CHAPTER 9

HISTOCHEMICAL OBSERVATIONS ON THE LOCALIZATION AND DISTRIBUTION OF PHOSPHORYLASE ACTIVITY IN THE PECTORALIS AND SUPRACORACOIDEUS MUSCLES OF SOME BIRDS

That the phosphorylase activity is high in the broad fibres and low in the narrow ones of the pigeon breast muscle has been histochemically demonstrated by Dubowitz and Pearse (1960). This was also confirmed by Vallyathan et al. (1964) in the same experimental material. Further, in a quantitative study of phosphorylase 'a' at different depths of the pigeon pectoralis, Vallyathan and George (1963) found the greatest enzymatic activity in the superficial part of the muscle than in any other region. Since the superficial layer of the pigeon pectoralis is known to contain a larger number of broad fibres, the above results indicate that the broad fibres also possess a greater concentration of phosphorylase than the narrow ones.

In a comparative study on the phosphorylase activity in the pectoralis muscles of different birds, Vallyathan and George (1963) also found that the fowl muscle which consists predominantly of white fibres, showed correspondingly, the highest enzyme activity than any of the other birds investigated. In the light of the above results an investigation on the histochemical localization of phosphorylase activity in the flight muscles of some representative flying and non-flying birds was thought worthwhile.

MATERIALS AND METHODS

The following birds were used in the present study:

Rosy Pastor (Sturnus roseus)

Purple Moorhen (Porphyrio porphyrio)

Indian Whitebacked Vulture (Gyps bengalensis)

Domestic Fowl

Guinea Fowl

Domestic Duck

These birds were brought alive to the laboratory and decapitated. A piece each from the pectoralis and supracoracoideus muscles was excised immediately, blotted free of blood, and quickly frozen. Fresh frozen hand sections (15 to 20µ thick) were cut and transferred to the incubation medium at 30°C for fifteen minutes. In order to demonstrate the phosphorylase activity, the method of Eränkö and Palkama (1962) was followed, using glucose -1- phosphate as the substrate. After incubation, the sections were rinsed in 40% alcohol, brought down to water and stained with Gram's iodine solution. They were then mounted in a mixture (1:1) of Gram's iodine and glycerine jelly and immediately photographed.

RESULTS

Rosy Pastor:

This bird being a migratory one, its pectoralis muscle was examined both in the pre- and postmigratory periods. In the post migratory period, a higher phosphorylase activity in the intermediate fibre (I type) and lesser activity in the red fibre (R type) was noticed with gradations among the R type fibres (Fig. 1). No reticular structure appeared to be prominantly stained. In the premigratory period, the phosphorylase activity showed the same pattern of localization as seen in the former period except that the sarcoplasmic reticulum was also stained (Fig. 2).

In the supracoracoideus, the broad fibre (W type), showed a high phosphorylase activity while the red fibres (R type) indicated a low activity during both the pre- and postmigratory periods.

Purple Moorhen:

In this bird, the fibre architecture of the pectoralis muscle is similar to that of the pigeon in that it possesses R and W types of fibres (Chapter 2).Thus distribution pattern of phosphorylase also showed a similarity to that of pigeon pectoralis in having a higher enzymatic activity in the W fibres and relatively lesser in the R type fibres (Fig. 3).

The supracoracoideus muscle too, has the same enzymatic pattern as that of the pectoralis.

Indian Whitebacked Vulture:

In this case the pectoralis consists of only the I type of fibres (Chapter 2). However, some of the fibres showed a higher phosphorylase activity than the others (Fig. 4), but on the other hand, the localization of fat and succinic dehydrogenase showed a uniform distribution in all the fibres (Chapters 2 and 4).

A higher phosphorylase content in the broad fibres (W type) and lesser in the intermediate fibres (I type) was observed in the supracoracoideus muscle (Fig. 5).

Domestic Fowl and Guinea Fowl:

These two birds have the same distribution of fibre types (W,I,R) in their pectoralis and in the supracoracoideus muscles (Chapters 2 and 3). The localization of phosphorylase was observed to be considerable in almost all the fibres of the pectoralis and supracoracoideus muscles irrespective of their fibre types with no significant gradations among the different fibres (Figs. 6 to 9).

Domestic Duck:

The pectoralis of the domestic duck belongs to the R, W,I type (Chapter 2). A distinctly higher phosphorylase level was noted in the broad fibres (W), whereas the rest of the fibres (R and I) showed less activity but some among them stained more than the others (Fig.10).

The supracoracoideus muscle which is of the W,I,R type, revealed an identical enzymatic distribution pattern as the pectoralis, except that among its I and R type of fibres, there was no distinct difference in the staining intensity as was observed in these fibres of the former muscle (Fig. 11).

DISCUSSION

On the basis of a number of histochemical and histophysiological studies (George and Naik, 1958; Dubowitz and Pearse, 1960; Vallyathan and George, 1963 and Vallyathan et al., 1964), it is well known that the white glycogen-loaded fibres of the pigeon pectoralis contain a higher concentration of phosphorylase in them. Thus these tetanic fibres possess the maximum capacity to metabolize the stored glycogen and are adapted for an anaerobic metabolism using glycogen as their major fuel. The red fibres on the other hand, although indicating their capacity to synthesize the little amount of glycogen present therein, utilize fat as their chief fuel for an aerobic metabolism.

In the Rosy Pastors which are migratory birds with a powerful flapping mode of flight, there is an appreciable amount of phosphorylase activity in the sarcoplasmic reticulum prior to migration (Fig. 1) in April. This is important since Bennett and Porter (1953) suggested that the sarcoplasmic reticulum represents the pathway for the internal conduction of excitation. Hence greater phosphorylase content in the reticulum might indicate the utilization of glycogen as fuel for the short flights when the bird is in the state of migratory restlessness. This also appears to be an instance of metabolic adaptation for increased glycogenolysis and formation of glucose which subsequently favours greater fatty acid synthesis by the formation of acetyl-Co A in preparation for migration (Vallyathan and George, 1964).

It could be mentioned, that in active fliers like Rosy Pastor, the pigeon and the Purple Moorhen, the broad white (W) fibres or the intermediate (I) fibres of the pectoralis as well as the supracoracoideus muscles with their greater phosphorylase activity are highly specialized towards glycogen utilization for quick phasic flights. This fact finds further support in the case of the Vulture (Fig. 4), a soaring bird, which although having only the I type of fibres in its pectoralis, yet these fibres manifest a disparity in their phosphorylase activity. The higher enzymatic content of some of the fibres show their greater ability to synthesize glycogen. This may be attributed to the fact that these heavy birds at the time of take off from the ground after a meal, require repetitive quick flappings of their wings to gain the required momentum for soaring.

The phosphorylase activity and its correlation with the functional adaptation of the flight muscles in the so called non-flying birds such as the Domestic Fowl, Guinea Fowl and Domestic Duck would be, no doubt, of immense significance. Thus in the former two birds with their W,I and R type of fibre distribution, which is a typical feature of the non-flying birds, and a considerable phosphorylase activity in almost all these fibres (Figs. 6 & 8) characterize them as those exclusively adapted for glycogen utilization. It has been established in our laboratory that for sustained muscular action fat is essential while glycogen is used as fuel only for quick and ready use by the muscle (reviewed by George and Berger, 1966). These birds use their wings only for quick flutterings to increase running speed and not for flight, wherein the muscle has to contract for a longer period. The Domestic Duck even though a nonflier, has still the R type fibres predominantly represented in both its flight muscles. This feature coupled with the fact that the distribution of phosphorylase is similar to that of a flying bird viz, pigeon, indicates that probably flightlessness in these Domestic Ducks may be a secondarily acquired character. It may also be mentioned that other members of the Anseriformes (Wild Ducks and Teals) are not only good fliers but some are migratory.

The findings that in a number of appendicular muscles of a flying bird, (pigeon) and a non-flying bird (fowl) (Nene and George, 1965); the abdominal muscles of these birds (Chinoy and George, 1964); the lateral line muscles of fishes (George and Bokdawala, 1964) and in mammalian muscles (Beatty et al., 1963; Hess and Pearse, 1961),the red fibres contain more glycogen than the white ones, suggests the possibility of increased glycogen synthesis by providing a continuous supply of oxaloacetate for increased fat utilization.

Figures 1 to 11.

Photomicrographs of the T. S. of the pectoralis and supracoracoideus muscles of some birds showing the localization of phosphorylase.

Fig. 1.
Rosy Pastor (*Sturnus roseus*)
Pectoralis in postmigratory period-August. X 432.

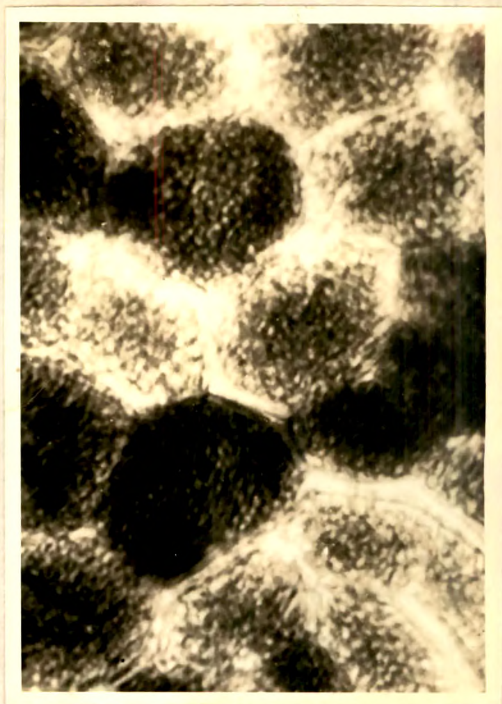

Fig. 2.
Rosy Pastor (*Sturnus roseus*)
Pectoralis in premigratory period-April. X 432.

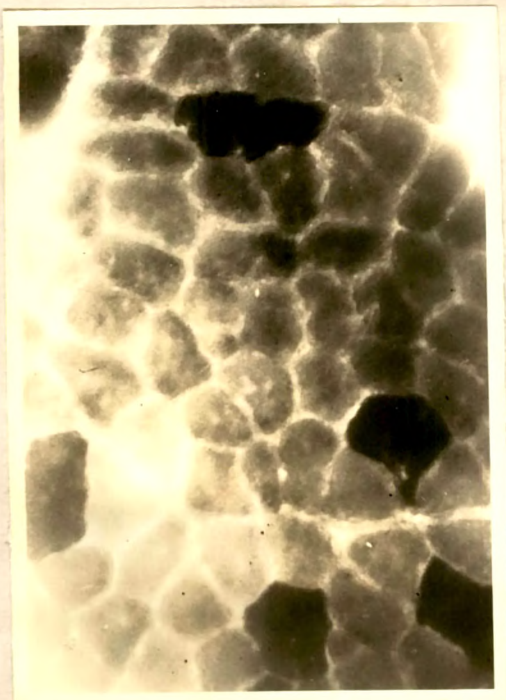

Fig. 3.
Purple Moorhen (*Porphyrio porphyrio*)
Pectoralis X 288.

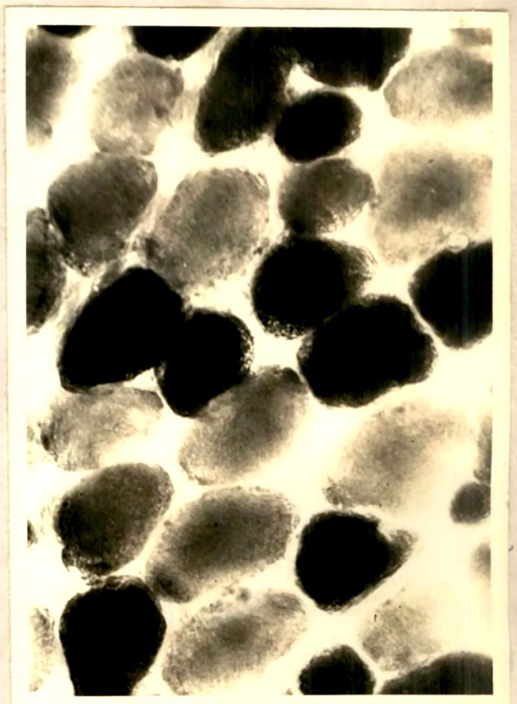

Fig. 4.
Indian Whitebacked Vulture
(*Gyps bengalensis*)
Pectoralis X 752.

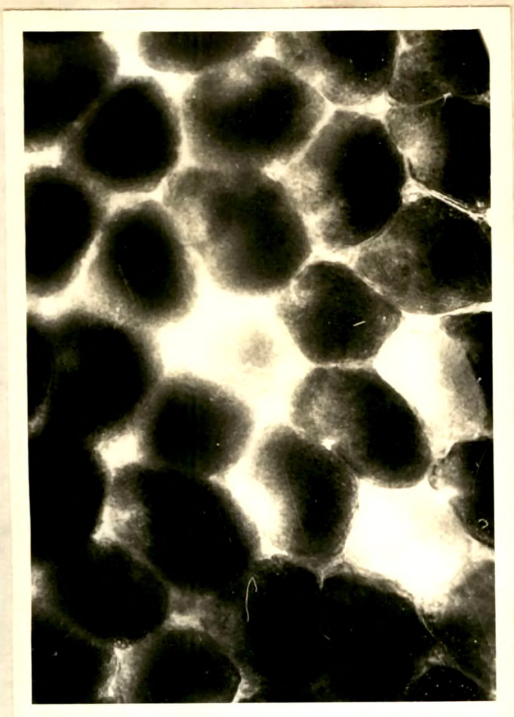

Fig. 5.
Indian Whitebacked Vulture
(Gyps bengalensis)
Supracoracoideus X 432.

Fig. 6.
Domestic Fowl
Pectoralis X 288.

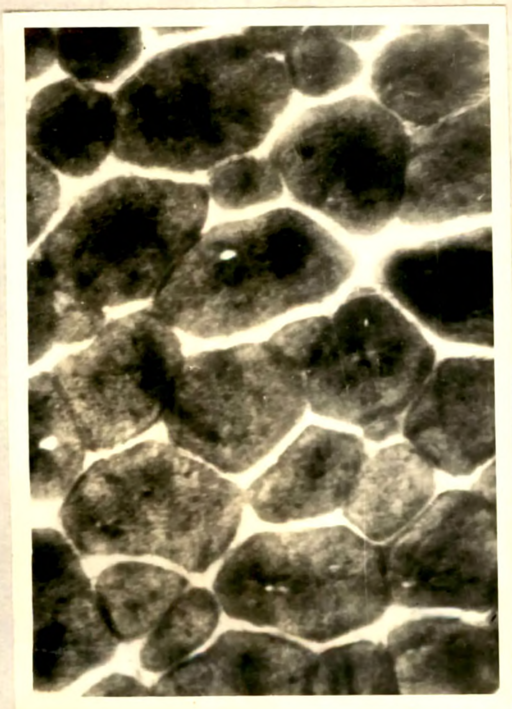

Fig. 7.
Domestic Fowl
Supracoracoideus X 288.

Fig. 8.
Guinea Fowl
Pectoralis X 288.

Fig. 9.
Guinea Fowl
Supracoracoideus X 288.

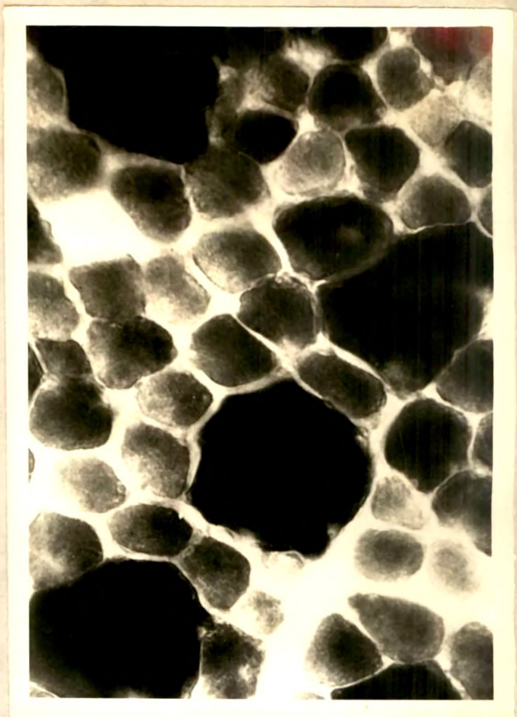

Fig. 10.
Domestic Duck
Pectoralis X 432.

Fig. 11.
Domestic Duck
Supracoracoideus X 432.