CHAPTER 9

ALTERATIONS IN CERTAIN METABOLITES OF BLOOD PLASMA AS INFLUENCED BY SEX HORMONES

Blood is a dynamically balanced fluid vehicle for distribution of substances from one organ to the other. Any abnormality which results in variation in the blood picture is usually to be ascribed to alterations in metabolic patterns of the tissues themselves. Varied and extensive changes in the blood of homeothermic vertebrates, especially the mammals, in the wake of multiferious physiological and pathological conditions and processes have been well documented. Previous findings on hepatic lipids (Chapter-1) and Glycogen content (Chapter-3) after castration indicated systemic response in the levels of liver lipids and carbohydrates. It is a common practice, under certain experimental situations, to gauge hepatic glycogenolysis/glycogenesis indirectly by assessing changes in the blood glucose levels. Regarding the lipids, it is certain that practically no synthesis or oxidation of lipids occurs in the blood itself. The levels of several lipids in the blood mirror well the conditions which are obtained in the tissues. Ziversmit *et al*. (1948) concluded that increase in specific levels

of plasma phospholipids are reflections of corresponding increase of their precursors in the liver. It is an accepted fact that the blood helps to regulate the intermediary metabolism of lipids by rendering the same readily available at the site of utilization. The most potent factors which influence metabolism of lipids are the secretions of endocrine glands (Deuel, 1955). Deuel (1955) reported on the influence of some synthetic sex-hormones on blood lipids. In the light of the above mentioned facts it is desirable to look for possible changes occurring in the blood plasma of animals under castration experiments. It is deemed that such a study may not only enable one to understand the obligatory changes undergone by blood plasma in response to the lack of male sex-hormone, but also would add to the data obtained in the present inquiry concerning the hepatic tissue of rats.

MATERIAL AND METHODS

Healthy male albino rats (Rattus norvegicus albinus), weighing about 120-160 gms were utilized in the present study. Bilateral castration was carried out under light ether

anaesthesia. The hormone used in the present study for replacement therapy was Testosterone propionate (TP). The TP (Sigma chemicals) was dissolved in pure tributyrin (Sigma chemicals) to give the concentration of 0.2 mg/ml and this solution was used for intramuscular injections. 0.1 mg of the hormone was administered intramuscularly to each animal.

The animals were divided into four groups. Group I was of normal intact animals. The castrated animals formed group II. The individuals of this group were sacrificed at selected intervals of 24, 48 and 120 hrs respectively. Individuals of the group III were sham-operated animals. They were tested after 24 hrs. of operation. Those of the group IV were animals which have been castrated for 48 hrs. and then injected with TP. A single dose was administered and the blood was collected at the regular intervals of 1,2 and 4 hours. All the rats were given regular balanced diet and water ad libitum. All treated rats were housed in separate cages.

Blood was collected in heparinized tubes from jugular vein of unanaesthetized animals. It was then

centrifuged to collect blood plasma. This was utilized to obtain values for total lipids, total cholesterol, phospholipids and glucose. Total lipids were estimated by gravimetric method of Folch et al. (1957). Quantitative evaluation of phospholipids and cholesterol were carried out as per the method of Dittmer and Wells (1969) and Crawford (1958) respectively. Levels of blood glucose were measured following the method of Folin and Malmros (1929).

RESULTS

Results obtained are presented in Table I, showing the percentages of different parameters measured under the influence of male sex hormone.

1. Effect of castration:

(a) On glucose: After 24 hrs. of castration there was an apparent drop in glucose content but when viewed in the light of results obtained with sham-operated animals this decrease could easily be seen as an increase in glucose content over the levels obtainable after sham-operation. A considerable increase was obtained after 48 hrs. of operation leading to values above normal levels.

It remained higher even at 120 hrs. post-operative interval.

(b) On total lipids: It was observed to increase only slightly after 24 and 48 hrs. of gonadectomy. Whereas, a sharp decline in its content was noted after 120 hrs. This fall registered in plasma lipids was approximately 50% of the normal values.

(c) On total cholesterol: These values, when calculated as mg per 100 ml of plasma, showed little decrease after 24 hrs. of castration; nearly similar values were obtained for sham-operated animals also. It remained at the same level by 48 hrs. A deep fall was obtained 120 hrs. post-operatively. If the values were calculated as percentage of total lipids, lower values were obtained by 24 hrs. (even when compared to sham-operated animals) which registered an increase after 48 hrs., but still remained lower than the normal cholesterol values. Thereafter, values were depleted again at the interval of 120 hrs.

(d) On total phospholipid: Comparisons of the data on the basis of the different terms of expression revealed

different pictures. 24 hrs. after castration, total phospholipid content did not show any variation when measured as concentration per 100 ml of plasma. At 48 hrs. interval there was a little rise which declined after 120 hrs. of experiment so as to reach the values below normal levels. When these were calculated as percentage of total lipids, a rise was noted 24 hrs. after gonadectomy, which was found to be insignificant on the basis of values obtained in sham-operated animals. It was depleted after 48 hrs reaching almost the normal level. This was depleted further, below normal, after 120 hrs. of castration.

2. Sham-operation:

These animals showed almost a 47% fall in the blood glucose content after 24 hrs. of operation, a little depletion of total lipids and the plasma cholesterol level (with either way of expression). The values of phospholipid remained steady when measured in terms of 100 ml of plasma, whereas, a rise was obtained when measured in terms of percentage of plasma total lipids.

3. Replacement therapy:

(a) Glucose: Immediately one hour after the TP injection, significant reduction in plasma glucose was obtained. Two and four hours after the replacement of the hormone, a steady rise was noted, yet, it still remained lower than the normal concentration.

(b) Total lipids: Lipid values showed an increase one hour after the replacement. This decline was continued upto two hours of hormone administration and remained almost at the same level even after four hours of the injection. These values were not far removed from the normal.

(c) Total cholesterol: The results obtained for this metabolite, under one hour of TP influence, denoted a significantly diminished levels (with either way of expression). An increase was noted thereafter upto four hours of injection, nevertheless, the values were still below normal.

(d) Phospholipids: As in case of the total cholesterol, phospholipids also registered a notable fall one hour after TP injection. An increasing trend was registered by two

Table I : Showing percentage values of glucose, total lipids, total cholesterol and phospholipids in the blood plasma of the male albino rats, obtained under various experimental conditions.

	Normal animals	Sham-operated animals 24 H*	Castrated animals			24 H Castrates injected with 0.1 mg TP		
			24 H*	48 H*	120 H*	1 H@	2 H@	4 H@
Glucose mg/ 100 ml of Plasma	81.12 ± 8.35	43.04 ± 2.42	77.67 ± 1.82	96.30 ± 6.52	99.99 ± 1.28	39.50 ± 3.15	46.00 ± 2.68	62.58 ± 2.16
Lipids gms/100 ml of Plasma	1.39 ± 0.395	1.02 ± 0.298	1.55 ± 0.191	1.40 ± 0.370	0.70 ± 0.194	1.76 ± 0.444	1.21 ± 0.194	1.22 ± 0.081
Cholesterol mg/ 100 ml of Plasma	186.0 ±13.60	125.9 ±17.18	121.3 ± 0.0	122.7 ±16.55	73.03 ± 3.51	81.9 ±12.00	115.5 ±12.90	123.8 ± 9.21
Cholesterol as % of total Lipids	13.50 ± 1.45	11.27 ± 1.98	7.13 ± 0.9	11.03 ± 0.57	9.57 ± 0.12	6.31 ± 0.097	9.54 ± 0.098	10.72 ± 0.278
Phospholipids mg/100 ml of Plasma	143.9 ±12.54	142.0 ± 8.31	146.6 ±13.69	162.2 ±12.91	119.7 ± 8.52	118.0 ± 5.16	116.8 ± 5.23	120.5 ±13.30
Phospholipids as % of Total Lipids	12.48 ± 1.99	16.27 ± 1.08	15.25 ± 0.731	12.38 ± 0.745	8.80 ± 0.813	5.50 ± 0.041	8.56 ± 0.078	8.23 ± 1.20

Each reading is the mean value of twelve different samples.

*Post-operative interval in hours.

@Hours after TP administration

and four hours of intervals, however, the values (both ways of expression) remained lower than normal.

DISCUSSION

Changes in the plasma glucose level obtained herein, after various time-intervals of castration in male albino rats appeared to reflect more or less faithfully the pattern of variations in the glycogen content of the liver. From the data obtained it became clear that 24 hrs. subsequent to sham-operation, a distinct hypoglycemic condition was registered. The possible reason for the hypoglycemic condition might have been the result of increased rate of uptake of glucose by the hepatic tissue. Compared to this a rise was noted after 24 hrs. of castration (Table I). Under similar experimental conditions reduction in the glycogen content (with reference to sham-operated animals) of the liver was observed earlier (Chapter-3). It is well known that the glycogenolysis in the liver is one of the reasons for increase in the glucose level of the plasma. Hence, the changes brought about by castration, within as short a period as 24 hrs., are decidedly significant and were certainly not due to surgical assault. Influence of castration actually

reverted that of sham-operation as far as carbohydrate metabolism was concerned. As the time-lapse after castration was increased a gradual rise in plasma glucose was obtained. The liver glycogen registered a gradual and parallel fall at these time intervals (Chapter-3). This clearly indicated increasing rate of glycogenolysis in the hepatic tissue; leading to release of glucose in the plasma. Further, Ligrele and Sutter (1972) also observed an increase in glucose level after 20 days of castration in rats. The degrees of perceptible hyperglycemic levels observed herein lends credulity to the observation that carbohydrate catabolism is accelerated as a result of lack of sex-hormone.

The investigation depicted a fluctuating pattern of changes as far as the levels of plasma lipids were concerned. 24 hrs. subsequent to castration the total lipid content showed an increase, whereas, cholesterol and phospholipids were found to be depleted (comparing the data with sham-operated animals). This suggested the possibility of increased triglycerides in blood plasma. Masoro (1960) had reported a general lipemia

subsequent to infliction of injury to mammals, which was noted to be chiefly a rise in neutral fat content with negligible variation in phospholipids and cholesterol of the plasma. It is obvious from the data obtained that the initial variations of plasma lipid constituents were the specific responses to the castrated condition.

A later noteworthy feature is the depletion in the levels of all the three lipid components after 120 hrs. of castration. The fall in plasma total lipids alongwith total cholesterol and phospholipids will lead to hypolipemic condition. It would be worthwhile to recall here the observations of Brown and Gass (1975); where a correlation between testosterone and serum lipid levels in human population was made out. A direct correlation was found between testosterone levels and the lipid components, indicating a possible sex hormone-lipid relationship. Further Kral and Tisell (1976) also had observed a significant reduction in blood triglycerides after castration of adult male rats. In the light of the above observations it could be seen here clearly that removal of gonads led to reduced level

of testosterone which in turn resulted into reduction in blood lipids.

Replacement with TP was found to bring about readjustment in plasma levels. Most significant effect of castration was manifested 48 hrs. post-operatively hence it was more logical to expect information of greater significance that could be obtained after administration of an appropriate dose of TP to 48 hr. castrates. Within such a short period as 60 minutes, after administering 0.1 mg of TP, a markedly sharp fall in the plasma glucose level was registered. Among lipid components the cholesterol and phospholipid concentrations too showed reducing trends quite clearly whereas that of total lipids exhibited an increase. This pattern of changes in the blood plasma indicated a significant flux reflecting reduced hepatic phosphorylase activity (Chapter-11) and perhaps greater uptake of plasma glucose by peripheral tissues. It also suggested removal of cholesterol and phospholipids from the plasma by the hepatic tissue to facilitate reparative changes occurring there. In short, the blood plasma picture is a reflection of altered physiological states of hepatic and peripheral tissues

under the influence of administered hormone.

From the tabulated data for next two intervals of 2 and 4 hrs. after TP administration it was obvious that as the time lapsed there was a tendency in all the parameters under consideration to shift towards normalization. Strong initial hypoglycemia really needs to be taken care of immediately and that was the case, yet it is not possible to explain this early induction of hypoglycemia beyond suggesting that it might represent a sort of a shock or stress. This would obviously mean that a single injection of hormone brings about only a transient change in blood plasma not lasting more than few hours though, it does faithfully reflect the alterations. Later, homeostatic processes prevail upon early changes of blood plasma; unless they could be held in abeyance by further doses of hormone, which would naturally influence the concerned tissues to respond again.

The present inquiry, unfortunately, has remained inadequate to allow much insight into systemic significance of early effects on plasma constitution. It is hoped that the existing lacunae will be filled up at the earliest opportunity to extend the study beyond present limits.