INTRODUCTION

Vertebrate skeletal muscle is a complex physico-chemical system in which series of chemical reactions take place synthesizing and burning the fuel to produce energy for the working of the physical system. This dynamic system has been the subject of intense research. In the recent years great strides have been made in our knowledge of its ultra-structure, bichemistry and physiology. The present series of studies have been carried out with the hope of contributing some new information regarding the metabolism of fat, a powerful source of energy of which very little is known.

It is well known that when a muscle is under disuse or subjected to motor denervation atrophic and degenerative changes are produced. The characteristic changes in the atrophied muscle may be regarded as a special problem in muscle physiology. In such cases quantitative studies on enzymes and metabolites may give useful information on the normal and abnormal functioning of the muscle.

A study on the effect of disuse atrophy on the pectoralis muscle and blood in the pigeon was carried out. Atrophy of the breast muscle was induced by applying a plaster cast on the wings, which were tied in a dorsally extended position. Lipolytic activity of the breast muscle and blood serum was determined at different intervals from one day to two months. A higher enzyme activity in the muscle and a corresponding fall in the enzyme activity in the blood serum were observed during the

first week of atrophy. Fat and water content in the muscle were also estimated. During the first two weeks there was an increase in the water content of the muscle. A sudden increase in fat content was observed on the first day of atrophy. Later the fat content gradually decreased and by the end of two months the fat content was found to be less than that in the normal pigeon.

Further observations on the changes in the succinic dehydrogenase, phosphorylase and glycogen levels in the atrophied pigeon breast muscle were quantitatively estimated at different intervals from one day to two months. Succinic dehydrogenase activity was found to be decreasing in the muscle soon after the immobilization of the muscles. Phosphorylase activity and glycogen levels in the muscle showed fluctuations in the beginning but during the later period of atrophy they were found to be steady and increasing slightly. These enzymatic fluctuations and changes in the concentration of the metabolites were more profound in the early stages of atrophy, but lateron the muscle became adapted to the changes taking place due to disuse.

It has been shown by earlier workers that the flight muscles of birds and other flying animals utilize fat as the chief fuel for the energy during long and sustained muscular activity. It has also been shown that the <u>pectoralis major</u> muscle of the pigeon consist of two types of fibres, a broad, glycogen-loaded white variety with **kerysfewshitochondrias** and was

narrow fat-loaded red variety with large number of mitochondria adapted for an anaerobic and aerobic metabolism respectively. The existence of such a contrasting system side by side in the pigeon breast muscle makes it an ideal material for studies on certain biochemical adaptations and enzyme levels in the muscle. Studies on the breast muscle of certain other birds and a bat were also conducted for the sake of comparison.

A quantitative study on the phosphorylase activity was carried out in the different layers of the pigeon breast muscle based on the distribution pattern of the two types of fibres. It was found the enzyme concentration is high in the superficial layers of the muscle where the number of broad white fibres are maximum in number. The enzyme activity was also assayed in a few other birds exibiting different modes of flight, and a bat.

A comparative study on the lipase activity in the blood sera of three representative birds having different modes of flight, was determined. The enzyme concentration in the blood sera was found to be proportional to the activity of the bird.

It is well known that a number of chemical changes take place in the muscle and blood when a muscle is exercised. It was also observed by earlier workers that there is a striking decrease in the fat content of the muscle and liver when the muscle was electrically stimulated. If fat is the chief fuel during long and sustained muscular activity, fat

in the muscle should get depleted during such prolonged activity of the muscle as in the case of migration. If so, the fat store in the muscle has to be reinforced by a regular supply from the adipose tissue or elsewhere. This aspect of fat metabolism was investigated in a series of experiments conducted on the pigeon before and after the muscle was electrically stimulated. Quantitative estimations of free fatty acids was carried out in the muscle, liver, adipose tissue and blood plasma before and after the electrical stimulation of the muscles. These studies showed that during activity of the muscle fat is being extracted by the muscle from the blood in the form of free fatty acids and that there is a regular supply of fat from the adipose tissue depending on the metabolic demand.

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Fatty acids are oxidized through the oxidative pathways involved in the citric acid cycle. Succinic dehydrogenase is an important enzyme linked with oxidative metabolism. Phosphorylase on the other hand plays the initial catalytic role in the breakdown of glycogen before utilization. A study on the activity of these enzymes in the muscle before and after the electrical stimulation of the muscle would indicate if there is any considerable enhanced utilization of the metabolite. It was observed that both these enzyme activities increase after stimulation of the muscle. The higher activity of succinic dehydrogenase suggests that there is an acceleration of the citric acid cycle and hence a higher fat metabolism. The phosphorylase activity was found to increase even after a short stimulation. But this increase could be mainly due to the conversion of the inactive form 'b' to the active form 'a' during the stimulation of the muscle.

Bird migration is an intriguing and fascinating problem. The dynamic physiological and biochemical adaptations acquired by birds before migration is not well understood. Migratory birds are known to store fat in their body before migration and are also known to utilize fat as the chief fuel during migratory flight. Lipase which is present in the muscle is responsible for the breakdown of fat which is the initial step in the utilization of fat. Even though the significance and the presence of lipase in the flight muscles of birds is known, the localization and subcellular distribution of this enzyme has not been known in any migratory bird. The oxidative enzyme succinic dehydrogenase also is known to have a major role in the metabolism of fat. Hence these two enzymes were studied in the different subcellular fractions of the Rosy pastor breast muscle in the post -migratory and pre-migratory periods during the early morning and evening in order to understand the exact role of these enzymes in the breakdown and biosynthesis of fat. From the observations on the enzymatic changes in the microsomes and supernatant in the two different seasons it was found that there is an augmented synthesis of fat during the pre-migratory period. It was also observed that the synthesis of fat is more in the night. These studies also suggest that

fat utilization in the bird by the muscle is considerably diminished during the pre-migratory period.

Studies on the capacity of the muscle for fatty acid (butyrate) oxidation during the post-migratory and premigratory periods showed that during the pre-migratory period fat is not oxidized by the muscle. A study on the seasonal changes in the phosphorylase, glycogen, fat and lipase were also carried out in these birds. A striking increase in the glycogen and phosphorylase were observed before migration which is suggestive of an increased glycogenolysis. Fat content of the muscle was also found to be increasing considerably during the pre-migratory period. A low lipase activity in the pre-migratory period was also observed.

The results obtained in the present investigations are discussed in the respective chapters.

Finally the author wishes to point out that all chapters have been written with a view to publish them as individual papers. So with the comprehensiveness as individual papers, some repetitions could not be avoided.

The following preliminary reports have been published in joint authorship with Professor J. C. George, my guiding teacher.

 Effect of disuse atrophy on the pectoralis muscle and blood in the pigeon. <u>Am. J. Physiol.</u>, 202: 268-272, 1962.
 A comparative study of the lipase activity in the blood sera of three representative birds. <u>J. Anim. Morph. Physiol.</u>, 8: 48-52, 1961.

- 3. Phosphorylase 'a' in bird and bat breast muscles. J. Anim. Morph. Physiol., 1963.
- 4. On the lipid content and lipase activity in the breast muscle of the Rosy pastor (<u>Sturnus roseus</u>).
 Accepted for publication in <u>Pavo</u>, Vol. 4: 1963.