# ~MATERIAL AND METHODS~

## Animal Models:

### 1) Bank myna (*Acridotheres ginginianus*)~

A grey coloured gregarious bird with a short tuft of feathers on the forehead that keeps together in a flock all throughout including breeding season. Takes a variety of food *i.e.* fruits, grubs, insects, ticks picked off from cattle's and eatables flicked from handcart vendors. It is a resident species of continental and peninsular India and found in neighbourhood of human habitations from market places of towns and cities to outskirts of villages. Breeds in colony made on vertical earthen banks of rivers or adapted to concrete holes made under bridges.

### 2) Brahminy myna (*Sturnus pagodarum*)~

A crested myna with rich buff colour over the body and black tuft of hairs on the head so called black headed myna, keeps together in associations with other mynas at roosts. Feeds on fruits, flowers, nectar and insects and is widespread in most of India, found in open deciduous forest, scrub jungle and near human habitations (Ali, 1979; Ali and Ripley, 1983; Grewal, 1995). Individual hole nester using abundant holes of wood pecks and also adapted to nesting in artificial condition of nest boxes and holes in urban conditions on electric poles or buildings etc.

The birds used for the present study were procured from a local bird supplier. The birds were kept in well-maintained aviary of Department of Zoology with free access to food and water. They were sacrificed within a day or two during the early morning hours to minimise the effect of caging. They were anaesthetized with ether, and then sacrificed by decapitation.

The birds were weighed after anaesthetisation and weighed on open balance to the nearest of 1 gm. After sacrificing, the birds were dissected and the tissues taken out were blotted free from tissue fluid and weighed accurately in a single pan Mettler Balance nearest to 0.1mg. The tissues for enzyme assays were homogenised using pre-chilled mortars and pestle in chilled redistilled water, except for the assay of Glucose-6-Phosphatase, where citrate buffer was used. Dilution was done according to the weight of the tissue *i.e.* 20 mg /ml. A fixed part of intestine was taken, was cut open and washed thoroughly with 0.9% saline to remove the undigested food.

**GLYCOGEN ~**

For the estimation of tissue glycogen content, method of Seifter *et al.,* (1950) was employed using Anthrone reagent. A pre-weighed piece of liver, a part of intestine of about 1/2 cm long and part of right kidney, were added in 30% KOH and were kept in boiling water bath till the tissue were digested. Then the test tubes were cooled and 2 ml of 100% alcohol was added to it to precipitate glycogen. The tubes

were warmed till first bubble appeared. The contents were mixed thoroughly and kept in refrigerator for 30 minutes for the precipitates to settle down. After 30 minutes the test tubes were centrifuged at 3000 rpm for about 10 minutes. Supernatant was poured off carefully & the precipitates were dissolved in redistilled water to make desired dilutions. The 2ml of aliquot was treated with anthrone reagent prepared in conc. $H_2SO_4$ to develop colour. The tubes were kept in boiling water bath for 4 minutes. The resultant colour obtained cool and then was measured spectrophotometrically at 620 nm & expressed as mg glycogen /100mg wet tissue.

## GLYCOGEN PHOSPHORYLASE ~

### E.C. 2.4.1.11 *1,4-α-D-Glucan: Orthophosphate, α-D-glucosyl transferase*

Phosphorylase activity was estimated by the modified version of the Cori *et al.* (1943) as followed by Cahill *et al.* (1957) using incubation medium containing 0.1 M sodium citrate buffer pH : 5.9 (0.2ml), 0.154 M potassium fluoride (0.3ml) and 0.2M dipottassium salt of G-I-P (.05ml) as substrate. 0.5 ml of homogenate was added to the tubes containing above reagents. The tubes were incubated for 30 minutes at 40°C. The reaction was stopped using 1ml of 10% TCA & the mixture was centrifuged at 3000 rpm for 10 minutes. The inorganic phosphate produced in supernatant was assayed by the

method of Fiske & SubbaRow (1925). This was carried out by adding 0.4 ml of 10N $H_2SO_4$ and 0.8 ml of 2.5% Ammonium molybdate to the clear supernatant. The assay medium was kept aside for 20 min and ASNA (amine napthol-sulphonic acid) was added as a reducing agent and this was diluted with redistilled water till 10 ml mark in the centrifuge tubes and kept aside for 10 minutes. The colour developed was measured at 660 nm on spectrophotometer and the enzyme activity was expressed as μg of phosphate released /mg protein /30 minutes / 40° C (unit/ mg protein).

## GLUCOSE-6-PHOSPHATES (G-6-Pase) ~

### E.C. 3.1.3.9 (*D Glucose-6-Posphate Phosphohydrolase*)

The activity of G-6-Pase was assayed by the method of Harper (1963) using 0.1 M (pH: 6.5) Citrate buffer & 0.08M disodium salt of glucose-6- phosphate as the substrate. 0.5 ml of homogenate was added to the tubes contain above reagents. This was incubated for 15 minutes at 40°C. The reaction was stopped using 2ml of 10% TCA. This was centrifuged at 3000 rpm for 10 minutes and the supernatant was used to estimate inorganic phosphate released employing the method of Fiske & SubbaRow (1925). The assay medium contains 0.002 N acidic ammonium molybdate solutions and the supernatant. The tubes were kept aside for 10 min. Then ANSA was added as a reducing agent. The colour developed was measured on

spectrophotometer at 660nm and the activity was expressed as μg of phosphate released /mg protein /15 minutes / 40° C (unit/mg protein).

## SUCCINATE DEHYDROGENASE (SDH) ~

### E.C. 1.3.99.1 *Succinate (acceptor) oxido-reductase*

SDH activity was estimated employing the method of Kun & Abood (1959) using INT [2- (4- iodophenyl) 3- 4 nitrophenyl 5- phenyl tetrazolium chloride] dissolved in DMF as an electron acceptor. The incubation medium contained 0.5 ml of 0.1 M phosphate buffer (pH 7.4), 0.5 ml of sodium succinate (0.5 M), 0.5 ml of INT (0.2%) and homogenate 0.2ml. The tubes were incubated at 40° C for 30 minutes, and the reaction was stopped by adding 7ml of acetone in each tube. The tubes were centrifuged for 3 minutes at 3000 rpm. The clear supernatant was drawn and the amount of formazan formed was measured at 420 nm on colourimeter. The enzyme activity was expressed as μg of formazan formed /mg protein / 30 minutes / 40°C (unit/ mg protein).

## ADENOSINE TRIPHOSPATE (AT Pase) ~

### *E.C. 3.6.1.3*

ATPase was assayed by the method of Umbreit *et at.* (1957). The assay medium contain 0.1 ml Tris buffer (7.2 pH), 0.1ml of NaCl (100mM), 0.1 ml of KCl (20mM), 0.1ml of $MgCl_2$ (5mM), 0.1 ml of EDTA, homogenate 0.2 ml and 0.1 ml of the substrate ATP (4mM). The

tubes containing the above reagents were incubated for the 10 minutes at 40° C. The reaction was stopped using 1ml of 6 % TCA. This was centrifuged for 10 minutes at 3000 rpm & from the clear supernatant 1 ml was taken for the estimation of inorganic phosphate released, using the method of Fiske & SubbaRow (1925) using 10 N $H_2SO_4$ (0.4ml) and 2.5% ammonium molybdate (0.8ml). The assay medium was kept aside for 20 minutes and Amine napthol-sulphonic acid (ANSA) was added as reducing agent. Then these tubes were kept aside for 20 minutes. The colour developed was measured on spectrophotometer at 660nm & the enzyme activity and was expressed as μg of phosphate released /mg protein /10minutes /40°C (unit/mg protein).

## ACID PHOSPHATASE (ACPase) ~

### E.C. 3.1.3.2 *ortho phosphoric monoester phosphohydrolase*

Acid phosphatase activities were measured according to the method described in Sigma Technical Bulletin No.104, using PNP (P-nitrophenyl phosphate) as substrate. For the above assay 0.5 ml of citrate buffer (pH: 4.8), 0.5 ml of PNP (0.005 M) and 0.2 ml of homogenate were taken, mixed well and incubated for 30 minutes at 40° C. The reaction was stopped by adding 4 ml of 0.1 N NaOH. The colour developed was measured spectrophotometrically at 410nm and was expressed as μmoles of P-nitrophenol released /mg protein /30 minutes /40° C.

## ALKALINE PHOSPHATASE (AlkPase) ~

### E.C. 3.1.3.1 *Ortho phosphoric monoester phosphohydrolase*

Alkaline phosphatase activities were measured according to the method described in Sigma Technical Bulletin No.104, using PNP (P-nitrophenyl phosphate) as substrate. For the above assay to 0.2 ml of homogenate, 0.5 ml of 0.05 M glycine buffer (pH: 10.5) and 0.5 ml of PNP (0.005 M) were added, mixed well and incubated for 30 minutes at 40° C. The reaction was stopped by adding 10 ml of 0.02N NaOH. The colour developed was measured spectrophotometrically at 410nm and was expressed as μmoles of P-nitrophenol released /mg protein /30 minutes /40° C.

## PROTEIN ~

The total protein content was assayed by the method of Lowry *et al.,* (1951) using Bovine Serum Albumin (BSA) as standard and Folin phenol as colour reagent. To the homogenate (0.1ml) 0.9ml of chilled redistilled water was added. To the above assay medium 5 ml of freshly prepared Reagent C [Reagent A (2% $Na_2CO_3$ in 0.1 N NaOH, 50ml of NaOH): Reagent B (0.5% $CuSO_4$ .$5H_2O$ in 1% Na-K tartarate, 1ml of $CuSO_4$)] was added and after 10 minutes, 0.5 ml Folin Phenol Reagent (Diluted with redistilled water in 1:1 ratio) was added. After 30 minutes the optical density or percentage transmission of the

colour obtained was read at 660nm and amount of protein in tissues was expressed as mg protein /100 mg wet tissue.

**TOTAL LIPID ~**

To find out lipid contents in tissues, studied method of Folche Lee et al, (1957) was used. Pre-weighed pieces of liver, intestine and kidney were taken in a test tube and crushed with sand granules. To this 5 ml of Chloroform : Methanol mixture (1:1 V:V) was added and this tissues were churned again to extract lipids. To this 2 ml of Calcium chloride solution was added and kept overnight. The upper layer was removed next day with an aspirator and the remaining assay medium was filtered in graduated tubes through Wattmann Filter Paper No.1 and the volume was brought to 6 ml by adding Chloroform: Methanol mixture. 2 ml of this was taken in the preweighed lipid tubes and kept in oven for drying at 60° C. The extracted lipids were measured gravimetrically and expressed as mg /100 gm of wet tissue.

**CHOLESTEROL ~**

Total cholesterol in liver, intestine and kidney was measured employing the method described by Crawford (1958). For cholesterol estimation 2ml of the dried lipid extract was used. To this 3ml of Ferric chloride, colour reagent was added. This was boiled for 5 minutes and kept in ice bath to add 2 ml of concentrated Sulphuric acid ($H_2SO_4$).

The colour developed was read colorimetrically at 540nm and expressed as mg /100 mg of wet tissue.

**STATISTICAL ANALYSIS ~**

The results are expressed as mean ± standard error of mean (SEM). The data were subjected to students 't' test with 95% confidence limit.