I N T R O D U C T I O N

Evidences from experimental embryology explain development as essentially a sequence of chemical and physical changes which have modifying effect on the differentiating cells. The chemical differences between differentiating and non-differentiating elements during organogenesis and histogenesis seem to arise gradually and progressively leading, eventually, to morphological distinctions. It has been recognized that the process of organogenesis and histogenesis must involve certain basic chemical and physical changes and there is every reason to believe that these changes are brought about under the control of enzymes. Extensive work has been done on the physiology and biochemistry of skeletal and cardiac muscles of different vertebrates but comparatively very little has been done as far as the smooth muscles are concerned. The muscle tissue forms the most dynamic systems in the vertebrate body having the important function of motility and much is known about its cellular heterogeneity, growth, differentiation and innervation. Skeletal muscle, especially, has been the subject of intense morphophysiological and biochemical studies in the recent times (Berger, 1953, 1954; Fisher & Goodman, 1955; Hanson & Canzilloth, 1955; Nair, 1952; Sullivan, 1962; Chinoy, 1963; George & Type, 1960; George & Jyoti, 1953, 1955b; George & Naik, 1958a, 1958b, 1959; George & Talesara, 1961a, 1962a; Stein & Padykula, 1962; Aleksakhina, 1953; Gasper, 1957; Ogata & Mori, 1964a, George & Berger, 1966). These studies revealed that the pigeon pectoralis muscle is composed of two distinct types of fibres; a broad white type containing a high concentration of glycogen as fuel and the other, a narrow red variety loaded with fat as the main fuel and adapted for aerobic metabolism. Chandra-Bose (1967) reported a third intermediate type of fibres which showed characters between the above two types of fibres in the skeletal muscle of certain representative birds. It is stated that smooth muscles are a group of muscles which are adapted to a variety of functions and therefore, differ widely in their physiological properties and that though they are considered to be primitive, they are actually highly specialized for a variety of functions and are found also in lower forms of animals like coelenterates (Bozler,1960). In the vertebrates, the study of smooth muscle has been confined to higher groups of animals such as birds and mammals though scanty literature is available regarding the smooth muscle physiology of pisces, amphibians and reptilians. Although the avian and mammalian alimentary canal have been the subject of intense physiological and biochemical investigations, recent studies have been centered upon the electrophysiology of that system. There exists an elaborate literature dealing with the enzyme histochemistry of the alimentary canal of different vertebrates but comparatively very little is known on this aspect of the avian gizzard, which is chiefly composed of smooth muscles. Since in birds, the alimentary canal in general and gizzard in particular, varies structurally and functionally according to food and feeding habits, there may be differences in the gross morphological structure and general biochemical and physiological activities of the smooth muscle of that system. It was in the wake of these above mentioned informations and for a comparison with the work on the skeletal muscle of pigeon conducted in this laboratory in the past two decades, it was thought worthwhile to explore some of the histophysiological aspects of the gizzard of pigeon, Columba livia, during its post-natal development and adult condition. The study was also extended to certain representative birds which show morphological and functional variations of the gizzard due to differences in their food and feeding habits.

The sequence of post-hatched development process of pigeon gizzard, from the day of hatching till the adulthood is reached, could be arbitrarily divided into different periods in days viz., 1, 5, 10, 15, 20, 25 and 30; and adult. This grouping becomes necessary as the gizzard contents gradually varied from "pigeon milk" on the day of hatching to very small partially digested grains on the 10th day. Semidigested grains could be identified in the lumen of 15 day old pigeon gizzard whereas fragmented grains and solid unbroken grains could be identified in its lumen during the remaining periods. Pebbles of different sizes are also found along with the grains on or around 10th day of development. The epithelial lining of the gizzard gets gradually keratinized and this keratinization becomes prominent on the 10th day of development coinciding with the change in the state of food. Eventhough there is no rigid distinction between the successive phases during the second half of development, such an arbitrary division of the continuous developmental process is made merely for the convenience of description.

To understand the histomorphological features of development and also to lay a basic ground work for the evaluation and elucidation of the various metabolic aspects aimed and planned at by the application of suitable histophysiological techniques, a morphophysiological and anatomical account has been made on the developing pigeon gizzard and also on that of adult representative birds.

Extensive investigation on the distribution of metabolites like glycogen and lipid, and associated hydrolytic enzymes and dehydrogenases have been undertaken in the skeletal and cardiac muscles of vertebrates in general and aves in particular (George & Berger, 1966; Stein & Padykula, 1962; Ballard & Oliver, 1963; Rossi *et al*., 1963; George & Iype, 1963; George & Bhakthan, 1963; George & Bokdawala, 1963, 1964; Adams & Finnegan, 1965; Goldberg & Wuntch, 1967; Rinaudo & Giunta, 1967; Nene & George, 1965, 1965d; Cherian, 1967; Moyer *et al*., 1968; Penney & Cascarano, 1970). In this regard, there is a ~~complete~~ lack of literature as far as the smooth muscles are concerned. This lack prompted a series of studies on the developing and adult pigeon gizzard and also that of adult representative birds so as not only to understand the metabolic aspects underlying the development of this part of the alimentary canal but also to account for a comprehensive knowledge of the changes in the enzyme activities and the preference and utilization of metabolites that occur during this process.

Studies on metabolites like glycogen and lipid in the developing pigeon gizzard revealed certain fluctuations, though not very high, during the different phases of its post-natal development. Glycogen was found to be lesser in concentration than lipid, all throughout the process of development and also in the adult stage. A similar type of work has been carried out by Grillo (1969) in the gizzard of chick during its post embryonic development. A quantitative and histochemical study on the presence of the above mentioned metabolites in the developing and adult pigeon gizzard and also in that of certain adult representative birds were, therefore, undertaken to characterize the significance of these metabolites in the overall metabolic adaptations of the gizzard.

Since carbohydrates and lipids serve as the main energy yielding sources for living tissues, it was deemed worthwhile to study some enzymes that play prominent roles in the anabolism and catabolism of these metabolites as well. Aldolase and lactate dehydrogenase (LDH), the two most important enzymes of EMP pathway, can be taken as indicators of anaerobic glycolysis by which, glucose and/or glycogen is converted into lactate. During this sequence of reactions the synthesis of adenosine triphosphate(ATP) takes plac and thus provides the main source of energy for muscular contraction. A histochemical study of these two enzymes viz., aldolase and LDH was therefore carried out and the variations in the concentrations of these enzymes observed are discussed in the present thesis.

Several workers have studied the importance of Krebs cycle enzymes in the metabolism of skeletal and cardiac muscles (George & Talesara, 1961; Ogata & Mori, 1964a; Germino et al., 1965; Bokdawala, 1965; George & Berger, 1966; Rinaudo & Giunta, 1967; Buno & Germino, 1958; Nene, 1966; Dubale & Muraleedharan, 1970; Klicka & Kasper, 1970; Penney & Cascarano, 1970). Quite recently Grillo (1969) has given a detailed account of some enzymes concerned with the oxidation of metabolites through Krebs cycle and has correlated their importance in ~~to~~ smooth muscle metabolism. Succinate and malate dehydrogenases (SDH & MDH) are the best indicators of Krebs cycle activity. Hence a histochemical study on the presence, distribution and variations of these enzymes in developing and adult pigeon gizzards and also that of adult representative birds was undertaken and the results are discussed in relation to smooth muscle metabolism.

The fact that lipids are utilized for various metabolic activities of the smooth muscle becomes conclusive with the present study of lipase and esterase in the developing and adult pigeon gizzard and also in certain adult representative birds. For further confirmation of the enzymatic adaptation and to understand the metabolism of lipid in the developing and adult gizzards, a comparative study with respect to the enzymes such as glucose-6-phosphate, α-glycero-phosphate and β-hydroxybutyrate dehydrogenases (G6PDH, αGPDH and BDH) has been undertaken. The activity of G6PDH which denotes the production of abundant reduced coenzyme II for lipogenesis and also the production of pentose sugar necessary for the synthesis of nucleic acids (Glock & McLean, 1954; Abraham & Chaikoff, 1959; Siperstein, 1958; Abraham, <u>et al</u>., 1954; Levy, 1961; Muscatello & Anderson-Cedergren, 1961; Wise & Ball, 1964; Ogata & Mori, 1964a; Beaconsfield & Carpi, 1964a; Beaconsfield & Reading, 1964b; Shah & Ramachandran, 1973) showed a progressive increase reaching a maximum on the 15th day and since then showed a reduction during the rest of the developing periods. BDH was found to be highly active during the different periods of development and also in the adult gizzard indicating its association in the fatty acid break down so as to facilitate its entry in the Krebs cycle. The activity of α GPDH could be attributed to its possible role and participation in the reversible reaction between glycerol and glycolytic pathway as per need in glycerol catabolism or anabolism in the developing as well as adult gizzards.

Phosphatases are known to play significant roles not only during the lytic processes and in phosphate metabolism but also in the synthesis of specific proteins such as collagen, fibrous protein and keratin (Verźar & McDougal, 1936; Moog, 1946; Marchant, 1949; Fell & Danielli, 1943; Vorbrodt, 1958; Mori, 1963; Shah & Chakko, 1966; Morton, 1965; Cobb & Bennett, 1969; Radhakrishnan, 1972). Since the development of gizzard encompasses all the above mentioned aspects, a study of phosphatases such as acid and alkaline phosphatases was deemed fit and hence a histochemical investigation was carried out on these phosphatases.

Finally it has been proved by the study conducted by Bennett & Cobb (1969) that nerve supply plays an important role in initiating and maintaining the development of the chick~~en~~ gizzard. From this point of view a histochemical study of acetyl and butyryl cholinesterases (AChE & BuChE), which could indirectly indicate the activities of nerves in the gizzards of developing pigeon as well as in those of the adult representative birds having different dietary habits and morphological features of the organ, was undertaken and the results obtained are discussed.