CHAPTER 2

HEPATIC FREE FATTY ACIDS IN CASTRATED AND TESTOSTERONE TREATED RATS

Interest in the possible causal relationship between disorders of lipid metabolism and castration, particularly in liver, has stimulated much work that could help to provide some understanding of such disorders. Further, our previous study has demonstrated that, the liver lipids are increased after castration in male albino rats (Chapter-1). Increase in total lipids could either be due to increased rate of synthesis or decreased rate of utilization (Cantarow and Schepartz, 1962). Increased synthesis of lipids may be a reflection of increased de novo synthesis in liver or an indication of higher rate of uptake from circulating free fatty acids (FFA). In any case there will be a disturbance in FFA pool of the hepatic tissue. In addition to this, it was also apparent from the data obtained previously that levels of cholesterol and phospholipids did not register any increase after castration. This clearly suggested an increase in the glyceride fraction of the hepatic lipids which could account for a rise in total liver lipids. Hence, knowledge about FFA levels alongwith those of total glycerides is of interest in

understanding overall patterns of liver lipid metabolism, as altered by castration.

There is substantial evidence that the liver occupies a central position in the regulation of metabolism of both FFA and triglycerides (Fredrickson and Gordon, 1958; Fritz, 1961); through uptake of excess of circulating FFA (Bragdon and Gordon, 1958; Rohim and Spitzer, 1958; Fine and Williams, 1960; McElory et al., 1960) and by synthesizing lipoproteins. The fate of FFA, however, is also known to be modified by the nutritional and hormonal states of the animal. Varied hormonal influences on plasma and liver FFA levels are very well established (Sirek et al., 1967; Basso and Havel, 1970; Froberg et al., 1975; Inc and Thorpe, 1975; Thapliyal et al., 1975), but those of sex hormones on mammalian liver have not been studied to a desirable extent. Hence, the purpose of this investigation was to evaluate further the effects of sex steroids on hepatic levels of free fatty acids.

MATERIALS AND METHODS

Healthy male albino rats, reared under laboratory conditions were chosen for the experiments. They were

bilaterally castrated through scrotal sacs, under mild ether anaesthesia. Simultaneously, sham operations were also performed to serve as controls. At regular intervals viz., 24, 48 and 120 hrs. after castration, animals were sacrificed by decapitation. Sham operated animals were sacrificed after 24 hrs. of operation. The median and Spigelian lobes of liver were quickly excised, trimmed off of adhering connective tissue and blotted to remove blood. The pieces from respective lobes were weighed separately and utilized for the estimation of FFA. The assay was performed by the method of Smith (1975) using 1-nitroso-2-naphthol as an indicator for the colour development. The values of the total glycerides were obtained by deducing the total phospholipid and total cholesterol from the gravimetrically determined total lipids as described by Reddy et al. (1972).

The alterations in the FFA and total glycerides were also measured after replacement therapy. The details of the different doses of the hormone and the experimental conditions are presented in Tables I and II. Testosterone propionate (TP) was injected intramuscularly, dissolved in 0.5 ml of tributyrin. A single dose was administered in each case.

RESULTS

The values of FFA and total glycerides under different experimental treatments are presented in Tables I and II. The figures are the mean values of several replications of each type of treatment. As may be noted, hepatic FFA level changed significantly to higher values as compared to the normal after 48 hrs. of castration, and decrease was observed thereafter at 120 hrs. of operation. At the interval of 24 hrs. the rise was observable only in the median lobe of the liver, whereas, Spigelian lobe did not show significant alteration (Table I, Fig. 1). The total glycerides calculated, showed increased value in both the liver lobes by 24 hrs., but a gradual decrease was noted thereafter in Spigelian lobe which reached to almost normal values after 120 hrs. of castration. On the other hand, the values for the median lobe, remained higher than that of the normal values even after 120 hrs. of castration (Table 1, Fig.2).

Treatment with TP produced a dose related response in total FFA of the liver lobes. As an immediate response, the hepatic tissue registered a fall in FFA content with the minimal dose of TP (0.05 mg) (Table II; Fig. 3). The level of FFA showed an elevation as the dose was increased to 0.1 mg. A further rise in FFA of the liver lobes was

Table I : Changes in the concentration of hepatic FFA and total glycerides following castration of rats

	Lobes of the liver	Normal Animals	Sham-operated animals	Castrated animals		
			24 hr*	24 hr*	48 hr*	120 hr*
FFA uEqv./mg of fresh tissue weight	Median lobe	123.65 ± 3.00	114.50 ± 4.83	135.14 ± 7.24	174.00 ± 6.62	68.02 ± 4.92
	Spigelian lobe	111.65 ± 5.56	103.80 ± 5.09	100.55 ± 3.74	123.33 ± 4.03	96.70 ± 4.55
Total glycerides % of fresh tissue weight	Median lobe	2.28 ± 0.091	2.35 ± 0.035	3.48 ± 0.048	3.19 ± 0.049	3.16 ± 0.028
	Spigelian lobe	4.14 ± 0.077	4.07 ± 0.021	5.27 ± 0.044	4.65 ± 0.041	4.23 ± 0.011

Each reading is a mean value of twelve different samples obtained from different animals.

*Post-operative interval in hours.

Table II : Alterations observed in concentration of liver FFA and total glycerides following TP treatment to castrated rats.

	Lobes of the liver	24 H castrated animals treated with TP			120 H castrated animals treated with TP
		0.05 mg*	0.1 mg*	0.5 mg*	0.1 mg*
FFA µEqv./mg of fresh tissue weight	Median lobe	104.20 ±10.43	128.72 ± 8.8	150.78 ± 6.99	62.70 ± 4.38
	Spigelian lobe	86.65 ± 8.81	116.95 ±12.39	121.17 ± 9.71	87.70 ± 7.79
Total glycerides % of fresh tissue weight	Median lobe	2.70 ± 0.089	2.15 ± 0.045	1.93 ± 0.030	3.67 ± 0.030
	Spigelian lobe	4.84 ± 0.088	4.19 ± 0.066	3.08 ± 0.040	4.13 ± 0.098

Values represented are the mean figures of at least ten samples obtained from different animals.

*Dosages of TP injected with 0.5 ml of tributyrin.

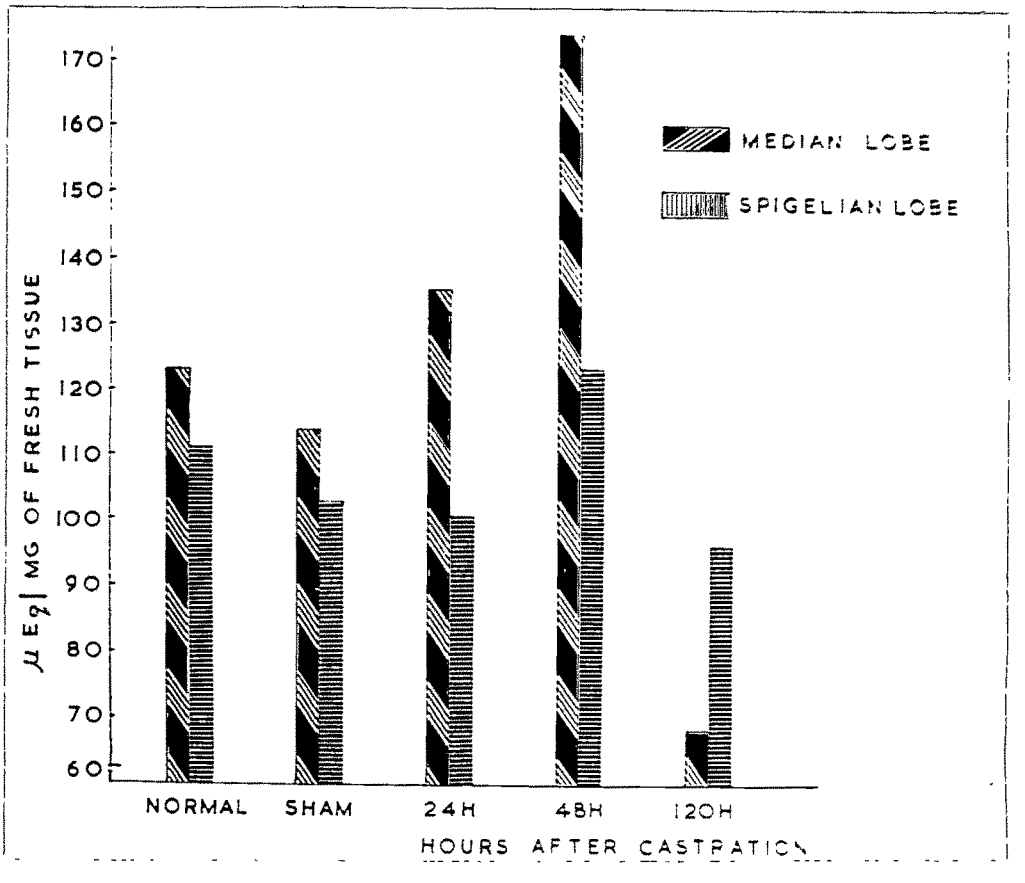

Fig. 1. Graphic representation of hepatic levels of FFA in normal, sham-operated and castrated rats.

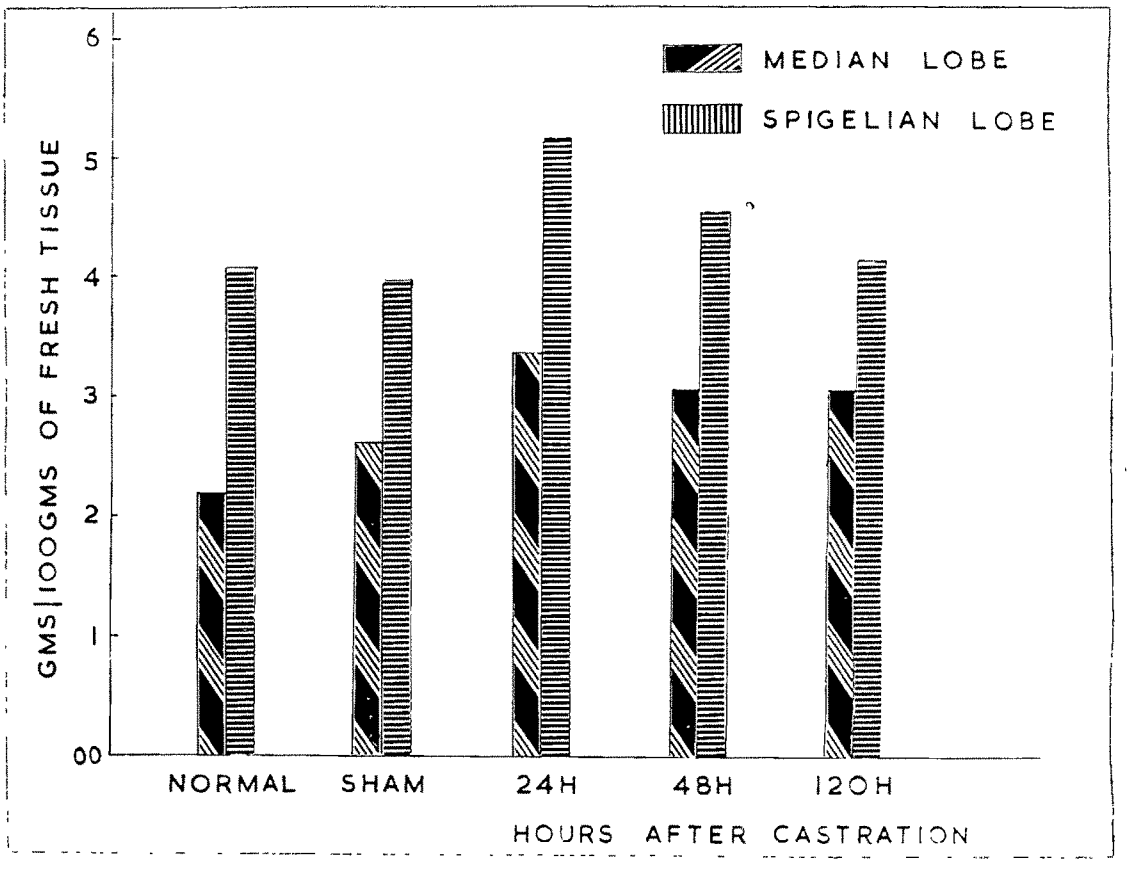

Fig. 2. Graphic representation of hepatic levels of total glycerides in normal, sham-operated and castrated rats.

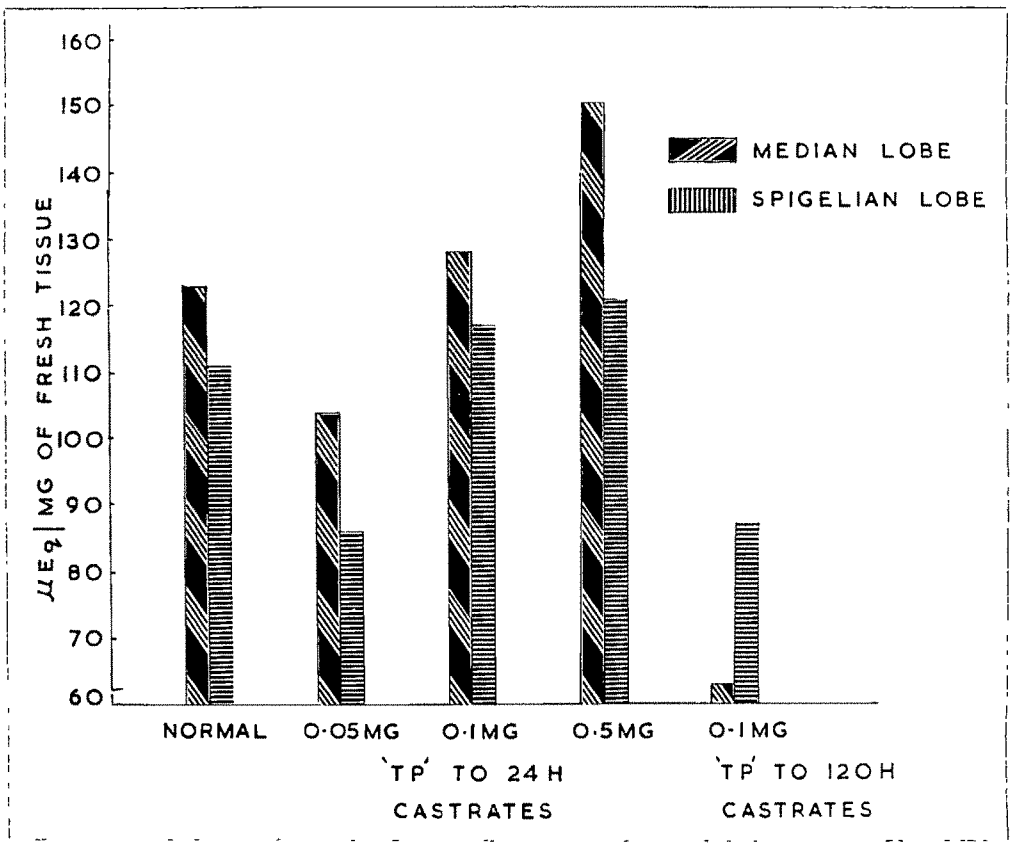

Fig. 2. Showing variations in the hepatic concentrations of FFA under the influence of TP injection to castrates.

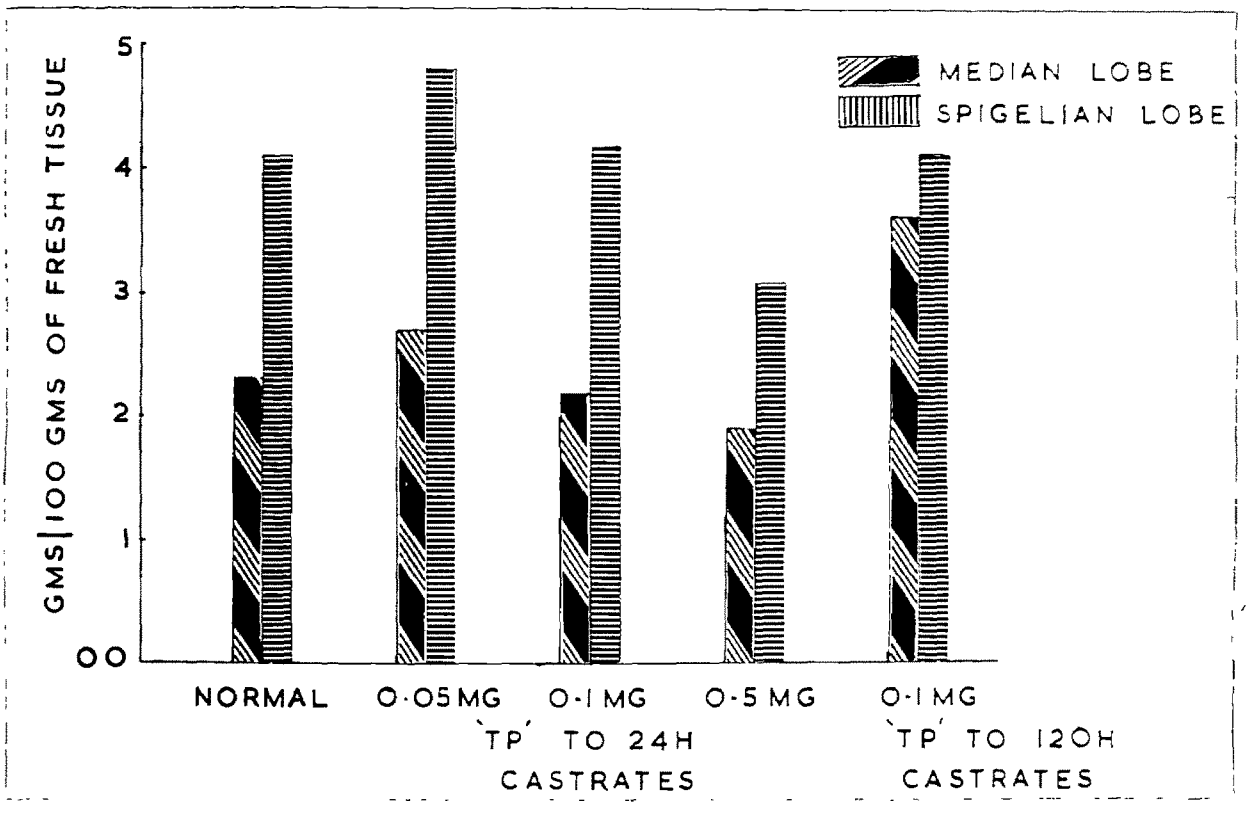

Fig. 4. Variations in the hepatic concentrations of total glycerides under the influence of TP injection to castrates.

obtained with the higher dose of TP (0.5 mg). Both the lobes were found to reach near normal levels with 0.1 mg of TP administration. Total glyceride content, on the other hand, showed a smooth decline with increasing hormone levels. Here also 0.1 mg of TP was found to effectively bring back glyceride levels to normal range (Table II; Fig. 4).

The selected effective dose of 0.1 mg TP, when administered after 120 hrs. of castration, showed a significant fall in FFA content of the liver lobes. The glycerides of the Spigelian lobe were found to be almost normal, but of the median lobe remained higher than normal (Table II; Figs. 3 & 4).

DISCUSSION

Increase in liver FFA levels observable within 24 hrs. of castration could point towards reduced rate of its utilization or increased rate of fatty acid breakdown or increased rate of transport from depots or increased de novo synthesis. Since, the total glycerides calculated at this hour of castration, showed higher values, the possibility for increase in the rate of fatty acid

breakdown in hepatic tissue becomes less plausible, Additionally, it was observed that the rate of fatty acid oxidation was reduced after 24 hrs. of gonadectomy (Chapter-8). The level of plasma FFA was also seen to be little below normal. Regarding the role of plasma FFA, it is known that circulating FFA, enter the liver where they get rapidly esterified (Laurell, 1959; Stein and Shapiro, 1959). Taking into account all these available data the only plausible explanation could be that the rate of lipid utilization is reduced significantly. As an alternative source; the plasma glucose can also be utilized for fatty acid synthesis in the liver. Leveille et al. (1968) have used labeled glucose and acetate as substrates and have studied the lipid synthesis in chick liver and adipose tissues. They showed the rapid incorporation of both these into fatty acids of liver and adipose tissue. Further, glucose was also utilized for glyceride-glycerol formation. The plasma glucose levels obtained after 24 hrs. following castration were below normal (Chapter-9). This obviously shows that there is no possibility of uptake and metabolic conversion of plasma glucose into fatty acids in the liver.

By 48 hrs. after gonadectomy the FFA level was found to be further increased in the hepatic tissue. The level of total glycerides remained higher in the median lobe, whereas, Spigelian lobe showed almost normal value. There was a little increase in plasma FFA, but it was still below normal level (Chapter-8). At this stage (48 hrs. postoperative interval), the increase in liver FFA could be due to two possible reasons that is increased uptake and/or decreased breakdown (as reported in Chapter-8, there was first an elevation in depot FFA and then a reduction was obtained by 48 hrs.). It is evident from the work of Vaughan and Steinberg (1965), that the plasma FFA originate from depots due to mobilization and liver FFA are derived from the circulating plasma FFA (Fredrickson and Gordon, 1958). Observing the data on depot fat, obtained during the course of present study (Chapter-8), an increased rate of fatty acid mobilization is evident. Further, it was also noted that there was decrease in the rate of liver fatty acid oxidation (Chapter-8). Hence, it is evident that there was an increased rate of transport of fatty acid from the depots alongwith reduced rate of its breakdown in liver, which was manifested clearly in the observed higher values for hepatic FFA. Circulating FFA are known to be

picked up from the blood and then incorporated into the glycerides of the liver (Laurell, 1959; Stein and Shapiro, 1959; Feigelson et al., 1961; Carlson et al., 1965). Presently observed increase in the total glycerides, therefore, could be accounted for.

At 120 hrs. postoperative interval, the liver lobes exhibited a fall in the FFA component, whereas the total glyceride fraction remained almost at the same level (Table I). Though the rate of fatty acid oxidation exhibited a slight increase, it was yet lower than the normal (Chapter-8). There was also a deep fall in plasma FFA. The depot FFA level was found to be higher (Chapter-8). From the increase in depot FFA level and the deep fall in both liver and plasma FFA, it is obvious that there is a high rate of FFA uptake by the adipose tissue from plasma and liver for esterification. This can finally lead into increased fat content of adipose tissue. DeSmet (1953) also had observed an increase in fat content under the skin and around the kidneys following the castration of male rats. Eaton et al. (1969) reported that in man as much as 80% of the plasma FFA are taken up by different tissues of the body where they are either oxidized or incorporated into lipid esters. Such a report points towards the

possibility of FFA uptake by other tissues including the adipose tissues.

Summing up the effect of castration on liver FFA and glyceride, it could be mentioned that at first there is a reduced rate of hepatic fatty acid oxidation combined with increased rate of mobilization and transport of FFA from depots leading to increased levels of FFA as well as glycerides of the liver. At a later stage- about 120 hrs. the tendency is almost reversed in the sense that there is some mobilization from the liver and a considerable rise in the rate of removal of FFA from plasma by adipose tissue. This is manifested by very high levels of FFA there, possibly indicating also an increased rate of esterification. Such a phenomenon of increase in adiposity in the castrates after few weeks has been reported earlier (DeSmet, 1953).

The observations on replacement therapy with three different dosages pointed out a dose related response of the liver. 0.1 mg of TP was found to be effective in bringing about normality in the contents of FFA and glycerides in case of 24 hrs. castrated animals. With higher dosages an abnormal increase in both the liver lobes was registered. As far as the glycerides were

concerned, a fall was obtained with higher dosages. The values observed after replacement with TP in 120 hrs. castrates suggested that the effective dosage (0.1 mg) when administered after longer intervals became subminimal. However, to evaluate these findings, a further detailed study is necessary.