CHAPTER 5

INFLUENCE OF TESTOSTERONE PROPIONATE ON HEPATIC ASCORBIC ACID LEVELS

Hepatic tissue is known to be the site of ascorbic acid (AA) production in mammals (except in primates and guinea pigs), whereas it is the kidney in reptiles and birds (Grollman and Lehninger, 1957; Roy and Guha, 1958). Normal rat liver has been known to store good amount of ascorbic acid (Hassan and Lehninger, 1956). Certain organs, other than liver, like adrenals, spleen and bone marrow are also known to store this vitamin in varied concentrations (Burns et al., 1951). Since ascorbic acid readily undergoes oxidation and reduction it was suggested that it may participate in the oxidation-reduction processes of the tissues (Meiklejohn, 1953). Possible metabolic significance of ascorbic acid levels in different avian, insect and mammalian tissues has been discussed by Chinoy (1969, 1970 and 1972a), wherein a correlation has been brought forth between the higher levels of ascorbic acid in the reproductive tissues and their greater metabolic turnover. Further, deficiency of this vitamin is known to disturb carbohydrate, lipid and iron metabolism (Rusch and

Kline , 1941; Banerjee and Ghosh, 1947; Mazur et al., 1961 and Banerjee and Ganguli, 1962). A general interrelationship between the metabolism of ascorbic acid and circulating testosterone levels has been suggested (Dieter, 1969; Majmudar and Chatterjee, 1974; Chinoy and Parmar, 1975 and Chinoy et al., 1975a,b). Stubbs et al. (1967), on the basis of the results obtained after extirpation of pituitary gland and gonads, have proved that the level of testicular secretion significantly influences the maintenance of tissue levels of ascorbic acid in the liver of male rats. In the light of the literature cited above on varied roles of vitamin C in several physiological processes, it was thought desirable to study the early effects of castration and testosterone propionate (TP) replacement therapy on ascorbic acid levels of the hepatic tissue of male rats.

MATERIAL AND METHODS

Adult male albino rats (Rattus norvegicus albinus) selected for the present study were more of less of uniform weight (120-160 gms.). They were maintained on

a balance diet and water ad libitum. Experimental animals were divided into different groups, viz.,

1. Normal animals
2. Castrated animals
3. Sham-operated animals
4. Castrated and TP administered animals.

Bilateral castration was performed through scrotal sacs, under light ether anaesthesia. They were then sacrificed at the selected time intervals of 24, 48 and 120 hrs. Sham-operated animals were sacrificed after 24 hrs. of operation for comparison. For replacement therapy, a single injection of TP was given intramuscularly. Here two different sets of experiments were conducted. In the first, three different dose levels of TP viz., 0.05, 0.1 and 0.5 mg were administered 24 hrs. after castration. They were then sacrificed by decapitation after 24 hrs. The different dosages of TP were administered in 0.5 ml of tributyrin in every case. In the next set of experiment on replacement therapy, the hormone was administered after 120 hrs. of castration. Here, only one dose viz., 0.1 mg TP in 0.5 ml tributyrin was injected. These animals were decapitated after 1, 2, 4 and 24 hrs. of

injection. In all of the experiments at least 12-15 rats were employed. From the earlier observations of the present study, it was found that the Spigelian lobe of the liver differed in its constituents from the rest of the liver lobes. In the present investigation, therefore, pieces of median and Spigelian lobes were assessed separately for ascorbic acid. Weighed pieces of liver lobes were homogenized in 6% trichloroacetic acid in pre-chilled mortars. Aliquots of these extracts were utilized for the determination of ascorbic acid by employing dinitrophenyl hydrazine method of Roe (1954).

RESULTS

The results obtained for different experimental conditions are presented in Tables I-II.

Under normal condition, the level of ascorbic acid was always found to be higher in the Spigelian lobe than that obtained for the median lobe.

24 hrs. after the removal of gonads, significant variation in the ascorbic acid (AA) content of the liver was noted. It was found to be increased in both the lobes.

Conversely, sham-operated animals showed decreased levels of AA (Table I; Fig. 1). By about 48 hrs. after operation levels of AA were observed to be decreasing gradually upto 120 hrs. Though, the levels of AA in both of the liver lobes dropped considerably, interestingly enough, these values were found to be higher than the normal ones. In addition to this, it was also observed that, whatever variations were there, the Spigelian lobe always showed a higher concentration of AA than that found in the case of median lobe.

During first experiment on replacement therapy, a dose dependent variation was observed. With 0.05 mg of TP a comparatively lower value of AA than normal liver was observed (Table I; Fig. 2). The AA content was found to be increased when 0.1 mg TP was administered. Here, AA content in the Spigelian lobe attained a higher level than that of normal whereas, in case of median lobe it could not even touch the normal level. With a further increase to 0.5 mg of TP a decrease in AA concentration was obtained in both the liver lobes. This drop in the AA values, however, did not bring them down as low as that obtained with 0.05 mg dose.

Table I : Effects of castration and TP replacement on ascorbic acid content (mg/100 gms of fresh tissue) of hepatic tissue of white rats.

Liver lobes	Normal animals	Sham-operated animals	Castrated animals			Castrated injected animals			
			24 hr*	48 hr*	120 hr*	0.05 mg@ 24 hr*	0.1 mg@ 24 hr*	0.5 mg@ 24 hr*	0.1 mg@ 120 hr*
Median	31.66 ± 3.024	21.87 ± 2.359	48.80 ± 2.224	46.18 ± 3.102	38.36 ± 1.774	23.27 ± 2.971	28.03 ± 3.030	27.31 ± 3.011	21.37 ± 2.112
Spigelian	34.52 ± 3.335	26.93 ± 2.566	54.38 ± 2.648	52.28 ± 2.774	42.53 ± 3.817	29.53 ± 3.207	40.30 ± 4.274	36.70 ± 2.728	21.38 ± 1.319

Each reading is the mean value of at least twelve different samples.

*Post-operative intervals

@Dosages of TP administered

Table II : Rapid effects of TP administration on ascorbic acid content (mg/100 gms of fresh tissue) of hepatic tissue of 120 H castrated male rats.

Liver lobes	Normal animals	120 H Castrates	120 H Castrates-injected with 0.1 mg of TP		
			1 H*	2 H*	4 H*
Median	31.66 ± 3.024	38.36 ± 1.774	23.75 ± 2.070	23.61 ± 2.400	25.14 ± 2.089
Spigelian	34.52 ± 3.335	42.53 ± 3.817	27.16 ± 2.292	24.55 ± 1.810	27.16 ± 1.794

Readings are mean values of at least twelve different samples.

*Hours after TP injection

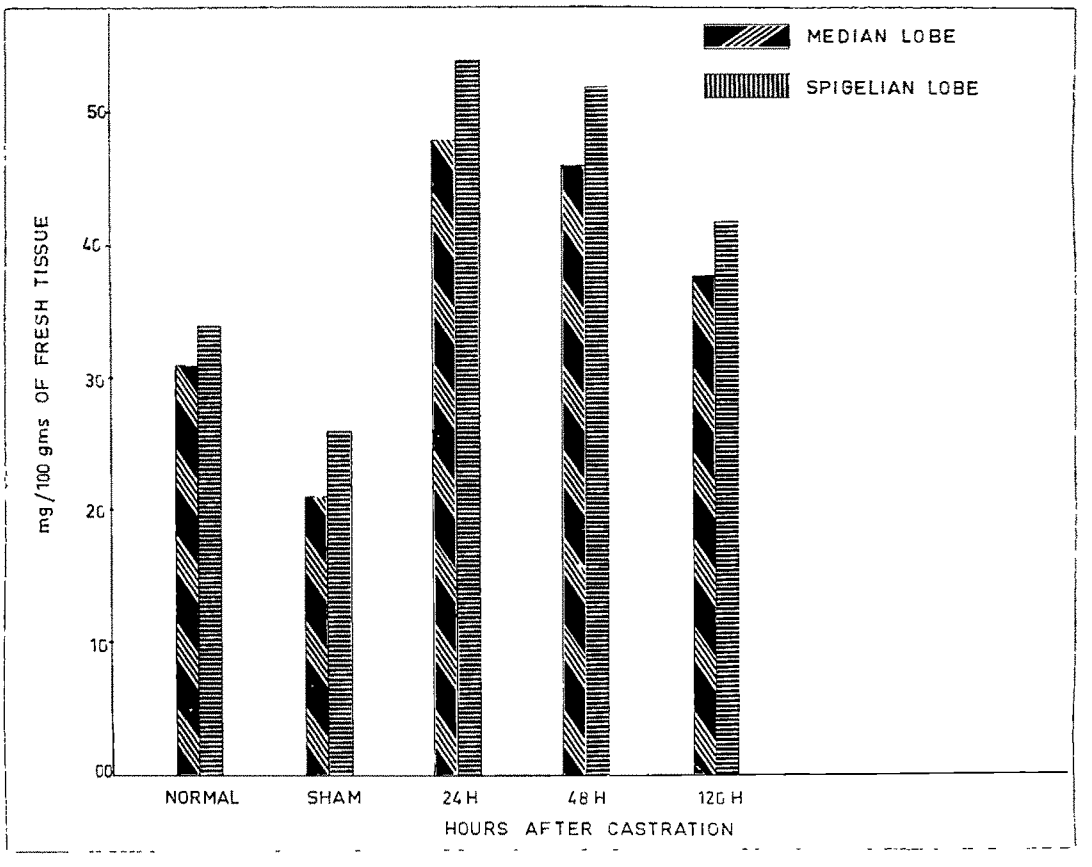

Fig. 1. Levels of AA (mg/100 gms of fresh tissue) in median and Spigelian lobe of the liver of normal, sham-operated and castrated animals.

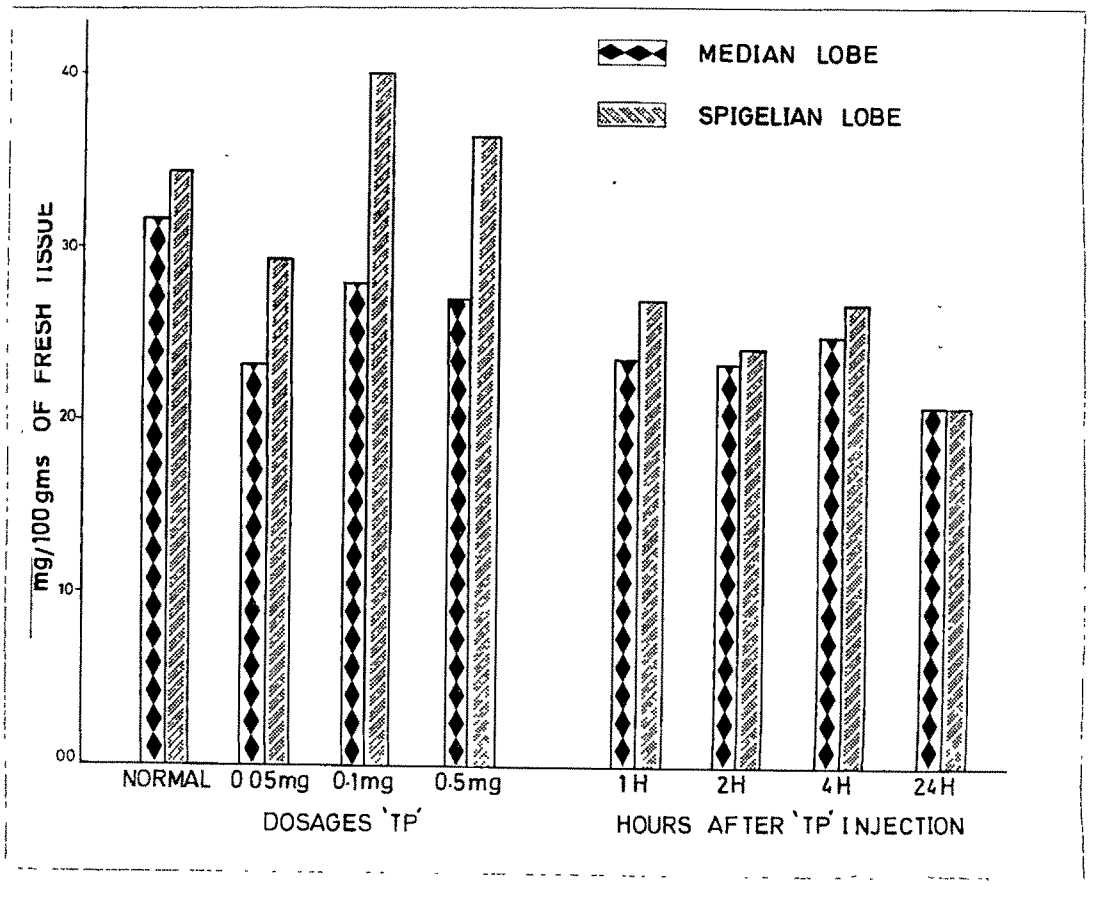

Fig. 2. Representation of the influence of three different dosages of TP, injected to 24 H castrates and the immediate effect of 0.1 mg TP administered to 120 H castrates on AA levels of median and Spigelian lobe of the liver.

In the experiments where 0.1 mg TP was administered after 120 hrs. of castration, a significant decrease in AA content in the hepatic tissue was observable. These values were lower than even the normal liver concentrations recorded (Table I & II; Fig. 2).

DISCUSSION

Since liver is known to be an important organ involved in the overall metabolism of the body; a number of factors are likely to influence its functions. Age and diet are known to influence ascorbic acid metabolism (Ehmke et al., 1956). These two factors were taken care of by keeping them constant throughout the present study. The effects observed could, therefore, be attributed to the different experimental treatments involved. Metabolism of L-ascorbic acid is known to alter under various abnormal conditions including that of adrenalectomy and hypophysectomy. Salmon and Stubbs (1961) observed, diminished synthesis of AA in hypophysectomized rats. Decreased synthesis of AA, and the increased degradation of AA in liver and kidney of adrenalectomized rats was observed by Nathani et al. (1971). Effects of castration on this vitamin have been studied by many workers in male rats (Stubbs and McKernan, 1967;

Stubbs et al., 1967 and Khandwekar et al., 1973). According to these authors concentration of hepatic AA was lowered due to castration and this finding had been correlated by them with the deficit of testosterone. The values obtained by these authors were those assessed few weeks after castration. The present investigation is an attempt to understand the immediate effects of castration on hepatic AA concentration. The results obtained here (Table I; Fig. 1) indicated that castration did induce a rise of AA in the liver 24 hrs. after removal of gonads. On the other hand in case of sham-operated animals a distinct decrease in AA level was noticed (Fig. 1). Hence, it is obvious that the rise in AA of the liver lobes 24 hrs. after castration is certainly not a result of operation trauma. A decline in AA levels was observed thereafter through 48 and 120 hrs. of intervals. But these values were significantly higher than the normal levels. It is a known fact that AA is involved in steroidogenesis (Szent Gyorggii, 1957; Bacq and Alexander, 1961; Biswas and Deb, 1970 and Chinoy, 1972a, b). The work conducted by Stubbs et al. (1967) has proved that normal testicular secretion is required for the maintenance of the tissue level of AA and the concerned enzyme system.

This suggests that the normal testicular secretion has got a certain influence on the rate of synthesis and its concentration in hepatic tissue. When the gonads were removed, this source of influence was removed and the rate of AA synthesis was observed to be enhanced immediately after few hours of castration. Nevertheless, at 48 and 120 hr. postcastration intervals a gradual decline was observed.

It is clear from the observations of other workers (loc. cit.) that at 4-6 weeks post-castration intervals there is a significant reduction in the concentration of hepatic AA levels. From all these evidences it appears that the initial response of hepatic tissue to extirpation of testes is quite unexpected and enigmatic. A plausible suggestion could be that circulating androgen levels may effect a sort of regulation on the enzymic system concerned with AA synthesis and its retention and if that regulatory agency is withdrawn the earliest response of the hepatic tissue is manifested in higher AA values observable. Later on homeostatic processes set in favouring reduced rate of synthesis and retention. Replacement experiments reported here show that after castration the hepatic tissue responds to TP administration. It required a certain specific

amount of the hormone to bring back the normal level of ascorbic acid. Higher dosage did not lead to further beneficial action whereas, the lower dosage was found to be insufficient within first 24 hrs. of injection. This again suggests that certain optimal level of TP is required for maintenance of normal tissue ascorbic acid concentration. If the post-castration interval is prolonged upto 120 hrs. then the previous effective dose (0.1 mg of TP) was found to be insufficient. The data on time-response study here suggested that the amount of TP injected could be catabolised within a short time. Hence, even after 1-4 hrs. of injection the level of ascorbic acid obtained was comparatively only slightly more than that at 24 hrs. Agamemnon (1971) also has suggested, on the basis of a study on rat liver, that a single dose of steroid disappeared rapidly from the perfusion fluid; with a half time of less than two minutes. It appears, therefore, that the hepatic tissue requires a certain minimal level of circulating androgens to maintain proper concentration of ascorbic acid, and more amount of TP need to be injected as more time elapses after castration.