CHAPTER I

M E T H O D S

Adult pigeons (Columba Livia) weighing about 250-300 grams, of both the sexes maintained in laboratory conditions on a balanced diet were used for the experiments. The birds were sacrificed by decapitation and immediately kidney was taken out, total weight was noted and used for quantitative estimations of enzymes and protein, glycogen and lipid contents.

Enzyme Assays :

Acid and Alkaline phosphatases:

Acid (E.C. 3.1.3.2) and Alkaline (E.C. 3.1.3.1) phosphatase activities were measured according to the method described in Sigma Technical Bulletin No.104, using p-nitrophenyl phosphate as substrate. Enzyme activity was expressed as μ moles of p-nitrophenol released/mg protein/30 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 as described by Bergmeyer and Bernet (1965). The amount of 'hydrozone' formed by oxaloacetate or pyruvate formed in enzymatic reactions was measured photometrically at the wavelength of 505 mμ. With an 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 (O.D.) of the colour developed was read in "Spectronic-20" Spectrocolorimeter, the Karmen unit was taken from the standard table and the activity was expressed as Karmen units/mg protein/min.

Na^{+}-K^{+}-ATPase :

Na^{+}-K^{+}-ATPase activity was assayed according to the method described by Stanstny (1971), using ouabain as inhibitor and substracting the value obtained for Mg^{++} ATPase activity. The inorganic phosphate released was measured according to the method of Fiske and Subbarao (1925). The readings were taken at 660 mμ on Klett-Summerson Photoelectric Colorimeter. Protein content was estimated by the method of Lowry et al. (1951) and the enzyme activity was expressed as μg of phosphorus released/mg protein/ 10 minutes.

Phosphorylase:

Phosphorylase (E.C.2.4.1.1.) activity in kidney was assayed by a modification of method of Cori et al. (1943) as adapted by Cahill et al. (1957). Glucose-1-Phosphate (dipottasium salt, Sigma Chemical Co.) was used as a substrate and the inorganic phosphate released was measured according to the method of Fiske and Subbarao (1925). The readings were taken at 660 mμ on a Klett-Summerson Photoelectric Colorimeter. Protein content was estimated by the method of Lowry et al. (1951) and the enzyme

activity was expressed as µg of phosphorus released/mg protein/ 10 minutes.

LDH :

The activity of lactate dehydrogenase (E.C.1.1.1.27) was assayed employing colorimetric method of King (1971) as described by Varley (1975) using sodium lactate as substrate and NAD^+ as co-factor. The optical density of the colour developed was read at 440 mµ on Spectronic-20 Colorimeter and enzyme activity was expressed as µ moles of lactate oxidized/mg protein/15 minutes.

G-6-Pase :

G-6-Pase (E.C.3.1.3.9.) was assayed by the method as described by Harper (1963), using disodium salt of G-6-P (Sigma Chemical Co.). Phosphate released was estimated according to the method of Fiske and Subbarao (1925) and the enzyme activity was expressed as µ moles phosphate released/mg protein/minute.

Acetylcholinesterase (AChE):

Kidney was removed, blotted and homogenized in cold 0.9 % solution of NaCl. Homogenates were centrifuged in cold at 3000 RPM for 10 min. to remove cell debris. The Supernatant was mixed with 5:5-dithiobis-2-nitrobenzoate (DTNB) Buffer solution. AChE activity was measured colorimetrically by the method of Gaunther and Klaus (1970). The reaction was initiated by addition of acetylthiocholine iodide as substrate, at $30^{o}C$. Control tubes were treated in a similar manner by adding one or two

drops of prostigmine (injectable preparation). Colour intensity was measured at 420 mμ in a photoelectric colorimeter. Activity was expressed as μM ACh hydrolyzed/mg protein/10 minutes.

Protein :

Protein content in the samples of homogenates used for the different enzyme assays were estimated by the method described by Lowry et al. (1951) using bovine serum albumin as the standard, and expressed as gm percentage of wet tissue.

Glucose :

The estimation of glucose in the blood (0.1 ml) was carried out by micromethod as described by Folin and Malmros (1929) and the concentration is expressed as mg glucose/100 ml blood.

Glycogen :

A small piece of kidney was used for the estimation of glycogen. The glycogen content was estimated employing the anthrone method as described by Seifter et al. (1950) and expressed as gm/100 gm wet tissue.

Total lipid :

A piece of fresh kidney of known weight was kept in an air oven at 60°C for complete drying. After getting the constant weight, lipid was extracted from a known quantity of powdered tissue employing the method described by Folch et al. (1957)

using a mixture of chloroform : methanol (2:1 v/v). The extracted lipid was measured gravimetrically and expressed as gm/100 gm dry tissue.

Statistical Analysis :

The data presented were subjected to Student's 't' test and the significant levels are expressed as P values.

Table of abbreviations used, unit of activities of enzymes and values of metabolites

Parameters	Abbreviations	Units
Protein %		gm % of wet tissue
Alkaline Phosphatases	Alk Pase	µ moles of p-nitrophenol released/ mg/protein/30 min.
Acid phosphatases	Ac Pase	µ moles of p-nitrophenol released/mg protein/30 Min.
Aspartate amino-transferase	GPT	Karmen units/mg protein/ minutes.
Alanine amino-transferase	GOT	Karmen units/mg protein/ minutes.
Na^+-K^+-ATPase	++	µg of phosphorus released/ mg protein/10 minutes.
Phosphorylase	-	µg of phosphorus released/ mg protein/10 minutes
Glucose-6-Phosphatase	G-6-Pase	µ moles phosphate released/ mg protein/10 minutes.
Lactate dehydrogenase	LDH	µ moles of lactate oxidized/mg protein/15 minutes.
Acetylcholinesterase	AChE	µ moles of ACh hydrolysed/ mg protein/10 minutes
Glucose	-	mg glucose/100 ml blood
Glycogen	-	gm/100 gm wet tissue
Lipids	-	gm/100 gm dry tissue

ABBREVIATIONS USED IN TEXT

ACh	-	Acetylcholine
AChE	-	Acetylcholinesterase
Ac-Pase	-	Acid phosphatase
c-AMP	-	Adenosine 3'-5' cyclic Monophosphate
CR	-	Cortical region
CV	-	Central veins
DTNB	-	5:5-dithiobis-2-nitrobenzoate
E	-	Epinephrine
F.1-6 DP	-	Fructose 2,6,diphosphatase
F.2-6 DP	-	Fructose 2,6,diphosphatase
G-1-P	-	Glucose-1-Phosphate
G-6-P	-	Glucose-6-Phosphate
G-6-Pase	-	Glucose-6-Phosphatase
GFR	-	Glomerular filtration rate
GOT	-	Glutamate - oxaloacetate transaminase
GPT	-	Glutamate-pyruvate transaminase
ICT	-	Intralobular collecting tubule
I.P	-	Intra Peritoneal
LDH	-	Lactate dehydrogenase
MC	-	Medullary cone
MCT	-	Medullary collecting tubule
MT	-	Medullary tubule
MR	-	Medullary region
MTN	-	Mammalian type nephron

Na^+-K^+-ATPase	-	Sodium-Potassium activated adenosine-5'-triphosphatase
NE	-	Norepinephrine
PCT	-	Perilobular collecting tubule
PEP	-	Phosphoenol pyruvate
PEPCK	-	Phosphoenol pyruvate carboxy kinase
PFK	-	Phospho fructokinase
PK	-	Pyruvate kinase
PRA	-	Plasma renin activity
RDW	-	Redistilled water
RP	-	Renal portal veins
RNA	-	Ribo nucleic acid
RTN	-	Reptilian type nephron
T_3	-	Triiodothyronine
T_4	-	Thyroxine
6-OHDA	-	6-hydroxy dopamine