CHAPTER 1

MATERIALS AND METHODS

PART - 1

SLICE-EXPERIMENT

Adult pigeons (Columba livia), weighing 180-250 grams, maintained in laboratory conditions on balanced diet were used for the experiments. Animals were sacrificed after an over night starvation. The liver was perfused with cold Krebs Ringer Bicarbonate Buffer (KRB buffer) and then quickly excised. The liver was placed on ice and cut into slices weighing around 40-50 mg. The weighed liver slices were incubated in 10 ml flask, with 5 ml KRB buffer, containing glucose and albumin. The liver slices were incubated for 90 min., at 37^{o}C in a water bath-shaker with 120 oscillations/min. The slices were incubated in media of different categories with different additives.

After the incubation, the slices were quickly washed with chilled KRB buffer and were digested in KOH for glycogen estimation. The glycogen was estimated in fresh slices removed from liver as well as in the slices after incubation. Glycogen was estimated by Anthrone method of Seifter et al. (1950). The glucose concentration was determined in the medium before and after incubation and the differences were calculated. Glucose was estimated by Folin-Malmros (1920) micro method.

The slices were quickly washed with chilled KRB Buffer before and after incubation and homogenised. The homogenate was used for the assay of various enzymes as well as protein.

Composition of KRB buffer

Nacl	-	0.73 %
KCl	-	0.035 %
$CaCl_2$	-	0.028 %
KH_2Po_4	-	0.16 %
$MgSo_4$	-	0.028 %
$NaHCo_3$	-	0.16 %
Glucose	-	3 mg/ml
Albumin	-	2 mg/ml

AChE :

Liver was removed after incubation, 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. Supernatant was mixed with DTNB Buffer solution for enzyme assay. AChE activity was measured colorimetrically by the method of Guenther and Klaus (1970). The reaction was initiated by addition of acetylthiocholine iodide as substrate, at 30°C. Control tubes were treated in a similar manner but the acetylcholinestrase activity was inhibited by adding one or two drops of prostigmine (injectable preparation). Colour intensity was measured at 420 mμ in a Klett.

$Na^+ - K^+$-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 from total ATPase activity.

Acid and Alkaline Phosphatases:

Acid and Alkaline 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/100 mg protein/30 min.

SDH :

SDH activity was quantitatively estimated according to the method of Kun and Abood (1949), using TTC as the electron acceptor, and measured the colour of the formazan formed at 420 μm; enzyme activity was expressed as μg formazan formed / mg protein/30 min.

LDH :

The activity of lactate dehydrogenase (E.C.1.1.1.27) was estimated employing the colorimetric method of Kings as described by Varley (1975).

Phosphorylase:

Phosphorylase activity in liver was assayed by a modification of the method of Cori et al. (1943) as adapted by Cahill et al. (1957). Glucose-1-phosphate (dipotassium 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 Klett Summerson photoelectric colorimeter. Protein content was estimated by the method of Lowry et al. (1951) and the enzyme activity expressed as μg of phosphorus released/mg protein/10 min.

Phospholipid:

Phospholipid was assayed in liver according to the method of Dittmer and Wells (1969). Inorganic phosphate was determined at 660 u colorimetrically, adopting the classical method of Fiske and Subbarao (1925).

Protein:

Protein was estimated using the same homogenate according to the method of Lowry et al. (1951). Protein is expressed as mg protein/30 min.

PART - II

The surgery (Vagotomy) was performed as follows.

The pigeons were anaesthetized with ether and 5 cm incision on the dorsal side of the cervical region was made. The vagal trunk was separated from the surrounding tissues and jugular vein and an approximate 10 mm section of it was removed. Thereafter the incision was closed by suturing. Sham vagotomy involved a similar procedure except that the vagal trunk was lifted and left back. Penicillin was administered after surgery (both sham and vagotomized). The sham-operated and vagotomized pigeons were maintained for 48 hr or 72 hr without food.

The hormones were administered to normal (overnight starved), sham-operated (48 hr. and 72 hr.) and vagotomized (48 hr. and 72 hr.) pigeons as per following.

Schedule	Time	Route of Administration
Choline chloride	8.00 A.M.	Intravenous
ACh	8.00 A.M.	Intravenous
Insulin	8.00 A.M.	Intravenous
Glucagon	8.00 A.M.	Intravenous
Glucocorticoid (DXM)	8.00 A.M.	Intravenous

The pigeons were sacrificed at intervals of 0, 30, 60, 90 and 120 min. after hormone administration, by decapitation under mild anaesthesia.

For the study of effect of cholin chloride on GTT and enzymes in the liver, normal (overnight starved), sham-operated (48 hr. and 72 hr.) and vagotomized (48 hr. and 72 hr.) pigeons were injected with choline chloride as per the following schedule.

	Time	Route of Administration
Glucose alone	8.00 A.M.	Intravenous
Choline chloride alone	8.00 A.M.	Intravenous
Choline chloride + Glucose	8.00 A.M.	Intravenous

The birds were sacrificed at regular intervals of 0, 30, 60, 90 and 120 min. after injection. The blood samples drawn by cardiac puncture were utilized for glucose estimation. The liver pieces exiced were subjected to estimations of glycogen, phospholipid and enzymes such as glycogen-synthetase, acid phosphatase, ATPase and LDH according to the methods given above.