CHAPTER 1

EXPERIMENTAL PROTOCOL, MATERIAL AND METHODS

The aim of the present investigation was to observe the very early effects, interms of few hours, of orchidectomy, replacement therapy as well as androgen administration to intact normal animals on the hepatic metabolism in adult male albino rats (Rattus norvegicus albinus). Rats weighing 120-160 gms. body weight and maintained in the laboratory on ad libitum food and water, served as the experimental animals.

EXPERIMENTAL DESIGN : PART - I

Bilateral orchidectomy was performed on normal healthy male albino rats, under ether anesthesia. Experimental animals were maintained for 48-hours post operatively and then sacrificed for data collection. Normal males of the same strain and similar body weight were sham operated, which served as reference controls. Replacement therapy was performed by injecting 0.1mg testosterone propionate (TP) Sigma.chem.Co. to 48-hours orchidectomised animals. Tributyrin (Sigma.Chem.Co.) was used as the solvent. A single intramuscular injection per head in 0.5 ml volume was given 48-hour post orchidectomy. The animals were weighed and sacrificed after first, second, and fourth hour after TP

administration. Hepatic tissue and blood samples were utilized for various quantitative biochemical investigations.

PART - 2

The results of the earlier study carried out in our laboratory (Gangaramani, 1979), and those of the present investigation prompted the study of the early influence of androgen administration to intact normal adult male albino rats. Therefore, in the second phase of the present study, normal rats (120-160 gms. weight) were given a single intramuscular injection of 0.1 mg TP and were sacrificed for data collection at 30, 60, 90 and 120 minutes after TP administration. Later, it was also thought necessary to carry out a dose dependent study, and hence two more doses of TP viz.- 0.25mg and 0.5mg were administered to normal animals. The time intervals after hormone injection were same as selected for 0.1mg dose of TP administration.

QUANTITATIVE ANALYSIS :

Hepatic tissue was quickly removed, trimmed free of adhrent connective tissue, blotted and weighed for quantitative estimations. Median and Spigelian lobes (nomenclature according to Green 1959) were dealt with separately for different estimations. Weighed liver pieces were separately homoginized in cold distilled water using chilled mortars

and the homogenate was prepared according to the experimental requirements. Blood sample was collected from internal jugular vein in an anti-coagulant coated tube so as to obtain plasma for glucose estimation. Whole blood sample was used for Ascorbic acid (A.A.) estimation.

METHODS EMPLOYED :

Glucose :

The estimation of plasma glucose level was carried out by micro method as described by Folin and Malmros (1929) and the concentration is expressed as mg glucose/100ml of plasma.

Glycogen :

Hepatic glycogen content was estimated employing the anthrone method as described by Seifter et al. (1950) and the concentration is expressed as gm/100gm wet tissue, (% of fresh tissue).

Phosphorylase :

Phosphorylase (E.C.2.4.1.1.) activity in the hepatic tissue was assayed by the modified method of Cori et al.(1943) as adapted by Cahill et al. (1957). Glucose-1-phosphate (dipotassium salt, Sigma chem.Co.) was used as substrate and the inorganic phosphate released was measured according to the method of Fiske and Subbarow (1925). The readings were taken at 660 mμ on Klett-Summerson photoelectric colorimeter.

Protein content was estimated according to the method of Lowry et al. (1951) and the enzyme activity is expressed as µg of phosphorus released/ mg protein/ 10 minutes.

Glucose-6-Phosphatase (G-6-Pase)

G-6-Pase (E.C.3.1.3.9.) activity was assayed by the method of Harper (1963) and the phosphorous released was measured by the classical method of Fiske and Subbarow (1925). Activity is expressed as µ Moles of phosphorous released/ mg protein/ 15 minutes.

Glycogen Synthetase :

Glycogen synthetase (E.C.2.4.1.11.) was assayed according to the method of Leloir and Goldemberg (1962) and expressed as µ Moles of UDPG formed/ mg protein/ 10 minutes.

Transaminases : (GOT, GPT)

Aspartate aminotransferase (GOT.E.C.2.6.1.1.) and alanine aminotransferase (GPT.E.C.2.6.1.2.) activities were assayed according to the method of Bergmeyer and Bernet (1965). The amount of 'hydrazone' formed by oxaloacetate or pyruvate formed in enzymic reactions was measured photometrically at the wave length 505 mµ. With increase in pyruvate or oxaloacetate and a concomitant decrease of α -ketoglutarate, the resulting increase in absorbance is proportional to pyruvate or oxaloacetate that is produced. The optical density (OD) of the colour developed was read in 'Spectronic-20' Spectro colorimeter. Karmen units were reckoned from the

standard table and the activity is expressed as Karmen units/ mg protein/ minute.

5'-Nucleotidase :

5'-Nucleotidase activity was assayed by the method of Jaffrri and Mustafa (1976) and the phospherous released was measured by the classical method of Fiske and Subbarow (1925). The activity is expressed as mg phosphate released/ 100 mg protein/ hour.

Phosphodiesterase :

Phosphodiesterase activity was measured according to the method described by Butcher and Sutherland (1962). The enzyme activity is expressed as µg phospherous released/ mg protein/ 20 minutes.

Nucleic acid contents :

Nucleic acid contents (DNA and RNA) were assayed spectrophotometrically as suggested by Schneider (1957) and are expressed as µg/ mg of fresh tissue weight.

Protein content :

Total protein content of the hepatic tissue was estimated by the method of Lowry et al. (1951) using boving serum albumin as the standard, and expressed as gm % of fresh tissue weight.

Ascorbic acid (A.A.) content :

A.A.content of the liver was estimated after homogenizing the tissue in 6% trichloro acetic acid in pre-chilled mortars. Aliquotes of these extracts were used for the determination of A.A. by employing 2,4 dinitrophenyl hydrazine method of Roe et al. (1954). The values are expressed as mg/ 100 gms of fresh tissue.

Statistical Analysis :

The values for mean, standard deviation and standard error were calculated as per routine statistical methods. Students 't' test was employed to determine the statistical significance and is expressed as P values.

Abbreviations used in Text

T P	Testosterone propionate
48-Hr	48-hours
c-AMP	Cyclic Adenosine mono phosphate
A.A.	Ascorbic acid
Pr	Protein
DNA	Deoxy ribose nucleic acid
RNA	Ribose nucleic acid
GOT	Aspartate aminotransferase
GPT	Alanine aminotransferase
i.m.	Intramuscular
min.	Minutes
M	Median lobe of liver
Sp	Spigelian lobe of liver
Cas	Castrated