

## SUMMARY

During the course of present investigation on the early influences of sex-hormone on the metabolic patterns of liver tissue, male albino rats were employed as the experimental animals. Bilateral castration was performed through scrotal sacs making a single incision under the mild ether <sup>a</sup>anesthesia. The castrated rats were maintained on a normal balanced diet and water ad libitum for arbitrarily selected shorter intervals of 24, 48 and 120 hours. They were then sacrificed by cervical dislocation. For reference and control normal and sham-operated animals of the same strain and similar weight-range were studied simultaneously. The sham-operated animals were always sacrificed after 24 hrs. of operation.

Replacement therapy was carried out by intramuscular injection of testosterone propionate (TP) dissolved in tributyrin. The regimen was as follows:-

(1) At first, three different dose levels of TP viz., 0.05, 0.1 and 0.5 mg were administered by way of a single shot, to 24 hr. castrates. The injected animals were kept for 24 hrs. and sacrificed thereafter. The 0.1 mg

dose turned out to be the most effective one to counteract the alterations that occurred due to castration. Hence, later on, only this dose was chosen for further experiments on replacement therapy.

(2) A single intramuscular injection of 0.1 mg of TP was given to 120 hr. castrates. These animals were also kept for 24 hrs. after injection before being sacrificed. To find out the early effect of the hormone, few of the rats of this experimental category were sacrificed after 1, 2 and 4 hrs. of hormone administration.

(3) Since, most of the parameters studied here, showed significant variations at 48 hr. of castration, the later replacement experiments were conducted on 48 hr. castrates. These rats were injected with a single dose of 0.1 mg of TP. They were then sacrificed after 1, 2 and 4 hrs. to study the rapid action of the hormone.

The details of the variations obtained and their possible significance are described below as chapterwise summaries.

## CHAPTER 1 :

Quantitative studies on different components of the total lipids in the hepatic tissue of normal, sham-operated, castrated and hormone replaced male rats were carried out. Castration resulted into a gradual increase in 'total lipids' contents from 24 hrs. to 120 hrs. of experiment. Total cholesterol and phospholipid values were found to be reduced very significantly within 24 hrs. of castration. Both these parameters registered elevation by 48 and 120 hr. post-operatively, yet the level remained below normal only.

Replacement with three different dosages of testosterone propionate (TP) indicated that 0.1 mg dose was sufficient enough to bring about normalization of lipid components of 24 hr. castrated rats. The lower dose was found to be ineffective, whereas, the higher dose of TP elevated the lipid components of the hepatic tissue. Hormone replacement study with the effective dose on 120 hr. castrates suggested that as the time interval after castration was prolonged the hepatic tissue required greater amount of the hormone to restore normality of lipid components.

During this preliminary study, an attempt was made to find out possible lobe-wise differences-if any. Spigelian lobe was found to differ from rest of the lobes in having higher concentrations of lipid components and it was found to be more sensitive to the sex-hormone.

## CHAPTER 2 :

A study on the total glycerides and free fatty acids (FFA) of the liver lobes (median and Spigelian) was conducted. Both these parameters were found to be elevated within 24 hrs. of castration. The total glyceride content denoted a fall thereafter upto 120 hrs., but the level remained above normal only. The FFA was found to be decreased by 120 hrs. of gonadectomy. The increase in total glyceride level was observed to be the result of reduced rate of fatty acid breakdown. Immediate increase in the levels of FFA was noted to point to an increased uptake of lipids mobilized from fat depots, whereas, the later decrease at 120 hrs. indicated possible release into circulation and utilization of this fraction by extrahepatic tissues. Study with hormone replacement showed a dose dependent variation in the above parameters.

0.1 mg TP was found to be the effective one. Higher dose resulted into an increase in FFA and a decrease in glyceride levels. As the time interval after castration was prolonged, the effective dose was observed to be subminimal.

### CHAPTER 3 :

In vitro study of the carbohydrate metabolism of the liver (median and Spigelian lobes) revealed a significant increase in the level of glycogen after injury, whereas, removal of circulating male sex-hormone, in comparison, resulted into decreased glycogen level.

The phosphorylase enzyme activity showed a rise concomitant with the fall in glycogen level after castration. The sham-operation reduced the activities of oxidative dehydrogenases (succinate dehydrogenase - SDH and glucose-6-phosphate dehydrogenase - G-6-PDH) within 24 hrs. denoting reduced rate of glycolytic as well as direct oxidative utilization of glucose by hepatic tissue. Castration per se led to counter-effects. However, by 24 hr. post-operatively, the activities of dehydrogenases, particularly that of

G-6-PDH, was very much reduced. A single injection of TP (0.1 mg), at this hour of castration was found to be capable of counteracting these adverse effects to a significant level.

A noteworthy point during this study was about the difference in glycogen content amongst the median and Spigelian lobe of the liver after castration and subsequent hormone injection. Though the normal intact rats did not show a lobe-wise variation, a notable difference in the sensitivity of the two lobes to sex-hormone administration was distinctly observable after castration.

#### CHAPTER 4 :

Castration was noted to induce in its early stages (1 to 5 days) an increase in the levels of total protein and nucleic acids (DNA-and RNA) in the hepatic tissue. Replacement with TP indicated that within one to two hours of hormone injection the level of total protein turned towards normalization, but later by about 24 hrs. stimulation of protein synthesis occurred. The hormone required approximately four hours to bring about normalization in

the nucleic acid contents. Exceptionally, in the median lobe the DNA content could not attain the normal level even upto 24 hrs. of hormone administration. The results obtained were discussed on the basis of protein synthesis and possible occurrence of polyploidy in the hepatic tissue after gonadectomy of male rats. The lobe-wise comparison indicated higher values for total protein as well as both nucleic acids in the Spigelian lobe under all the experimental conditions.

#### CHAPTER 5 :

The normal intact animals showed a distinct difference in the concentration of ascorbic acid (AA) between the Spigelian and the median lobe. Spigelian lobe indicated higher AA concentration. During the course of investigation an unexpected and contradictory result was obtained in regard of the AA level of the hepatic tissue of male rats soon after gonadectomy. After 24 hrs. of castration a significant rise in the level of AA was observed. Conversely, the sham-operated animals showed decreased AA levels. However, 48 hrs. post-operatively, the concentration of AA was observed

to exhibit declining tendency. When the castrated animals were treated with TP, a reduction in AA content was obtained. It was noticed during the study that the Spigelian lobe of the liver always differed from the median lobe in respect of higher AA level. The results obtained due to castration and replacement therapy were discussed in relation to the importance of physiological levels of androgens in the regulation of AA biosynthesis and subsequent retention of the vitamin.

#### CHAPTER 6 :

Castration resulted into lowering of the adenosinetriphosphatase (ATPase) activity of liver after 24 hrs., but the same was found to restore to near normal level by 120 hrs. post-operatively. The altered activity of the enzyme in the liver of 24 hr. castrates, was found to attain normality with hormone administration. 0.1 mg of TP was found to be sufficient to bring about restoration. The study revealed that this enzyme which is crucially important in the overall physiological welfare of the animal, readjusted itself without much difficulty, unlike some other parameters under investigation, though perhaps in an altered way.



## CHAPTER 7 :

Normal rat liver exhibited non-specific phospho-monoesterase enzyme activity, mainly in the acidic range. There was no significant difference between the two lobes of the liver as far as this enzyme activity (at acidic and alkaline pH) was concerned. The activity of alkaline phosphatase was increased following 120 hrs. of castration, whereas, the acid phosphatase activity remained almost normal. With the administration of three different doses of TP, a dose-dependent variation in the activity of alkaline phosphatase was noted. With the lower dosage (0.05 mg) the activity remained stimulated and the higher dosage (0.5 mg) led to a depletion of the enzyme activity. Activity of the enzyme of acidic range did not show significant variation in response to TP injection. Activity of the enzyme was found to be reduced when the 0.1 mg hormone was injected to 120 hr. castrates. These observations hinted at existence of a sex-influence on alkaline phosphatase activity while the acid phosphatase activity remained more or less unaffected.

## CHAPTER 8 :

The capacity for fatty acid oxidation (FAO) of the liver lobes (median and Spigelian) and the levels of free

fatty acids (FFA) in the blood plasma and omental adipose tissue were determined quantitatively in adult male albino rats. The rate of FAO was noticed to be reduced immediately after 24 hrs. of the removal of gonads. Maximum depletion was noted by 48 hrs.. After 120 hrs. of gonadectomy a slight elevation in the rate of FAO could be noted but that was still less than normal rate. One hour after the hormone replacement in 48 hr. castrates, normality in the rate of FAO in Spigelian lobe was evident, whereas, the median lobe took about 2 to 4 hrs. to attain normality.

The level of plasma FFA was depleted very significantly (50% of normal value) after 120 hrs. of operation. Four hours after TP replacement it reached above normal levels. Omental fat depot registered a sharp increase in the FFA concentration immediately 24 hrs. after castration. It decreased to subnormal levels after 48 hrs. and again went higher up by 120 hrs. of castration. Hormone replacement led to a significant rise in the level of FFA in 48 hr. castrates. The significance of these alterations were discussed as a correlation between the rate of FAO by hepatic tissue

and the rate of transport of FFA from the adipose tissue to the hepatic tissue.

#### CHAPTER 9 :

Variations in the levels of total lipids, total cholesterol, phospholipids and glucose in the blood plasma have been measured. An increase in plasma glucose level was noted after castration, reflecting the diminishing level of hepatic glycogen. Contrastingly enough, the sham-operated animals (24 hrs.) registered a distinct hypoglycemia. The level of total lipids registered an increase, initially by 24 hrs. of castration, whereas, the levels of total cholesterol and phospholipid showed a distinct fall. By 120 hrs. post-operatively the levels of all the three lipid components were observed to be decreased causing hypolipemic condition.

The replacement therapy was carried out by administering 0.1 mg TP to 48 hr. castrates. The effects of hormone were studied after 1, 2 and 4 hrs. of injection. Within 60 minutes of the hormone administration, a significant fall in the plasma glucose was noticed. As

the time lapsed after injection, a tendency to shift towards normalization was observed in the glucose level. All the three lipid components also showed near normal values within 4 hrs. of hormone replacement. The implications of this influence of sex-hormone on the blood plasma profiles were discussed in the light of homeostatic mechanisms involving storage and mobilization of two physiologically important metabolites viz., carbohydrates and lipids.

#### CHAPTER 10 :

Gonadectomy was observed to reduce the activity of hepatic cAMP-specific phosphodiesterase. Administration of TP led to further sustained depression in the activity of this enzyme. The results were discussed in relation to mediator-like action of intracellular concentration of cAMP as influenced by variations in the activity of the specific phosphodiesterase under the influence of sex-hormone on the hepatic tissue - a non-target gland.

#### CHAPTER 11 :

A study on the rapid effects of TP replacement on various parameters of liver, blood plasma and omental fat

depot was conducted on 48 hr. castrated male rats. The effects of the hormone were studied after 1, 2 and 4 hrs. The parameters under investigation were chosen in such a manner, on the basis of all previous results, that a study of them would enable one to get an overall picture of metabolic alterations of lipids, carbohydrates and proteins and their reflection in the blood plasma. It was observed that within 60 minutes of hormone administration, hepatic phosphorylase activity was reduced, a distinct hypoglycemia was induced, level of FFA was lowered and free fatty acid oxidising capacity was restored to normality. cAMP-specific phosphodiesterase activity was suppressed, protein biosynthesis was accelerated and SDH activity was reduced significantly. All these alterations, except the last two, were noticed to counteract castration-induced changes. Regarding the last two observations, which were contrary to theoretical expectation, much could not be said presently except that initial immediate influences indicate something that needs further intensive investigation for a better understanding of the problem.