# CHAPTER 7

## PHOSPHORYLASE 'a' IN BIRD AND BAT BREAST MUSCLES

It has been shown by earlier workers that the pectoralis major muscle of the pigeon consists of two distinct types of fibres, a broad glycogen-loaded white variety with a few mitochondria and a narrow fat-loaded red variety with a large number of mitochondria (George and Jyoti, 1955; George and Naik, 1958a; 1958b). It has been established that the narrow fat-loaded variety with a higher concentration of lipase (George and Scaria, 1958a) and oxidative enzymes (George and Scaria, 1958b; George and Talesara, 1960), predominantly utilize fat as energy fuel while the broad glycogen-loaded white variety utilize carbohydrates as the chief fuel. Dubowitz and Pearse (1960) demonstrated histochemically the presence of phosphorylase activity in the pigeon breast muscle and other vertebrate skeletal muscles and has shown that the activity of the enzyme is higher in the broad white fibres and very low or negligible in the narrow red ones. Based on these findings it was thought desirable to conduct a quantitative assay of this enzyme phosphorylase in the two types of fibres of the pigeon breast muscle to confirm the histochemical observations. The study was further extended to the pectoralis of other birds and a bat in order to find out if there is any correlation between the concentration of the enzyme and the type of muscle, its metabolite load and its mode of activity.

Owing to the technical difficulties in separating

the two types of fibres and then estimating the enzyme activity an indirect method based on the knowledge of the relative distribution of the two types of fibres in the pigeon pectoralis worked out by George and Naik (1959a) was adopted. They found that the number of the broad, white, fibres are least in fasciculi situated in the middle of the dorso-ventral axis of the muscle while it tends to increase above or below this mark, reaching the maximum in the superficial and deepest layers of the muscle. This indirect method of enzyme assay in the two types of fibres has been employed in this laboratory for the study of certain other enzymes (George and Talesara, 1960; 1961).

Phosphorylase plays a strategic role in the glycolytic sequence since it is the initial catalytic reaction in the chain of chemical events that leads to the phosphorylative degradation and utilization of glycogen (Stetten and Stetten, 1960). Therefore, phosphorylase activity in a tissue or changes in its activity would apparently indicate the rate of glycolysis. When a muscle contracts stored chemical potential energy must be mobilized and made available to the system in a readily utilizable form. Phosphorylase exists in the muscle in two forms. An active form 'a' and an inactive form 'b', the active form predominating in a contracting muscle (Krebs and Fisher, 1955). It is also known that the active form 'a' increases during contraction of the muscle and decreases during rest (Cori, 1956; Rulon et al, 1961).

In the present investigation the active form 'a'was estimated in the different muscle layers of the pigeon breast muscle and in the case of other animals it was estimated in the whole muscle.

## Materials and Methods

Freshly shot wild pigeons of both sexes weighing from 320 to 350 gm. were used. The bird was immediately decapitated and the blood drained off. A muscle strip of nearly 2 $cm^2$ cut along the orientation of the fibres was taken from the middle of the muscle in its entire thickness. After quickly blotting off the blood with a filter paper the muscle piece was placed on the stage of a freezing microtome and freezed with the ventral side up so as to cut sections of desired thickness starting from the ventral side. When the muscle was freezed the epimysium was removed by a superficial stroke of the microtome knife and serial sections of 0.5 to 1 mm thick were cut as required. These sections were transferred to chilled mottars and homogenized in cold distilled water. A similar piece of muscle from the other side of the breast muscle was preserved in the deep freeze chamber of the refrigerator. Later sections of this muscle piece were cut and the fibre count of broad white fibres per square mm counted according to the method described by George and Naik, (1959a).

The Rosy pastor (Sturnus roseus), Myna (Aeridotheres tristis) and the Bat (Hipposiderous sperois) were either trapped or shot dead and a piece of the pedtoralis major muscle was

immediately homogenized in a chilled mortar with ice cold distilled water. The Fowls (Gallus domesticus) used were domesticated laboratory birds.

Phosphorylase activity was determined by a modification of the techinique of Cori, Cori and Green (1943) as adapted by Cahill et al, (1957). A 2% homogenate of the muscle was prepared in cold distilled water and was used as the enzyme material. Samples of 0.1 ml. of this fresh homogenate were added to a cold mixture in a test tube containing 0.2 ml. sodium citrate buffer (0.1 M) at pH 5.9 and 0.3 ml. of potassium fluoride (0.154 M) and 0.05 ml. of glucose-1-phosphate (0.2 M) as the substrate. The samples were incubated for 15 minutes at 30°C and the reaction was terminated by the addition of 1 ml. of 10% trichlorøacetic acid (TCA). In some experiment glycogen was added to give a 1% final concentration in the incubation mixture. However, it was found that the adding of extra glycogen did not make any change in the activity of the enzyme since there was enough glycogen in the homogenate to serve as a nidus for further glycogen formation during incubation.

Controls were run with each experiment by adding 1ml. of 10% TCA in the incubation mixture before the addition of the homogenate and incubated along with the samples. Blanks were prepared by the addition of 1 ml. of 10% TCA in the incubation medium. After the incubation period the samples and the controls were filtered into 10 ml. graduated test tubes by several washings of the filter paper to ensure complete

recovery of the phosphate. The filtration procedure was carried out in a cold water bath (10 to 15°C) due to high room temperature which was found to be interfering with the hydrolysis of glucose-1-phosphate. The inorganic phosphate liberated from the glucose-1-phosphate by the enzyme activity was estimated by the method of Fiske and Subbarow (1925) and the optical density was read on a Klett-Summerson photoelectric colorimeter using 660 mμ filter. The controls were substracted from the samples to determine the phosphate moiety released by the enzymic action. Enzyme activity was calculated as μg phosphorous released per 10 mg. dry weight of the muscle at 30°C for 15 minutes.

## Results

Phosphorylase activity and the number of broad and narrow fibres per square millimeter in the different layers of the pigeon breast muscle are shown in Table 1. Table 2 represents the activity of the enzyme in the white and red fibres as derived from the equation of,the regression line. The results clearly indicate that there is a relationship between the activity of the enzyme and the number of broad fibres in the different regions of the muscle. The activity of the enzyme was found to be low in the deeper layers of the muscle where there are few broad fibres compared to the superficial layers where the broad fibres are more in number.

Figure 1 gives a graphic representation of the ratio of the broad and narrow fibres to the activity of the enzyme

Table 1

Showing the distribution pattern of phosphorylase activity in the different layers of the pectoralis major muscle of the pigeon and its relation to the number of broad white and narrow red muscle fibres.

| Depth of the muscle starting from the superficial side | Number of fibres $mm^2$ | | Phosphorylase activity ug phosphorous/10 mg. dry muscle/15 minutes | | | |
|---|---|---|---|---|---|---|
| | Broad | Narrow | | | | |
| mm 0 - 1 | 109 ± 4 | 260 | 333.7 | ± | 58.17 | (7) |
| 1 - 2 | 86 ± 8 | 387 | 274.5 | ± | 43.30 | (7) |
| 2 - 3 | 68 ± 3 | 497 | 235.8 | ± | 27.20 | (6) |
| 3 - 4 | 54 + 5 | 576 | 192.1 | ± | 33.50 | (6) |
| 4 - 5 | 44 ± 3 | 636 | 163.3 | ± | 40.00 | (6) |

Numbers in the parentheses indicate number of experiments in each case.

phosphorylase in the pigeon breast muscle. George and Naik (1959a) showed that in the pigeon breast muscle per unit area cross section of the muscle at any particular depth, the number of broad white fibres is inversely proportional to the number of narrow red fibres and derived the formula

$$Y = -5.75\ X + 890.01$$

(where Y stands for the number of narrow fibres and X for the

number of broad fibres). It means that when X is zero the total number of narrow fibres per square millimeter of the area would be 890, i.e., all narrow fibres and when Y is zero, the total number of broad fibres per square millimeter of area is nearly 155 i.e., all broad fibres only. The regression equation $Y = 0.2641\ X + 4.6904$ was obtained and derived the enzyme activities in the individual red and white fibres by extending the regression lines on either side as indicated by broken lines(Fig. 1).

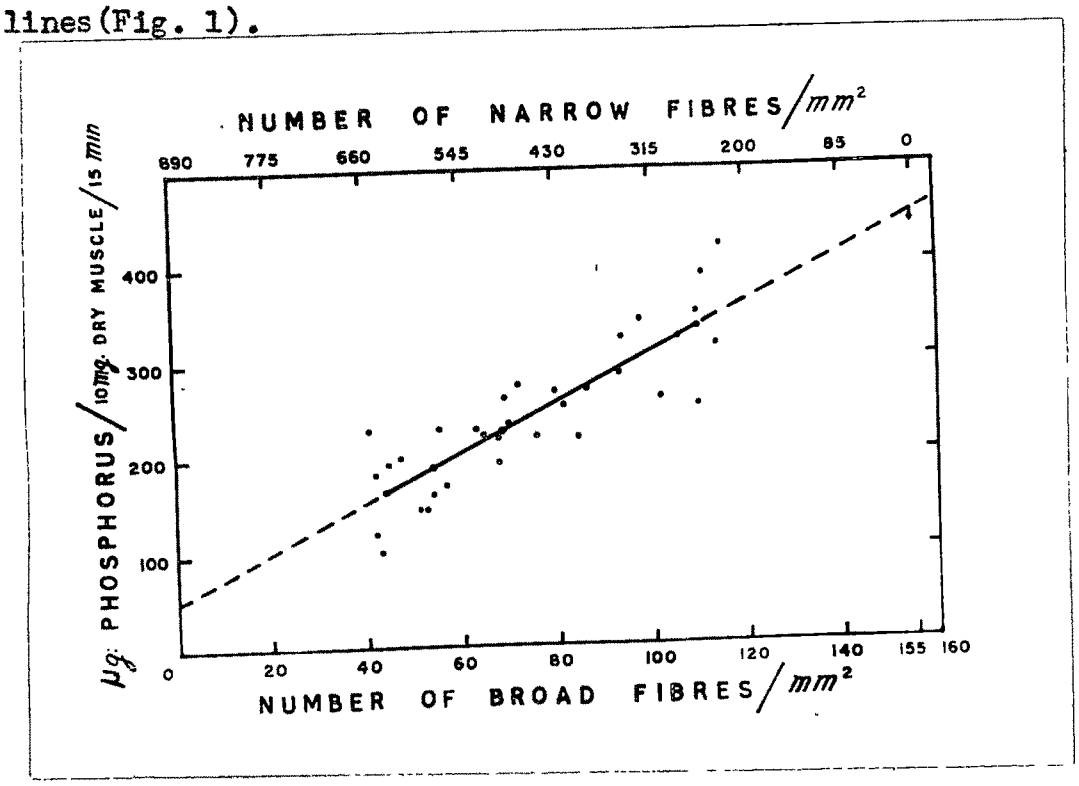


Figure 1. A graphic representation of the relation between the number of broad and narrow fibres per square millimeter and phosphorylase activity as obtained at different depths of the breast muscle of the pigeon.

Table 2

Showing the activity of phosphorylase in the red and white fibres of the pectoralis major muscle of the pigeon as derived from the equation of the regression line

| Muscle | Phosphorylase activity µg phosphorous/10 mg. dry muscle/15 minutes |
|---|---|
| White | 456.1 |
| Red | 46.9 |

Y = 0.2641 X + 46.904 S. E. of byx = 0.028

X = Mean fibre count Y = Enzyme activity

Table 3 represents the activity of the enzyme in the breast muscle of other birds and the bat studied. In these animals the enzyme activity was determined in the whole muscle since there is no clear morphological differentiation of the fibres into two types and there is no definite pattern of distribution as in the pigeon breast muscle.

From the results obtained it could be noticed easily that the major enzyme activity in the pigeon breast muscle is confined to the broad white fibres. In the case of the other animals the highest enzyme activity was found in the breast muscle of fowl and the next in myna and Rosy pastor and the least in the breast muscle of bat.

Table 3

Phosphorylase activity in the pectoralis major muscles of other birds and a bat studied

| Animal | Phosphorylase activity µg phosphorous/10 mg. dry muscle/15 minutes | | | No. of Expts. |
|---|---|---|---|---|
| | Mean | | S. D. | |
| Fowl | 293.7 | ± | 77.6 | 5 |
| Myna | 285.3 | ± | 68.6 | 9 |
| Rosy pastor* | 221.0 | ± | 30.3 | 7 |
| Bat | 49.8 | ± | 18.6 | 10 |

*These experiments on the Rosy pastors were conducted in the post-migratory period (December and January).

## Discussion

Glycogen is the primary fuel for muscle contraction in most muscles. It is an established fact that an increase in the phosphorylase activity is associated with increased glycogenolysis (Cahill et al, 1957). Cori has attempted to correlate the rate of glycogen breakdown and the rate of activation of phosphorylase during muscle contraction.

A higher concentration of the enzyme phosphorylase in the broad white fibres of the pigeon breast muscle suggests the presence of a highly specialized system utilizing carbohy-

drate as the chief fuel for energy in this type of muscle fibres during muscular contraction. George and Naik (1958a; 1958b) have shown that the broad white fibres of the pigeon breast muscle are rich in glycogen and poor in fat and mitochondria whereas the narrow red fibres are fat-loaded and have a low glycogen content. Further studies on the oxidative enzymes and a lipase (George and Talesara, 1961) and DPN-linked dehydrogenases and aldolase, (George and Talesara, 1962) in these two types of fibres of the pigeon breast muscle have revealed that the narrow red fibres have predominantly an oxidative metabolism and the broad white fibres a glycolytic metabolism. Dubowitz and Pearse (1960) have demonstrated histochemichally the enzyme phosphorylase in the pigeon breast muscle, but the enzyme concentration in the narrow red fibres was shown to be very low. The present quantitative study on the two types of fibres shows that there is a considerable activity of the enzyme in the narrow fibres too. The failure in demonstrating histochemically this enzyme in the narrow red fibres of the pigeon breast muscle may be due to the density of high granular inclusions in this fibre. This is interesting because it is known that a muscle capable of sustained activity like the heart muscle also contains a high concentration of the enzyme phosphorylase(Rall et al, 1956). The heart is known to utilize fat for its major energy supply (Bing, 1956). It has also been shown that during sustained muscular activity the pigeon breast muscle utilizes fat as chief fuel (George and Jyoti, 1957). Nevertheless, the present

investigation on the distribution pattern of the enzyme suggests that the narrow red fibres of the pigeon breast muscle are also adapted to a certain extent for the utilization of carbohydrates as the heart muscle. However, major phosphorylase activity is confined to the broad white fibres which indulge in tonic contractions and have a glycolytic metabolism.

Among the other birds studied the breast muscles of the Myna and Rosy pastor and the Fowl showed very high concentrations of the enzyme activity. As regards to the breast muscle of Fowl with its tonic broad white fibres and high concentrations of glycogen one might expect to find extremely high activity of the enzyme but the concentration of the enzyme was more or less same as in the breast muscle of Myna. This may be attributed to the fact that the concentration of this enzyme is not strictly proportional to the metabolite load but to the activity of the muscle. In the Bat the enzyme level was found to be extremely low. This may be due to the fact that the animals were sacrificed during the day time which is the resting period for these animals. In the resting muscle the activity of phosphorylase 'a' is shown to be low(Krebs and Fisher, 1955; Chapter 5). It has also been shown by Leonard et al, (1959) that glycogen and phosphorylase levels increased in the muscle of bat after the arousal of the animal from hibernation. Increase in the enzyme activity due to electrical stimulation of the muscle was reported by Cori (1956); Rulon et al, (1961) and also in Chapter 5.

Phosphorylase is maintained at a higher level in active

muscle. A higher level of this enzyme is associated with accelerated glycogen breakdown whether in vitro (Cahill et al, 1957) or in vivo (Sutherland, 1951). But it is not always possible to correlate phosphorylase activity with glycogen concentration in the muscle as is seen in the present study. On the other hand in the case of certain other enzymes e.g., lipase, succinic dehydrogenase and cytochrome oxidase in the two types of fibres (red and white) of the pigeon breast muscle studied in this laboratory, it was found that the concentration of the enzyme was proportional to the metabolite load (fat and glycogen respectively). The reason why the same is not true for phosphorylase may be due to the fact that this enzyme is chiefly extramitochondrial (cytoplasmic) and is in a continous state of flux as could be visualized happening in fermentation.