CHAPTER 3

A QUANTITATIVE STUDY OF THE DISTRIBUTION PATTERN OF IRON AND PROTEIN IN THE RED AND WHITE FIBRES OF THE PIGEON BREAST MUSCLE

George and Naik (1959a) studied the fibre architecture of the pectoralis major muscle of the pigeon in detail and showed 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 that 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. Based on this finding the distribution of succinic dehydrogenase activity was studied quantitatively in the different layers of this muscle by recording the distribution pattern of the narrow red and the broad white fibres side by side (Chapter 1). It was found that the main bulk of the enzyme activity is confined to the narrow red fibres. In a further study (Chapter 4) the extent of the activity of some oxidizing enzymes and a hydrolyzing enzyme, lipase, were assessed quantitatively in the different layers of the pigeon breast muscle and the respective enzyme concentrations in the individual red and white fibres were estimated using the statistical derivation employed as mentioned in Chapter 1. Such a procedure was adopted because as already stated it is extremely difficult to separate out the fibre types of a mixed muscle such as this and then estimate directly the various biochemical constituents. Moreover, there are certain labile constituents which could neither be studied histochemically nor biochemically and could not be estimated even if the separation of the fibre types was realized. Hence the present chapter deals with a study undertaken to assess quantitatively the respective iron and protein contents of the red as well as the white fibres of the pigeon breast muscle, by using an indirect method as mentioned above.

Material and Methods

The pectoralis major muscle of well fed, and fully grown, normal laboratory pigeons (Columba livia), was used throughout the study.

For each experiment a bird was decapitated and the blood completely drained off. A muscle strip of nearly 2 square cm. area cut along the direction of the fibres, was removed from the middle of the body of the pectoralis in its entire thickness. Another piece with exactly the same topography was cut quickly from the muscle of the other side. In every case the muscle strip removed was only from this particular region. In each set of experiments one such piece of the muscle was used for the estimation of iron or protein and the other for recording the distribution pattern of the broad and narrow fibres per square mm. at different depths of the muscle. Slices of the desired thickness were cut on a freezing microtome and the relative distribution of the fibre types per square mm. in the different depths was assessed according to the procedure already referred to (Chapter 1). The only change adopted in the present study was that each of the slices cut, was transferred immediately to weighing bottles and dried in an air oven at 100° C. till a constant dry weight was obtained. In the case of the estimation of iron, however, it was found necessary to pool slices from 3 or 4 muscle pieces taken from different pigeons. Thus after obtaining the constant dry weight of the muscle slices, the iron and protein contents were determined by the methods given below:

The Micro-Kjeldahl method of Wong as described in Hawk et al (1954), for the estimation of total proteins in the blood plasma was adopted for the estimation of protein in the muscle slices. The intensity of the colour developed was read on a Klett-Summerson photoelectric colorimeter.

Kennedy's method as described in Hawk et al (1954) was followed for the determination of the iron content. The muscle was digested in concentrated H_2SO_4 in the presence of perchloric acid and potassium thiocyanate was added to a part of this. The colour developed was extracted with amyl alcohol and the intensity was read on the same colorimeter.

Results

The iron and protein contents were found to vary at different depths of the muscle as is shown in Table 1. The iron and protein contents were also determined in the 0.5 mm. thickness of the most superficial layers starting from the ventral face of the muscle where the broad fibres are relatively much more concemtrated than in the 1-mm. thick layer (Table 1). The relative distribution pattern of the broad and narrow fibres at a particular depth or layer is also recorded in the same table. These data obtained clearly indicate that the variation in the chemical content is in accordance with the variation in the ratio of the two types of fibres in their distribution pattern in the different layers. The same conclusion was arrived at in the case of metabolites like glycogen and fat (George and Naik, 1959a) and oxidative enzymes and a lipase (Chapters 1 and 4).

Table 1

Showing the distribution pattern of iron and protein in the different layers of the pectoralis major muscle of pigeon and their relation to the number of muscle fibres. (The figures indicate the average values of five sets of experiments in each case)

Expt.	Depths of the muscle in mm. starting from the superficial side (ventral face)	No. of fibres per square mm.		Iron in mg. per 100 gm. dry muscle	Protein in gm. per 100 gm. dry muscle
		Broad	Narrow		
1	0 - 0.5	119 ± 11	214	14.64	88.57
	0.5 - 1.0	88 ± 9	385	23.20	86.60
2	0 - 1	102 ± 8	301	19.53	85.40
	1 - 2	78 ± 6	471	26.56	85.02
	2 - 3	56 ± 7	567	30.74	84.20
	3 - 4	44 ± 3	640	33.90	82.93
	4 - 5	34 ± 6	696	38.06	80.84

The first observation which can be made from this study is that unless the ratio of the distribution pattern of the different types of fibres that constitute the muscle under investigation is taken into account while assessing its biochemical constituents no accurate values could be obtained as have been the case in the numerous investigations hitherto made. The average values obtained in the earlier studies might give a broad average for the muscle as a whole which certainly does not constitute the true picture of the muscle and its cellular components.

Table 2

Iron and protein content of the red as well as the white fibres of the pigeon pectoralis major muscle as derived from the equation of the regression line

Muscle	Iron in mg./100 gm. dry muscle	Protein in gm./100 gm. dry muscle
Red	44.57	78.42
White	4.97	92.61

The results obtained for the different layers in this study indicate that with the increase in the number of narrow over the broad fibres in a particular layer, the iron content also increases whereas there is a slight decrease as far as the protein content is concerned. It is also interesting to note that the iron content is maximum and protein content the minimum in the layer taken somewhere from the middle of the dorso-ventral axis where the number of broad fibres is the least. Iron content tends to decrease above this mark and is the least in the most superficial layer where the number of broad fibres is maximum. Protein follows somewhat a reverse pattern but the increase is not very sharp. There is a slight increase towards the superficial side. A graphical presentation (Fig. 1) of the distribution of narrow and broad fibres per square mm. in a particular layer against the values of iron show that the major amount of iron is confined to the narrow fibres whereas the broad fibres have considerably less. A similar type of graph (Fig. 2) showing that the protein content of the broad fibres is slightly more than that of the narrow ones, is obtained.

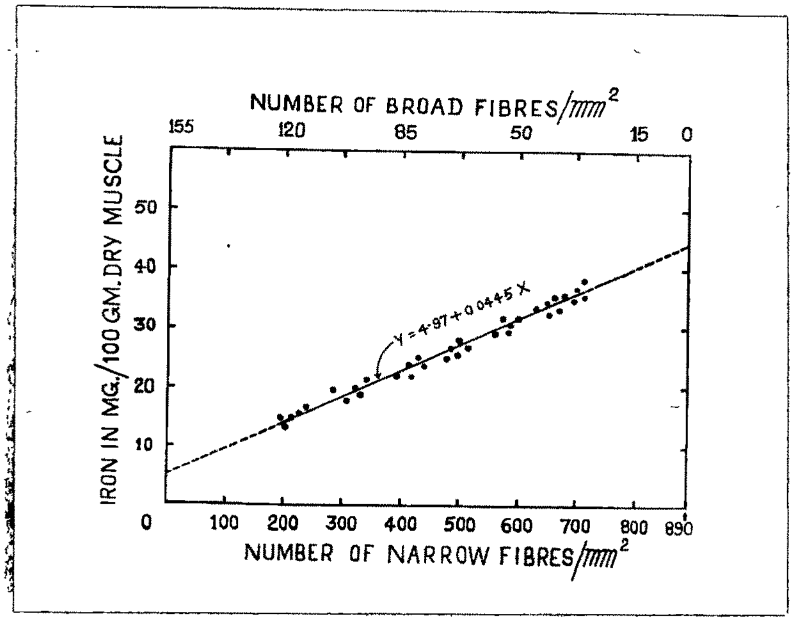

Fig. 1. Graph showing the relation between the number of narrow and broad fibres per square mm. and the iron content as obtained at different depths of the *pectoralis major* muscle of the pigeon.

George and Naik (1959a) 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). It means that when X is zero the total number of narrow fibres per square mm. area would be 890 i.e. all narrow fibres and when Y is zero, the total number of broad fibres per square mm. area nearly 155 i.e. all broad fibres. It should be mentioned, however, that this is merely a theoritical assumption and such a condition never exists in this muscle in any region. However, considering the nature of the regression lines (Figs. 1 and 2), which show a linear pattern, it is possible to derive the iron and protein contents in the individual fibre types, when this line is extended on either sides as shown by broken lines (Figs. 1 and 2).

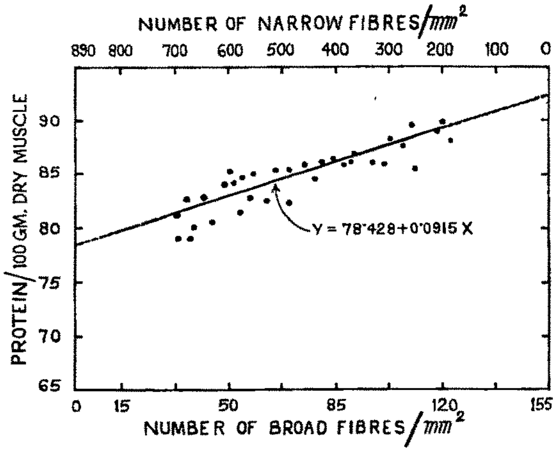

Fig. 2. Graph showing the relation between the number of narrow and broad fibres per square mm. and the protein content as obtained at different depths of the *pectoralis major* muscle of the pigeon.

The values so derived for the individual fibre types are shown separately in Table 2. It should be noted, however, that the values derived by this indirect method may not be absolute, but nevertheless, does provide a reasonably accurate method for a quantitative determination of the various constituents specially certain labile constituents and enzymatic activities which cannot be revealed either histochemically or biochemically in the two fibre types of this muscle without isolating them. On this basis the values of a number of oxidizing enzymes and a lipase for the red and white fibres respectively, have been derived (Chapter 4).

Discussion

The considerably higher content of iron in the narrow red fibres of the pigeon breast muscle indicate that these fibres are rich in myoglobin. Higher concentrations of this pigment are known to be present in the muscles having a high aerobic metabolism and the strong affinity of myoglobin for oxygen enables it to act as an intramuscular oxygen store for tissue oxidation. Lawrie (1952, 1953a, and 1953b) in his biochemical studies on different types of muscles has shown that there is a parallelism in muscle between the percentage of myoglobin, the activity of cytochrome oxidase and a high capacity for aerobic synthesis of high energy phosphates. Thus the very low concentrations of oxidizing enzymes (Chapters 1, 2, 4 and 5) and myoglobin as indicated by the low iron content in the broad white fibres do indicate a poorly developed respiratory metabolism in them as against the narrow red fibres of the pigeon breast muscle.

The protein content of the broad white fibres is slightly higher than that of the narrow red fibres (Table 2, Fig. 2) and could be clearly observed in histochemical preparations (Pishawikar, Unpublished). This difference may perhaps be due to the difference in the mode of contraction of the two types. The broad white fibres which appear to be adapted for quick and sudden but vigorous contraction, though for a short duration, may have some structural protein, as its major component as compared to the narrow red fibres. It has been shown (Chapter 2) that a large volume of the narrow fibres is occupied by sarcoplasm, embedded with numerous granules and fat droplets whereas in the broad white fibres, these inclusions are extremely sparse and the closely packed myofibrils form the major components of the contractile unit.

Nair (1952) reported highest protein content and lowest water content in the pectoralis major muscle of the domestic fowl, among the pectoralis major muscles of the various birds studied. It should, however, be noted that the pectoralis major muscle of the fowl consists of quick contracting, large pale fibres basically resembling the broad white fibres of the pigeon breast muscle. It is also likely that the water content of the broad white fibres of the pigeon breast muscle is less than that of the narrow red fibres. The difference in protein content in the two fibre types, may be associated with the mode of action of the contractile system. The considerably higher content of iron in the red fibres is suggestive of their high capacity for aerobic metabolism in sharp contrast to the broad white fibres.