CHAPTER 4

EFFECTS OF CASTRATION AND TESTOSTERONE PROPIONATE ADMINISTRATION ON THE LEVELS OF NUCLEIC ACIDS AND TOTAL PROTEIN OF THE LIVER OF WHITE RATS

Mammals exhibit a basic ability to adapt to environmental alterations through intricate adjustments in their general metabolic patterns. The regulatory mechanisms respond to all external and internal variations, and this in turn is reflected in metabolic alterations. Regulation of protein metabolism is achieved by mechanisms operating at the subcellular level and also by coordinated actions between cells and tissues. Hormones are the principal regulators of biosynthesis of specific proteins in mammalian systems.

According to Barrington (1964) and Bern and Nicoll (1968) different tissues of an organism may respond differently to the same hormone. However, it is clear that hormones are not directly essential for particular processes per se but rather may serve either to accelerate or decelerate it.

In many cases metabolic responses to endocrine secretions are organ- or tissue-specific. Leathem (1970)

pointed out that certain hormones will inhibit protein metabolism in some organs while stimulatory effect may be observable in others.

It is a well established fact that nucleic acids are associated with protein synthesis and growth (Riddiford, 1960; Hay and Fischman, 1961; Osteen and Walker, 1961). Cellular systems engaged in protein-synthetic activity invariably show a concommitant rise in the level of ribonucleic acid. It is a proven fact that protein synthesis is intimately connected with the synthesis of ribonucleic acid and that ribonucleic acid participates directly in protein synthesis. Levels of nucleic acids have been reported to be altered by adrenocorticotrophins (Boyadjieve and Hadjidov, 1971); glucocorticoids (Feigelson and Feigelson, 1963); androgens (Kochakian, 1969); progesterone (Trams et al., 1973) etc. Anabolic effect of male sex hormones (on accessory sex glands) is well documented fact (Kochakian, 1964; Mainwaring and Wilce, 1972; Liang and Liao, 1975; Rajalakshmi and Prasad, 1976). Further, from the studies of Leathem (1958), it could be seen that adult rats have

a limited capacity to respond to androgens; as the animals normally are in a state of positive nitrogen balance and protein stores may be sufficiently adequate. As far as the rat liver is concerned, no marked effect of androgens could be observed (Aschkenasy-Lelu and Aschkenasy, 1959; Nimni and Bavetia, 1961). These authors have also noted that total protein, nucleic acids and the rate of incorporation of amino acids into liver were not influenced by androgens. Similarly, the action of estrogens on protein metabolism have been extensively reviewed by Aschkenasy-Lelu and Aschkenasy (1959). They pointed out that the action of these hormones on the nitrogen balance of rat varies with dosages. At low dose levels, estrogens have a slightly anabolic action, but at higher dosages they are distinctly catabolic.

According to Munro (1970) hormones play an important role in the integration of adaptive changes in protein metabolism of the whole animal. He has further suggested that each cell or tissue is competent to synthesize and degrade its own constituent proteins; the extent of these reactions is regulated largely by processes taking place

within the cell. Munro (1970) has also suggested that liver proteins, together with the plasma proteins synthesized by the liver, comprise a major part of labile protein reserves and deposition or loss of proteins is faster in the liver than in any other organ, except in the pancreas.

It was, therefore, thought desirable to study the possible influence of androgenic hormones on the nucleic acids and proteins of the hepatic tissue so as to gain some clear understanding concerning possible responsiveness of a non-target, yet metabolically an important tissue - the liver.

MATERIALS AND METHODS

All work was done on hepatic tissue of adult male albino rats, weighing about 120-160 gms. Animals were killed by cervical dislocation. The liver lobes (Chapter-1) were dissected out and weighed immediately. Liver protein content was measured according to the Folin-phenol method as described by Lowry et al. (1951) and nucleic acids were assayed Spectrophotometrically as suggested by Schneider (1957). Similar measurements were made after castration

and replacement therapy. Experimental procedures were similar to those described in 'Chapter-1'. The details of the experimental conditions are presented in Tables I & II.

RESULTS

In normal intact animals, higher values for proteins and nucleic acids were observed in the case of Spigelian lobe of liver than those obtained for median lobe (Table I). After castration total protein levels were found to be increased by about 120 hrs. The sham-operated (24 hrs.) animals showed higher protein values for median lobe, but the Spigelian lobe showed lower one than the normal value. In comparison to this, 24 hr. castrated animals showed decreased protein content in median lobe, whereas, increased level was observed in Spigelian lobe of the liver. Thereafter, by 48 and 120 hrs. post-operatively, a smooth rise was noted in both the lobes (Table I; Fig. 1).

When the results of nucleic acids were studied, it was noted that deoxyribonucleic acid (DNA) level increases immediately after 24 hrs. of castration but thereafter by

120 hrs. a decrease towards the normal level was observed. Though it was found to be decreasing than earlier intervals, it still showed values higher than those noted for normal intact animals (Table I; Fig. 2).

Sham-operated animals showed almost negligible change in DNA level of median lobe, but the Spigelian lobe registered a fall in its content. When these results were compared with 24 hr. castrated animals, Spigelian lobe was found to show almost 60% rise, which was much more than that obtained in median lobe of the liver. The other noticeable difference between the response of these two lobes was that, the median lobe showed further increase at 48 hr. interval, whereas, the level of DNA remained almost similar to 24 hr. interval in case of Spigelian lobe (Table I; Fig. 2).

Ribonucleic acid (RNA) of the hepatic tissue also showed an elevated level immediately after 24 hrs. of gonadectomy. Here also the sham-operated animals showed a decreased content in both the liver lobes. Increase observed, therefore, in the Spigelian lobe was higher than the median lobe (Table I). By 48 hrs. the level of RNA declined in both the lobes showing values nearer to normal

ones. However, again an increase by 120 hr. interval could be noted (Table I; Fig. 2). Comparison of the ratios of DNA/RNA, clearly indicated a differential response of the two lobes of liver. In case of median lobe, the DNA values were found to be less than RNA in normal condition. It still showed the same trend after 24 hrs. of castration. But at 48 hr. interval, the DNA content was higher than the RNA, showing a continued rise of DNA content whereas a distinct fall in RNA content at this hour was obvious. At 120 hr. post-operative interval this ratio was found to attain more or less original pattern. Of course, the values for both the nucleic acids were higher than the normal (Table II).

Considering the Spigelian lobe, almost 1:1 ratio was observed in normal and 24 hr. castrated animals. Here also, at 48 hr. interval, the DNA level went higher up than RNA. This was due to the actual fall in RNA content of the liver lobe. By 120 hrs. of castration the ratio was again restored to almost 1:1 (Table II).

Studies with hormonal replacement indicated a dose dependent response of total protein content of the hepatic

tissue. With 0.05 and 0.1 mg of testosterone propionate (TP), an increase was observed in both the lobes. But, when dosage was increased further to 0.5 mg, median lobe registered a fall, whereas, Spigelian lobe continued to show further increase (Table II; Fig. 3).

Nucleic acids on the other hand, were found to be decreased with the minimum dose (0.05 mg) of TP administered. Higher dosages resulted in increased levels so much so that the levels were above the normal values with 0.5 mg. Median lobe did not show much difference in its nucleic acid content with 0.05 and 0.1 mg TP but with 0.5 mg it also registered an increase above normal (Table II; Fig. 4). Considering the differences in the values of DNA and RNA, it was observed that DNA was higher than the RNA in the Spigelian lobe, whereas, it was low in median lobe and only showed slight increase than RNA with administration of 0.5 mg TP (Table II).

Hourly responses of the liver lobes to the 0.1 mg of TP after 120 hrs. of castration, denoted a gradual fall in the protein content upto 4 hrs.. After 24 hrs. of injection it was found to be increased so as to reach the levels that were above the normal and castrated levels (Table II; Fig. 3).

Table I : Data on total protein and nucleic acids of rat liver after castration.

	Normal Animals		Sham-operated Animals		Castrated Animals					
					24 H*		48 H*		120 H*	
	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp
Protein % of fresh tissue wt.	14.41 ±1.67	17.55 ±2.73	18.42 ±3.42	16.69 ±3.10	15.59 ±0.75	17.38 ±0.69	18.24 ±0.74	19.11 ±0.58	19.45 ±0.89	20.20 ±0.80
DNA µg/mg of fresh tissue wt.	0.044 ±0.0106	0.064 ±0.0099	0.041 ±0.0087	0.049 ±0.0063	0.052 ±0.0010	0.081 ±0.0013	0.060 ±0.0090	0.080 ±0.0076	0.053 ±0.0028	0.071 ±0.0080
RNA µg/mg of fresh tissue wt.	0.050 ±0.0095	0.060 ±0.0140	0.037 ±0.0013	0.039 ±0.0030	0.064 ±0.0011	0.080 ±0.0040	0.055 ±0.0031	0.062 ±0.0042	0.068 ±0.0050	0.075 ±0.0057

Each value is the mean of at least ten different samples.
M - Median lobe of the liver.
Sp - Spigelian lobe of the liver.
*Time interval in hours after castration.

Table II : Hepatic protein and nucleic acid concentrations as observed after TP replacement in castrated rats.

	24 H Castrated/injected with TP						120 H Castrated/injected with 0.1 mg TP							
	0.05 mg*		0.1 mg*		0.5 mg*		1 H@		2 H@		4 H@		24 H@	
	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp
Protein % of fresh tissue wt.	23.75 ±0.971	20.35 ±0.984	20.17 ±0.587	22.10 ±0.224	18.44 ±0.409	22.98 ±0.569	19.30 ±3.17	16.60 ±1.28	13.00 ±1.08	15.50 ±1.46	13.00 ±0.409	14.80 ±0.[illegible]	22.00 ±0.65[illegible]	22.52 ±0.695
DNA µg/mg of fresh tissue wt.	0.037 ±0.0047	0.064 ±0.0060	0.036 ±0.0051	0.074 ±0.0076	0.063 ±0.0031	0.086 ±0.0089	0.060 ±0.0095	0.061 ±0.0014	0.058 ±0.0031	0.055 ±0.0052	0.068 ±0.0083	0.065 ±0.0052	0.058 ±0.0064	0.079 ±0.0068
RNA µg/mg of fresh tissue wt.	0.045 ±0.0074	0.048 ±0.0050	0.049 ±0.0040	0.054 ±0.0010	0.061 ±0.0030	0.068 ±0.0010	0.040 ±0.0013	0.042 ±0.0035	0.039 ±0.0073	0.050 ±0.0024	0.045 ±0.0075	0.044 ±0.0066	0.052 ±0.0055	0.058 ±0.0036

Each value is the mean of at least ten different samples.
M - Median lobe of the liver.
Sp - Spigelian lobe of the liver.
*Different dosages of the TP
@Time interval after injection of TP.

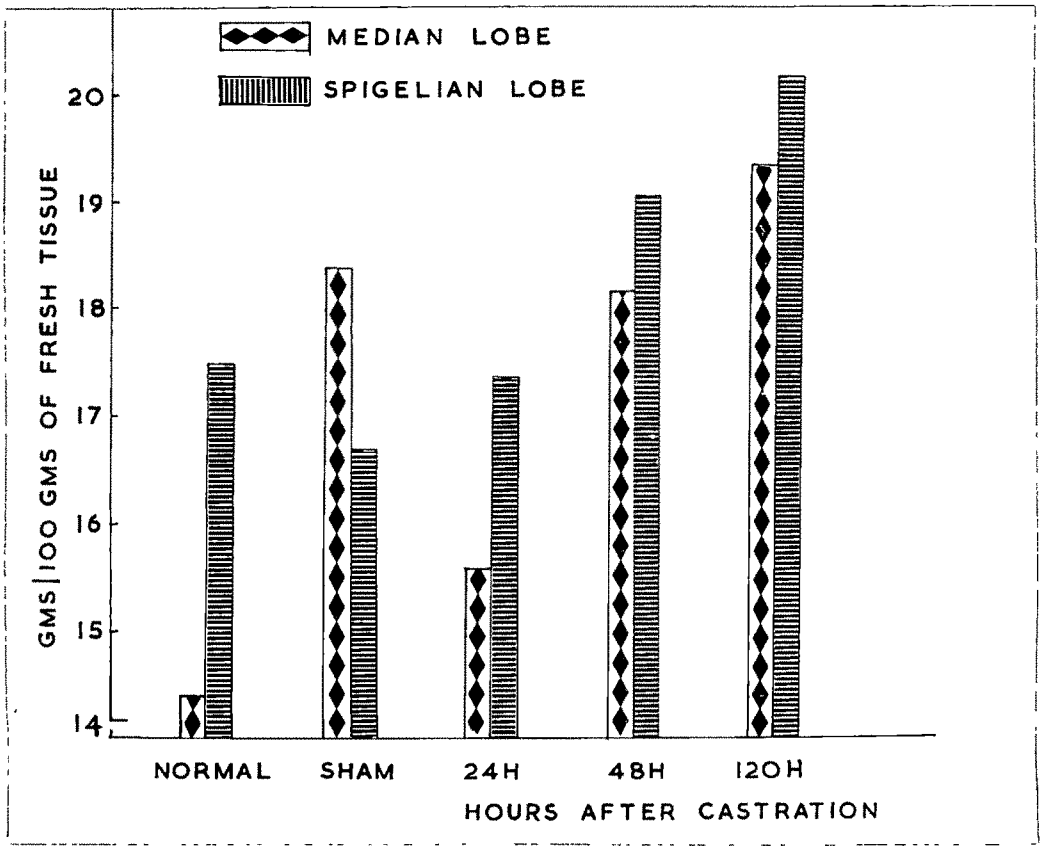

Fig. 1. Variation in total protein level, expressed as gm. percent, in the median and Spigelian lobes of the liver due to castration.

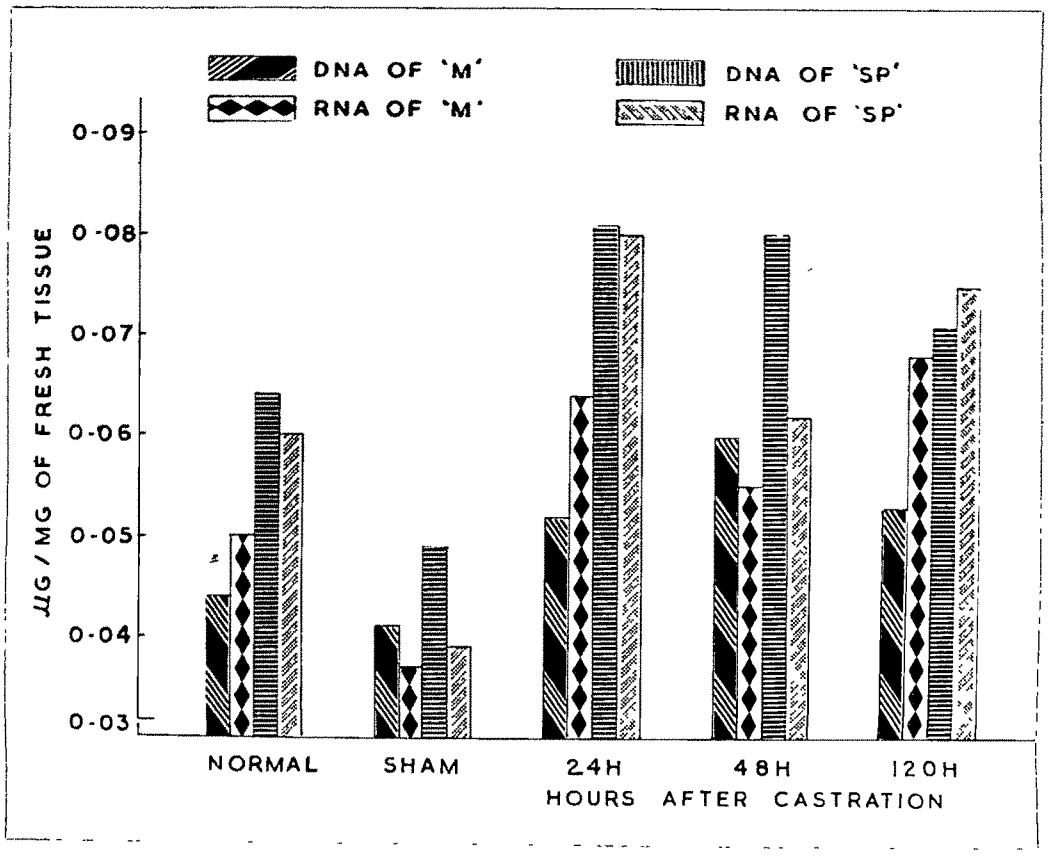

Fig. 2. Concentrations of nucleic acid (DNA and RNA) expressed as µg/mg of fresh tissue in median and Spigelian lobes of the liver of normal, sham-operated and castrated animals.

M - Median lobe of the liver.

Sp - Spigelian lobe of the liver.

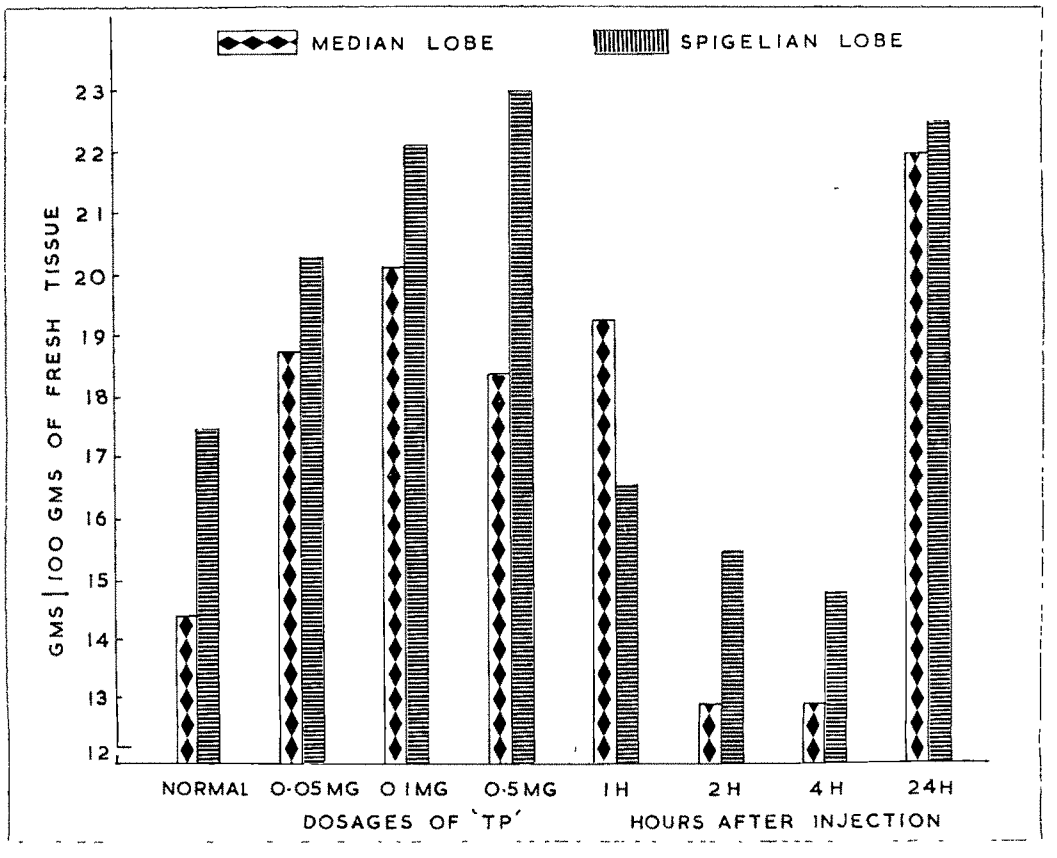

Fig. 3. Influence of TP administration to castrates- three different doses after 24H of castration and four time intervals in 120 H castrates- on total protein contents of the two liver lobes.

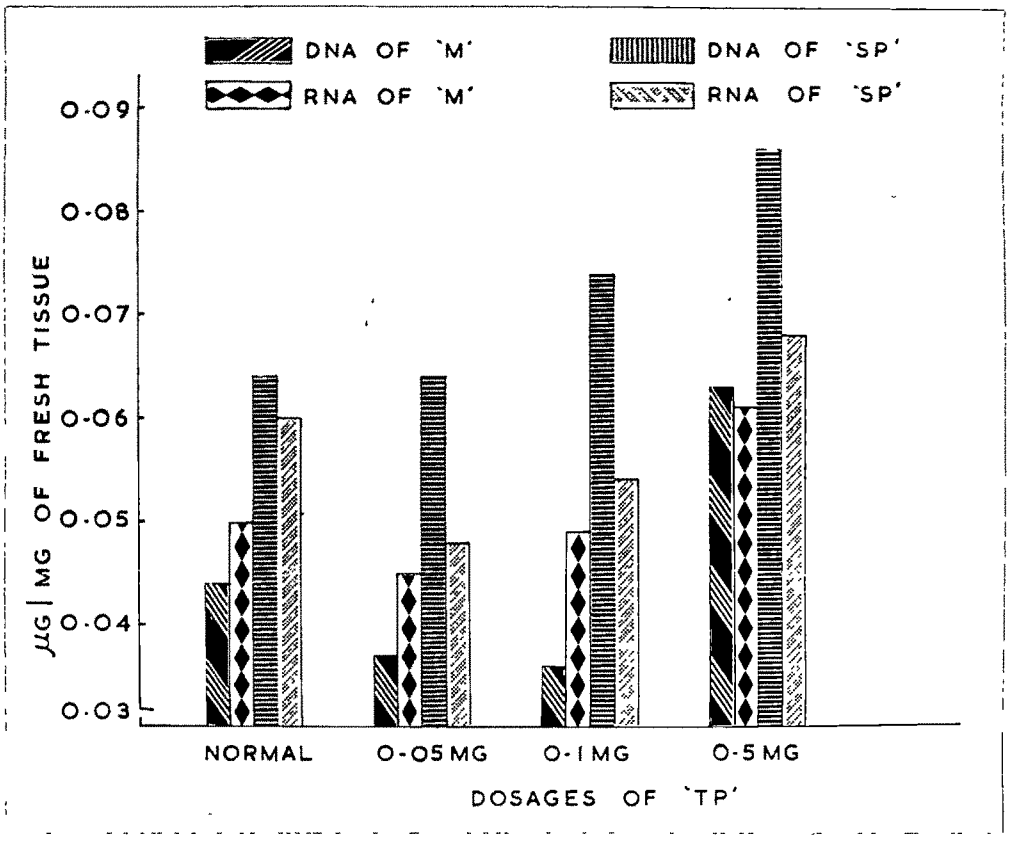

Fig. 4. Effects of administration of three doses of TP to 24 H castrates on the nucleic acids of the median and Spigelian lobes of the liver.

M- Median lobe of the liver.

Sp- Spigelian lobe of the liver.

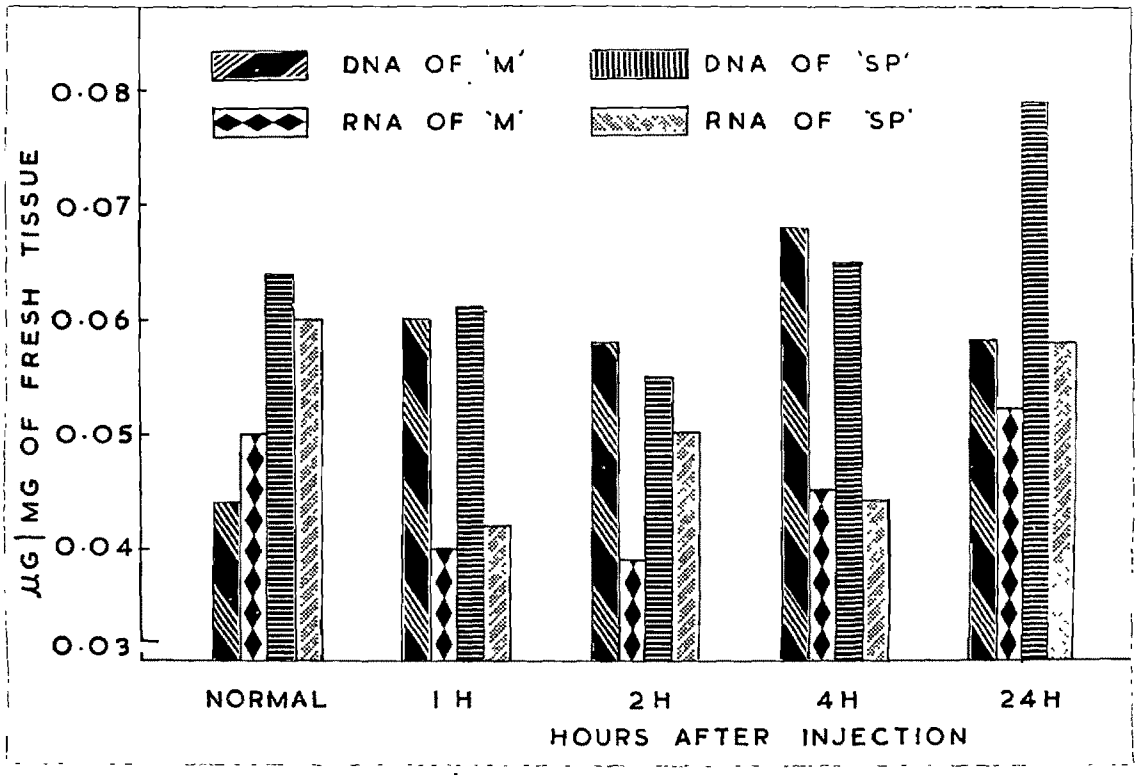

Fig. 5. Shows the rappid effect of 0.1 mg dose of TP to 120 H castrates.

M - Median lobe of the liver.

Sp - Spigelian lobe of the liver

DNA level declined in the Spigelian lobe at one hour and furthermore at two hrs.. By about 4 hrs. of injection it reached almost the normal value. It shot up high after 24 hrs. of TP administration (Table II; Fig. 5).

RNA of the Spigelian lobe also decreased after one hour of hormone injection. It registered ups and downs by 2 hrs. and 4 hrs. and finally increased to nearly normal level after 24 hrs. of hormone replacement (Table II; Fig. 5).

The RNA content was low in the median lobe at one and two hour intervals after TP. administration. By about four hrs. it started going back to normal level but reaching that level only after 24 hrs.. The DNA of the median lobe was first found to be increased by about four hrs. and then it decreased after 24 hrs. but still remained higher than the normal (Table II; Fig. 5).

DISCUSSION

At the very outset, it may be noted that the increase in the total protein content of the liver after castration,

was the result of the increase in RNA content. Contrastingly enough, the nucleic acids were found to be decreased in case of sham-operated animals, whereas, the protein content was found to be increased. It appears that the rise in protein level in sham-operated animals might be due to an acceleration of pre-existing protein synthesizing enzyme systems. Enhanced formation of proteins due to any acute stress, including surgery, that did not involve liver, have been reported by Majumdar et al. (1967).

24 hr. castrated animals, after hormone replacement experienced a fall in RNA content of the hepatic lobes with minimal dose (Table II), substantiating the results obtained with the castrated animals (Table I). Increase in dosages resulted into increase in RNA content. There are reports in which contrary effects of higher dosages of hormone were observed. Aschkenasy-Lelu and Aschkenasy (1959) observed an anabolic action of estrogen with low dose levels but at higher dosages it was found to be distinctly catabolic. Further, Kochakian (1946) reported that androgens have anabolic effect in castrated as well as intact animals. The results obtained in the present study were again confirmed by replacement with testosterone propionate after 120 hrs. of castration. Here also immediately one hour

after TP administration the liver lobes registered a decrease in RNA level (Table II), and could reach approximately normal levels only after 24 hrs. of hormone injection.

The protein content showed a concomitant decrease after two hours of hormone replacement and reached values higher than normal after 24 hrs. (Table II). The responses shown by hepatic protein to dosage of hormone after 24 hrs. of castration were found not to be parallel with the RNA response. The reason for which is not clear. Only observable point in protein content was that higher dosage (0.5 mg) (Table II) led to a decrease in median lobe whereas Spigelian lobe showed almost no change except with the 0.1 mg dose level, which was found to be the most effective one.

Results obtained immediately (24 & 48 hrs.) after gonadectomy revealed an increase in DNA content of the liver. Generally, the DNA content of the cell is known to remain constant. One of the possibilities for increase in DNA could be an induction of polyploid condition. Konopkova and Nedvidek (1972) have observed a high degree of polyploidy in liver after castration of male rats.

Wiest (1974) has reported stimulation of DNA synthesis in rat liver after adrenalectomy. The explanation given by him was that the increase was due to high rate of polyploidy. Further, by taking into account the ratio of DNA/RNA, particularly at 48 hrs., it could be suggested that the rise in DNA content after 24 and 48 hrs. of castration was due to induction of polyploidy. Thereafter, by 120 hrs. this nucleic acid was found to decrease but, still remaining higher than normal levels. This suggested that the hepatic tissue tries to attain the normal level. The liver cell might undergo nuclear amitosis without cytosomal division leading to diploid and polyploid condition. The RNA was found to be higher under these experimental conditions, which could be due to its increased synthesis in the hepatic tissue, ultimately leading to increased protein content.

Replacement therapy induced declining trend (below normal in the median and almost normal in the Spigelian lobe) for DNA with the minimal dose (Table II). Increasing the dose level resulted into increased DNA content which was found to be above normal levels. The response observed in the case of Spigelian lobe with different

dosages (Table II) suggested its greater sensitivity to hormone administration than the median lobe (the higher value for DNA in median lobe was obtained only with 0.5 mg dose of TP). The results obtained were further analysed by administering the hormone to 120 hr. castrated animals. This showed that after 4 hrs. of hormone administration the Spigelian lobe attained almost normal DNA level (Table II), whereas, the median lobe could not reach the normal level at all the different intervals studied. The overall picture obtained for nucleic acids and protein contents of the liver suggested that they are influenced by the male sex-hormone in rats.