CHAPTER 7

ALTERATIONS IN NON-SPECIFIC PHOSPHOMONOESTERASE ACTIVITY UNDER THE INFLUENCE OF SEX HORMONES IN RAT LIVER

Phosphatases have drawn considerable attention in recent times due to their importance in various aspects of cellular metabolism. Relatively little specific information is available regarding biological functions of phosphatases despite their ubiquitous distribution. These enzymes are necessary during the hydrolysis of phosphate esters. Phosphatases are classified into phosphomonoesterases, phosphodiesterases and pyrophosphatases. The most widely distributed phosphomonoesterases are acid and alkaline phosphatases. Acid phosphatase activity accelerates hydrolysis of phosphate esters optimally at acidic pH and has been identified with a special group of cytoplasmic particles, the lysosomes (Duve et al., 1962). Catalytic activity of alkaline phosphatase is similar to that of acid phosphatase, except that the hydrolysis of phosphate esters takes place optimally at an alkaline pH. Since these phosphatases hydrolyze a variety of phosphate esters, they are termed as non-specific phosphatases. Because of their non-specificity, it is difficult to ascertain any specific role played by either acid- or alkaline-phosphatase activity

present in a particular tissue or cell. Usually, therefore, such an enzyme activity is correlated with the specific functions of a particular tissue or cell type. Some of the prominent functional attributes assigned to alkaline phosphatase activity are its association with transmembrane transport mechanisms, process of calcification and growth and differentiation as suggested by many chemical and histochemical studies reviewed by Moog (1946), Sols (1949) and Bradfield (1951). Various functional events such as synthesis, transport of substances and other metabolic regulations and activities requiring the mediation of phosphatase usually depend to a great extent on alkaline phosphatase. Raekallio (1970), however, suggested that the functional correlation of alkaline phosphatase activity with transport of metabolites across cell membranes, to be a more acceptable contention. Acid phosphatase activity has been identified in a very large number of plant and animal tissues (Reviews by Schmidt and Laskowski, 1961; Hollander, 1972). Many of these reports have been concerned mainly with the enzyme activity in crude extracts or with histochemical localization. Of recent, acid phosphatase activity has gathered some interest as a result of apparent alterations in its total activity or distribution patterns

of its isozymes in a number of pathological conditions. Some of the important functions ascribed to acid phosphatase activity are:- as the hydrolytic enzyme (Heinrikson, 1969), in the regulation of pyridoxal phosphate requiring enzymes (Andrews and Turner, 1966), involvement in steroid transport, in Vit.B6 metabolism (DiPietro and Zengerle, 1967), and in lipid metabolism (Blank and Snyder, 1970).

Several studies have been conducted on these enzymes in the sex accessory organs (Kugler et al., 1956; Bialy and Pincus, 1967; Chinoy et al., 1973; Chinoy and Sheth, 1977; Sheth and Chinoy, 1977). But the activities of the enzymes could also differ in the non-accessory tissues. Hence, an investigation on the phosphatase activities in hepatic tissue under the influence of sex hormone was undertaken.

The influence of the sex hormone could be better understood by removing the sex glands and then administering the hormone(s). Since the removal of the source of hormone is an artificial phenomenon and also negative in itself, it can result in significant alterations. Information available on phosphatase

activities in the hepatic tissue, particularly in relation to sex hormones, is relatively scarce. Hence, it was thought desirable to investigate these enzyme activities as altered due to the castration.

MATERIALS AND METHODS

Liver pieces of both median and Spigelian lobes, were weighed and processed separately for the quantitative determination of acid and alkaline phosphatase activity. The animals used and the experimental conditions applied were similar to those described previously in Chapter-1. Enzyme activities were measured according to the methods as described in Sigma technical bulletin No. 104 using p-nitrophenol phosphate (4-nitrophenyl phosphoric acid of Hopkin and Williams-Searle Comp.) as the substrate. The readings for both the phosphatase activities were obtained at 410 μ after incubating for 30 minutes at 37°C. These readings were then computed for the levels of enzyme activities with reference to the standard curves and expressed in terms of μg of phosphate liberated per mg of total protein. The determination of protein was carried out, using biuret reagent, according to the method of

Layne (1957). Comparisons were made, in case of both the phosphatases, between normal, castrated and sham-operated animals. Data obtained after replacement of hormone were compared with those for castrated (24 hrs./ 120 hrs.) and normal intact rats.

RESULTS

Results obtained for the normal intact animals, indicated that the rat liver exhibits phosphatase enzyme activity mainly in the acidic range, while very little alkaline phosphatase activity was noticeable. There were no marked differences between the two liver lobes (median and Spigelian) as far as the activities of acid- and alkaline-phosphatases were concerned. In case of castrated rats, alkaline phosphatase was found to be affected more than the acid phosphatase. After 24 hrs. of castration the activity of this enzyme was found to be higher than the normal value, but when this was compared with sham-operated animals (after 24 hrs.) it was apparent that the rise in enzyme activity could not be merely due to castration. The alkaline phosphatase activity was seen to be decreased after 48 hrs. of castration. Hence,

the rise during 24 hrs. castration could not be attributed to the effect of removal of gonads. After 120 hrs. of orchidectomy again a rise in the alkaline phosphatase activity was observed, which showed higher values than that obtained in normal (Table I; Fig. 1).

The level of acid phosphatase activity was found to decline immediately 24 hrs. post-operatively. The activity of this enzyme was found to attain almost the normal levels by 48 and 120 hrs. intervals after the removal of gonads (Table I; Fig. 2). In sham-operated animals (24 hrs. interval) the rat liver did not exhibit any significant variation in acid phosphatase enzyme activity.

With hormone replacement therapy, a dose dependent effect on phosphatases was noticed. 24 hrs. after the single injection of 0.05 mg of TP, alkaline phosphatase activity was seen to be higher than the normal level as well as that obtained for 24 hrs. castrates. When 0.1 mg of TP was injected, the activity increased further, but when 0.5 mg TP was given intramuscularly, a decrease was noticed as compared to previous two doses (0.05 and 0.1 mg). The activity of alkaline phosphatase as influenced by

Table I : Levels of acid- and alkaline-phosphatase activity in liver under the influence of castration in male rats.

	Normal Animals		Sham-operated Animals		Castrated Animals					
			24 H*		24 H*		48 H*		120 H*	
	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp
Alkaline phosphatase μ M of p-nitrophenol liberated/ mg PR/30 min.	0.0905 ±0.0046	0.0936 ±0.0016	0.1531 ±0.0195	0.2291 ±0.0237	0.1587 ±0.0266	0.1763 ±0.0302	0.0854 ±0.0119	0.1027 ±0.0193	0.1285 ±0.0269	0.1358 ±0.0172
Acid phosphatase μ M of p-nitrophenol liberated/ mg PR/30 min.	1.022 ±0.197	0.953 ±0.120	0.923 ±0.031	0.994 ±0.058	0.849 ±0.024	0.829 ±0.008	0.957 ±0.039	0.936 ±0.077	0.989 ±0.051	0.985 ±0.044

Each value represents the mean of at least 15 readings.
M - Median lobe of the liver.
Sp - Spigelian lobe of the liver.
*Post-operative intervals in hours.

Table II : Influence of TP administration on levels of acid- and alkaline-phosphatase activity in hepatic tissue of castrated male rats.

	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*	
	M	SP	M	Sp	M	Sp	M	Sp	M	Sp
Alkaline phosphatase /uM of p-nitrophenol liberated/ mg PR/30 min.	0.0905 ±0.0046	0.0936 ±0.0016	0.1810 ±0.0181	0.2349 ±0.0258	0.1784 ±0.0188	0.1765 ±0.0178	0.1354 ±0.0113	0.1740 ±0.0140	0.1006 ±0.0178	0.1200 ±0.0097
Acid phosphatase /uM of p-nitrophenol liberated/ mg PR/30 min.	1.022 ±0.197	0.953 ±0.120	1.013 ±0.046	0.986 ±0.040	1.077 ±0.019	1.105 ±0.064	0.957 ±0.027	0.997 ±0.029	0.737 ±0.025	0.785 ±0.049

Readings represented are mean values of 15 different samples.
M - Median lobe of the liver.
Sp - Spigelian lobe of the liver.
*Dose levels of TP injected.

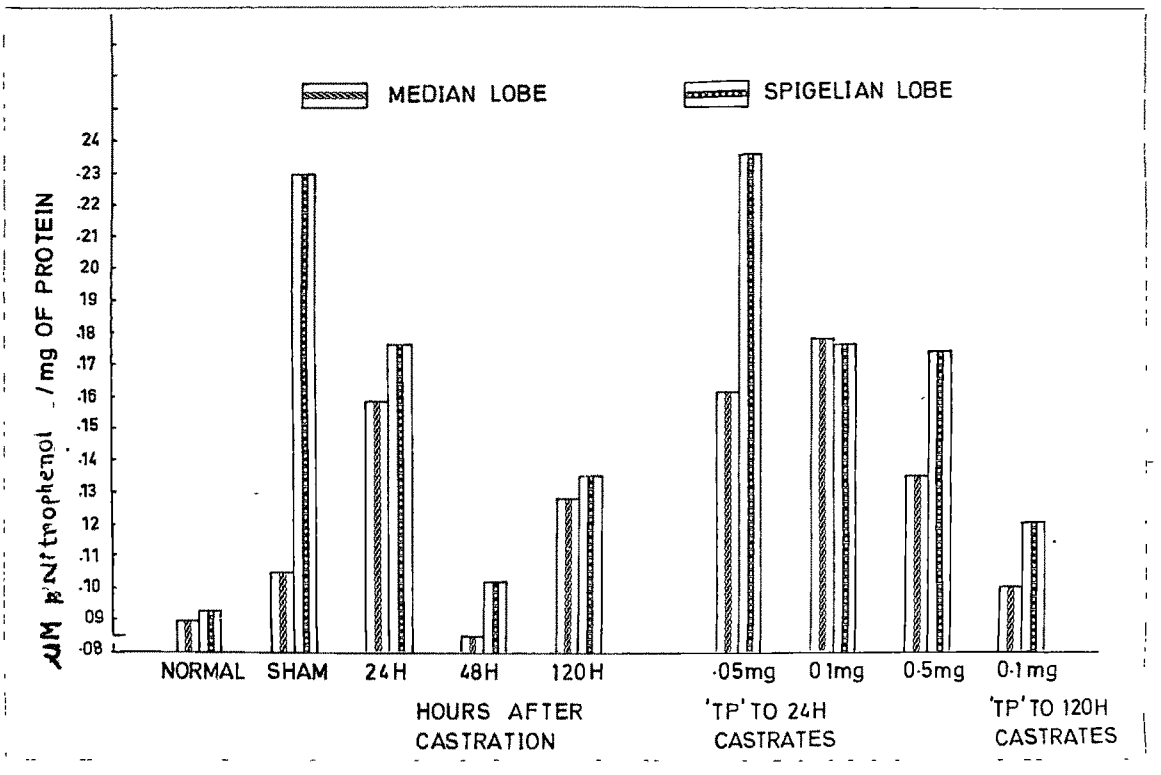

Fig. 1. Histograms showing the levels of hepatic non-specific phosphomonoesterase enzyme activity at alkaline pH in the normal, sham-operated, castrated and hormone replaced rats.

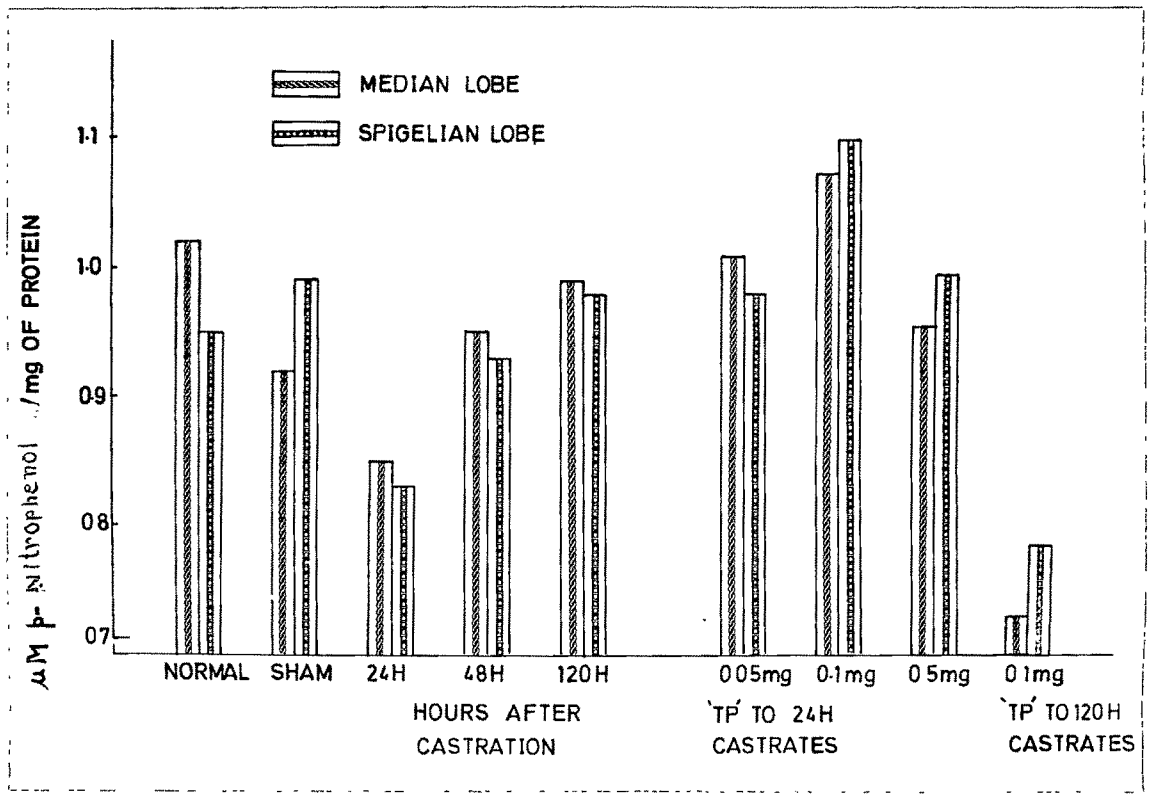

Fig. 2. Histograms showing the levels of hepatic non-specific phosphomonoesterase enzyme activity at acidic pH in the normal, sham-operated, castrated and hormone replaced rats.

0.5 mg of TP was found to be less than that recorded for 24 hrs. castrated animals but remained higher than the normal (Table I; Fig. 1). The 120 hrs. castrates also, when injected with TP, showed above-normal activity in both the lobes.

The activity of acid phosphatase resulted into an increase with 0.05 mg in comparison to 24 hrs. castrated rats. The level was found to be very close to normal in both the lobes. Increasing the dose level further to 0.1 mg, it exhibited a continued increase in its activity. But with 0.5 mg TP a fall was observed. The castrated animals which were given the hormone injection after 120 hrs. (0.1 mg TP) showed a reduced enzyme activity (Table I; Fig. 2).

DISCUSSION

The present study indicated that due to the removal of gonads alkaline phosphatase activity of rat liver was affected comparatively more than the acid phosphatase activity. In the normal condition, acid phosphatase activity was found to be higher than the alkaline phosphatase

activity. High acid phosphatase activity and low alkaline phosphatase activity in mammalian liver was reported by Thorbeck et al. (1960) and Wachstein (1963). Thorbeck et al. (1961) also pointed out, in their study on mouse liver during various conditions modifying the activity of reticuloendothelial system, that changes in alkaline phosphatase activity occur earlier than those in acid phosphatase activity. In the present study also the alkaline phosphatase activity was found to be altered immediately after castration. There was a depletion after 48 hrs. in this enzyme activity which increased again by 120 hrs. Rogers (1960) has hypothesised the possibility of alkaline phosphatase playing a role in phosphate transfer in DNA metabolism. An increase in DNA content of the liver lobes was observed in the present work as an early effect of castration (Chapter-4). Chevremont and Firket (1953) also had suggested the possible involvement of alkaline phosphatase in nucleic acids synthesis. In the present study also the increase in alkaline phosphatase could be due to its involvement in the nucleic acid synthesis. Since the enzyme is a non-specific one, there could be no specific functions performed by it. Hence, the functions of phosphatases

will vary from tissue to tissue and under different experimental conditions. Membrane related alkaline phosphatase has been associated with phospholipid metabolism, phosphate transfer and transport of blood glucose across the cell membrane (Allen and Slater, 1956). Taking all these facts into account, it could be said that the effect of hormone on metabolites could result from modifications of enzyme activities that are either directly or indirectly involved in the transport processes. Alkaline phosphatase was found to be associated with the passage of metabolites across the cell membranes (Danielli, 1954). It is pertinent in this context to note that as early as 1944, Kochakian and Fox, in their studies (Kochakian and Fox, 1944) on different tissues of castrated mice, observed no change in alkaline phosphatase activity of the liver. Thereafter, Kochakian and Robertson (1950) observed an increased alkaline phosphatase activity after administration of testosterone to the castrated rats. Though the work of Kochakian and Fox (1944) suggested different results in the case of alkaline phosphatase activity as affected by male hormones, the present findings were observed to bear predictable relations with glycogen balance of the

liver. Glycogen was found to be decreased after 120 hrs. due to castration. The depletion of liver glycogen will naturally result into an increased blood glucose level (Chapter-9). It is therefore, possible that hepatic alkaline phosphatase activity is associated with transport of glucose molecules across the cell membranes. It should be noted here that the alkaline phosphatase activity increased at 120 hrs. From the studies of many workers (Cori and Cori, 1952; Cusworth, 1958; Duncan, 1959 and Rosenthal et al., 1960) it has been shown that the glycogen storage diseases were characterised by phosphatase deficiency. Hence, the apparent increase in alkaline phosphatase activity associated with the removal of gonads could be associated with the decreased glycogen level.

The acid phosphatase activity was found to exhibit a minor decrease immediately 24 hrs. after castration. This was found to be reaching almost the normal levels by 48 and 120 hrs. post-operatively. These results would indicate that the acid phosphatase activity was not altered significantly due to lack of the male sex hormone. Acid phosphatase is known to be a lysosomal enzyme of strong hydrolytic nature (Pokrovskii et al., 1974). Therefore, the presence of this enzyme in the 'Kupffer

cells' is understandable. Ratzlaff and Tyler (1973) also found acid phosphatase in the 'Kupffer cells' of avian liver. Barka (1960) and Wachstein (1963) opined that the degree of acid phosphatase in 'Kupffer cells' reflected the functional state of reticuloendothelial cells in the liver. As a result of castration in rabbits, Nathaniel and Nathaniel (1966) observed retraction of reticulo-endothelial cell-processes which resulted in reduced surface area. Presently obtained diminished enzyme activity (24 hrs.) could well have been due to similar influence on 'Kupffer cells' rather than on hepatocytes shortly after castration.

When influence of replacement therapy was studied a dose-dependent variation was observed in alkaline phosphatase activity. Immediately after 24 hrs. of injection with 0.05 mg TP, the alkaline phosphatase was found to be increased. With a slightly higher dose (0.1 mg TP) the activity increased further in the median lobe of the liver but that of the Spigelian lobe showed depletion. As the dose level was increased further to 0.5 mg TP, the activity of alkaline phosphatase fell in both the lobes of the liver, yet it was higher than normal (Table I; Fig. 1). Kochakian and Robertson (1950)

had observed an increase in alkaline phosphatase with administration of testosterone to intact rats. Hence, the increased activity found after replacement in the present work could be the effect of sex hormone administration, whereas, the increase observed due to lack of sex hormone could possibly be due to the involvement of this enzyme in various other physiological mechanisms. Replacement therapy in case of 120 hr. castrates virtually showed a decrease with reference to 24 hr. castrate level. It, therefore, appeared that this enzyme activity responds to TP administration only after an interval of five days post-castration.

When acid phosphatase was studied with replacement therapy, it was found not to respond to any of the three dosages of TP. Remarkably enough, administration of 0.1 mg of TP to 120 hr. castrates was observed to induce a significant reduction in the acid phosphatase activity; an effect that was synergistic with that of castration. It was not easy to explain this synergism on the basis of the observation available. What could be suggested at this stage is only that both the acid- as well as alkaline-phosphatase activities exhibit sensitivity at a well cognizable intensity only after about 120 hrs. of castration.

Hence, no rapid effects of either castration or hormone replacement could be of obvious significance in early stages. Possibly this spectrum of non-specific phosphomonoesterase activity may come into effective play at later stages only.