INTRODUCTION

In the recent years the pigeon breast muscle has been extensively used by biochemists and physiologists for studies in cell metabolism and what is known from these studies is for the muscle as a whole and not with respect to its heterogenous cellular components. It has been shown by earlier workers that the <u>pectoralis major</u> muscle of the pigeon consists of two distinct types of fibres, a broad glycogen-loaded white variety with few or no mitochondria and a narrow, fat-loaded red variety having a large number of mitochondria in them. The co-existence of two such distinct heterogenous components in the same muscle with striking dissimilarities in structure and biochemical properties, have prompted the present investigations in order to understand the mutual relationship of these fibres, their mode of action and the problem of energetics for contraction.

It is being realized more and more, that the flight muscles of birds and other flying animals utilize fat during long and sustained activity because of the high requirement of energy needed for flight. Therefore the breast muscle of the pigeon in particular, and the breast muscles of a few representative types of birds exhibiting different modes of flight, were chosen for the present study. Some histo-physiological and biochemical observations have been made on certain aspects of the glycolytic system, oxidative enzymes and fat metabolism in the two types of fibres of the pigeon breast muscle and the flight muscles of birds in general. This constitutes the major part of the present thesis.

Quantitative biochemical investigations on the two types of fibres are by no means easy since the complete isolation of these fibres without damaging them, appears extremely difficult if not impossible. Hence an indirect method viz. of estimating the various metabolites and the activity of the enzymes in different layers of the pigeon breast muscle, where the broad and narrow fibres vary in number, was adopted. It has been shown that the least number of broad fibres is found in the fasciculi situated in the middle of the dorso-ventral axis of the pigeon breast muscle and the number tends to increase above or below this mark reaching the maximum in the most superficial and in the deepest layers. Based on this finding the quantitative determinations of the relative distribution pattern of the activity of succinic dehydrogenase, using a tetrazolium salt, and the activity of a number of other enzymes such as lipase, cytochrome oxidase, succinoxidase, malic-oxidase and lactic dehydrogenase using the conventional methods with the Warburg apparatus, were made in the different layers of the pigeon breast muscle. The relative distribution of the number of the red and white fibres in different layers was also recorded side by side in each case to correlate the enzyme activity. The data so obtained in each case showed a dissimilar pattern of enzyme activity in the different layers. Of the various enzymes studied, the activity was found to be the maximum in each case, in the layer taken from the middle of the dorso-ventral axis of the muscle while the activity was found to decrease above or below this mark reaching the minimum in the most superficial and in the deepest layers. These data indicate that the enzyme activity in the different layers varies in each case depending more or less upon the distribution pattern of the two types of fibres and also that the major bulk of all the enzymes studied is confined to the narrow red fibres whereas the broad white fibres have comparatively very low concentrations of these enzymes. Apart from enzymes, protein and iron were also determined quantitatively in different layers of the pigeon breast muscle. Protein was found to increase from the middle of the dorso-ventral axis towards the superficialmost layer whereas the iron content showed the reverse pattern. Thus based on the values obtained for the different layers, an indirect approach was made to derive the values of different enzymes and other constituents in the red as well as white fibres separately with the help of the regression line.

The contention that the broad white fibres of the pigeon breast muscle like the white fibres of the other vertebrate skeletal muscles too possess a low concentration of oxidizing enzymes and hence a few mitochondria, was further supported by demonstrating histochemically, succinic dehydrogenase, cytochrome oxidase and various DPN-linked dehydrogenases, in these fibres, using more specific and modified methods. These oxidizing enzymes which were not detected earlier histochemically in the broad white fibres, were demonstrated by using modified incubation mixtures. The size, localization and distribution pattern of the histochemically demonstrable diformazan and indophenol blue granules showed more or less a close resemblance with the mitochondrial staining in the red as well as white fibres. The occurrence of high oxidative metabolism in the narrow red fibres was revealed by the presence of a large number of spherical diformazan and indophenol blue granules arranged linearly in between the myofibrils. On the other hand the presence of a few such smaller granules in the broad white fibres is nevertheless indicative of the fact that these fibres too possess mitochondria where some measure of oxidative activity howsoever little, does take place and that they are to be considered as analogous to the white fibres of the other vertebrate skeletal muscles. It is also suggested that these granules in the pigeon breast muscle are to be considered as mitochondria in the general sense and that the distinction into sarcosomes and mitochondria as proposed by previous authors needs reconsideration.

Certain oxidizing enzymes such as succinic dehydrogenase, lactic dehydrogenase, malic dehydrogenase and cytochrome oxidase were demonstrated histochemically by improved methods in the breast muscle of the rosy pastor, goose and fowl. Rosy pastor muscle showed a very high concentration of these enzymes as compared to the goose and fowl whereas, the goose though a poor flier but with mixed type of muscle, showed a higher concentration of these enzymes than that in the fowl.

Succinic dehydrogenase activity was determined quantitatively in the <u>pectoralis</u> <u>major</u> and <u>minor</u> muscles of a few representative types of birds exhibiting different modes of flight. Rosy pastor a migratory form showed higher activity as compared to the sparrow, parakeet, pigeon and bat, a flying mammal, whereas the non-flier fowl showed the minimum activity. Besides this, the enzyme activity was found to some extent, in the birds studied, more or less inversely proportional to the fibre diameter of the fibres of the pectoralis major muscle as well as to the ratio of the body weight to the muscle (pectorlis major) weight. In each individual bird and the bat the pectoralis major muscle showed higher enzyme activity than that of the pectoralis minor which support the fact that the former muscle is much more metabolically active and highly evolved. Succinic dehydrogenase activity was found to be sufficiently increased in the case of the rosy pastor during the premigratory period. From these observations it can be concluded that the concentration of succinic dehydrogenase in particular or other oxidizing enzymes in the breast muscle of the various birds studied, is an index of the overall respiratory metabolism and hence the activity of the muscle in each case. Similar results were obtained as regards the concentration of lipase in the flight muscles of a few birds studied by other workers. This perhaps shows that wherever fat is utilized the concentration of succinic dehydrogenase or oxidizing enzymes and lipase run parallel at least in the case of the flight muscles of birds and bats.

Apart from the oxidizing enzymes, the relative distribution pattern of the enzyme aldolase which is involved in glycolysis was demonstrated histochemically in the two types of fibres of the pigeon breast muscle. The enzyme concentration was observed slightly higher in the broad white fibres which are rich in glycogen.

The occurrence of lipase was shown in the breast muscle of the birds by earlier workers and also that fat is the chief fuel for energy during long and sustained contraction. Based on these facts an attempt was made to assess the lipase and succinic dehydrogenase activity in the different particulate fractions of the pigeon breast muscle, such as myofibrils, mitochondria, microsomes and the supernatant. A study of the in vitro oxidation of triglycerides of long as well as short chain fatty acids by pigeon breast muscle mitochondria and whole homogenate, was attempted in order to find out whether or not and if so at what rate and extent, the different triglycerides are oxidized.

Having thus obtained an overall picture of the relative distribution of the various oxidizing enzymes, lipase, iron, protein, mitochondria, cytochrome c and other metabolites in the different layers of the pigeon breast muscle and in the individual fibre types, it could be visualized that the metabolically most active centres lie in the fasciculi situated somewhere in the middle of the dorso-ventral axis of the muscle where the number of broad fibres is the least. The arrangement of the two types of fibres in this remarkable fashion with a definite distribution pattern of various metabolites, enzymes and other constituents, is undoubtedly an intriguing problem. Nevertheless it is probable that such an unique fibre architecture may be associated with some sort of mechanical and physiological efficiency concerned with the problem of general metabolism and energetics for contraction in this muscle.

The following preliminary reports have been published (or in press) in joint authorship with Dr. J. C. George, my guiding teacher:

1. Studies on the structure and physiology of the flight muscles of birds. 9. A quantitative study of the distribution pattern of succinic dehydrogenase in the <u>pectoralis major</u> muscle of the pigeon. <u>Biol</u>. <u>Bull</u>., 118, 262, (1960).
2. Histochemical observations on the succinic dehydrogenase and cytochrome oxidase activity in pigeon breast-muscle. <u>Quart</u>. <u>J</u>. <u>Micr.</u> <u>Sc.</u> (In press).
3. A quantitative study of the distribution pattern of iron in the pigeon <u>pectoralis</u> <u>major</u> muscle. <u>J</u>. <u>Anim.</u> <u>Morph.</u> <u>Physiol</u>. (In press).
4. Histochemical demonstration of certain oxidizing enzymes in the <u>pectoralis</u> <u>major</u> muscle of rosy pastor, goose, and fowl. Accepted for publication in the <u>J</u>. <u>Anim.</u> <u>Morph.</u> <u>Physiol.</u>

The following abbreviations are used in the text:

ATP (Adenosine triphosphate); Co A (Coenzyme A); DPN (Diphosphopyridine nucleotide); LDH (Lactic dehydrogenase); NT (Neotetrazolium chloride); SDH (Succinic dehydrogenase); TPN (Triphosphopyridine nucleotide); and TTC (Triphenyl tetrazolium chloride).