CHAPTER 8

SOME ASPECTS OF FATTY ACID METABOLISM IN MALE ALBINO RATS UNDER THE INFLUENCE OF SEX HORMONES

Previous investigations (Chapters-1 & 2) have revealed changes of significant nature with respect to hepatic levels of total lipids, cholesterol, phospholipids and free fatty acids (FFA) after brief periods of gonadectomy in male albino rats. Having established these facts, it would be more pertinent to extend further studies on lipid metabolism, so that not only the involvement of sex-hormone on these factors could be ascertained, but also could arrive at a certain conclusive idea thereof regarding general metabolism of the hepatic tissue.

The studies conducted on liver lipids indicated an accumulation of total lipids, whereas, concentrations of total cholesterol and phospholipids were found not to be increased. FFA content was found to be below normal. Hence, increased accumulation of triglycerides can be the result of castration in hepatic tissue. In general, the fatty livers may result from increased net transport into the liver, or increased hepatic synthesis of lipids, or reduced hepatic oxidation of lipid (Cantarow and Schepartz, 1962).

Further, fatty infiltration of this organ is known to be associated with injury or disease-states in man (Lombardi, 1965 - a review article). Hence, the purpose of the present study is to ascertain the underlying mechanisms resulting into alterations in lipid metabolism of liver under castration experiments. The increased rate of transport to liver or the increased rate of liver lipid synthesis could be studied by measuring the free fatty acids of depot fat, blood and the hepatic tissue itself. Whereas, the decreased rate of mobilization from liver could be evaluated by estimating the rate of fatty acid oxidation. The importance of FFA in lipid metabolism was demonstrated by Dole (1956) and Gordon and Cherkes (1956). They assumed that this lipid fraction is primarily concerned with the supply of fats to tissues for oxidative metabolism. Taking into account these observations a quantitative evaluation of plasma and omental fat FFA content was undertaken along with the measurement of rate of fatty acid oxidation.

MATERIAL AND METHODS

Adult male albino rats (120-160 gms); maintained under uniform standard husbandry conditions were used.

A number of animals were castrated bilaterally and sacrificed after periods of 24, 48 and 120 hours. Another group of rats was castrated and injected with 0.1 mg of testosterone propionate (TP) (Sigma Chem. Co.) after 48 hrs. once only and sacrificed after 1, 2 and 4 hours. TP was administered intramuscularly in 0.5 ml of Tributyrin (Sigma Chem. Co.). Requisite number of normal and sham-operated control animals were used. For estimating the rate of fatty acid oxidation (FAO) Warburg's direct method (Dixon, 1943; Umbreit et al., 1957) was employed. The FFA levels were assayed colorimetrically as per the method of Smith (1975) in blood plasma and omental fat depot.

RESULTS

The data on quantitative analysis are presented in Table I.

The rate of fatty acid oxidation in both the liver lobes (Median and Spigelian) decreased considerably so as to show a fall of about 60% in 24 hr$. castrated rats. It decreased further by 48 hrs. However, an increased rate was obtained in both the liver lobes after

120 hrs. of gonadectomy, yet it was found to be lower than the normal rate (Fig. 1). In 24 hr. castrates, the FFA level of omental fat was several fold higher, while that of plasma exhibited a relatively low level than that of the normal animals. A sudden drop in FFA of omental tissue was obtained after 48 hrs. of operation, which increased again to reach above-normal levels 120 hrs. post-operatively (Fig. 2). Plasma FFA, in contrast, increased at 48 hr. interval but remained below normal whereas at 120 hrs. a 50% reduction in its concentration was obtained (Fig. 2).

TP increased the rate of fatty acid oxidation immediately one hour after therapy in both the liver lobes. Increase in case of Spigelian lobe was very sharp, almost to reach normal level; whereas, the median lobe showed but a little rise in its capacity to oxidize fatty acids (Table I; Fig. 1). It continued to increase even after 2 hrs. of hormone replacement. At this stage, Spigelian lobe showed negligible increase, whereas, median lobe now exhibited a significant rise. This rate was increased further in the median lobe after 4 hrs. of injection, though it was still below the normal value. Spigelian lobe at this hour of therapy showed a

Table I : Rate of fatty acid oxidation and levels of FFA under the influence of sex hormone in male albino rats.

	Normal animals	Sham-operated animals	Castrated animals			48 H Castrates-injected with 0.1 mg of TP		
			24 H@	48 H@	120 H@	1 H*	2 H*	4 H*
FAO in median lobe of liver /u102/mg of fresh tissue	1.322 ±0.131	1.145 ± 0.151	0.565 ± 0.077	0.377 ± 0.196	0.588 ± 0.090	0.549 ± 0.041	1.008 ± 0.095	1.168 ± 0.157
FAO in Spigelian lobe of liver /u102/mg of fresh tissue	1.454 ± 0.064	1.482 ± 0.186	0.502 ± 0.015	0.393 ± 0.032	0.602 ± 0.039	1.312 ± 0.042	1.370 ± 0.129	1.082 ± 0.125
FFA of blood plasma /u Eq/litre of plasma	761.02 ±12.56	721.00 ±11.07	701.35 ±14.96	741.36 ±11.24	376.93 ±16.67	616.60 ±12.65	646.10 ±17.80	958.82 ±16.81
FFA of omental fat depot /u Eq/mg of fresh tissue	35.93 ± 3.063	32.08 ± 5.04	82.83 ± 4.427	20.11 ± 3.061	67.70 ± 4.843	23.49 ± 1.671	22.04 ± 3.321	51.63 ± 3.932

Each value represents the mean of at least ten different samples.

@Time period in hours after castration experiment.

*Time period after injection of TP.

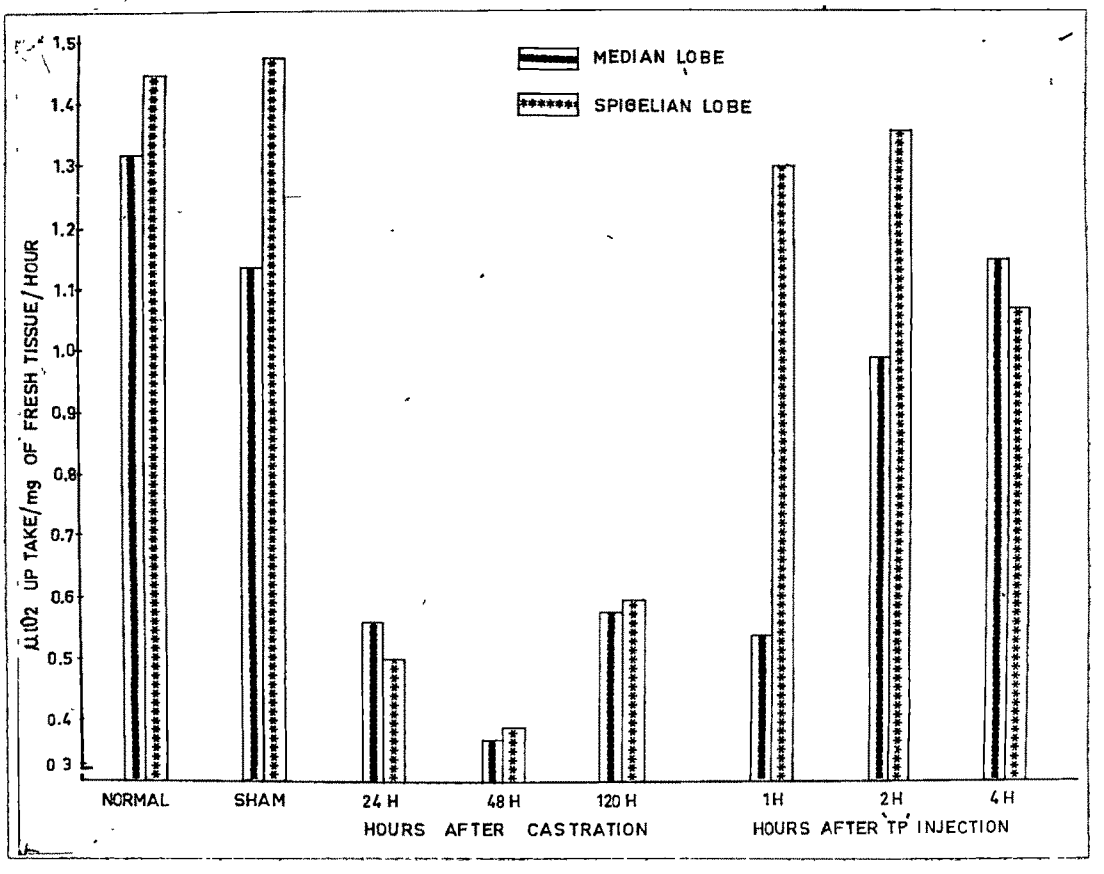

Fig. 1. Histogram representing the influences of castration and 0.1 mg TP administration to 48 H castrates at 1, 2 and 4 hours, on the rate of fatty acid oxidation of two liver lobes (median and Spigelian). The values are expressed in terms of μlO_2/ mg fresh tissue/ hour.

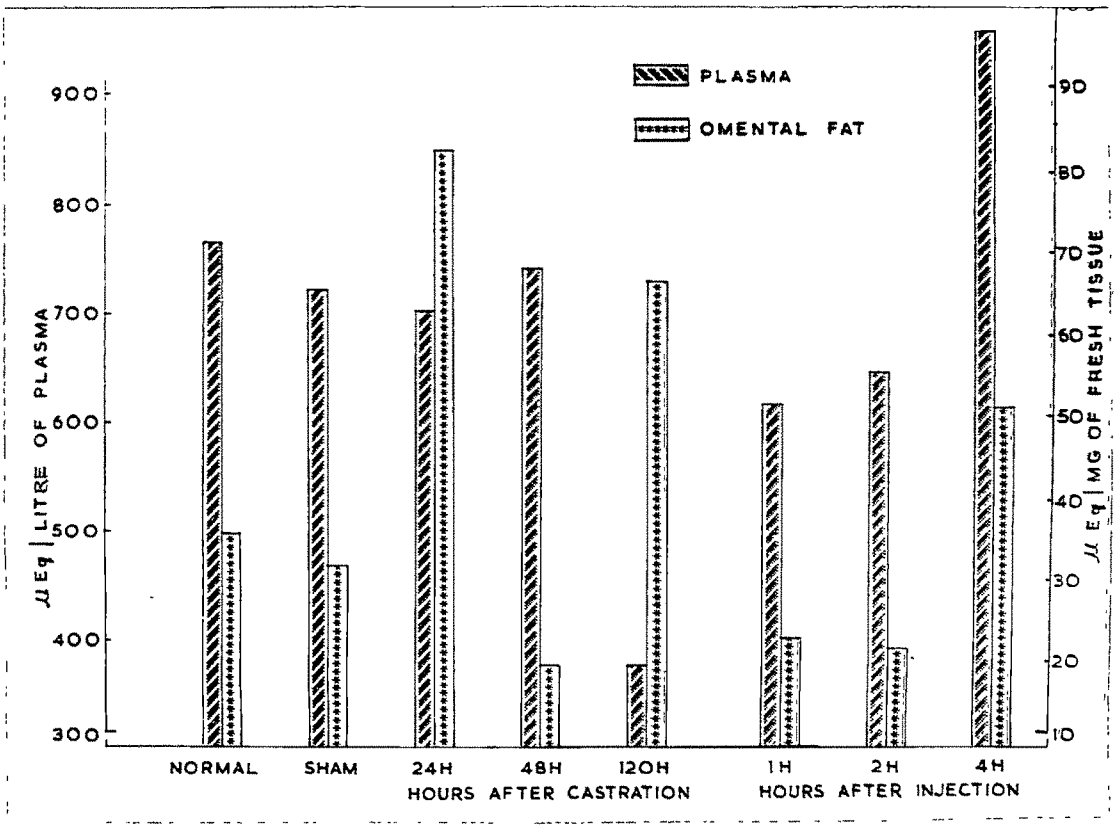

Fig. 2. Graphic representation of FFA levels in the blood plasma and omental fat depot of normal, sham-operated, castrated and 0.1 mg TP injected 48 H castrates.

little depletion (Fig. 1).

The level of plasma FFA was found to be decreased after one and two hours of injection, than the 48 hrs. castrated animals in which the hormone was replaced. After four hrs. of injection, a sudden increase was obtained leading to above-normal values (Fig. 2). The values obtained for omental FFA, after 1 and 2 hrs. of TP injection, did not show significant alteration when compared with 48 hrs castrated animals. The level was below normal. Interestingly enough, it was found to be elevated after four hrs., resulting in values higher than normal animals (Fig. 2).

DISCUSSION

The changes observed in the hepatic tissue in the present study clearly point to a very crucial and significant influence of sex-hormone on the lipid metabolism. A decrease in the rate of fatty acid oxidation was observable 24 hrs. after castration. The total lipids of the liver were found to be increased at this stage of castration (Chapter-1). Concentration of FFA was found to be diminished in the blood plasma,

whereas, elevated values were obtained for omental fat depot after 24 hrs. of gonadectomy (Table I; Fig. 2). Similarly, a higher level of hepatic FFA was also noticed (Chapter-2). The variations in the levels of FFA in these three different tissues could mean that lipids are getting increasingly mobilized from the fat depots, that are then taken up by hepatic tissue from the blood plasma which could finally lead to increase in the concentration of liver lipids. Simultaneous reduction in the rate of fatty acid oxidation of hepatic tissue, as noted here, would also complement this phenomenon. The same pattern must be true after 48 hrs. of castration. Here also, the rate of FAO was reduced. The FFA pool of depot was depleted whereas, that of plasma was increased. It may be recalled here that an increase in the level of hepatic FFA was recorded under these conditions (Chapter-2). Hence, it could be surmised that FFA are being released at an enhanced rate from depot fat into the blood stream, which are then transported and picked up by the hepatic tissue at more or less same rate. The studies conducted by Feigelson et al. (1961) and Carlson et al. (1965) on dogs with noradrenaline, also suggested a precursor-product relationship between metabolism of plasma FFA and the rate

of synthesis in the liver. They observed that an increased plasma FFA level resulted into an increased concentration of triglycerides in the liver. Apart from this, it is also well known that the plasma FFA are derived from fat depots (Norcia and Evans, 1964; Vaughan and Steinberg, 1965). Correlating all these facts with present observations, the transport mechanism, along with reduced rate of hepatic oxidation, could be suggested to lead to increased hepatic lipid content. Such a mechanism should result into a several-fold increase in liver lipids. Observations on liver lipids (Chapter-1) did not show a sharp increase in total lipids, though a slight increase was observable. However, the increase in FFA pool of liver and plasma may help to increase the concentration of lipids in other body tissues as well. To substantiate these findings, further studies are necessary.

The studies after 120 hrs. of castration, reported a fall in the levels of plasma FFA and a rise in that of depot FFA. Corresponding to this a significant fall in hepatic FFA (Chapter-2) was also observed. It should be emphasized here that under these circumstances alterations in the rates of lipid mobilization are not the important causal factors after 120 hrs. of gonadectomy, but it is

the reduced rate of oxidation per se that leads to increased lipid levels in hepatic tissue. Further, a striking decrease in the concentration of total blood lipids (Chapter-9) at 120 hrs. after gonadectomy also points to reduction in the rate of liberation of lipids from the liver. Tinoco et al. (1964), in their study on choline deficient rats, also suggested that decrease in serum lipids was due to impaired mobilization from liver and an accumulation of triglycerides in liver. In a similar manner, it is well established that several of the experimentally induced fatty livers result from a block in the release of hepatic triglycerides into plasma in the form of plasma lipoproteins (Lombardi, 1965).

During the course of studies on effects of castration, it was noted that most significant changes occurred after 48 hrs. of castration, hence further experiments involving replacement therapy were conducted on animals after 48 hrs. of gonadectomy.

The liver lobes responded to TP with an actively stepped up rate of fatty acid oxidation as compared to castrated animals. Spigelian lobe was observed to

settle down to the normal level within one hour of hormone replacement, though with slightly lower than normal values. On the other hand, the median lobe took four hours to come to a similar stage. Changes in the FFA content of plasma and omental fat obtained herein during various periods of replacement showed the significance of time response. An elevation in both tissues could be seen only after four hours. The hepatic lobes also showed higher values of FFA at this hour of TP replacement (Chapter-11). In the light of these observations it would be pertinent to say that fatty acid mobilization from depot tissue results into an increased liver lipid synthesis after sex-hormone injection to castrates; the rate of liver fatty acid oxidation remaining normal. Of relevance in this context are the reports of Miller et al. (1961) wherein sex-hormones have been shown to influence accumulation of liver lipid in choline deficient rats. Choline deficient livers are also fatty due to reduced mobilization in liver. From these results it appears that the absence of sex-hormones exert a significant influence on the process of degradation of liver lipids, whereas, administration of TP increases the lipid mobilization from depots resulting into an increase in the liver lipids.