CHAPTER 4

OBSERVATIONS ON THE FIBRE ARCHITECTURE OF THE PECTORALIS MAJOR MUSCLE OF THE PIGEON

It will be realised from the data so far presented that a basic understanding of the nature and disposition of the two distinct fibre components of the pectoralis major muscle as a whole is called for. The present study, therefore, is an attempt to provide a comprehensive picture of the pattern of fibre distribution and the nature of the metabolite load in the different regions of the muscle.

Material and Methods

In order to obtain uniformly well developed pectoralis major muscle, only fully grown wild pigeons, either shot or trapped from a single locality, were used throughout for the present study.

Mapping the distribution of the two types of fibres in the muscle:

Due to the bipectinate arrangement of the fasciculi, it was found convenient to divide the muscle into twelve regions, each one extending to 10 mm. in length along a hypothetical line, drawn midway between the origin of the muscle fasciculi and the centrally placed tendon (as shown in fig.4.1). From each of these regions at the level of the aforesaid line, fresh frozen transverse sections were cut on a freezing microtome. Subsequently, the sections were treated in the following manner. Transferring a fresh frozen section into distilled

water or even saline or isotonic sucrose solution resulted in uneven curling up of the section and, owing to the large size of the muscle piece handled, some difficulties encountered in the begining in obtaining a good entire section, were completely avoided by transferring the section directly into chilled 50 % glycerol and mounting on a microslide in the glycerol solution. In the preparations thus made, the arrangement of the fibres in the section, however large, was faithfully maintained with no distortions taking place. The glycerol impregnated sections were thus found to be ideal to manipulate. Moreover, the sections left in glycerol solution maintained at 0°C., can remain for more than a week without any perceptible deffect and thus could be utilised for future observations.

The desired region of the mounted section was projected on the screen of a microphotographic camera at a magnification of X47 and the photographic printing paper exposed directly to the image. 'Normal' bromide papers were found suitable. Using the sliding vernier on the stage of the microscope, continuous photographic records of the distribution of the broad fibres were made. From such records, by the method of random sampling, the mean value of the number of broad fibres per square millimeter was determined for every millimeter depth of the muscle. A survey of all the twelve regions was thus completed and a graph plotted illustrating the continuous distribution of broad fibres per square millimeter at a distance of every five millimeter along the line 0 - 120 (fig.4.1).

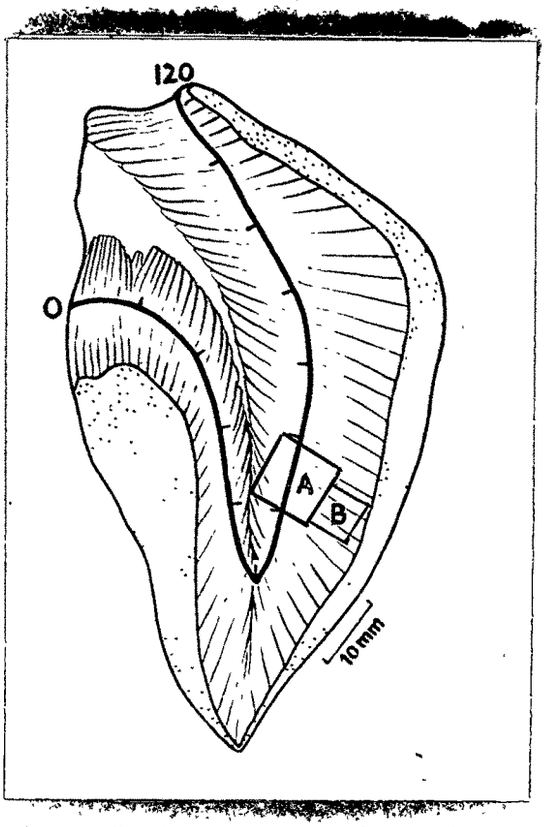

Fig.4.1 Dorsal view of the pectoralis major muscle of the pigeon showing the hypothetical line 0 - 120 along which the distribution of broad fibres is recorded in figure 4.2. The squares A and B indicate the regions of the muscle used for studying the variation in metabolite load and the structure at different depths of the muscle.

The lines demarcating the areas containing 30-50, 50-70, 70-90, 90-100, 100-120 and 120-140 and 120-150 broad fibres per square millimeter were drawn. The entire procedure was repeated on the pectoralis of three pigeons. The results obtained are summarised in a graphical representation as shown in figure 4.2. Since the individual variations in the pectoralis of different pigeons are considerable, the lines demarcating different areas in the figure are not claimed to be absolute,

but they do show the generalised pattern of the distribution of the broad fibres in the pectoralis major muscle of the pigeon.

For counting the broad as well as the narrow fibres in one and the same region, the same procedure was adopted, except that the image of the section projected on the screen was magnified to about 100 times and that the sections from the different typical regions of the muscle were used.

Estimation of fat and glycogen at different depths of the muscle:

For the sake of convenience, the region of the muscle (marked A in figure 4.1), on the posterior most end of the keel was used throughout. In this region the thickness of the muscle is only about 10 millimeters and the variation in the distribution of the broad fibres at the different depths of the muscle is gradual. From this region A, a piece of about 10 cubic millimeters in size was cut out for the estimation of glycogen and a somewhat bigger piece for the estimation of fat. From a region B lateral to A, another piece was cut out and transferred to the deep freeze chamber of the refrigerator and used later for studying the distribution of broad fibres in this region by the method already described.

The muscle piece cut out from region A, was mounted on the stage of a freezing microtome so as to obtain horizontal sections. It was frozen hard, the outermost epimysium was peeled off with a pointed forceps or sliced off by a superficial stroke of the microtome knife, and one millimeter thick

slices of the muscle were serially cut. Since, all these horizontally cut sections were of uniform and known thickness, each could be said to represent the nature of the muscle tissue at a known depth. The thickness of the sections were not actually measured since the microtome used was a brand new 'Sartorius' model and all the possible precautions, such as avoiding the fluctuations in the temperature, were taken so as to obtain sections of uniform and accurate thickness. Each frozen section was immediately transferred to a weighing bottle and dehydrated. The sections to be used for the estimation of glycogen were dehydrated in a vacuum-desicator, vacuumed at one atmosphere pressure and maintained at 0°C., whereas for fat extraction sections were dehydrated in an air-oven at 80°C., and finally in vacuum.

The dehydrated sections were weighed and their glycogen content was estimated according to the method of Kemp et al (1954). For the quantity of the muscle used for estimation (about 20-30 mg. per dry weight) it was found necessary to dilute the glycogen extract in the deproteinising solution to 10 ml. The colour developed was measured on the Beckman Spectrophotometer (DU model) at 520 μ. For the estimation of fat the dehydrated material was ground and after weighing, transferred to a fat extraction thimble. The fat was extracted with 1:1 ethanol-ether mixture. About 70-100 mg. of dehydrated muscle was used for each estimation.

The estimation of glycogen in the two types of fibres:

Small pieces from the breast muscle of a decapitated pigeon were cut out and dropped in previously chilled 80 % methanol and left undisturbed at -10° C for 24 hours. The fibres from the muscle, thus preserved, were teased out in methanol under a binocular dissection microscope with watch-maker's forceps. The two types of fibres were isolated and transferred into two separate containers containing methanol and fitted with air tight glass lids and stored in refrigerator. Sufficient number of fibres (which would yield about 2 - 5 mg. in dry weight were isolated and collected for each estimation. These fibres were then removed from the methanol solution, dehydrated in vacuum and weighed on a microbalance. Glycogen was estimated as already mentioned, by the micro-method of Kemp et al (1954).

Results

In each muscle fasciculus the broad fibres are mainly concentrated towards the periphery. This pattern is maintained throughout the muscle. The regions of the muscle where there are larger numbers of broad fibres, or lesser numbers of narrow fibres, the fasciculi have a smaller cross sectional area with broad fibres closely packed along their borders without any intervening narrow fibres. The regions where the narrow fibres are (comparatively) more abundant, the fasciculi are with larger cross sectional area. The number of broad fibres per square millimeter in the different regions of the muscle is shown

in figure 4.2. The relation of the number of broad fibres to (that of) the narrow ones per square millimeter is shown in figure 4.3. From both of these the number of broad fibres, as well as the narrow fibres per square millimeter in any region of the muscle, could be approximately determined.

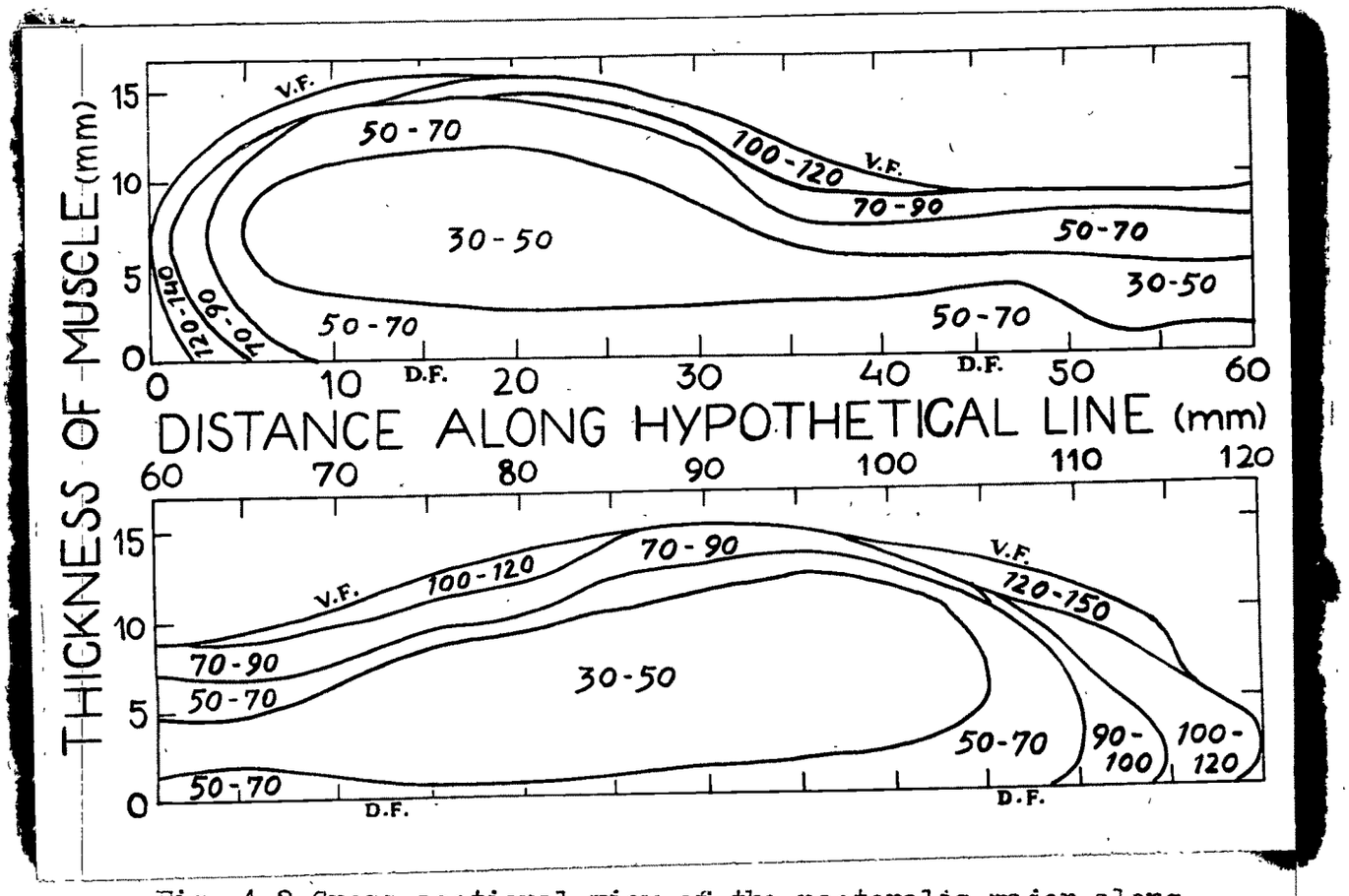

Fig. 4.2 Cross sectional view of the pectoralis major along the line 0-120 drawn in figure 4.1. The figures inside the chart show the number of broad fibres per square millimeter.
D.F., Dorsal face of the muscle; V.F., Ventral face of the muscle.

The variation in the metabolite load and the number of broad fibres per square millimeter at different depths of

the muscle is indicated in table no.1, while in figure 4.3 the same data are utilised to show the relation between the structure of the muscle and the metabolite load. The number of narrow fibres for the corresponding number of broad fibres was calculated by using the formula of regression line $y = -5.75x + 890.01$ in figure 4.3 and the ratio of the area occupied by the broad fibres to that of the narrow fibres in a square millimeter was determined by using the mean value of the diameter of these fibres. The diameter of the broad fibres is $69 \pm 14.00\ \mu$ (1000) and that of the narrow fibres - $30.11 \pm 6.56\ \mu$ (2000). The figures given in parentheses brackets indicate the number of fibres measured from the fresh frozen sections taken from the various regions of the muscle.

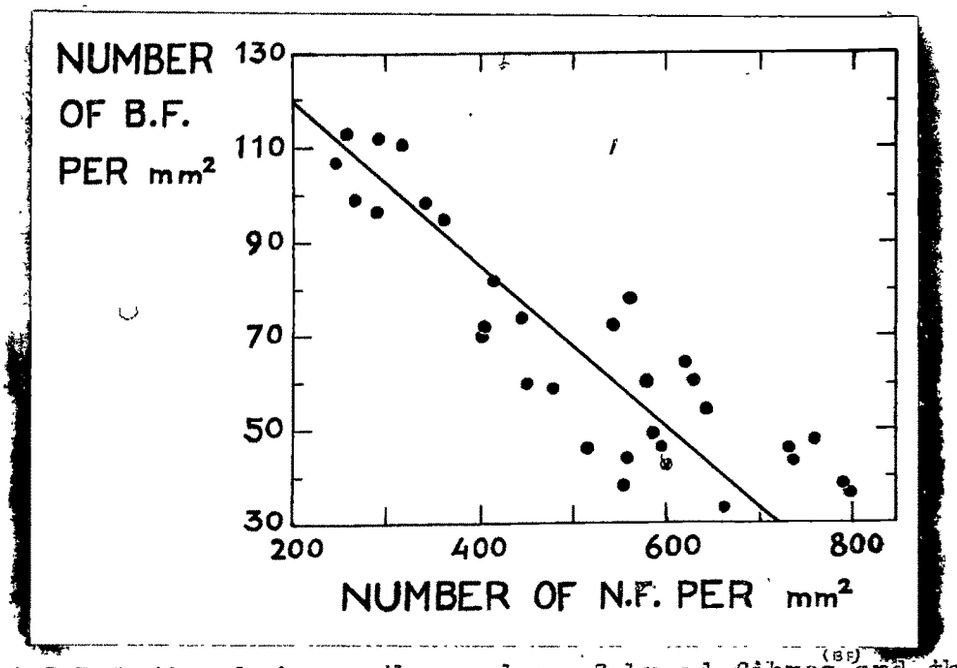

Fig. 4.3 Relation between the number of broad fibres (B.F.) and the number of narrow fibres (N.F.) per square millimeter of the muscle.

Table 1

The number of broad fibres per square millimeter and the percentage of fat and glycogen at different depths of the breast muscle of the pigeon

(The portion of the muscle marked A and B in figure 4.1 were used. The figures indicate the average values of six sets of readings.)

Depths of the muscle mm. (Starting from the ventral face)	Number of broad fibres per square mm. ± S.D.	Percentage per dry weight of the muscle ± S.D.	
		Glycogen	Fat
0 - 2	90 ± 14	3.655±0.28	10.289±1.94
2 - 4	63 ± 8	3.475±0.05	12.095±1.06
4 - 6	48 ± 3	3.102±0.13	14.632±1.75
6 - 8	51 ± 4	3.409±0.18	13.250±0.57
8 - 10	72 ± 9	3.588±0.24	11.743±0.57

The values of the glycogen content of the broad and narrow fibres, calculated on the dry weight of the muscle preserved in methanol are as fellows:

The broad fibres 10.240 ± 0.093 per cent

The narrow fibres 2.464 ± 0.311 per cent

(Each value is the mean of three readings)

Methanol removes much of the fat (mainly from the narrow fibres) and some of the amino acids.

Discussion

It is known that in many active muscles, the muscle fibres towards the periphery become larger in diameter and lighter in colour, compared to those in the interior. In such muscles, even in the individual fasciculus, the light fibres are situated towards the periphery. In the pigeon breast muscle the white broad fibres and the red narrow ones show a somewhat similar distribution pattern, but these fibres differ from the light and dark fibres of the other muscles in that they are sharply differentiated into two distinct types without intermediate forms. It has already been shown that the broad fibres are glycogen-loaded, having negligible amounts of mitochondria, whereas the narrow fibres are fat-loaded, having high mitochondrial content but poor in glycogen.

In a single muscle, uneven distribution of metabolites has been long realised. To reduce such localised variations to the minimum, customarily large pieces of muscle are utilised for the estimation of metabolites. Present work shows that in a muscle like the pectoralis major of a pigeon having heterogenous cellular elements, variations in metabolites in the different regions of the same muscle and even in a single fasciculus are quite large. Needless to say that what also? applies to glycogen and fat equally applies to other chemical constituents, such as lipase (George and Scaria, 1957), dehydrogenases (George and Scaria, 1958), ATP, creatine phosphate and some of the free amino acids (George and Pishawikar, unpublished),

in which two types of fibres differ.

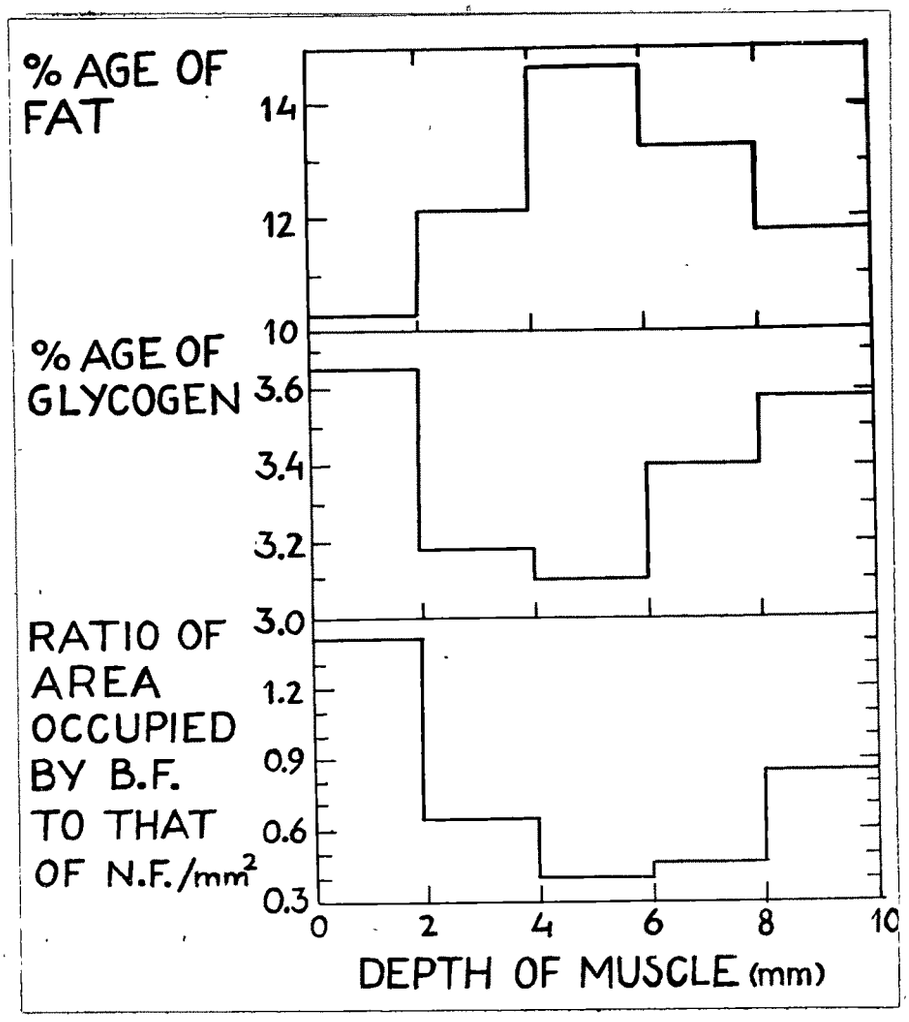

Fig. 4.4 Variation in the percentage of glycogen and fat in relation to the ratio of the area occupied by the broad fibres to that of the narrow fibres per sq.mm., at different depths of the muscle. The portion of the muscle marked A and B in figure 4.1 were used.

A general belief that the muscle fibres towards the periphery of the muscle are more active than those in the interior and that due to higher activity they increase in diameter, does not hold good (at least) in the case of the pectoralis

of the pigeon. Undoubtedly the red fibres of the pigeon breast muscle, due to their remarkably well developed enzyme systems, play a major role in effecting the sustained contraction of the muscle. In the white fibres on the other hand, the oxidative processes are not developed or developed only to a negligible extent, in that the dehydrogenase activity in these fibres, as shown by histochemical methods, is negligible or nil (George and Scaria, 1958). Nevertheless All the same, the white fibres are not inactive elements of the pigeon breast muscle. In the normal animal they show no sign of atrophy. A glycerinated white fibre of pigeon breast muscle contracts in the same manner as a glycerinated red fibre of the same muscle on the addition of ATP. The study on the reactions of these two types of fibres to experimentally induced disuse atrophy as has been already noted in the previous chapter has yielded significant results, which suggests the possibility of some differences in the mechanical properties of the two types of fibres in which case some physical factor may underlie the distribution pattern of the two types of fibres in the muscle.

Denny-Brown (1929), has shown that a single nerve in the breast muscle of the pigeon can innervate both, the red as well as the narrow fibres. Since the activity of these muscle fibres must be conditioned by the fundamentally different chemical systems in them, it is difficult to believe that the amount and the mode of activity performed by these two types of fibres is the same. In what exact manner the

white fibres contribute to the activity of the muscle is far from clear and, as a prelude to such an understanding, an extensive study of these fibres is essential. For such a study figure 4.2 can be an useful guide. Moreover, the method used in the present work to study the variation in the metabolite load in relation to the variation in the fibre [illegible] make up of the muscle, can be used for studying the distribution of various constituents, such as enzymes, amino acids, and minerals in the muscle.

Summary

1. The relative distribution pattern of the red and white muscle fibres in the breast muscle of the pigeon is presented/studied.

2. There exists a direct relation between the distribution of metabolites and (that of) the two types of fibres in the different regions of the muscle.

3. Quantitative estimation of glycogen in the two types of fibres, confirms the higher concentration of glycogen in the white fibres.