# CHAPTER 6

## EFFECT OF CASTRATION ON $Mg^{++}$ DEPENDENT ADENOSINE TRIPHOSPHATASE (ATPase) ACTIVITY OF RAT LIVER

A great deal of attention has been given to the general problem of energy transductions in biological systems. In the recent past, interest has been centering around the problem of mechanisms coupling metabolic energy with the movement of substances across biological membranes against concentration gradients. Assessment of ATPase activity as an indication of the active transport mechanism has been validated by several investigators (Scholefield, 1964; Wolfe, 1964; Stein, 1967). Various studies have been conducted on different types of ATPase activities in different tissues with respect to the effects of $Mg^{++}$ and $Ca^{++}$ ions, pH optima, substrate concentration as well as responses to activators and inhibitors (Newman et al., 1950; Maengwyn-Davies et al., 1952; Chappell and Peny, 1955; Padykula and Herman, 1955; Freiman and Kaplan, 1960; Azzone et al., 1961; ). Though occurrence of magnesium dependent ATPase activity has been found to be quite common, yet its assessment could easily give an insight into the general mechanics of energy metabolism that go on in any particular

tissue.

One of the primary mechanisms underlying the influence of steroid hormones on their respective target tissues involves an alteration of the rate of transport of substances across the membranes. A wide range of hormones influence the transport of water, inorganic ions and organic compounds into and out of the cells. A cell or tissue will respond as an integrated unit to a given hormone. A part of the hormonal regulation of metabolism may depend upon the availability of substrates or cofactors to the enzyme systems; an access determined by the rate of transport across the cell membranes. For this reason, it is apparently necessary to examine the influence of hormones on the overall energy flux as well as on the rate of transport across membranes, when the total effect of the hormone action is to be understood. Riggs (1964), in a review article, explained that a single hormone, such as oestradiol, will influence the transport of not one but many substances. Further, he suggested that the influence on the rate of transport of amino acids and glucose observed with aldosterone, oestrogens, androgens and corticosteroids, was in keeping with the major physiological activities of these hormones. These observations clearly show that there

are certain enzymatic systems which on hormonal stimulation cause accelerated transport activity, which in its turn may perform the function of metabolic pace-setting. Several androgenic steroids have been shown to increase the uptake by different muscles of inorganic phosphate (Fleischmann and Fleischmann, 1952-1953), the amino-acids (Metcalf and Gross, 1960; Riggs and Wegrzyn, 1966; Mills and Spaziani, 1968); and sugar (Mills and Spaziani, 1968). Most of these measurements have been made in vivo after repeated injections of hormone, or after treatment for several days. Hepatic tissue is considered to be a non-target tissue, and therefore, the relation between its energy metabolism and the action of sex-hormones on it has been reported explicitly. Nevertheless, our previous observation on lipids, carbohydrates, ascorbic acid etc., did show some discernible effects of castration within 24 hrs., on concentrations of certain metabolites of the hepatic tissue of albino rats. The purpose of the present study was to examine the possible effects of removal of gonads, and thereafter, of hormone replacement therapy on general energy metabolism of liver. Hence, an investigation on alterations of ATPase activity under these experimental conditions was carried out.

## MATERIALS AND METHODS

The adult male albino rats weighing about 120 to 160 gms. were used as subjects. They were acclimated to the laboratory conditions for few days prior to experimentation. They were maintained on a balanced diet and water ad libitum. For experimental use, the surgical procedure was either bilateral castration or sham-operation. The hormone treatment consisted of Testosterone propionate (TP) administration of three different dosages viz., 0.05, 0.1 and 0.5 mg per animal. The hormone was taken in tributyrin and administered intramuscularly (volume-0.5 ml in every case). The experimental groups and other conditions are detailed in (Table I and II). The rats were sacrificed by decapitation. Assessment of ATPase activity was made by employing the method of Umbreit et al. (1957). A substrate blank control was used to measure the enzyme activity.

Earlier observations on different parameters have clearly shown that the Spigelian lobe differs significantly from the rest of the liver lobes. Hence, the Spigelian lobe and median lobe (as a representative of other lobes) were assessed severally for comparison in the present study.

Readings were taken at 660 mμ on a Klett-Summerson colorimeter. The values are expressed as μg of phosphorus liberated per mg of protein per 10 minutes period. Protein was estimated by the biuret method (Layne, 1957).

## RESULTS

### I. Castration:

The median and Spigelian lobes of the normal intact animals presented a significant difference in the levels of enzyme activity. The Spigelian lobe showed higher values than the median lobe. The enzyme activity was altered due to castration and changes were obvious at the end of 24 hrs. (Table I, Fig. 1). In both the liver lobes a depletion was noted. The two lobes at this hour exhibited similar levels of enzyme activity. It is significant that the depletion in Spigelian lobe was comparatively much more than that of the median lobe of the rat liver. The sham operated animals, at this interval showed values higher than those found for experimental animals. This observation clearly indicates that the reduction in the enzyme activity observable after 24 hrs. of castration is not due to the surgical stress but, appears to be the result of depletion of circulating sex-hormones.

At 48 hrs. interval the enzyme activity levels were found to be restoring. When the time interval after castration was extended upto 120 hrs., the activity was found to be very much near the normal level, yet it was slightly subnormal (Table I, Fig. 1). Spigelian lobe was found to be responding very sensitively as compared to the median lobe. It regained its comparatively high level of enzyme activity by the end of 120 hrs.

II. Replacement:

Among the three different doses of TP administered 24 hrs. after castration, that of 0.1 mg was found to be more effective dose that could restore the enzyme activity to normal levels. Rats which have been castrated and given the 0.05 mg TP injection 24 hrs. post-operatively also showed a response. Here, the enzyme activity was found to be increased in both of the liver lobes, as compared to the levels obtained for 24 hr. castrates (Table I & II). With 0.1 mg TP further increase was noted. These values were similar to those obtained for normal rat liver. When the dosage was increased to 0.5 mg, the Spigelian lobe registered a slight depletion whereas the

Table I : Changes in ATPase activity of rat liver after castration
( µg phosphate radicals liberated/mg protein/10 min)

| Lobes of the liver | Normal animals | Sham-operated animals 24 H* | Castrated animals | | |
|---|---|---|---|---|---|
| | | | 24 H* | 48 H* | 120 H* |
| Median lobe | 79.20 ± 1.38 | 80.94 ± 3.90 | 75.01 ± 4.72 | 77.51 ± 2.08 | 77.38 ± 4.18 |
| Spigelian lobe | 104.80 ± 6.10 | 94.066 ± 5.31 | 75.07 ± 4.49 | 87.148 ± 6.56 | 98.65 ± 4.80 |

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

*Hours after operation.

Table II : ATPase activity in liver after administration of TP to castrated animals. (µg phosphate radicals liberated/mg protein/10 min).

| Lobes of the liver | Normal animals | 24 H Castrates injected with TP | | | 120 H Castrates injected with TP |
|---|---|---|---|---|---|
| | | 0.05 mg* | 0.1 mg* | 0.5 mg* | 0.1 mg* |
| Median lobe | 79.20 ± 1.88 | 76.14 ± 2.85 | 87.25 ± 4.32 | 89.60 ± 7.51 | 84.42 ± 5.73 |
| Spigelian lobe | 104.80 ± 6.10 | 91.88 ± 4.05 | 103.72 ± 5.08 | 99.36 ± 5.17 | 82.43 ± 4.37 |

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

*Dosages of TP administered.

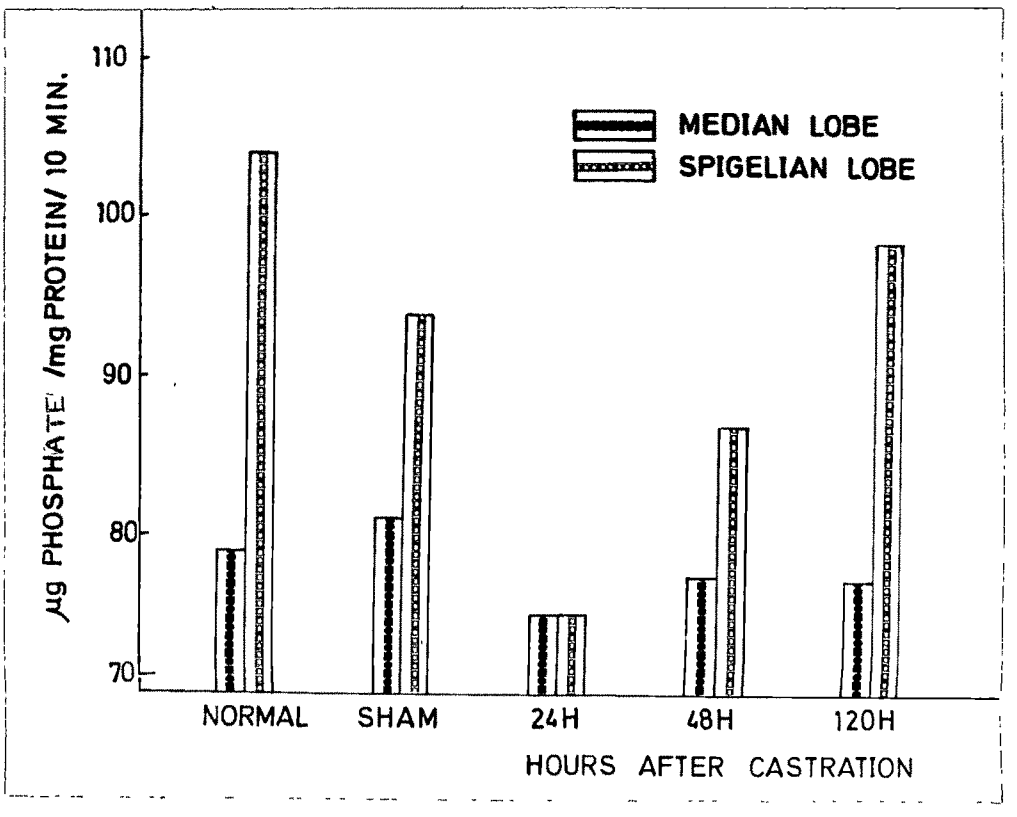


Fig. 1. Adenosine triphosphatse activity of median and Spigelian lobes of the liver of normal, sham-operated and castrated rats.

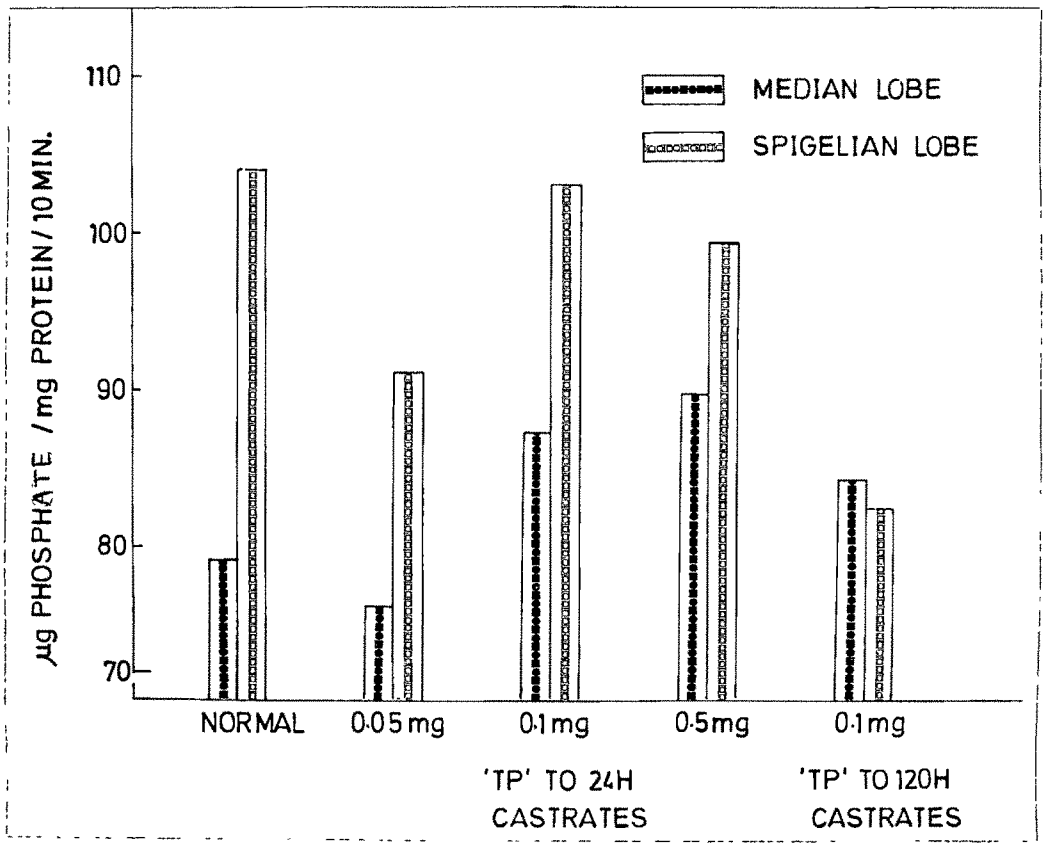


**Fig. 2. Depicts the effects of TP administration on the enzyme (ATPase) activity of the liver: a) 24 H castrates injected with three different doses of TP. b). 120 H castrates injected with 0.1 mg TP.**

median lobe continued to show further increase (Table II).

As noted here, by about 120 hrs. of castration, the levels of ATPase activity returned to more or less normal values. It would, therefore, be interesting to know the effect of TP administration after 120 hrs. With this view another experiment was performed. In this case, only the effective dose of the hormone (0.1 mg) was administered. The results obtained are presented in Table II. Altogether a different picture emerged in this series. ATPase activity of the median lobe exhibited a value higher than those obtained for the normal as well as 120 hr. castrates (Fig.2), apparently indicating a stimulation of the enzyme. On the other hand, the Spigelian lobe showed a distinct decrease inspite of the fact that the dose of TP administered here was found to be the effective one in the previous series.

## DISCUSSION

The observations recorded here provide enough evidence to show that the hepatic lobes of the rat do exhibit fluctuations in the enzyme activity when the animal is deprived of the male sex hormones emanating from the testes. A discernible influence on enzymatic levels of liver lobes

of the castrates was apparent after 24 hr. interval. Since a reduced enzyme activity was observed, a lower energy flux by the tissue is suggestive. This points to a low clearance of adenosine diphosphate (ADP) meaning that there exists a state of lower rate of turnover of energy rich phosphate bonds, hence a reduced energy flux, in general, within 24 hrs. of castration. With further time lapse after castration, the enzyme activity almost reached normal levels. It appears that the animals attain an initial recovery at the physiological level by about 120 hrs. post operatively. It is apparent from the vast amount of literature on castration effects that after about four weeks of castration again new physiological manifestations appear that lead ultimately to more or less normal pattern of hepatic metabolism.

The observations reported in the present study on replacement with TP (Table II), revealed that 0.1 mg of TP was an effective dose and 0.5 mg was found to be superfluous for Spigelian lobe when administered after 24 hrs. of castration. Contrastingly, this same effective dose (0.1 mg) turned out to behave differently for Spigelian lobe when administered 120 hrs. post operatively. It could be said that, by 120 hrs. after castration,

animal settles down to a steady state, showing more or less normal ATPase activity, hence, the dose level required to bring about the normalization of this enzyme appears to be lower than that required after 24 hrs. Apparently the stimuli for the immediate alterations in the metabolism of ATP are most probably related to altered membrane permeability and possible changes in the consequent synthesis of macromolecules that characteristically follow gonadectomy. ATPase activity is also known to be helpful for active transport (Klein and Boyer, 1972; Simoni and Shallenberger, 1972). According to Metcalf and Gross (1960), steroids (TP and other synthetic hormones) caused an increased uptake of amino acids in levator ani muscle of rats. This would necessitate involvement of ATPase activity. Mills and Spaziani (1968) have shown that testosterone does increase the transport of amino acids and glucose in case of prostate gland, seminal vesicle and levator ani muscle of castrated rats within 6-18 hrs. in vivo. In the present study, during the replacement therapy after 24 hrs. of castration, the rate of enzyme activity was found to be enhanced. Testosterone propionate may accelerate the rate of hydrolysis of ATP in the liver mitochondria. Wade and Jones (1956) reported

that there was no effect of testosterone on liver mitochondrial ATPase activity isolated from intact rats. However, they observed, that progesterone increased the ATPase activity. It may be recalled here that castration might alter overall general membrane permeability including that of mitochondrial membrane and hence the response to consequent administration of TP. Moreover, interconversions of steroidal hormones in the hepatic tissue might also be contributing to this.

The present findings do not seem to point to any significant lasting effects of castration on the $Mg^{++}$ activated ATPase activity of the rat liver. However, it does indicate that there are certain subtle changes that occur within 48 hrs. of gonadectomy which are obviously governed by the male sex hormones. Nevertheless, $Mg^{++}$ dependent ATPase activity being important to the general economy in the overall physiological welfare of the animal appears to readjust itself, though under obviously altered endocrine milieu, during later periods for the benefit of the individuals. Since the early changes are of no apparent consequence at later stages to the well-being of an animal they constitute at least under present state of knowledge, an assemblage of information of pure academic interest.