CHAPTER 1

A QUANTITATIVE STUDY OF THE DISTRIBUTION PATTERN OF SUCCINIC DEHYDROGENASE IN THE PECTORALIS MAJOR MUSCLE OF THE PIGEON

Denny-Brown (1929), while studying the red and white muscles of vertebrates, made some observations on the 'light' and 'dark' muscle fibres in the breast muscle of the pigeon. The later works on these two types of fibres have been reviewed by George and Naik (1957a). More recently George and Naik (1958a, 1958b) have shown that the pectoralis major 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. George and Scaria (1958c) in a histochemical study of dehydrogenases (succinic, malic, lactic and glycerophosphate dehydrogenases) in this muscle could not detect the presence of any of these dehydrogenases in the broad white fibres, by the methods employed. They therefore concluded that these fibres in the pigeon breast muscle are a unique system in which none of the oxidative processes concerned with the above enzymes takes place and therefore cannot be considered as analogous to the white fibres of the other vertebrate skeletal muscles studied. These observations should naturally raise some important questions as well as doubts regarding the exact role of the broad white fibres in this muscle and also the problem of energetics for their

contraction. It should be mentioned, however, that the above conclusions were based solely on histochemical observations and can be considered decisive only if supported by quantitative studies. Here in this chapter is reported the results of a quantitative study of the distribution pattern of the SDH activity in the pectoralis major muscle of the pigeon.

Material and Methods

It is technically impossible by ordinary methods to estimate directly the activity of any enzyme in the two types of fibres in the pigeon breast muscle after isolating the fibre types separately. But the indirect method, viz. of estimating the activity of the enzyme in the different layers of the muscle, where the broad and narrow fibres vary in number, was adopted in the present study. The fibre architecture of the pectoralis major muscle of the pigeon has been worked out in detail by George and Naik (1959a). They have found that the least number of broad fibres is found in the fasciculi situated in the middle of the dorso-ventral axis of the muscle and the number tends to increase above or below this mark, reaching the maximum in the most superficial and in the deepest layers of the muscle. They also showed that per unit area of cross-section of the muscle at any particular depth, the number of broad fibres is inversely proportional to the number of narrow 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) in order to find out the number of narrow fibres in any one particular square mm. area, X being known. Throughout the study this formula was applied for finding out the number of narrow fibres in a particular layer of muscle which was used for the estimation of the succinic dehydrogenase activity.

The method employed in the estimation of SDH was the one according to Kun and Abood (1949) which makes use of the principle that colourless TTC is reduced to a red insoluble compound, formazan, by the enzymic oxidation of succinate to fumarate. The colour developed was extracted in acetone and the intensity measured in a colorimeter. The amount of formazan formed was directly read from a standard curve plotted by reducing completely known quantities of TTC by sodium hydrosulphite. Since the reduction of TTC by hydrosulphite was found to be reversed, the colour was stabilized by adding acetone within one minute of the addition of hydrosulphite to the TTC solution, during which time the TTC was completely reduced.

In order to ensure that only uniformly well developed muscle was used in the study, well fed and fully grown, normal laboratory pigeons (Columba livia) were used throughout.

In each experiment the bird was decapitated and the blood completely drained off. Pieces of the muscle from the regions shown in Fig. 1 (A and B) were cut off for each set of

experiments. A portion (1) of this muscle piece A was immediately mounted on the stage of a freezing microtome with the superficial (ventral) side facing up, so as to obtain serial horizontal sections of the desired thickness starting from the superficial side of the muscle. The remaining portion (2) of the same piece was set apart for counting the broad and narrow fibres. The same procedure was followed for the muscle piece B. In some cases it was found necessary to freeze the

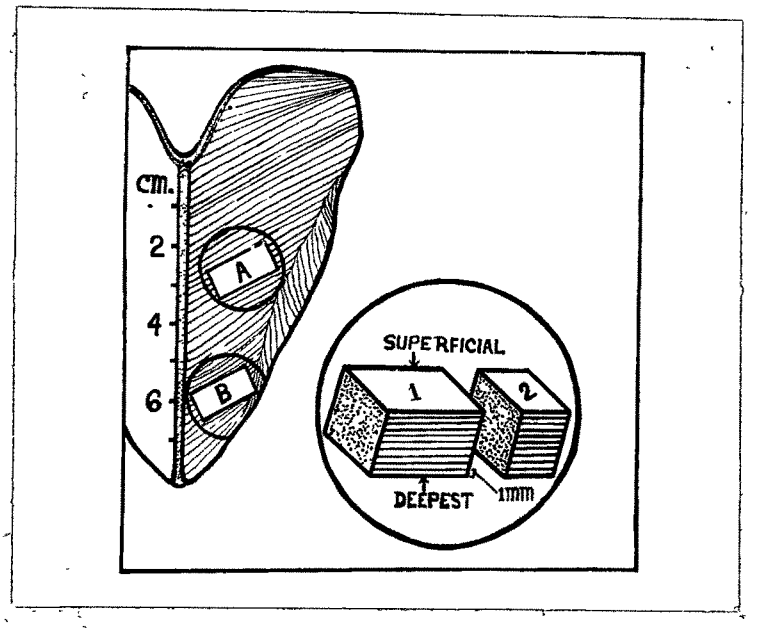

Fig. 1. Illustrating the topography of the muscle pieces used for the study of SDH activity in the pigeon breast muscle.

muscle block first with the superficial (ventral) face down and after trimming the deeper (dorsal) side it was reversed so as to render the surface even and parallel to the edge of the knife. When the block was frozen hard the epimysium was

removed by a superficial stroke of the microtome knife and serial sections of 0.5 mm. or 1.0 mm. thickness as required were cut. The sections were immediately transferred to weighing bottles and the weight determined. They were then chilled and each of the sections was homogenized in 10 ml. of cold distilled water. The SDH activity of this homogenate was then determined using the method as mentioned below.

The incubation mixture in each case contained 0.5 ml. of 0.1 M phosphate buffer of pH 7.4, 0.5 ml. of 0.2 M sodium succinate, 1.0 ml. of the muscle homogenate and 1.0 ml. of a freshly prepared 0.1 % solution of TTC in a total volume of 3 ml. The TTC solution was added last, and after shaking, the tubes were incubated for 15 minutes at 37° C. Seven ml. of acetone were then added to each tube. The tubes were tightly stoppered, shaken well and centrifuged for about three minutes at 3000 r.p.m. The clear supernatant was drawn off and the intensity of the colour read at 420 mμ on a Klett-Summerson photoelectric colorimeter. The readings were corrected for a blank with zero time incubation. Inhibition experiments with malonate confirmed that the colour production was solely due to SDH.

Part (2) of the original piece of the muscle was sectioned as shown in Fig. 1. The section was mounted in 50 % glycerine and the number of broad fibres per square mm. counted according to the method described by George and Naik (1959a). From the broad fibre count, the number of narrow fibres in the particular area was calculated using the formula already

referred to.

Results

The SDH activity is expressed as the number of micrograms of formazan produced by 100 mg. wet weight of the muscle for 15 minutes at 37° C. under the conditions of the experiment mentioned above. It is not claimed that the values given are absolute because it is likely that freezing and thawing of the tissue in the process of cutting sections might destroy a certain amount of its enzyme activity. However, this loss of activity could not be considerable and could be even neglected in view of the fact that the purpose is to compare the activity at the different depths and in relation to the number of the fibres per square mm. area.

The SDH activity and the number of broad and narrow fibres per square mm. in the different layers of the regions A and B of the breast muscle of the pigeon is shown in Table 1. The results indicate that there exists a relationship between the activity of the enzyme and the number of narrow fibres per square mm. in the different layers of the muscle. It was also decided to find out the activity of the enzyme in the 0.5-mm. thickness of the most superficial layers of A and B where the broad fibres are relatively much more concentrated than in the one-mm. thick layer (Table 1).

From the results obtained the regression line is drawn using the equation

$$S = 0.3 + 0.11478\ Y,$$

where S stands for the SDH activity and Y for the number of

narrow fibres in one square mm. area. If the value of Y is zero the value of S will be 0.3 μg. of formazan, which is an infinitely small measure of activity. If the values obtained for the SDH activity are plotted against the number of narrow fibres per square mm. a linear graph illustrating this relationship is obtained using the equation of the regression line (Fig. 2). Similarly plotting the dehydrogenase activity and the number of narrow fibres in the different layers from A and B almost a similar pattern is obtained (Fig. 3). In the most superficial region, which consists mostly of broad fibres, the SDH activity more or less corresponds to that of the number of narrow fibres.

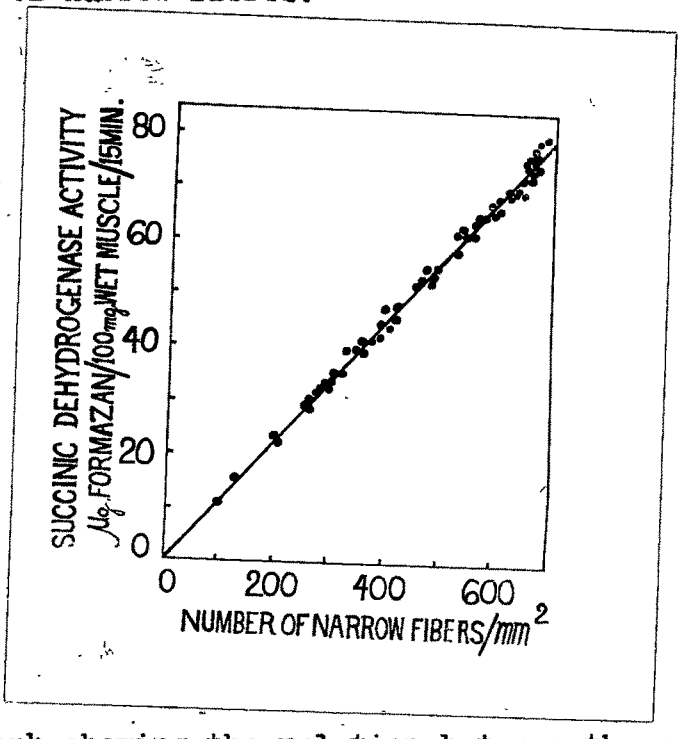

Fig. 2. Graph showing the relation between the number of narrow fibres and SDH activity in the different layers of the <u>pectoralis</u> <u>major</u> muscle of the pigeon.

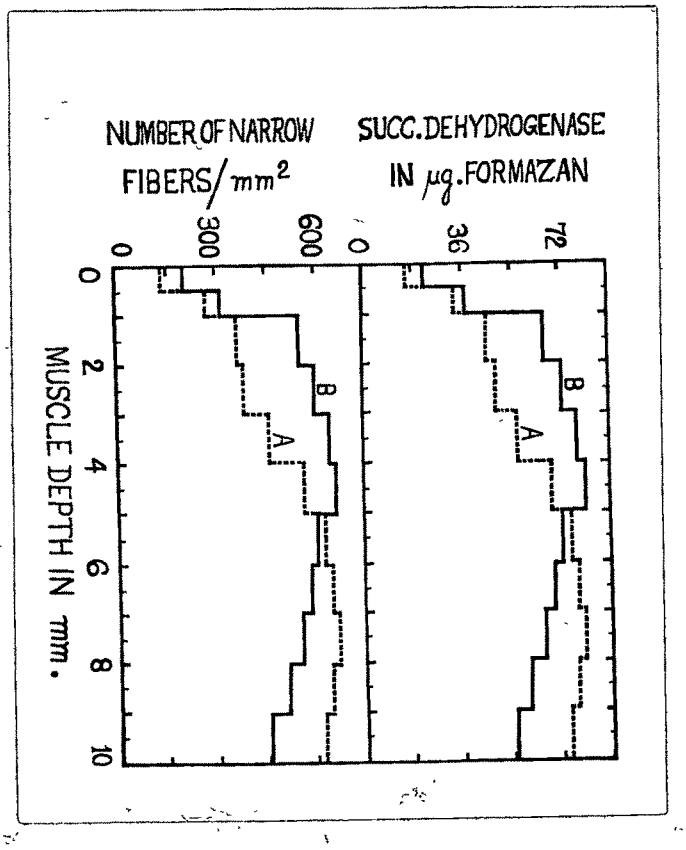

Fig. 3. Showing the number of narrow fibres and the corresponding SDH activity in the different layers of the two regions A and B of the <u>pectoralis major</u> muscle of the pigeon.

Discussion

From the data presented above it appears that there exists a relationship between the number of narrow fibres and SDH activity in the different layers of the pigeon breast muscle. This relationship can be traced to the fact that the activity of the enzyme in the broad fibres is negligible. By substitution of the values in the formula mentioned above it is found that

$$S = 0.11478\ (890.01 - 5.75\ X),$$

(where S stands for the SDH activity in μg. of formazan per

100 mg. of wet weight of the muscle taken from any region parallel to the surface of the muscle, per 15 minutes under the conditions of the experiment mentioned earlier, and X stands for the average number of broad fibres per square mm. area of cross-section of the muscle). In other terms

$$\frac{S}{(890.01 - 5.75\ X)} = K,$$

(where K is a constant, the value of which is 0.11478). This indicates that S in any particular layer of the pigeon breast muscle is a function of the number of narrow fibres. The conclusion that can be drawn from this study is that in the pigeon breast muscle the main bulk of the SDH is confined to the narrow fibres and that the broad white fibres possess only a negligible concentration of the enzyme.

George and Scaria (1958c) and George, Susheela and Scaria (1958a) studied, using histochemical methods, the activity of certain dehydrogenases in the breast muscle of the pigeon, fowl and bat and the leg muscle of the fowl and frog, and observed that the concentration of these oxidative enzymes has a relationship with the colour and the mitochondrial content of the individual muscle fibres in the sense that the red narrow fibres possess a high concentration of these enzymes and mitochondria, in sharp contrast to the broad white fibres. Similar observations were made by Nachmias and Padykula (1958) in the skeletal muscle of rat. It should be pointed out here that within the limitations of the methods

employed in all the above mentioned muscles, all the fibres in every muscle, except the broad ones of the pigeon breast muscle, dehydrogenase activity was detectable. Nevertheless the activity was varying in the different muscle fibres according to their diameter, highest activity being in the smallest fibres. In the pigeon breast muscle, however, where the same histochemical methods were employed the broad white fibres did not show the presence of any of the dehydrogenases mentioned earlier (George and Scaria,1958c). The quantitative data presented here show the distribution of the SDH activity in the different layers of the pigeon breast muscle and also that the main bulk of the enzyme is confined to the narrow red fibres.