CHAPTER I

Material and Methods

I Experimental Procedure

a. Animal model

Day old female chicks of domestic fowl *Gallus gallus domesticus* (Rhode Island Red variety) were procured from Government Hatchery, Baroda. They were divided at random into two groups of 30 each and kept in metal coops of 150 x 100 x 75 cm with 14/10, light/dark cycle. The chicks in both the groups were provided with water and standard commercial starter diet *ad libitum*.

b. Treatment schedule

Group 1 (Experimental): Chicks in this group were provided through intragastric route 1 ml of fluoridated water of appropriate concentration so as to make a dose of 15.4 mg F^-/kg b.w. (1/5 of LD_{50}) daily. [Fluoridated water was made by dissolving commercial grade sodium fluoride- Sigma Chemical Co., St. Louis, MO- in doubly distilled water according to Standard Methods (APHA, 1989)]. The pollutant was administered at early morning hours (between 06 and 07 h). After fluoride administration food and water were withdrawn for 1 h.

Group 2 (Control): The birds in this group were treated similarly with distilled water.

On day 1, 5, 10, 20 and 30 following the commencement of experiment, six birds from each group were bled and killed by decapitation. The small intestine, gizzard, pancreas and liver were quickly excised and weighed using an electronic Mettler balance. For the initial readings six birds were sacrificed on the day of purchase. The blood was drained from jugular vein till day 10 and thereafter from basilic vein using sterile disposable plastic syringes and needles (21 gauge).

Left liver lobe and gastrocnemius muscle were used for various biochemical assays except for

the nucleic acids profiles where liver and a piece of small intestine immediately following duodenum were used.

II Analytical Methods

Total Red Blood Cells: The blood sample was diluted in a red blood cell pipette using erythrocyte diluting fluid. The enumeration of RBC were made using a haemocytometer with improved Neubauer ruling (Germany) and expressed per cubic millimeter.

Packed Cell Volume (PCV): The PCV was determined by the microhaematocrit method (Schlam, 1979) with a microhaematocrit centrifuge.

Iron and Haemoglobin: Both iron and haemoglobin were estimated according to the method of Wong (1928) and expressed as gram percent.

Plasma Glucose: Oxalated blood sample was centrifuged at 3500 rpm for 30 minutes to collect the plasma. Glucose was estimated by employing the method of Winkers and Jacob (1971) using o-toluidine reagent and expressed as mg/100 ml plasma.

Glycogen: The tissues were dissolved in 30% KOH for the estimation of glycogen employing the anthrone method of Seifter *et al.*(1950) and the glycogen content in the liver and muscles are expressed as mg/100 mg wet tissue.

Protein: The total protein content of the tissues were determined with the help of Folin Phenol method as described by Lowry *et al.* (1951) using bovine serum albumin as standard and expressed as mg/100 mg wet tissue.

Lipid: A pre-weighed piece of liver was dried in an air-oven maintained at 60° C. After achieving a constant weight the lipid was extracted from the tissue using the method of Folch *et al.* (1957) using a mixture of chloroform : methanol (2:1, v/v). The lipid extracted was

measured gravimetrically and expressed as mg lipid/100 mg non-fat dry tissue.

Phosphorylase (EC 2.4.1.1, 1,4-α-D-Glucan : Orthophosphate, α-D-glucosyl transferase): Assay of phosphorylase was made by the modified method of Cori *et al.* (1943) as adapted by Cahill *et al.* (1957), using dipotassium salt of G-1-P as substrate. The inorganic phosphate liberated was estimated by the method of Fiske and SubbaRow (1925). The absorbance was read at 660 nm on a spectrophotometer and the enzyme activity was calculated as μg phosphate released/mg protein/15 minutes.

Glycogen Synthetase (EC 2.4.1.11, UDPG α - glucan glucosyltransferase): This enzyme was assayed by employing the method of Leloir and Goldemberg (1962). The activity of glycogen synthetase was measured by the amount of UDP formed from UDPG in the presence of glycogen and dipotassium salt of G-6-P. The UDP estimation was carried out by using a preparation of pyruvate kinase which catalyses the transfer of phosphate from phosphopyruvate. The liberated pyruvate was measured at 520 nm and the enzyme activity is expressed as μM of UDP formed/mg protein/10 minutes.

Succinate Dehydrogenase (EC 1.3.99.1, Succinate: (acceptor) oxidoreductase): The activity of this enzyme was calculated by the procedure of Nachlas *et al.* (1960) using INT as electron acceptor and PMS as an intermediate electron carrier between reduced succinate dehydrogenase and INT. The enzyme acitvity is expressed as μg formazan formed/mg protein/15 minutes.

Lactate Dehydrogenase (EC 1.1.1.27, L-Lactate: NAD^+ oxidoreductase): Quantitative estimation of LDH was done by employing the colorimetric method of King (1959, 1965c) as described by Varley *et al.* (1980) using sodium lactate as substrate and NAD^+ as cofactor. The enzyme activity is expressed as μM lactate oxidised/mg protein/15 minutes.

Na^+-K^+ ATPase (EC 3.6.1.3): The Na^+-K^+ ATPase activity was measured according to the method of Post and Sen (1967), using ouabain as an inhibitor and substracting the value obtained from Mg^{2+} ATPase activity. The inorganic phosphate released was assayed according to the method of Fiske and SubbaRow (1925). Readings were taken at 660 nm and the activity is expressed as μg phosphorous released/mg protein/10 minutes.

Acid and Alkaline Phosphatases: The quantitative estimation of acid phosphatase [Orthophosphoric-monoester phosphohydrolase (acid optimum), EC 3.1.3.2] and alkaline phosphatase [Orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1] were done by the method of Linhardt and Walter (1963). The activities of both the enzymes are recorded by the formation of p-nitrophenol and expressed as μMoles of p-nitrophenol released/mg protein/30minutes.

Acetylcholinesterase (EC 3.1.1.7 Acetylcholine hydrolase): The AChE activity was assayed according to Ellman *et al.* (1961) using acetylthiocholine iodide as substrate. The rate of choline production was measured at 412 nm in a spectrophotometer and is expressed as μMoles of acetylthiocholine hydrolysed/mg protein/minute.

Nucleic Acids: The extraction of nucleic acids were made according to Schneider (1957).

a. Deoxyribonucleic acid: The quantitative estimation of DNA was made according to the improved diphenylamine method of Giles and Myers (1965). The absorbance difference at 595-700 nm was read against 595-700 nm blank and is expressed as mg/100 mg wet tissue.

b. Ribonucleic acid: The RNA was assayed according to Mejbaum (1939) using orcinol reagent. The extinction was read at 660 nm in a spectrophotometer and is expressed as mg/100 mg wet tissue.

III Statistical Analysis

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