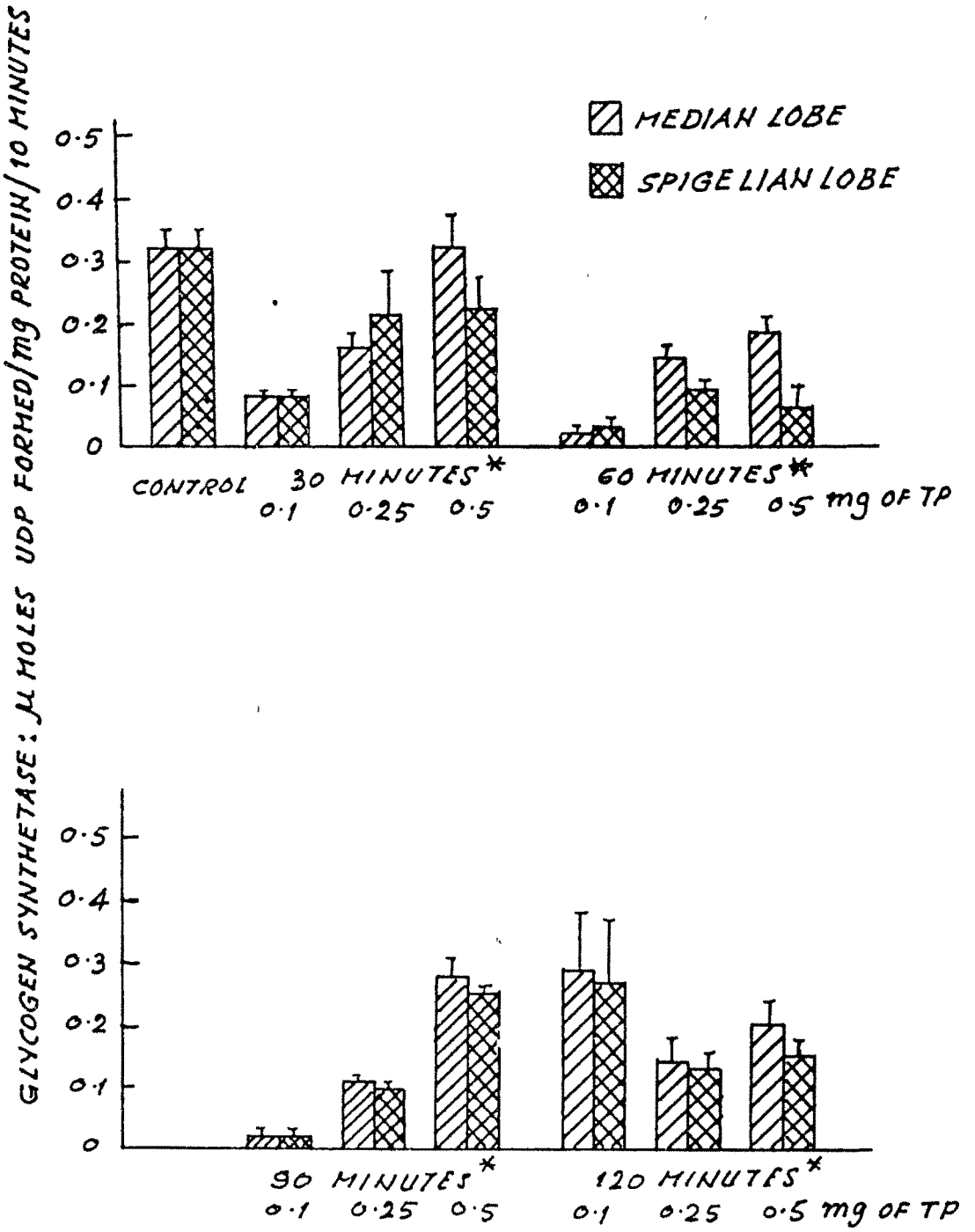
Normal hepatic G-6-Pase activity level $\mu$ Mole phosphorus released/mg protein/15 minutes.

| M (Median lobe) | Sp (Spigelian lobe) |
|---|---|
| 1.93 ± 0.13 | 1.57 ± 0.10 |

* P. < .05 ** P < .01 *** P < .005 **** P < .001 ***** P < .0005

± S.E.M. of at least eight animals.

## HEPATIC GLYCOGEN SYNTHETASE ACTIVITY LEVELS




* AFTER TP INJECTION.

Table 4.4 : Immediate influence of exogenous TP administration to white rats, with respect to hepatic glycogen synthetase activity level

| Post operative intervals | Normal animals injected with TP | | | | | |
|---|---|---|---|---|---|---|
| | 0.1 mg | | 0.25 mg | | 0.5 mg | |
| | M | SP | M | SP | M | SP |
| 30 minutes | 0.08 ***** ±0.01 | 0.08 ***** ±0.01 | 0.16 *** ±0.02 | 0.21 * ±0.07 | 0.32 ±0.05 | 0.22 * ±0.05 |
| 60 minutes | 0.02 ***** ±0.002 | 0.03 ***** ±0.004 | 0.14 *** ±0.02 | 0.09 ***** ±0.008 | 0.18 *** ±0.02 | 0.06 ***** ±0.03 |
| 90 minutes | 0.02 ***** ±0.004 | 0.02 ***** ±0.003 | 0.11 ***** ±0.002 | 0.10 ***** ±0.008 | 0.28 ±0.03 | 0.25 * ±0.01 |
| 120 minutes | 0.29 ±0.04 | 0.27 ±0.04 | 0.14 *** ±0.04 | 0.13 *** ±0.03 | 0.20 * ±0.04 | 0.16 *** ±0.03 |

Normal hepatic glycogen synthetase activity level μmoles UDP formed/mg protein/10 minutes

| M (Median lobe) | Sp (Spigelian lobe) |
|---|---|
| 0.32 ± .031 | 0.32 ± 0.031 |

* $P < .05$ ** $P < .01$ *** $P < .005$ **** $P < .001$ ***** $P < .0005$

± S.E.M. of at least eight animals

## HEPATIC PHOSPHORYLASE ACTIVITY LEVELS

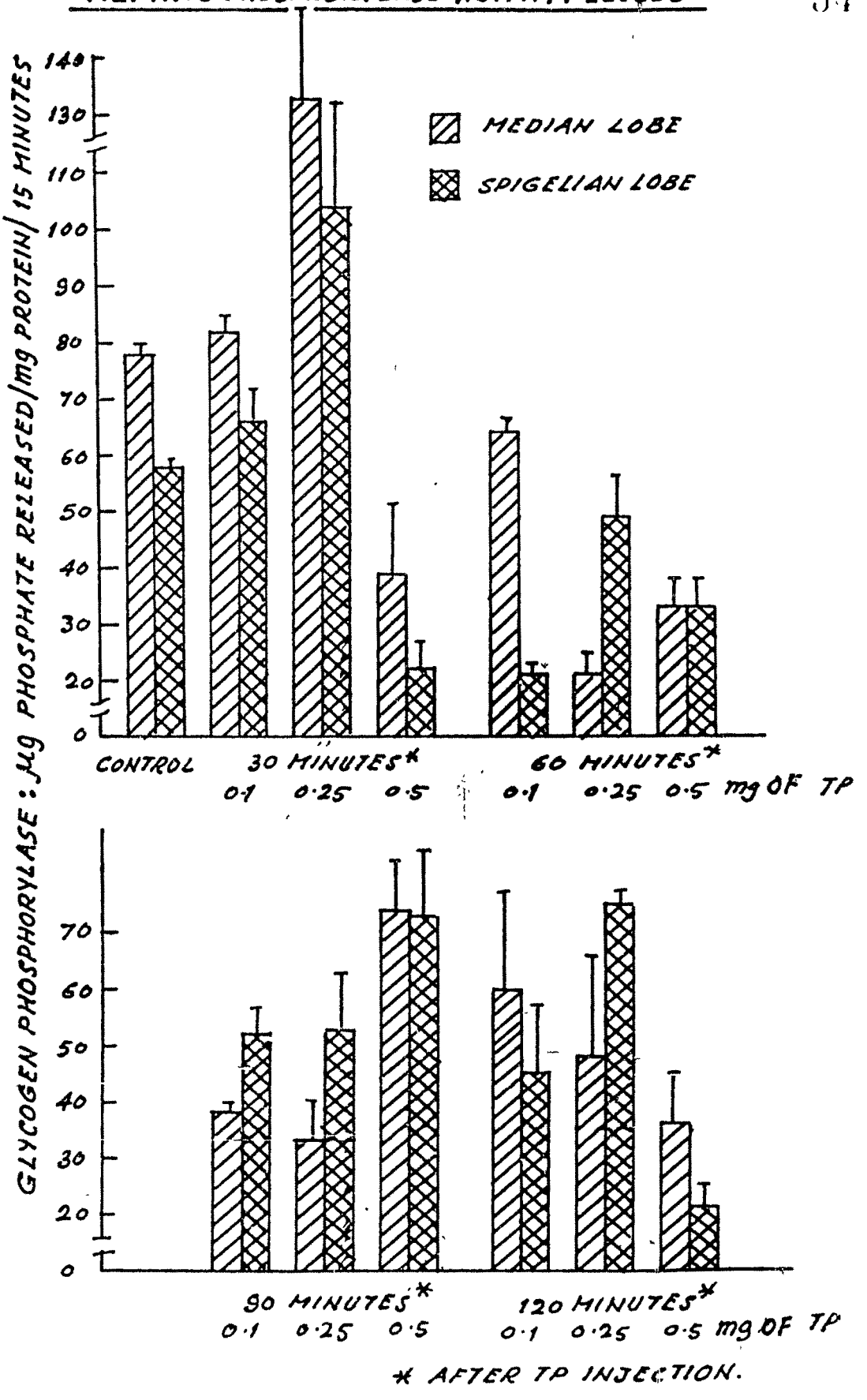


Table 4.5 : Immediate influence of exogenous TP administration to white rats, with respect to hepatic phosphorylase activity level

| Post operative intervals | Normal animals injected with TP | | | | | |
|---|---|---|---|---|---|---|
| | 0.1 mg | | 0.25 mg | | 0.5 mg | |
| | M | SP | M | SP | M | SP |
| 30 minutes | 82.14* ± 2.8 | 66.96* ± 0.7 | 133.0***** ± 16.0 | 104.0*** ± 18.7 | 39.2*** ±12.5 | 22.4***** ± 5.3 |
| 60 minutes | 64.54 ± 1.0 | 20.90***** ± 1.4 | 20.99***** ± 3.5 | 49.5 ± 7.3 | 33.6**** ± 5.4 | 33.8***** ± 5.8 |
| 90 minutes | 38.51***** ± 0.2 | 52.76 ± 0.5 | 33.88***** ± 5.0 | 53.2 ± 10.3 | 74.05 ±10.9 | 73.85***** ±15.4 |
| 120 minutes | 60.21* ± 7.0 | 45.5*** ± 5.1 | 48.2*** ± 10.8 | 75.25***** ± 2.7 | 36.4*** ± 9.9 | 21.45***** ± 4.2 |

Normal hepatic phosphorylase activity level

mg phosphurus released/mg protein/15 minutes

| M (Median lobe) | Sp (Spigelian lobe) |
|---|---|
| 78.82 ± 1.7 | 58.74 ± 1.3 |

* P < .05 ** P < .01 *** P < .005 **** P < .001 ***** P < .0005

± S.E.M. of at least eight animals

to hormonal variations than that of the median lobe, and that, once again 0.1 mg TP dose appeared to be physiologically better defined than the other two higher doses (Table 4.5).

Alterations observed in plasma glucose indicated a certain pattern worth noting. Initial immediate response of increase was apparent with all the three doses of TP. Noteworthy fact, however, pertains to homeostatic attempts with 0.1 mg dose, where restoration of normal level could be seen by 60 minutes whereas this could be observed with 0.25 mg dose as late as 120 minutes. No such attempt could be noticed when 0.5 mg of TP was administered. Another point is; though there could be recovery by 60 minutes in case of 0.1 mg dose, the same was observed to be not tenable at later intervals, so it seems that higher doses of TP increasingly countermand the capacity for restoration of plasma glucose level and further that this action is facilitated by increasing dose level as well as time lapse. It is, therefore, apparent that 0.1 mg dose is seemingly more compatible physiologically than the other two higher doses (Table 4.1).

DISCUSSION :

It is now well known that liver responds to the changes in the glucose content in portal circulation (Soling and Kleineke,1976; Stalman,1976), and hence measurement of plasma glucose levels is a good indicator of the status of hepatic glycogenolysis-glycogenesis-glycolysis. Hyperglycemic condition within the first 30 minutes of androgen administration

to the intact male albino rats irrespective of the dose levels, was obvious. This might be an indicator of the decreased glucose utilization by the peripheral tissues and/or an increased rate of release of glucose from the hepatic tissue as an early response to the higher titres of circulating androgen. The lowered hepatic glycogen level, increased G-6-Pase activity, lowered hepatic glycogen synthetase activity and increased phosphorylase activity were all mutually supportive indicators of the obviously altered pattern of hepatic carbohydrate metabolism. Even though this is the general physiological state; it should be noted that the response of the Spigelian lobe particularly at this early interval of 30 minutes and with 0.25 mg dose was the least as far as the hepatic glycogen content and glycogen synthetase activity were concerned, whereas that of phosphorylase was at its maximum. This suggests that the hyperglycaemia at 30 minutes with 0.1 mg TP dose was a result of combined interrelationships between all the parameters under study but that with 0.25 mg was seemingly more due to glycogen break down, as neither the plasma glucose level was raised as high as with other two doses of TP nor the G-6-Pase activity was enhanced significantly; particularly that of the Spigelian lobe. In fact Spigelian lobe was found to be influenced as late as 60 minutes after TP administration. Hepatic glycogen content, however, registered a marginal reduction. All these observations, point to the fact that

0.25 mg dose induces an atypical response as far as hepatic carbohydrate metabolism is concerned.

0.1 mg TP dose, when administered, induced hyperglycaemia within 30 minutes which was maintained upto 120 minutes. Simultaneously there also occured glycogen break down as well as reduction in synthesis corresponding with depletion in glycogen content. At the intervals of 60 and 90 minutes there occured a reduction in glycogen break down as well as synthetic activity. A trend towards normalization was seen at 120 minutes - glycogen level still being subnormal. Spigelian lobe though showed a similar pattern of response, a slight deviation at 90 and 120 minutes was evident. At 120 minutes the Spigelian lobe exhibited a metabolic state similar to that exhibited by the median lobe at 90 minutes. Thus 0.1 mg TP administration induces initial hyperglycaemia due to increased rates of glycogen break down as well as liberation of glucose. Response of Spigelian lobe is different only temporarilly.

With 0.25 mg dose the results indicated a different pattern of hepatic response. According to the work reported by Cahil et al. (1957) and Palasi and Larner (1969) there is a inverse relation between the levels of phosphorylase enzyme activity and glycogen content of a tissue. Whereas the results obtained at 30 minutes interval in the present investigation registered a highly significant enhancement of phosphorylase enzyme activity with moderate hyperglycaemia

and significantly reduced level of glycogen synthetase activity in both the liver lobes; yet total glycogen content was not altered from the normal. The only plausible explanation for this situation can be the rare capacity of hepatic tissue to synthesize glycogen through the activity of phosphorylase enzyme itself via G-1-P pathway as has been explained by (Satoskar and Bhandarkar, 1978).

Most remarkable feature noticeable at 60 minutes interval was highly significant reduction of the phosphorylase activity. Yet hyperglycaemia was to worsen further with some rise in G-6-Pase levels. At this juncture it is pertinent to note that in the presence of high titre of glucose, insulin is known to inhibit phosphorylase enzyme activity (Harper, 1977).

In the present context it is also worth noting that androgenic compounds are known to facilitate activities of several enzymes, also those involved in carbohydrate metabolism, by increasing intracellular concentration of cAMP in various accessory reproductive organs (Singhal and Valdares, 1968; Singhal et al., 1971; Mangan et al., 1973). On the other hand some other compounds like insulin, prostaglandins, adrenergics, etc. are known to decrease cellular cAMP levels and produce more or less opposite effects (Butcher, 1968). The present investigation also revealed that there is a reduction in cAMP concentration as evident from elevated specific phosphodiesterase activity (Chapter-5).

So it seems that the hepatic response to 0.25 mg dose at 60 minutes interval is noticeably different as was stated earlier.

Lastly when the highest dose of 0.5 mg TP used in the present investigation was administered it was found that the hyperglycaemic condition got aggravated by 60 minutes with no sign of improvement. At this stage there was increased stimulation of the G-6-Pase activity. The median lobe showed a decrease in glycogen break down along with nearly normal synthetic activity; however glycogen level was significantly low at 30 minutes. Also at 60 minutes, phosphorylase activity was low, with significant lowering of synthetic activity, accompanied by equally low levels of hepatic glycogen. This could possibly point towards a remote chance of the inhibition of branching enzyme (amylo-1;4 - 1,6 transglycosidase) with higher TP dose and hence glycogen content registers low values. However, there was no change in hyperglycaemia.

The metabolic pattern that emerged at 90 minutes interval was once again very different from the previous intervals. Here there was significant improvement of phosphorylase activity as well as that of glycogen synthetase and glycogen content; particularly more evident in respect of Spigelian lobe. However hyperglycaemia continued, but this time with concomitant significant rise in G-6-Pase activity.

This picture probably does not require any special explanation. On the other hand, by 120 minutes the metabolic patterns exhibited significant reduction in phosphorylase activity, and drastic increase in G-6-Pase activity. Glycogen synthetase level was only slightly reduced and glycogen content was also slightly lower. All this changes were comparatively more intense in case of Spigelian lobe. It appears from this that the hepatic tissue in all probability tries to normalize the metabolic activities after about two hours of hormone administration, nevertheless, it apparently does not succeed in this direction by this interval. Probably observations beyond this interval would have revealed such a picture.

Rest of the observations on hepatic response to various doses and different time intervals do not exhibit predictable patterns and hence data on hand do not permit any clear cut explanations or interpretations. This certainly means that only more extensive work could possibly throw some light on such enigmatic responses. It is obvious from the foregoing discussion that exogenous administration of androgenic hormone (TP) does induce alteration in the pattern of hepatic carbohydrate metabolism within first few hours. A dose of upto 0.1 mg of TP is perhaps less disturbing than any higher dose, as overall recovery and capacity for proper homeostasis could be observed in the liver by about 120 minutes after TP injection.

Hence, it could be suggested that lowest physiologically compatible dose level should be found out and instead of few heavy doses over a shorter period of time smaller doses at adequate intervals should be administered over longer periods of time if one wants to introduce least possible side-effects in case androgen treatment becomes necessary.