INTRODUCTION

All mammals have the fundamental ability to adapt to environmental changes which is manifested in the alterations of their metabolic patterns. The regulatory mechanisms respond to external as well as internal factors and, these inturn, are reflected in metabolic responses. The importance of hepatic tissue in the general economy of body functions is a well established fact. Voluminous literature, regarding multiferious activities of the liver, has been continuously piling up. However, there is one aspect of liver functions, that has yielded no significant information so far; concerning the possible influences of sex steroids on structural and functional aspects of the gland. Hence, the present study was undertaken to investigate some aspects of the influences of sex hormones on the hepatic tissue in adult male albino rats, Rattus norvegicus albinus.

The effects of sex steroids on the accessory reproductive organs are very well established (Doeg et al., 1972; Tuohimaa and Soderstrom, 1974; Brooks, 1976, Rajalakshmi and Prasad, 1976; Stormshak et al., 1976; Catelli and Baulieu, 1977; Dahnke and Mosebach, 1977). Different studies have been made and are still being carried out which have added to the knowledge of some aspects of

structure and functions of these organs. It has been a common practice to study the effects of hormones in two ways : under deficient conditions and with excess. The deficient condition could best be visualized by removing the chief internal source of hormone and the excess by exogenous administration of the respective hormone to the normal animals. Removing first the hormonal source and then administering the respective hormone thereafter, by artificial means would give a general idea about the importance of a particular hormone and, thereby, help in the elucidation of functional regulation of the concerned organ. Castration of the animal and subsequent replacement therapy has become a common practice in the study of the role of gonadal hormones and the physiological patterns of gonads. Effects of endogenous and exogenous androgens on the accessory reproductive glands have been reviewed by Moore as early as 1939, and thereafter, by many others (Price, 1947; Burrows, 1949; Dorfman, 1950; Dorfman and Shipley, 1956; Calvin and Williams-Ashman, 1967; Coffey, 1974; Liao et al., 1974; Ofner et al., 1974; Tuohimaa and Niemi, 1974). Modulations of metabolic processes that can be evoked by testicular androgens are extraordinarily diverse. They may occur in practically every tissue of

the vertebrate body. Some of the effects of removal of the testes on hepatic tissue have been recognized by many authors (Swartz et al., 1960; Fuji and Villee, 1968; Konopkova and Nedvidek, 1972; Kurtz et al., 1976; Moore et al., 1977). All these earlier attempts to uncover meaningful androgenic influences on intermediary metabolism of hepatic systems have paved the way for detailed studies of non-target tissues. The literature concerning the effects of these hormones on intermediary metabolic patterns of the hepatic tissue is enormous (Kochakian and Robertson, 1950; Dugal and Saucier, 1957; Swartz and Sams, 1961; Stubbs et al., 1967; Chandola et al., 1974; Thapliyal et al., 1975), yet, it is presently nearly impossible to weld the available bits and pieces of experimental information into anything approaching a coherent account of the action of testicular hormones on the over all physiology of the liver. Most of these studies were made several weeks after castration. The present investigation was mainly undertaken to study the early effects of castration on this non-target tissue. Hence, the time period selected for the influence of gonadectomy was 24, 48 and 120 hrs.

As a prerequisite, all the different lobes of the liver were tackled separately. This was an attempt to

investigate the suspected existence of regional differences and similarities amongst the different lobes of the liver. There are very few reports regarding lobe-wise differences in the hepatic tissue (Hems et al., 1972; Krishna Kantha, 1972; Tyagi and Mishra, 1977). These reports focus the light on the functional differences between the lobes of the liver. The preliminary findings of the present study, established the fact that the Spigelian lobe of the liver differs significantly from the rest of the liver lobes in regard to ~~their~~ its metabolic aspects. This was borne out further during subsequent studies. Hence, later on only the median lobe was selected as the representative of the rest of the lobes for comparison with the Spigelian lobe.

Changes after gonadectomy might be prevented or reversed by administration of sex hormones. To substantiate the effects of castration, the influence of replacement therapy with testosterone propionate (TP) was preferred. Different dose levels of the TP were administered to castrated males. This dose dependency study pointed out the effective dose of the TP which can bring back the hepatic gland towards normality, after castration experiments. Hence, in the next phase of the study, only the effective dosage was used to evaluate the castration effects. Further,

most of the studies referred to were made under continued injection regimes (Perry and Bowen, 1958; Swartz and Sams, 1961). In this context, an attempt to understand the possible changes in the physiology of the animal, in relation to single injection, could possibly provide additional information that would throw more light on the relation between gonadal hormones and the hepatic tissue.

The studies with radiolabelled compounds have revealed the importance of the time interval in the manifestation of the effects of the hormones. Balnave (1968), in his study on hormonal effects in immature chicks, pointed out that testosterone and progesterone produce rapid synthesis as well as degradation of liver lipids. This study revealed the action of hormones within minutes. It was with a view to investigate such immediate or rapid effects of the hormones that a study at 1, 2 and 4 hours was conducted in the later part of the present work.

Directly or indirectly hormones influence the functioning of every cell in the body. They may influence, at times markedly, metabolism of carbohydrates, fats, proteins, vitamins, water or electrolytes. Some of the

effects of removal of the testes in males have been recognized ever since castration was first practiced on man and domestic animals. To gain an insight into the normal metabolic pattern of the rat liver initially the concentrations of total lipids, glycogen, proteins, nucleic acids and vitamin C (ascorbic acid) were determined and later under castration and replacement therapy to understand their relation to male sex hormone in rats.

Investigation on the role of the gonadal hormones concerning lipid metabolism has received increased attention in recent years (Aftergood and Alfin-Slater, 1965; Nathaniel and Nathaniel, 1966; Doeg, 1968; Watkins et al., 1972; Pearce, 1977). Liver is known to function as an important centre for metabolism and accumulation of lipids. In this light a quantitative evaluation of the total lipids was carried out. From the results obtained in the present study, it could be noted that total lipid values were significantly increased due to lack of sex hormone. Such a variation could be due to some "particular" lipid component of the "total lipids". Hence, levels of total cholesterol and phospholipids were also measured (Chapter-1). An attempt has been made here to correlate these fluctuations with the rate

of lipogenesis and lipolysis. To make this study more extensive, the rate of fatty acid oxidation, total glycerides and free fatty acids (FFA) of the hepatic tissue were also assayed (Chapters-2 and 8). These observations brought out clearly the existence of certain interesting metabolic and biochemical changes due to castration experiments. The rate of fatty acid oxidation was found to be lowered and the total glycerides were increased. The role of FFA in the transport of lipids from one organ to other is very well known. The picture of FFA levels would facilitate in accounting lipid variations in the liver. It would give a guide-line on the rate of mobilization, synthesis and transport of lipids from the depots to the liver, and hence study of plasma free fatty acids along with depot tissues would be desirable (Chapter-8). The omental tissue was selected here to study the depot FFA, since it is assumed that omental fatty tissue is metabolically significantly dynamic, when numerous variables are to be considered; such as glucose uptake, glycogen deposition, rate of FFA esterification or release, lipogenesis as well as participation in shifts in body fats (Shafrir and Wertheimer, 1965). Blood

being an important participant in the internal homeostasis; any disturbance, normal or abnormal, in any part of the organism is bound to influence its constitution and show resultant changes either as supporting or aiding measure, if it be a normal disturbance or as a correcting measure, if an abnormal one. Varied and extensive changes in the blood of reptiles, in the wake of multiferous physiological and pathological conditions and processes, have been well documented (Shah et al., 1977a & b). Involvement of the blood in the distribution of metabolites from one organ to the other is a known fact. It helps to regulate the intermediary metabolism of lipids by influencing its availability at the site of action. It is virtually certain from Deuel's report (1956) that practically no synthesis of lipid occurs in the blood itself, and that the alterations observed in the blood reflect the metabolic alterations of distant tissues. Hence, it can serve as an excellent medium for investigation which could throw light on causative factors underlying the observed changes in the hepatic tissue as well. A beginning in this direction was made by obtaining the overall picture of the total lipids, total cholesterol and phospholipids of the blood plasma (Chapter-9) since the total lipids of liver depicted an increase in castrated animals.

It was the intention of the present study to get a preliminary idea about the alterations in hepatic metabolism induced by the lack of sex hormones. Liver being the central organ for almost all biochemical and metabolic changes; an insight into these aspects of metabolic alterations concerning carbohydrates (glycogen) and proteins (total protein values) could easily be gained. A study along these lines forms a part of this investigation.

An initial increase in the levels of glycogen was noted in both the castrated as well as sham operated (control) rats after 24 hrs. of operation, but the degree of increase was significantly higher in control ones. This logically means that the suppressed values found in castrates were mainly due to the influence of gonadectomy. The observation was extended to longer intervals of 48 and 120 hrs. A clear-cut decrease was observed at these intervals also (Chapter-3). Changes in phosphorylase enzyme activity would reveal the state of glycogen metabolism in the liver. The glucose level in the blood is known to be affected by glycogen depletion in the hepatic tissue. Hence, its level in the blood plasma was also estimated (Chapter-9). A quantitative study of the activity of

succinate dehydrogenase and glucose-6-phosphate dehydrogenase in liver was carried out to look for possible interrelationship of lipid and carbohydrate metabolism (Chapter-3).

Sex hormones have been implicated in an important way in the integration of adaptive changes in the protein metabolism of animals (Kochakian, 1964). Moreover, protein synthesis is intimately connected with the dynamics of ribonucleic acids. It was, therefore, thought desirable for better understanding of the aspects of protein metabolism of liver to assay the levels of protein and nucleic acids (deoxyribonucleic acid-DNA and ribonucleic acid-RNA). The observations on castrates over the period of experimentation revealed a general increase in the levels of these parameters. Induction of polyploidy in the liver cells due to castration (Konopkova and Nedvidek, 1972) could also induce changes leading to greater protein synthesis in the castrates. The confirmation of these findings was evaluated on the basis of replacement therapy (Chapter-4).

The liver is known to be the site of ascorbic acid production in mammals, except in man and guinea-pig

(Grollman and Lehninger, 1957). Ascorbic acid plays an important role in steroid biosynthesis (Stubbs et al., 1967). Earlier studies (Stubbs et al., 1967; Khandwekar et al., 1973) on castration in mammals have revealed a significant decrease in the ascorbic acid level, but the observations were made as late as two to six weeks after castration. The study of ascorbic acid levels immediately after castration was, therefore, thought desirable. During the course of the present investigation a definite picture on the requirement of testicular hormone for the maintenance of normal hepatic ascorbic acid level was obtained (Chapter-5).

To get a generalized idea about the total energy flux in the hepatic tissue, activities of certain enzymes were studied (Chapter-6). An analysis of magnesium dependent ATPase (adenosine triphosphatase) whose occurrence is of a general nature, could give an insight into the mechanisms of energy metabolism occurring in any particular tissue. Another significant aspect is the importance of ATPase in providing the requisite energy at the cell surface for the active transport of chemical moieties. An economic balance between the output and input of chemical energy is of importance for a metabolically

active tissue. Also, possible implications of variation found in the activities of acid and alkaline phosphatases were taken into consideration (Chapter-7).

Recent studies have made it clear that several hormonal influences on general metabolism of the tissues usually involve a mediator-like action of cAMP and hence the latter is referred to as a 'secondary messenger' (Robinson and Sutherland, 1972 - A review article). The enzyme cAMP-phosphodiesterase reduces the nucleotide to a non-cyclic form-5'AMP. Hence, any change in this enzyme activity could give an insight into the fluctuation occurring in the intracellular levels of cAMP. Such observations, it was thought, would help in assessing the mediator action of the cAMP that could be invoked by alterations of sex-hormone levels on hepatic tissue. Since, with the available facilities, it was difficult to measure directly the intracellular concentrations of cAMP, phosphodiesterase activity was measured, which was easily possible (Chapter-10). Effects of castration and rapid influence of TP administration were assayed in order to facilitate a more comprehensive understanding about the present problem.

Changes in all the above parameters, revealed after castration; were considered in the light of simultaneous variations observed in case of sham operated animals and under replacement therapy. Finally an attempt has been made to study the hourly response to TP administration in castrated rats. Osterberg and Tuohimaa (1975) have shown that the replacement of the hormone was much effective after longer periods of castration in sex-accessory organs. During the course of the present investigation hormone was initially injected after 24, 48 and 120 hrs. after gonadectomy. Nevertheless, certain parameters showed significant alterations after 48 hrs., therefore, hormone was injected at this stage of castration during later studies. Rapid effects of hormone replacement were studied after one, two and four hours of injection. This would add further to the understanding about the time required by different parameters to attain the normalization (Chapter-11).

CHAPTER 1

EFFECTS OF CASTRATION ON LIVER LIPIDS IN ALBINO RATS

Liver plays an important role in steroid hormone metabolism. Considerable information exists on the role of male hormones in the regulation of carbohydrate, lipid and protein metabolism of mammals (Gaunt et al., 1939; Lewis and Mccullagh, 1942; Mccullagh and Lewis, 1942; Mannerfelt, 1948; Dorfman and Shipley, 1956; Furman et al., 1957, 1958, 1963; Laron and Kowadlo, 1963; Kochakian, 1964; Robinson et al., 1964; Abell et al., 1965; Beck, 1969; Furman, 1969; Ono and Imai, 1971). Metabolism of various lipid components occurs in the liver. Although, under normal circumstances, the liver lipids comprise primarily of phospholipids and neutral fats; this organ may, under certain conditions, contain larger accumulation of cholesterol. Hepatic cells have the ability to synthesize the fatty acids (Bloch and Kramer, 1948) and cholesterol (Little and Bloch, 1950; Srene et al., 1950; Zabin and Bloch, 1950). Liver is also considered to be one of the important sites for phospholipid synthesis, though this may occur in a number of other organs (Perlman et al., 1937).

Diet and sex-difference are some of the major factors which influence the metabolic patterns of liver. Greisheimer (1931) was the first to point out that male rats fed on various diets had a higher level of liver glycogen and a lower one for liver lipids than that noted in the female animals. Deuel et al. (1934) were unable to demonstrate sex difference in the level of liver lipids of unfasted rats, nevertheless, they found increasingly higher values for liver lipids in the female, as compared to the males, during a subsequent fasting period. Similar variations in liver lipids have been recorded by Best et al. (1951). Okey et al. (1934) reported that in spite of the fact that the total lipids in the liver of female rats exhibit consistently higher levels than those of male animals the total cholesterol was higher in the males. The results obtained by Okey et al. (1934) have been confirmed by Barnes et al. (1941), who have further noted that the higher lipid values in the females, as compared with the males, were confined to the acetone-soluble fraction, while it was not observed in the phospholipid fraction. There have been numerous reports in the literature concerning sex-differences in lipid metabolism of the rats. For example, it has been observed that female rats have higher plasma

cholesterol but lower liver cholesterol levels than do male rats (Aftergood et al., 1957; Fillios, 1957). Methyltestosterone has been reported to exert a hypocholesterolemic effect in male rats (Abell and Mosbach, 1962). Similarly, Fillios et al. (1958) observed that, female animals have higher rates of hepatic cholesterol synthesis, and the administration of estradiol to males causes an increase in biosynthesis in the liver. Further, Coleman et al. (1958) reported that hepatic cholesterol biosynthesis is lower after gonadectomy of the female rats. Other studies have also shown effect of sex-hormones on lipid metabolism. Mitochondria from intact female rats oxidize cholesterol to a greater extent than those from intact males (Kritchevsky et al., 1963). The sex of the animal also affects fatty acid composition of liver lipids (Okey et al., 1961, 1962).

To study the role of sex-hormones in liver metabolism, castration and administration of sex-hormones were performed by many workers. Leathem (1948, 1951) showed that castration causes no significant change in liver weight, whereas Hall and Korenchevsky (1938) observed a shrinkage in size of the lobules. Further, the latter workers have noted that administration of testosterone to castrated rats exerts a

a lipotropic effect. Control of mitochondrial lipid biosynthesis by testosterone in male sex accessary gland tissue and liver of castrated rats was studied by Doeg (1968).

Most of the studies on effects of castration on metabolic patterns of hepatic tissue have been made after several weeks post-operatively. The present investigation was undertaken to study the early effects of castration. Therefore, an investigation was made on the influence of gonadectomy at intervals of 24, 48 and 120 hrs., on lipid metabolism of the liver of male rats.

MATERIAL AND METHODS

Adult male albino rats (Rattus norvegicus albinus), weighing 120-160 gms were employed as experimental animals. Prior to actual experiments, the rats were maintained on an ad libitum diet with free access to drinking water. For experimental work, normal, healthy male albino rats were bilaterally castrated under mild ether anaesthesia. The time intervals selected for sacrifice were 24, 48 and 120 hrs. after the operation. Normal males of the same strain and similar weight were sham-operated for reference controls. Replacement

therapy was performed by treating the castrated animals with Testosterone Propionate (TP) (Sigma Chem. Co.). Tributyrin (Sigma Chem. Co.) was utilized as the solvent. A single intramuscular injection was given in each case. At first, three different doses of TP (0.05 mg; 0.1 mg and 0.5 mg) were injected to three different sets of 24 hr. castrates. In every case a constant volume of 0.5 ml was injected. Later on 120 hr. castrates also were administered 0.1 mg of TP. All the treated animals were kept for 24 hrs. after injection.

At the end of selected intervals, the rats were weighed and sacrificed by cervical fracture. Pieces of liver were quickly removed, trimmed free of adherent connective tissue, and weighed. All the liver lobes were tackled separately for different estimations. Pieces of tissue of the following liver lobes were utilized : left, right, median and Spigelian (nomenclature of various lobes of the liver is according to Green, 1959). For determination of 'total lipids' weighed tissue pieces were ground separately with thoroughly washed sand and 2:1 chloroform-methanol mixture for extraction as per the method of Folch et al. (1957). Total cholesterol content

was measured employing ferric chloride reaction (Crawford, 1958). Phospholipids were measured by a method described by Dittmer and Wells (1969). The lipid content was calculated in terms of gms, percentage of fresh liver. Cholesterol and phospholipid were calculated in terms of percentage on fresh weight basis as well as percentage of total lipids.

RESULTS

The present investigation has sought to answer two fundamental questions; firstly, possible lobewise regional differences in the liver, and secondly, the relation between the sex-hormones and liver lipid metabolism. Results of the experiments are presented in Tables I and II and Figs. 1-5.

Normal animals:

In the beginning of the study, all the liver lobes were tackled separately for estimating lipid metabolites. There was no significant difference between the various liver lobes except the Spigelian, which differed in all the aspects considered here. Hence, later on, only the

median and the Spigelian lobes were used for comparisons. Spigelian lobe was found to show significantly higher values for total lipids. Level of total cholesterol did not show much of the variation amongst the liver lobes. Again, as far as phospholipids were concerned, a difference was noted between these two lobes. When the values of phospholipids were considered as percentage of fresh tissue no marked difference was apparent, but when the phospholipid values were considered as percentage of total lipids, significantly lower values were noted for the Spigelian lobe.

Castrated animals:

A gradual rise in the total lipid content of the liver lobes was observed after 24, 48 and 120 hrs. of castration.

Values of total cholesterol were observed to fluctuate. 24 hrs. after the castration, a drop in its level was observed which went up again by 48 hrs. The rise at this level was seen to reach almost the normal values. Again, 120 hrs. post-operatively, a slight decrease occurred in total cholesterol levels.

Nevertheless, these values were not far from the normal level when considered as percentage of fresh tissue. Whereas, as a percentage of total lipid the level of cholesterol at 120 hrs. after castration was lower than the normal values, but higher than the values observed at 24 hrs.

Concentration of phospholipids in the liver was also affected by castration. Significantly low level was observed after 24 hrs., which gradually rises at 48 and 120 hrs. Though there was an apparent tendency for restoration of the level of phospholipids after castration, it was observed that it remained lower than the concentration observed in the liver of intact animals. Here also, when the values were considered as percentage of wet tissue, it was noted that they reached almost the normal levels by 120 hrs., but when compared on the basis of percentage of total lipids the level of phospholipids remained still lower.

Sham-operated animals:

With a view to eliminate any possible effect of surgical shock on the animals, a few rats were sham-operated

and the effect was studied 24 hrs. after operation. In these animals no significant fluctuation was observed in the total lipid content of the liver after 24 hrs. of operation. Cholesterol value, showed a decrease after 24 hrs., but the level was found to be higher than that observed in the gonadectomized rats after 24 hrs. Content of phospholipid was found to be same as that of normal when considered on the basis of percentage of fresh tissue. Only when the data were considered as percentage of total lipids a lower level was observed after 24 hrs. which was, nevertheless, found to be higher than the value at 24 hrs. noted in castrated animals.

Castrated + hormone treated animals:

As a prerequisite, the replacement therapy was conducted to observe the effect of different dose levels rather than to obtain the normal condition. The results of this study showed an immediate rise in the 'total lipid' content with 0.05 mg TP (minimum dose applied) in both the liver lobes in comparison to normal as well as 24 hr. castrated rats. When the dose level was increased to 0.1 mg, both the lobes showed a little depletion. As a result of this depletion Spigelian lobe reached to almost

normal level (Fig. 3) and was below 24 hr. castrated values. On the other hand, median lobe exhibited values which were nearer to 24 hr. castrates and at the same time above normal (Fig. 3). With higher dose of 0.5 mg TP, median lobe registered further depletion but was found to maintain still above normal level. Spigelian lobe presented an elevation with this higher dose of TP, as a result of which it attained the level above 24 hr. castrates (Tables I & II).

Examination of total cholesterol content revealed its lower level with the minimum dose of TP (0.05 mg) when expressed on percentage of total lipid, whereas, a rise was discernible when the values were calculated in terms of percentage of fresh tissue weight. With higher doses elevation was obtained in both the liver lobes (Table II; Fig. 4).

The recorded data for liver phospholipids denoted higher levels in both the lobes with all the three different dosages of TP (when expressed in terms of percentage of fresh tissue weight). There was no significant variation between the two lobes with different dosages of the hormone, as was the case with intact rats.

When the results were considered in terms of percentages of total lipids, the values recorded with 0.05 mg TP were found to be below normal in both the liver lobes. An increase to above-normal level was obtained in Spigelian lobe with 0.1 mg, whereas, median lobe registered a fall in phospholipid content. With further increase in TP dose (0.5 mg), Spigelian lobe showed a depletion and the median lobe an elevation. This last variation brought the levels of phospholipid close to normal values in both the lobes of the hepatic tissue (Tables I & II; Fig. 5).

With 0.1 mg TP replacement in 120 hr. castrated rats, values higher than normal were obtained for total lipids. In case of cholesterol similar effect was observed when calculated on basis of fresh tissue weight. When it was measured on the basis of percentage of total lipid values, lower level in the median lobe and higher level in the Spigelian lobe were obtained. Similarly, phospholipids also showed elevated level in both the lobes if expressed in terms of fresh tissue. With the other way of expression, near-normal level was obtained in Spigelian lobe, whereas, median lobe was found to be below normal.

Table I : Effect of castration on lipid metabolites of 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
Total Lipids % on fresh tissue wt.	5.36 ±0.847	7.29 ±0.990	5.40 ±0.431	7.30 ±0.507	5.94 ±0.946	7.95 ±2.109	6.22 ±0.328	7.81 ±0.450	6.41 ±0.654	8.10 ±2.550
Total cholesterol % of lipid values	9.25 ±1.799	8.40 ±1.354	7.61 ±0.430	6.41 ±0.544	4.31 ±0.665	3.63 ±0.554	8.40 ±1.150	7.75 ±0.959	6.70 ±1.262	6.01 ±1.363
Total cholesterol % of fresh tissue weight	0.487 ±0.088	0.558 ±0.121	0.450 ±0.057	0.538 ±0.022	0.337 ±0.131	0.366 ±0.096	0.527 ±0.062	0.624 ±0.074	0.480 ±0.055	0.525 ±0.083
Phospholipids % of lipid values	51.3 ±2.68	39.7 ±7.33	43.2 ±0.45	32.7 ±3.00	33.5 ±3.51	26.1 ±1.55	42.4 ±6.60	36.0 ±1.09	42.5 ±8.49	31.68 ±7.20
Phospholipids % of fresh tissue wt.	2.6 ±0.405	2.6 ±0.340	2.6 ±0.057	2.7 ±0.022	2.1 ±0.265	2.3 ±0.313	2.5 ±0.441	2.5 ±0.396	2.7 ±0.416	2.6 ±0.397

Each reading is the mean value of at least ten different samples.
M - Median lobe; Sp - Spigelian lobe.
*Post-operative intervals in hours.

Table II : Effect of replacement therapy on liver lipid metabolites in 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
Total Lipid % on fresh tissue wt.	5.36 ±0.847	7.29 ±0.990	6.48 ±0.682	8.51 ±1.445	5.98 ±0.523	7.23 ±0.744	5.86 ±0.421	8.11 ±0.544	7.13 ±0.652	7.72 ±0.739
Total cholesterol % on lipid values	9.25 ±1.799	8.40 ±1.854	8.079 ±0.875	7.28 ±0.726	9.47 ±1.057	9.37 ±0.784	10.48 ±0.663	9.62 ±1.024	8.18 ±0.264	9.20 ±0.191
Total cholesterol % on fresh tissue wt.	0.487 ±0.088	0.558 ±0.121	0.561 ±0.086	0.657 ±0.096	0.620 ±0.051	0.680 ±0.069	0.613 ±0.047	0.785 ±0.087	0.581 ±0.038	0.618 ±0.042
Phospholipid % on lipid values	51.33 ±2.68	39.76 ±7.23	47.01 ±3.51	34.16 ±8.82	45.78 ±2.98	47.84 ±5.12	56.98 ±5.45	38.03 ±2.94	39.38 ±3.97	40.32 ±4.95
Phospholipid % on fresh tissue wt.	2.60 ±0.405	2.60 ±0.340	3.21 ±0.257	3.01 ±0.456	3.21 ±0.325	3.47 ±0.332	3.31 ±0.182	3.13 ±0.427	2.88 ±0.286	2.97 ±0.310

Each reading is the mean value of at least ten different samples.
M - Median lobe ; Sp - Spigelian lobe
*Dosages of TP injected

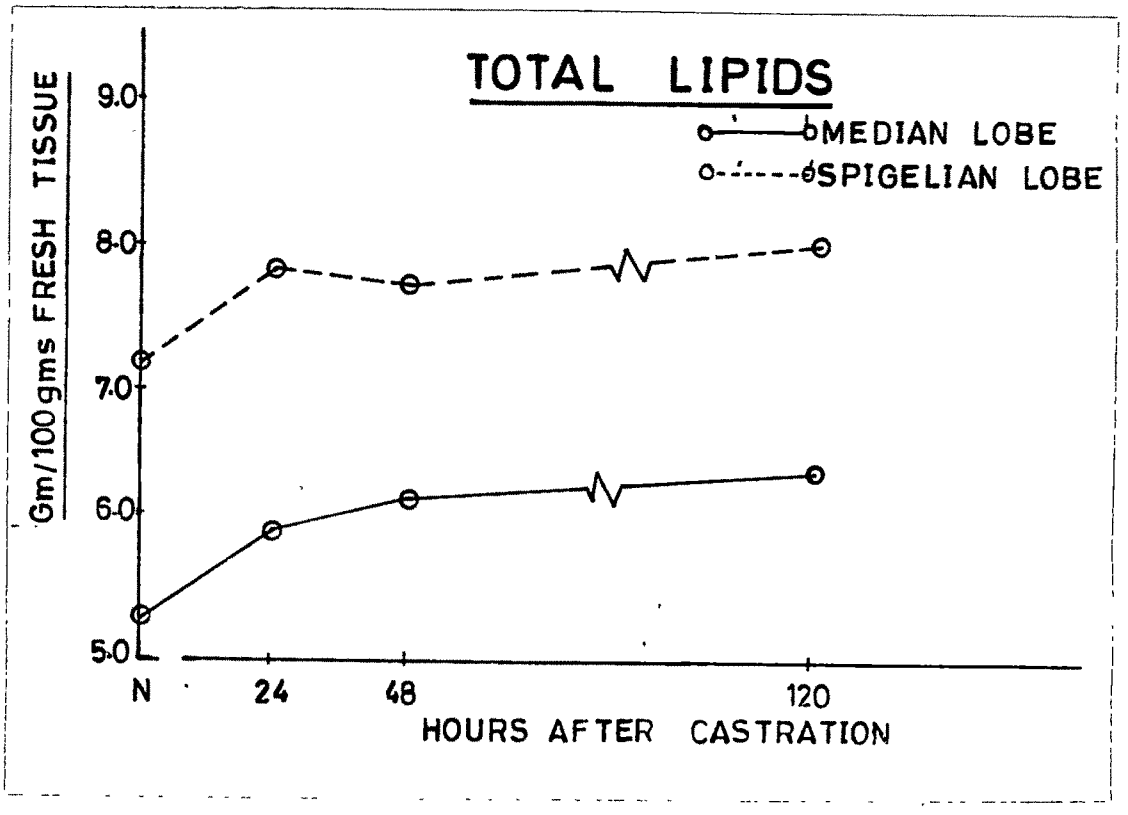

Fig. 1. Shows the effect of castration on total lipids in the liver lobes.

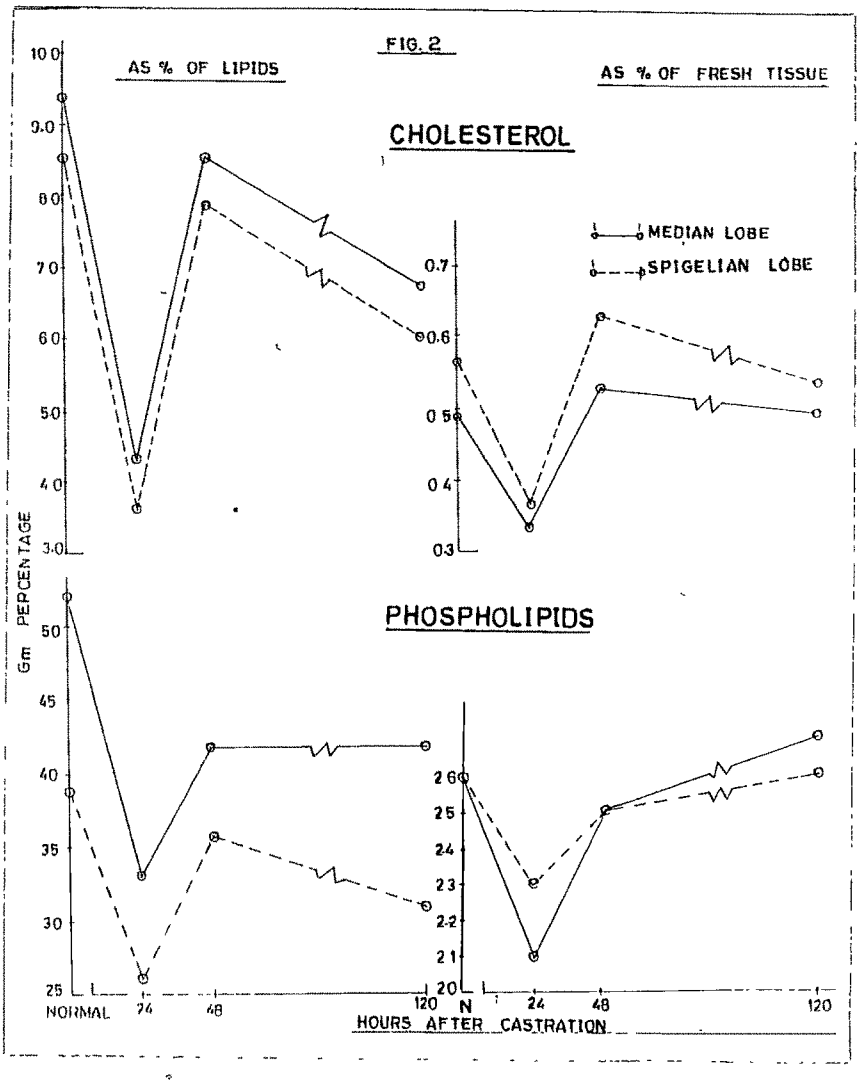

Fig. 2. Shows the effects of castration on cholesterol and phospholilids as percentage of total lipid and percentage of fresh tissue in liver lobes.

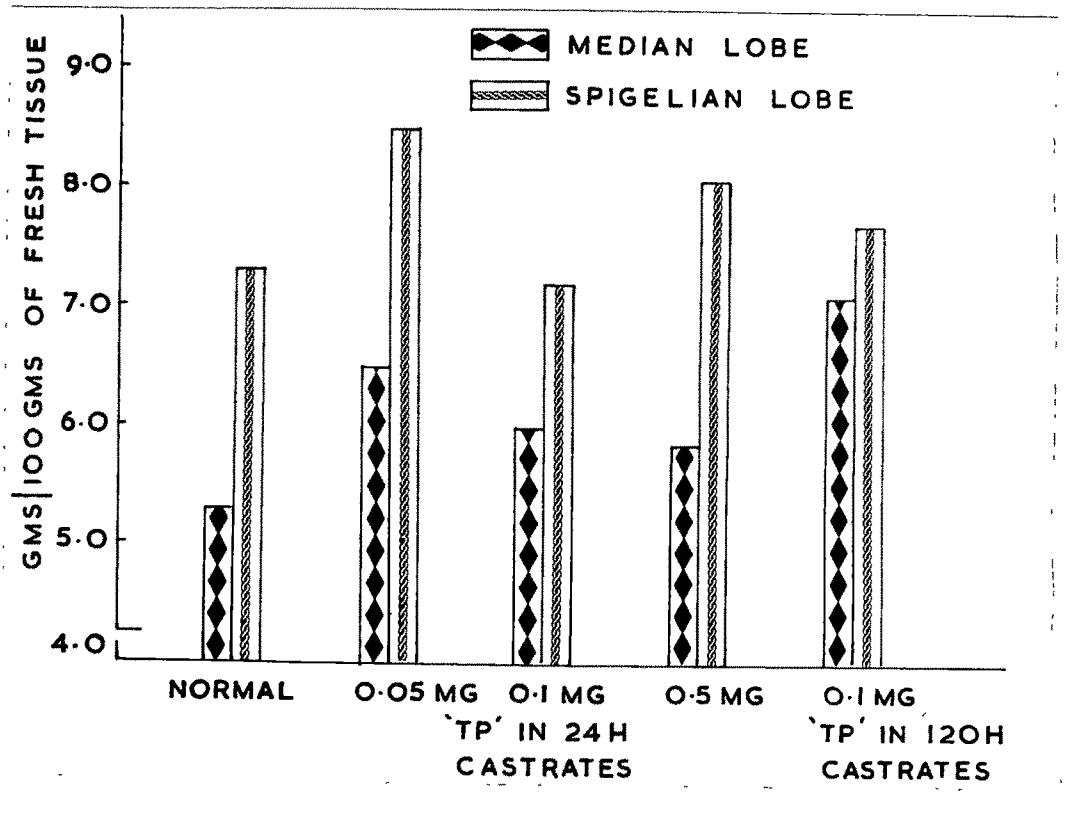

Fig. 3. Histogram showing concentrations of total lipids in the liver (median and Spigelian lobes) of 24 H castrates injected with three different doses of testosterone propionate (TP) and 120 H castrates injected with 0.1 mg of TP.

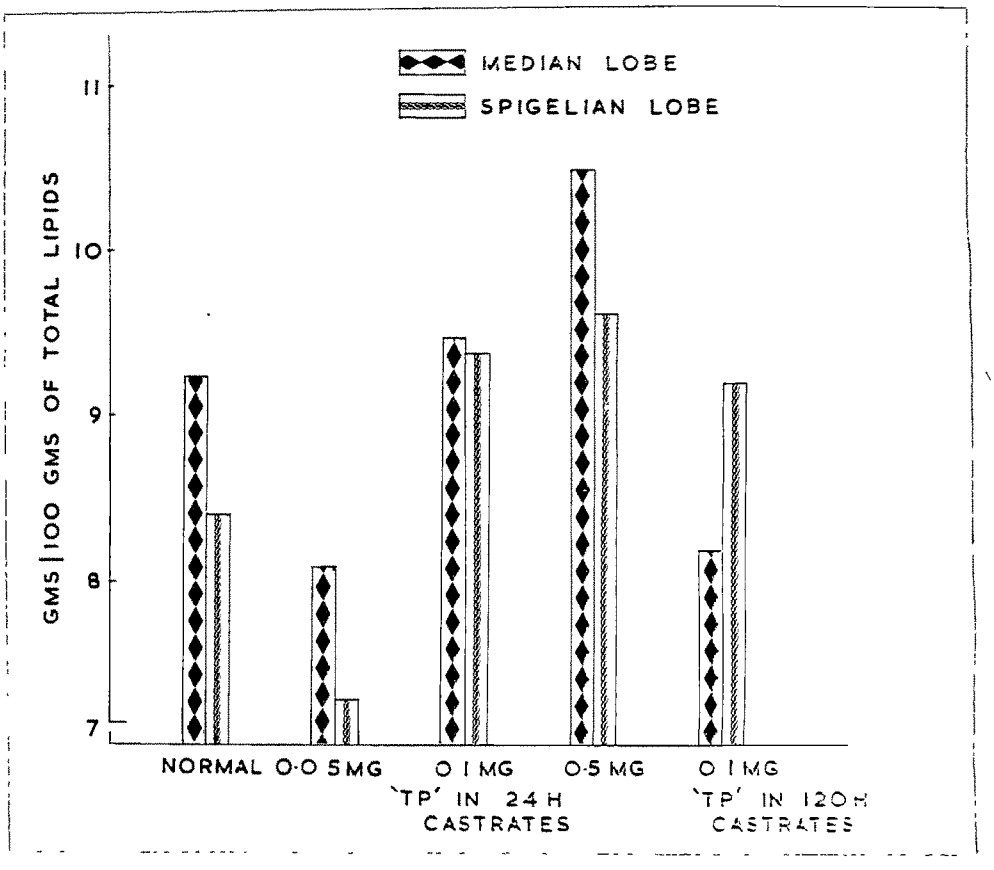

Fig. 4. Total cholesterol concentrations of the two liver lobes, expressed as percentage of total lipid content. Experimental details were similar to those given for Fig. 3.

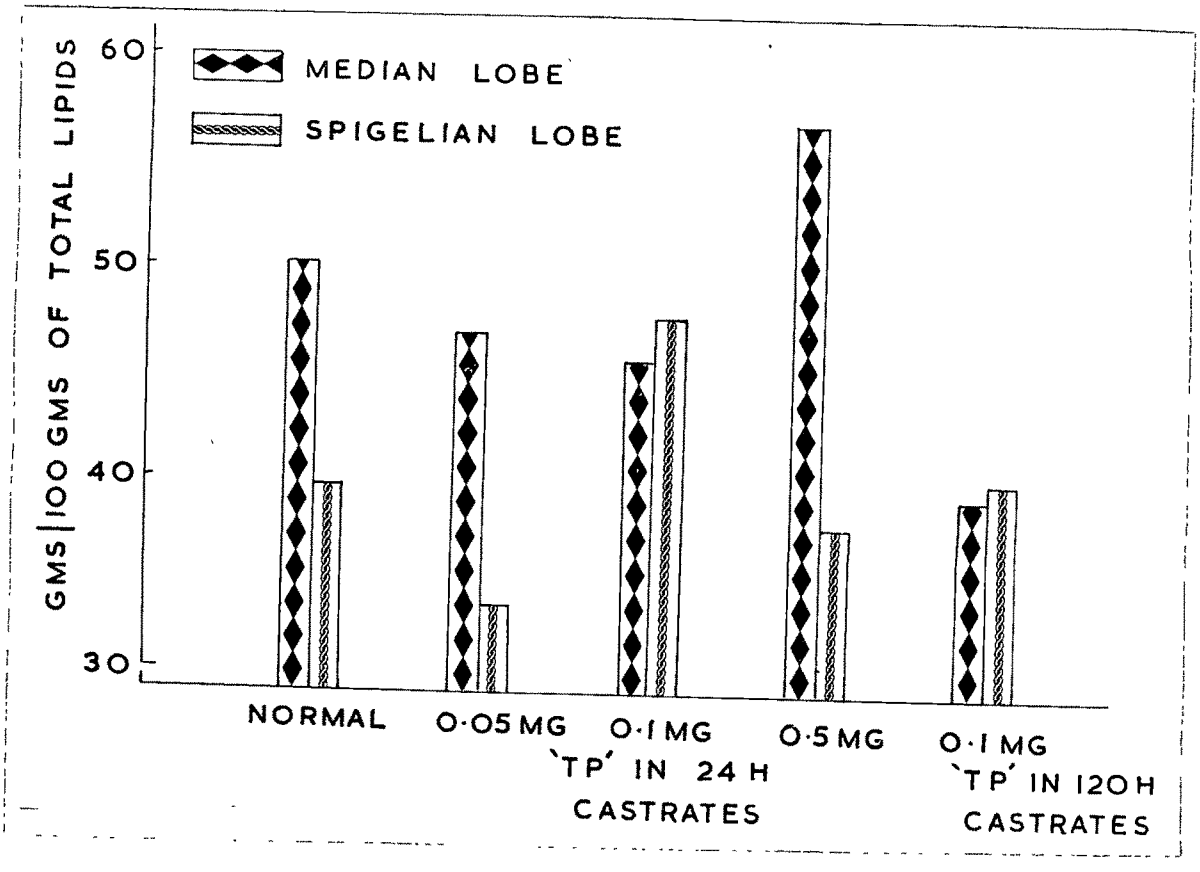

Fig. 5. Concentrations of phospholipids in two liver lobes. Experimental details were similar to those given for Fig. 3.

DISCUSSION

Investigation of the role of the gonadal hormones concerning lipid metabolism has received increased attention in recent years. Significance of lipid metabolites in various animal tissues has been well recognised (George and Jyoti, 1957, 1958; Fredrikson and Gordon, 1958; Rossiter and Strickland, 1960). DeSmet (1953a) observed that, following castration, male rats show an increase in the fat content under the skin and around the kidneys, while there is a concomitant decrease in abdominal fat. In a later report DeSmet (1953b) noted that, following castration in adult male rats, an initial decrease in total body lipids occurred; this was followed by an increase. Rapid deposition of large quantities of fat in the subcutaneous depots is one of the conspicuous changes after castration. Although sex dependent variation in lipid metabolism is a generalised phenomenon, the clearest expression of this variation is to be noted in the lipid composition of the liver. Best and Campbell (1936; 1938) reported an increase in liver lipids as a result of administration of anterior pituitary extracts. This increase in the liver fat content has been ascribed

to the process of mobilization of lipids from the depots. Nathaniel and Nathaniel (1966) also observed an increase in lipid content of the cells, during a study of cytological changes in the liver after gonadectomy of male rabbits. Watkins et al. (1972), in their study on rats, concluded that hepatic output of triglyceride is regulated by gonadal or gonadotropic hormones. The results obtained presently are in agreement with the above mentioned observations.

Scattered reports (Lanczos, 1935; LeBlond et al., 1939; Selye, 1939) in the literature indicated that exposure of laboratory animals to stress results in an increase in liver fat content. Such results can be interpreted to indicate that the increased concentration of ACTH and/or of adrenocortical steroids, induced by excitation of the pituitary-adrenal axis as a consequence of stress, is responsible for the mobilization of fat to the liver. Such an influence of surgical operational stress was not apparent in the present investigation as sham-operated animals did not show significant difference from the intact normal animals.

Higher values of lipids in the liver after castration, in all probability, are due to changes either in the rate

of lipogenesis or lipolysis. It is well known that, there exist endogenous rate-limiting factors governing the lipid metabolism of the liver. Such rate-limiting factors work at different enzymic levels concerning lipid metabolism. Hepatic lipids have three origins: dietary, intrahepatic synthesis and mobilization from stored body fats. Animals employed in this study were fed with standardized and balanced diet, hence variations due to dietary factors could be eliminated. This means that the higher values are either due to acceleration of intrahepatic synthesis and/or mobilization of stored fat. One of the important rate-limiting reactions exists at the level of acetyl-CoA carboxylase (Bortz and Lynen, 1963). Long chain acyl-CoA molecules competitively inhibit acyl-CoA carboxylase activity. Thus, if acyl-CoA's are esterified quickly enough, it may lead to increased lipogenesis. The removal of testis may influence the rate of lipid synthesis probably by hastening esterification process, as is evident from the gradual but continued increase in the hepatic lipid levels in both of the lobes upto 120 hrs. after castration (Table I; Fig. 1).

To substantiate these findings replacement therapy was conducted. Many workers have studied replacement of

the hormones in castrates to explain the effects of hormones (Giegal et al., 1971; Koths et al., 1972; Thapliyal et al., 1975; Morris and De Conti, 1976). It could be expected in the present study that with replacement of the sex-hormone the lipid values should come down to near normal levels. Contrary to such assumption, a noticeable increase with the minimum dosage of TP (0.05 mg) was observed in total lipid content of both the hepatic lobes (Table II). But 0.1 mg TP was found to effectively bring about a depletion in the lipid content. Spigelian lobe reached almost normal level, whereas, median lobe still retained slightly higher values. As the dosage was increased further, Spigelian lobe registered higher values but the median lobe showed a slight depletion. This would suggest that 0.1 mg of TP was sufficient to bring back the lipid levels closer to normal in Spigelian lobe, whereas, even 0.5 mg dose of TP was insufficient for the median lobe. It could be pointed out from these results that the lower dose employed here was ineffective in bringing about desirable response to hormone administration and hence, the tissue continued to show the effects of castration. However,

with 0.1 mg dose liver lipids showed near-normal concentrations (Table II, Spigelian lobe).

When the effective (0.1 mg) dose of the hormone was administered to 120 hr. castrated animals, it was found to be insufficient for median lobe, whereas, the Spigelian lobe did show a depletion though the level remained above normal. This would possibly suggest that as the time interval after castration is prolonged, liver requires greater concentration of the hormone to restore normality in total lipids. The replacement study could further suggest that the Spigelian lobe is more sensitive to TP levels than the median lobe.

From the results obtained in the present study, it can be seen that total lipid values increased due to lack of sex-hormone after castration. Such a variation may be due to some particular lipid component of the 'total lipid'. It was observed that by 24 hrs. both cholesterol and phospholipids decreased. This leads to the impression that the initial rise may be in the neutral fat component. Later, at 48 and 120 hrs. after castration, both cholesterol and phospholipid concentrations increased; indicating rising rates of biosynthesis

of these components.

Metabolism of cholesterol has been the subject of extensive research in the recent years and several excellent reviews including monographs have been published (Cook, 1958; Kritchevsky, 1958; Glover et al., 1959; Portman and Stare, 1959; Bergstrom et al., 1960; Olson and Vester, 1960; Papjak and Conforth, 1960; Kritchevsky, 1960; Goodman, 1963, 1965). Cholesterol production is closely related to the needs of the organisms, and in this, it is conditioned by nutritional, endocrine and neurohumoral influences. Current information on the effects of sex-hormones on cholesterol metabolism concerns mainly with the effect on serum cholesterol levels (Kritchevsky, 1958). According to Bortz (1973), biosynthesis of cholesterol and of the enzymatic machinary on which it is dependent is closely associated with the flow of fat and bile acids to the liver. He further suggested that fat serves to induce increased amounts of β-hydroxy- and β-methyl glutaryl-CoA (HMG-CoA)-reductase, and that the bile acids serve to repress further formation of this enzyme. Bortz and Steele (1973), observed that a rise in plasma free fatty acids was due to variation

in the activity of HMG-CoA reductase. A similar correlation was suggested for hepatic tissue by Bortz et al. (1973). From this and the work of Siperstein and Fagan (1966); Horton et al. (1970); Gregory and Booth (1975) it is now well understood that HMG-CoA reductase is the key enzyme in cholesterol biosynthesis. In the light of the literature cited above, it may be said that the low level of liver cholesterol at 24 hrs. and higher levels thereafter are possibly due to fluctuations in the activity of HMG-CoA reductase. Such fluctuations, in all probability, appear to be due to castration. Initial decline in the level of cholesterol in the liver might occur due to enhanced cholesterol oxidation (Kritchevsky et al., 1963). However, at the later intervals (48 and 120 hrs.) after castration, lack of male sex-hormones may lead to increased HMG-CoA reductase activity as manifested in higher cholesterol levels.

When TP was administered to 24 hr. castrated rats, 0.05 mg was found to be sufficient to elicit a restoring response in case of liver cholesterol levels. Higher

level of TP, on the other hand, increased the cholesterol values above the normal levels. Similarly, when 0.1 mg TP was injected to 120 hr. castrated animals, the liver lobes showed an increase in total cholesterol to reach the normality, but were still below normal. The above study pointed out that immediately after castration the lack of sex-hormone brings about a drop in total cholesterol, which may then rise toward normal level by 48 hrs. of castration. This process of normalization was observed to fail by 120 hrs. but administration of TP helped to hold normalization. Another fact was that with the effective dose hepatic tissue responds by settling down to normal levels, but a higher dose of sex-hormone increases the total cholesterol level beyond normal state, which is not desirable for normality.

The metabolism of phospholipids is intimately related to that of cholesterol (Glomset, 1962). During the course of the present investigation it was observed that variations in the levels of phospholipids showed a parallel pattern with that of the cholesterol corroborating the view of Glomset (1962). Initial drop in the level of hepatic phospholipids might have been due to increased

energy demands soon after castration. Later elevation of hepatic phospholipid level appeared to be mere reparative process that is certainly facilitated by hormone administration. From the limited observation available it was not possible to suggest probable mechanism of the observed results. The hormone injection to 24 hr. castrates could readjust the levels of phospholipids in both liver lobes towards normal with 0.5 mg TP. Similar effect was observed with 0.1 mg TP in the Spigelian lobe only, when injected to 120 hr. gonadectomized rats. For median lobe this dose was found to be less effective and hence, it remained below normal.

Present study showed that castration significantly altered the pattern of lipid metabolism of the liver. Replacement with 0.1 mg TP brought the tissue towards normal condition, whereas, doses higher than this resulted into an increase above normal level. Hence, physiologically normal levels of sex-hormones could be considered important for the maintenance of the normal physiological state of this non-target tissue.