CHAPTER 11

A STUDY OF RAPID EFFECTS OF TP ADMINISTRATION ON HEPATIC METABOLIC PATTERNS OF RAT

It is apparent from the observations reported in the previous chapters that hormone deprivation through castration leads to several significant metabolic changes as early as 24 hrs. after the operation and by about 120 hrs. various parameters under consideration re-establish an altered pattern that utlimately leads to characteristic castration changes, the latter have been reported by several workers. What was significant in the present study was that very prominent changes were observable around 48 hrs. post-operatively. Further, it was also recorded that very obvious alterations occurred, concerning hepatic carbohydrate and lipid metabolism. Hence, it was thought desirable to study more carefully such alterations in the hepatic metabolism. In order to achieve this, it was decided to study the influence of androgen deprivation after 48 hrs. of castration. Additionally, in the light of recent trends on rapid influences of hormones on metabolic processes, it was deemed appropriate to investigate the effects of hormonal replacement within first few hours

only. This conclusion was arrived at, on the basis of following reasoning:-

A variation in the sensitivity of the accessory sex glands to androgen replacement at different time of castration had been observed by Osterberg and Tuohimaa (1975) in male rats. Their results indicated that all accessory sex glands respond to testosterone administration with an increasing intensity as the time lapse after castration increases. From this it appears that it is imperative that the selection of the time of hormone replacement is very important and should be made with great precautions. In the present study, therefore, the interval of 48 hrs. was chosen as maximal changes were observable then. Balanave (1968) showed that testosterone and progesterone could induce changes in synthesis as well as degradation of liver lipids very rapidly after administration in immature male chicks. There are several other reports pointing out that different accessory reproductive tissue and other non-target tissues respond to hormone administration within hours (Liao et al., 1965; Fuji and Villee, 1968; Koths et al., 1972; Demers and Jacobs, 1973; Liang and Liao, 1975). Hence, in the

present investigation, it was thought desirable to undertake an hourly study of responses to hormone administration. It was decided to record the influence of testosterone propionate (TP) administration after 1, 2 and 4 hours.

Since the aim was to study alterations in the patterns of lipid and carbohydrate metabolism, it was thought appropriate to study the changes in certain related metabolites of the blood plasma as well as the levels of free fatty acids (FFA) of fat storage in the Omentum. These would naturally reflect the underlying fluctuations and help in arriving at more plausible deductions.

MATERIALS AND METHODS

Mature adult male rats weighing about 120-160 gms were bilaterally castrated under mild ether anaesthesia. They were then given a single intramuscular injection of 0.1 mg of TP after 48 hrs. of castration. The parameters selected for assessment of effects of hormonal replacement after 48 hrs. of castration were as under:

(a) Glycogen, phosphorylase, succinate dehydrogenase (SDH), total proteins, phosphodiesterase, free fatty acids (FFA) and fatty acid oxidation (FAO) of liver. As in all the previous studies, here also the tissue from the median and the Spigelian lobe were assessed separately to note the possible differences.

(b) Glucose, total lipids, total cholesterol, phospholipids and FFA of plasma.

(c) FFA of the omental fat depot.

The quantitative estimations of all the above parameters were conducted as per the methods mentioned in earlier chapters. The animals were sacrificed after 1, 2 and 4 hrs. of hormone injection.

RESULTS

The results obtained are presented in Tables I and II.

(a) Liver parameters:

The glycogen content was much higher than the normal level in both the liver lobes at all the intervals after TP injection. Immediately after one hour there was a

little depletion, in comparison to the level obtained in castrated rats in the Spigelian lobe. It showed further depletion by two hours, but by four hours a slight rise was noticeable. In case of median lobe, first there was an increase which resulted into a decrease by two hrs and remained at the same level after four hours also (Table I).

Phosphorylase activity registered a significant rise, over the normal value, in the castrated animals at 48 hrs. in case of the median lobe. A very remarkable lowering of this enzyme activity was obvious immediately after 1 hour of TP administration in both lobes. By two hours, a non-significant rise was seen that was again reduced a little by 4 hrs. These minor fluctuations in the enzyme activity were, no doubt, seen better in the Spigelian lobe (Table I).

The level of SDH was not different than the normal value after 48 hrs. of castration. However, it was found to be lowered to less than 50% of the normal level in both the liver lobes within one hour of TP administration. The median lobe showed a non-significant but gradual increase upto four hours. Yet, it did not rise above 50%

normal level. The Spigelian lobe registered a further fall by two hours. Only by fourth hour it did show a minor rise in the activity (Table I).

The total protein content of the liver lobes exhibited an increased level 48 hrs. after castration. This showed further increase with the TP administration.

Phosphodiesterase activity was found to be considerably lowered by castration as is apparent at 48 hours. Interestingly enough, influence of TP administration was not evident to any extent upto 4 hours (Table I).

It was observed that the hepatic tissue (both the lobes) suffered a significant reduction in its capacity to oxidize fatty acids by 48 hrs. of castration. The values were almost 1/4th the normal levels. The Spigelian lobe responded to TP administration within one hour by registering reversal of castration effect and reached the normal level. The median lobe was found to be slow in its response and showed a gradual increase through the period of experimentation to reach almost normal level (Table I).

Castration was seen to lead to a considerable increase in the levels of FFA content in both of the liver lobes by 48 hrs. However, this increase was more evident in the median lobe. Administration of TP induced a minor fall in FFA content of both lobes within an hour. This was, however, nullified by a good rise in FFA content by two hours. There was again a reduction of FFA level by four hrs. after TP injection. The FFA content, however, was always higher than the normal throughout the period of observation (Table I).

(b) Plasma parameters:

Castration led to a discernible rise in plasma glucose level. It was found to be very steeply lowered to about 1/3rd the castration level within one hour of hormone injection. Thereafter, a gradual increase upto 62.58 mg/100 ml was seen by 4 hrs., however, this value was considerably lower than the normal (Table II).

Total lipids of plasma exhibited a rise immediately within one hour of hormone replacement, but thereafter, there was a depletion that stayed upto four hours.

Total cholesterol concentration in the blood plasma was seen to undergo a distinct reduction due to castration. It showed a further fall of similar magnitude as an immediate response to hormone injection within one hour. Within the next hour of TP injection the pattern of cholesterol concentration was almost reversed with similarly intense change to bring the value from 81.9 mg/100 ml to 115.5 mg/100 ml. By 4 hrs. the cholesterol was similar to initial level of about 122 mg/100 ml (Table II).

Phospholipid concentration resulted in an increase after castration. Replacement of the hormone brought about a distinct reduction in its level within one hour. By two hrs., the level remained almost similar to the previous one, but an insignificant fall was noticed at this hour. By four hours a rise was observed which was again insignificant. With the result the level remained lowered than that of the normal concentration (Table II).

Castration did not induce any significant change in the FFA content of plasma, Injection of TP was observed to bring about a significant reduction in the first two hrs. but, the most remarkable influence was obvious by 4 hrs. wherein a very steep rise in plasma

Table I : Rapid effects of testosterone propionate administration on the different parameters of liver lobes of 48 hrs castrates.

	Normal Animals		48 H Castrated Animals		48 H Castrates injected with 0.1 mg TP					
					1 H*		2 H*		4 H*	
	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp
Glycogen gms/100 gms of fresh tissue	3.835 ± 0.395	3.660 ± 0.531	7.770 ± 0.500	8.170 ± 0.439	8.335 ± 0.0770	6.510 ± 0.0623	6.613 ± 0.0650	5.799 ± 0.0533	6.635 ± 0.1417	6.474 ± 0.1071
Phosphorylase /ug PO_4/mg protein/ 15 minutes	78.82 ± 5.41	58.74 ± 4.92	60.00 ± 4.49	59.74 ± 2.58	17.04 ± 2.054	23.56 ± 2.226	21.58 ± 1.698	22.20 ± 2.298	17.16 ± 1.750	17.78 ± 1.919
SDH /ug Formazan/ mg protein/ 30 minutes	11.29 ± 0.584	7.72 ± 0.581	11.52 ± 0.120	8.11 ± 0.321	4.24 ± 0.97	4.71 ± 0.78	5.72 ± 0.31	3.81 ± 0.61	6.29 ± 0.20	4.55 ± 0.59
Protein gms/100 gms of fresh tissue	14.41 ± 1.67	17.55 ± 2.73	18.24 ± 0.74	19.11 ± 0.58	20.96 ± 1.96	21.01 ± 1.43	20.44 ± 0.492	21.36 ± 0.756	24.16 ± 0.438	24.06 ± 0.622
Phosphodiesterase /ug PO_4/mg protein/30 min.	2.344 ± 0.135	2.321 ± 0.098	1.696 ± 0.052	1.579 ± 0.055	1.383 ± 0.187	1.883 ± 0.134	1.500 ± 0.130	1.540 ± 0.016	1.131 ± 0.079	1.143 ± 0.145
Fatty acid oxidation /u10_2/mg of fresh tissue	1.322 ± 0.131	1.454 ± 0.064	0.377 ± 0.196	0.393 ± 0.032	0.549 ± 0.041	1.312 ± 0.042	1.008 ± 0.095	1.370 ± 0.129	1.168 ± 0.157	1.082 ± 0.125
Free fatty acid /u Eq/mg of fresh tissue	123.6 ± 3.00	111.6 ± 5.56	174.0 ± 6.62	123.3 ± 4.03	145.0 ±11.63	117.0 ± 8.52	192.2 ± 9.44	144.6 ±10.79	173.3 ± 6.66	124.7 ± 8.34

Each reading is the mean of at least 10 different samples. M - Median lobe; Sp - Spigelian lobe.
*Hours after TP injection.

Table II : Rapid effects of TP administration on levels of glucose and lipids of blood plasma and FFA of omental depot in 48 H castrates.

	Normal animals	48 H Castrates	48 H castrates injected with 0.1 mg TP		
			1 H*	2 H*	4 H*
Glucose mg/100 ml of plasma	81.12 ± 8.35	96.80 ± 6.52	39.50 ± 3.15	46.00 ± 2.68	62.58 ± 2.16
Total lipids gms/100 ml of plasma	1.39 ±0.395	1.40 ±0.370	1.76 ±0.444	1.21 ± 0.194	1.22 ± 0.081
Cholesterol mg/100 ml of plasma	186.0 ±13.60	122.7 ±16.55	81.9 ±12.00	115.5 ±12.90	123.8 ± 9.21
Phospholipid mg/100 ml of plasma	143.9 ±12.54	162.2 ±12.91	118.0 ± 5.16	116.8 ± 5.23	120.5 ±13.30
FFA µ Eqv/liter of blood plasma	761.02 ±12.56	741.36 ±11.24	616.60 ±12.65	646.10 ±17.80	958.82 ±16.81
FFA of Omental fat µ Eqv/mg of fresh tissue	35.93 ± 3.063	20.11 ± 3.061	23.49 ± 1.671	22.04 ± 3.321	51.63 ± 3.932

Each reading is the mean of at least 10 different samples.
*Hours after TP administration.

FFA level was recorded (958.825 μEq/100 ml) (Table II).

(c) The FFA content of Omental fat showed a small increase within the first two hours. Whereas, after four hours it registered a sudden increase to the level that was found to be significantly higher than the normal values (Table II).

DISCUSSION

This inquiry about the immediate effects (within first four hours after injection) of TP replacement has revealed some very remarkable facts and also has brought forward equally confusing results.

Within an hour of TP administration the phosphorylase activity of the liver was observed to be drastically reduced though elevation in hepatic glycogen content was not commensurate. Similar trend was apparent in these two parameters under different experimental time schedules as reported in Chapter-3. On the basis of these observations it could be suggested that TP has a direct and immediate influence on the hepatic phosphorylase enzyme activity. There is no possibility of even making a guess about such

an influence on the basis of observations, which are tangential to the main problem, that are available. Variations in the glycogen concentration and phosphorylase activity by 2 and 4 hrs. after TP injection were of marginal magnitude and hence the initial influence may be said to last upto 4 hours. Another important change in this connection was the induction of a highly significant yet transient hypoglycemia within the first 60 minutes of hormone replacement as was apparent from the steep reduction in plasma glucose level from 81.12 mg/100 ml to 39.5 mg/100 ml. Plasma glucose level, however, was found to head towards normality by 4 hrs. of injection.

48 hrs. after castration there was depletion in the FFA content of the Omental fat store as well as that of blood plasma and at that time there was an increase in the liver. This could mean that the hepatic tissue exhibits a rapid acceleration of FFA uptake (vide also Chapter-2) leading towards adiposity characteristic of castration changes. Under these conditions there was a sharp drop in the capacity of the hepatic tissue to oxidize FFA. This fact, alongwith that of a rise in

glyceride content as noted in Chapter-2 , supports the contention of castration adiposity.

Within an hour of TP injection there was a reduction in liver FFA level and increase in rate of FAO. Omental FFA registered an increase whereas that in the blood plasma was depleted. This suggests an initiation of reversal process. However, two controversial findings that occur during next 3 hrs. need be mentioned. Whereas the changes in the FAO rate were in confirmity with reversal process, there was a transient increase in the FFA content of the liver by 2 hrs. and a highly remarkable rise by 4 hrs in the FFA content of the Omental fat pad as well as in the blood plasma. It is not possible to forward any plausible explanation for these occurrences, however, it may be said that these are foreshadowing effects of well known lipid mobilizing action of androgenic compounds.

Concerning the SDH activity of liver a very strange phenomenon came to light. TP administration to 48 hr. castrates lead to a highly significant reduction (about 50%) in this enzyme activity. This effect did last upto 4 hrs. with some fluctuations. As was apparent from earlier

observations (Chapter-3, The Spigelian lobe), the SDH enzyme activity was found to be lowered by 24 hrs. to a level where only 4.28 µg of diformazan could be released in first 30 minutes as opposed to 7.72 µg for normal. However, this was raised once again to 8.11 µg by 48 hrs. after castration. Present readings show that SDH activity remained at a low level within first 4 hrs. of hormone replacement. This shows that immediately after removal of the main source of androgens and also within first 4 hrs. of androgen administration SDH activity happened to be reduced by about 50% but at later stages it returned to almost normal levels. The work of Kalman (1952) and Rindani (1958) has shown that SDH activity of liver is increased due to castration and it was decreased after testosterone addition under in vitro conditions. What is important in the present context is that, inspite of this immediate reduction in SDH activity (first 4 hrs.), there is a definite increase in the fatty acid oxidizing capacity of liver during that time. In the circumstances, sustenance of higher rate of FAO becomes untenable with the reduction in the activity of one of the key enzymes of tricarboxylic acid cycle. What could be said, at this stage, is that only a very intense inquiry could prove or disprove this

observation. It is worth noting here that the Spigelian lobe of the rat liver did show a discernible higher level of sensitivity than the median lobe under all these experimental treatments.

Minor variations observed in the total lipid content of the blood plasma due either to castration or to hormone replacement appear to be non-significant. Plasma phospholipids were found to be elevated slightly due to castration but administration of TP could more than effectively reverse this elevation. As far as the cholesterol level of blood plasma is concerned a striking action of TP injection was revealed within first sixty minutes. Cholesterol level was reduced to less than half the normal level. However, in the next three hours this was observed to go towards normal value. This rapidly suppressing action of TP is certainly enigmatic and more work is necessary to throw light on this phenomenon.

An increase was evident in the protein concentration in both of the liver lobes, 48 hrs. after castration. With the hormone administration a discernible increase was noted after 4 hrs. Considering this information and a decrease in phosphodiesterase activity, it is logical to suggest that hormone action may lead to slowing down

of degradation of intracellular adenosine 3',5'-monophosphate (cAMP), which in its turn may increase the rate of protein synthesis. cAMP is known to increase the protein content of rat uteri (Hechter et al., 1967). Hence, with the result an anabolic effect of the hormone on the protein content of liver which was apparent upto 4 hrs., could be due to greater stimulating action of cAMP within the cells.

Though the present study was conducted to establish the clear picture of hormone action on liver, it has raised many questions.