MATERIALS AND METHODS

CHAPTER I

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I Experimental Protocol

Animal Models: Newly hatched chicks of Domestic fowl, *Gallus gallus domesticus* (Rhode Island Red variety) and Japanese quail, *Coturnix coturnix japonica*, were procured from Government Hatchery, Baroda and Mualia Farm, Dahod, Gujarat respectively. They were housed in a well maintained aviary. The young and adults were allowed free access to water and standard commercial starter diet *ad libitum*. Of the adult birds, Blue Rock Pigeon (*Columba livia*) was obtained from local animal dealer, House Swift (*Apus affinis*) and House Sparrow (*Passer domesticus*) were caught from the nests located in the premises of Zoology department, Baroda.

II Morphometric Studies

The birds under study were weighed and then killed by decapitation. Six birds each were sacrificed on day 0, 5, 10, 20 and 30 of age. The proventriculus, ventriculus and intestine were quickly excised and weighed using an electronic mettler balance. Readings were noted even of the proventriculus, ventriculus and intestine of all the adult birds. The body weight and organ weights were expressed in grams. The external dimensions of proventriculus and ventriculus of developing chicks and adult birds (as mentioned above) were measured using vernier caliper.

The measurements of outer layer, small and large multiple glands and diameter of proventriculus and the four layers of ventriculus *viz.*, koilin, mucosal, submucosal and muscle layers were made using micrometer.

III Biochemical Analysis

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 proventriculus and ventriculus are expressed as mg/100 mg wet tissue.

Protein: The total protein content in the tissue was determined by 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: Pre-weighed pieces of organs under investigation were dried in an air-oven maintained at 60°C. After achieving a constant weight the lipid was extracted in a 2:1 v/v mixture of chloroform:methanol (Folch *et al.*, 1957). The lipid, thus, extracted was measured gravimetrically and expressed as mg lipid/100mg 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 adopted 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 expressed as μg phosphorus released/mg protein/10 minutes.

Myoglobin: The myoglobin activity was measured according to the method of Reynafarge (1963). Homogenate was centrifuged in a cooling centrifuge and from the clear supernatant 3 ml was taken and CO gas was allowed to passed through it. After 8 minutes, the flow of gas was interrupted by adding a pinch of a dry dithionate to the solution and to ensure complete reduction of the pigment CO gas was again passed for 2 more minutes. The difference in optical density taken at 538 nm and 568 nm is multiplied by the factor 117.3 and the activity is expressed as mg/gm wet tissue.

Adenosine triphosphatase: The ATPase activity was measured according to the method of Umbreit *et al.* (1957). 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 (Acid EC 3.1.3.2 and Alkaline EC 3.1.3.1): The quantitative estimation of acid phosphatase (orthophosphoric-monoester phosphohydrolase -acid optimum) and alkaline phosphatase (orthophosphoric-monoester phosphohydrolase -alkali optimum)) were done by the method of Linhardt and Walter (1963). The activities of both the enzymes are

recorded by the formation of p-nitro phenol and expressed as μ moles of p-nitrophenol released/mg protein/30 minutes.

Cholesterol: The quantitative estimation of cholesterol (Total and free) was done by the method of Crawford (1958). The method depends on the interaction of ferric chloride and conc. H_2SO_4 with cholesterol in glacial acetic acid. The resulting (stable) reddish purple colour is compared photometrically with the colour produced by treating a cholesterol standard similarly. Free cholesterol is determined by the same method after precipitation with Digitonin. Readings were taken at 540 nm and the activity is expressed as mg/100 mg wet tissue.

IV Histochemical Analysis

The developing and adult birds were killed by decapitation. The proventriculus and ventriculus were quickly excised and blotted well to remove blood and other tissue fluids and a piece of each was fixed on a dry chuck of a cryostat microtome maintained at - 20°C. Fresh frozen sections of 12-15 μ thickness were cut and processed.

Myoglobin: For the histochemical observation the tissue sections were fixed in a mixture of 25% glutaraldehyde and 0.67 M phosphate buffer, pH (7.4) following the method of James (1968).

Acid and Alkaline Phosphatases: The acid and alkaline phosphatase activities were analysed by fixing the sections in 10% cold formalin following the method suggested by Burstone (1962).

In all the histochemical observation, the tissue sections incubated in a substrate blank medium served as controls.

V Statistical Analysis

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