CHAPTER 2

STRUCTURE OF THE PECTORALIS MAJOR OF THE PIGEON

In the first chapter it was pointed out that the pectoralis major of the pigeon is a mixed type of muscle. Mixed muscles are commonly found in most of the higher vertebrates including man. Though the structural and biochemical properties of red and white muscles have been extensively studied, very little of such work has been done on red and white fibres of mixed muscles. This is primarily because the refined histochemical procedures and micromethods for quantitative and qualitative determination of the chemical constituents of cells have been developed only recently. Moreover, there has been a tendency among anatomists and physiologists to equate the red and white fibres of a mixed muscle with those of the red and white muscles, with the result that the former have remained neglected and to a considerable extent obscure. On the other hand, since the red and white fibres of mixed muscles coexist in a single structural and functional system under identical conditions and the differences between them could be brought out with better advantage and should thus form excellent material for studying the diversities of the muscular tissue. Therefore, the need for extensive studies on red and white fibres of mixed muscles needs no special emphasis.

My interest in the mixed muscle has been chiefly

enhanced by the fact, as will be evident from the later part of this work, that the most important flight muscle, the pectoralis major, (in majority of birds) is a mixed muscle and the study of its two component types of fibres is pertinent to (get an insight into) the mechanism as well as energetics of flight. Again, the pigeon pectoralis in which the two types of fibres, namely red and white, are extremely specialised, and sharply defined divergent types with no intermediate forms, seemed to me worthy of special consideration.

Material and Methods

Several pigeons, laboratory bred as well as wild, were used. The pigeon was decapitated and small pieces of muscle were cut out from the pectoralis major and fixed in various fixatives, to study the structural details and distribution of glycogen, fat and mitochondria in the two types of fibres.

Histological structure:

For studying the structural details of the two types of fibres, some muscle pieces were fixed in Zenker's fluid (Guyer, 1930) and after embedding in paraffin, sections were cut at 10 μ and stained with haematoxylin. Other pieces, fixed in 10 % neutralised formalin for 24 hours, were washed in running water for 1 hour and embedded in 25 % gelatin after passing through graded gelatin solutions, according to the method of Clark (1947). The gelatin blocks were hardened

in formalin for 24 hours and frozen sections — l.s. as well as t.s. — were cut at 15 μ . Unstained sections mounted in distilled water were studied under phase contrast microscope.

Fat:

For the demonstration of fat, muscle pieces fixed in Baker's Calcium-Formol (1 gm of calcium chloride per 100 c.c. of 10 % formalin) and post-chromated for 18 hours at room temperature and 24 hours at 60° C in potassium dichromate solution (Baker, 1946) were embedded in gelatin and sectioned according to the method of Clark (1947). Sections were floated in 1 % gelatin over microslide and after draining off the excess solution, the mounted sections were exposed to formalin vapour in Couplin jar to effect perfect adhesion of the sections on the microslides. The sections were stained with Sudan Black B and mounted in glycerine jelly.

Mitochondria:

Mitochondria were demonstrated according to the method of Altmann (1890). Thin strips of muscle (pieces) were stretched on a filter paper and fixed in Altmann's fluid (equal parts of 1 % osmic acid and 2.5 % potassium dichromate). The paraffin-embedded sections were cut at 3 μ and stained with Aniline-Fuschin (20 % Acid Fuschin in saturated solution of aniline) on slide heated to steaming and differentiated with alcoholic picric acid (water 65, alcohol saturated with picric acid 35) on slide heated to steaming. Sections were

rapidly dehydrated and after clearing in xylol were mounted in Gum damar. Since bacteria are also stained by this method, throughout the process all the precautions as proposed by Gray (1954) were taken to avoid the settling of bacteria on the sections.

Glycogen:

For the demonstration of glycogen, thin strips of muscle were fixed in Rossmann's fluid (alcohol saturated with picric acid 90 ; formalin 10) maintained at 0° C. The fixed muscle pieces were dehydrated in series of alcoholic grades and cleared in toluol. In both the cases, previously chilled reagents were used. After embedding in paraffin, t.s. as well l.s. of muscle pieces were cut. The sections, stretched by floating on warm 70 % alcohol, were mounted directly on a clean microslide and were allowed to dry at room temperature. No adhesive was used for attaching the sections to slides. After removing paraffin from the sections in a normal manner, all the slides were transferred in a jar of absolute alcohol. Few slides to be used as controls were brought down to water through graded series of alcohols and incubated in two changes of saliva for 30 minutes at room temperature, to make the sections free from glycogen. Control slides were then dehydrated and after transferring them back to absolute alcohol, control as well as sample slides were coated with celloidin. Sections were stained with Best Carmine, then dehydrated and mounted in Gum damar in the usual manner.

Blood capillaries:

A Hypodermic needle attached to a glass syringe containing a solution of India ink was introduced into the body cavity of an anesthetised pigeon, through a slit made near the posterior margin of the sternum and the ink was injected into the left ventricle of the heart. After allowing the ink to circulate for a short time, the skin over the pectoralis major was removed and the entire pigeon was immersed in 10 % neutral formalin for an hour, at the end of which small pieces of pectoralis were cut out and transferred in fresh formalin solution and fixed for 24 hours. Muscle pieces were embedded in gelatin and sections, t.s. as well as l.s. were cut according to the method of Clark (1947).

Results

Histological structure:

Figure 2.1 shows the two types of fibres as they appear in fresh frozen sections. The granular inclusions are clearly visible in the narrow fibres whereas broad fibres are devoid of them. Few granules seen in the broad fibres are those displaced from the narrow fibres.

Figure 2.2 shows the haematoxylin stained transverse sections fixed in Zenker's fluid. All the broad fibres are intensely stained dark, giving a mosaic appearance. Some of the narrow fibres are faintly (or hardly) stained. Note the reduction in diameter of the fibres in this figure (fig.2.2)

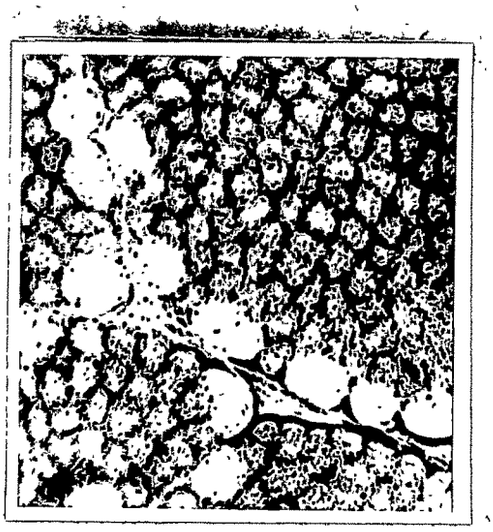

Fig.2.1 Microphotograph of t.s. of untreated pectoralis of the pigeon
×180

Fig.2.2 Microphotograph of t.s. of the fixed muscle. Haematoxylin
×180

in comparision to that in figure 2.1. Even after making allowance for shrinkage due to fixation, dehydration and paraffin embedding, it can be surmised that the original diameter of the muscle fibres in figure 2.2 must be (comparatively) smaller than that in figure 2.1. Figure 2.1 represents the section of the pectoralis of a wild pigeon, whereas figure 2.2, that of a laboratory bred one. From this observation, which was also verified by other observations made on several fresh frozen sections of the pectoralis of the wild and the laboratory-bred pigeons, it can be said that in comparision to wild pigeons, laboratory-bred pigeons have fibres with smaller diameter, but it appears that in both the cases, however, the relative difference between the diameter of the two types of fibres remains the same, i.e., in the laboratory-bred pigeon both the types of fibres are proportionately reduced in diameter.

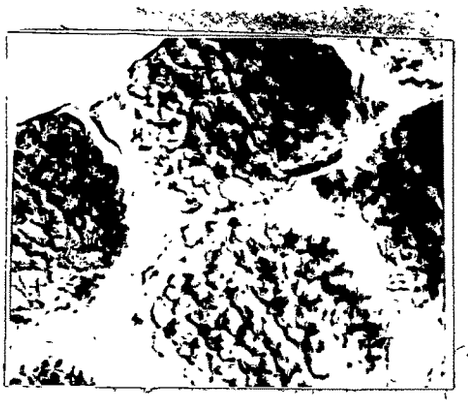

Fig.2.3 High power microphotograph of narrow fibres.- t.s. Haematoxylin
x 1700

Fig.2.4 High power microphotograph of a broad fibre. - t.s. Haematoxylin
x 1700

The interstitial cytoplasm in the narrow fibres forms a wide and irregular network extending inbetween the muscle columns. This 'intercolumnar' cytoplasm contains coarse inclusions with the result that in transverse sections of these fibres (fig.2.3) the areas of Cohnheim* appear very prominent but irregular. On the other hand, in the broad fibres the 'intercolumnar' cytoplasm is less coarse and forms narrow regular mesh and hence in t.s. figure 2.4 the areas of Cohnheim appear narrow and regular.

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* In current literature there is some confusion about the identity of Cohnheim's areas or fields. This subject has been amply reviewed by Bennett and Porter (1953) and Tiggs (1955). They explain Cohnheim's areas as follows: The cytoplasmic network in muscle fibres extends between the bundles of myofibrils - such bundles of myofibrils go to form (Kolliker's) muscle columns. In c.s. these muscle columns appear as areas of Cohnheim. I am in full agreement with their interpretation of the classical works of Cohnheim (1865) and Kolliker (1866).

Fig.2.5 Phase contrast micrpphotograph of l.s. showing the two types of fibres of the pigeon pectoralis - unstained - ×1200

The coarse inclusions in the narrow fibres are chiefly mitochondria and fat globules, due to which cross striations in these fibres appear poorly defined (fig.2.5). The broad fibres, being devoid of such inclusions, show cross striations quite clearly (fig.2.5).

Fat:

Knoll(1891), Denny-Brown (1929) and George and Jyoti (1955a) observed greater fat inclusions in narrow fibres (compared to the broad ones of the pigeon pectoralis. These observations were based on the sections of formalin-fixed material stained with osmic acid, Scharlach R or Sudan III (Knoll, 1891; Denny-Brown, 1929) or teased out fresh muscle fibres, stained with Acetocarbol Sudan III (George and Jyoti, 1955a). Except for osmic acid, all these stains colour only neutral fats, whereas staining with osmic acid has several limitations

and is not always specific for fat (Lison,1936; Cain,1950). Moreover, though fixation of tissue in formalin is adequate enough to preserve neutral fats, it is unsatisfactory for the preservation of phospholipids. In the present work Baker's method (1946) was employed for preservation of fat. Calcium chloride in calcium-formol prevents the emulsification of phospholipids; thus the latter is prevented from diffusing in the fixative, whereas postchromation with potassium dichromate renders fat resistent to some extent to even some of the fat solvents like alcohol (Baker, 1944,'46 and '56).

Fig. 2.6 T.S. of a fasciculus from the pectoralis of pigeon
Sudan Black B
×120

With Sudan Black B, I-bands, mitochondria and fat were demonstrated in muscular tissue by Demsey, Wislocki and Singer (1946), Baker (1944,'45). With the method used in the present work no fat could be demonstrated in the broad fibres. In narrow fibres the fat droplets were coloured deep blue-black and arranged in longitudinal streaks along the long axis of the muscle fibres, but they appeared to bear no relation with any transverse band of muscle fibre. The mitochondria* were stained light grey.

Fig. 2.7 L.S. of the narrow fibres of the pigeon pectoralis
2.8 L.S. of the broad fibre of the pigeon pectoralis
Aniline Fuschin - Picric acid
×1200

From the preparations made to demonstrate mitochondria* it was observed that the narrow red fibres are loaded with mitochondria (fig.2.7), which also are arranged

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* Retzins (1890) suggested the name sarcosomes for the granular bodies, earlier described by Henle (1841) and Kolliker (1857) as lying between the myofibrils of muscle fibres, to distinguish them from fat droplets. Chappell and Perry (1953) proposed the usage of the term sarcosomes, in its original sense as a general term to describe the lipoprotein granules, which lie between the myofibrils. But the larger lipoprotein granules of muscle, if qualitatively similar to those from the other tissues like liver, this term could include also mitochondria. The granules in the pigeon breast muscle since found to be very similar to the mitochondria of liver and kidney in their behaviour with the Krebs cycle intermediates, were referred to by them (Chappell and Perry, 1953) as mitochondria. Recently the term sarcosomes has been used by Cleland and Slater (1953) to describe these bigger granules in muscle fibres. Kityakara and Harman (1953), however, have used the term sarcosomes to describe the smaller granules found in the pigeon breast muscle homogenates, which they consider distinct from mitochondria. In the present work the term mitochondria will be used in the same sense as has been suggested by Chappell and Perry (1953).

in loggitudinal streaks like fat globules, but unlike the latter the mitochondria are in close association with myofibrils, probably at the level of A-band. In the broad fibres the mitochondrial content is negligible or nil (fig.2.8), though the presence in these fibres of granules too small to be resolved under light microscope is not ruled out.

Sections stained to demostrate glycogen show intense staining in the broad fibres, whereas the narrow fibres are stained light (figs. 2.9,2.10). In control sections treated with saliva, the muscle fibres remained unstained. Glycogen, when demonstrated by paraffin method,

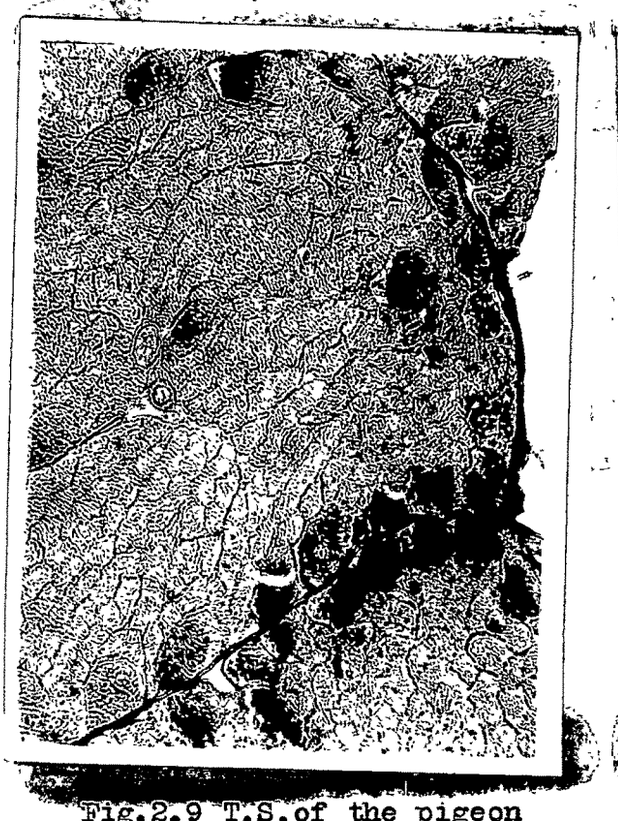

Fig.2.9 T.S.of the pigeon pectoralis - Best Carmine
×250

Fig.2.10 L.S.of the pigeon pectoralis - Best Carmine
×250

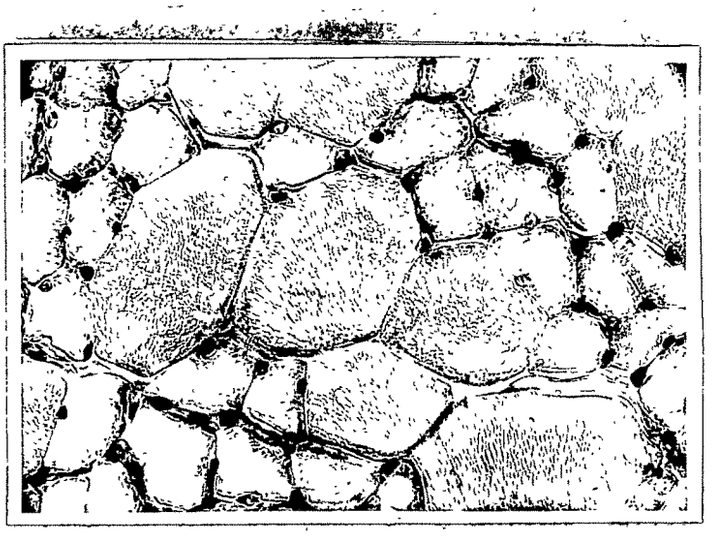

Fig.2.11 T.S. of the pigeon pectoralis injected with India ink to demonstrate blood capillaries ×430

many times appear polarised towards one margin of the cell, but with adequate precautions it could be minimised, as can be seen from figures 2.9 and 2.10. Glycogen appears to be uniformly distributed throughout the substance of the muscle fibres.

Figure 2.11 shows the distribution of capillaries as seen in cross section. In the fibres lying on the periphery of the fasciculus, the free border which is not in contact with any muscle fibre does not adjoin any blood capillary and since the broad fibres are mostly situated on the periphery of the fasciculus inspite of their bigger size, is surrounded by a few capillaries, on the average not more than those surrounding the narrow fibre.

Discussion

The coarseness of the sarcoplasm is much more

pronounced in the fibres of the avian flight muscles than in those of the mammalian muscles. In structural details the red fibres in the pectoralis of the pigeon, represent the typically characteristic muscle fibre of avian flight muscles, while, on the other hand, the white fibres resemble to a certain extent fibres of the mammalian muscle.

Differential staining of the two types of fibres with haematoxylin presents a complex problem. Denny-Brown (1929) observed that when treated with haematoxylin the narrow dark fibres take up more stain than the broad ones, while my results are to the contrary. The differences in fixing fluids or perhaps in the composition of the staining solutions might account for these divergent results. But nothing can be said with certainty because no information is available about the fixing fluid or the staining solution used by Denny-Brown (1929). However, it does point to the fact that even after dehydration in alcohol and the treatment with paraffin and xylol, some chemical difference in the two types of fibres does persist. Differential staining might be a direct result of the differences in the structural proteins of the two types of fibres.

Since Knoll and Haur (1892) first described the disappearance of fat in the pigeon breast muscle in starvation and which was later confirmed by Bell (1911), the fat globules in muscle fibres were taken as reserve store. Denny-Brown stimulated to fatigue the gastrocnemius and soleus of cat with the blood supply to the muscle cut off, could find no

evidence of disappearance of fat globules. Similar conclusion was (arrived at) earlier by Bell (1911). There is increasing evidence ~~at present showing~~ that fat can be utilised directly in the muscle (Krogh and Lindhard, 1920; Gemill, 1940, 1942 ; Weis-Fogh, 1952). George and Jyoti (1955a) demonstrated the disappearance of fat globules in the narrow fibres of the pigeon breast muscle after flight. George and Scaria (1958) histochemically demonstrated high lipase activity in the red fibres of the pigeon breast muscle. These findings suggest that the fatty acid oxidative system is well developed in the narrow red fibres of the pigeon breast muscle and that the fat deposition in these fibres, apart from being a reserve store, can also serve as energy source during long and sustained activity.

Chappell and Perry (1953) reported that in the breast muscle of the pigeon the mitochondria constitute about 20 % of the total nitrogen of the muscle. The present work shows that the broad fibres which make up not less than one-fifth the volume of the muscle are poor in mitochondria. Evidently, the main bulk of the mitochondria obtained by Chappell and Perry (1953) must have come from the narrow fibres. Studies on the cyclophorase preparation of skeletal muscle by Paul and Sperling (1956), show that there exists a correlation between granule count and oxidative activity. Chappell and Perry (1953) correlated the low oxidative activity of the rabbit muscle suspension with low granule content and suggested that this tissue (rabbit muscle)

must be largely anaerobic in function. From these it can be said that in the red fibres of the pigeon breast muscle, oxidative processes are well developed, whereas in broad fibres they are poorly developed.

The white fibres, with high glycogen store and insignificant amount of fat, seem to rely wholly on their glycogen store for the production of energy, which clearly means that in a single muscle two types of cells, viz., glycogen-loaded and ~~the~~ fat-loaded, ~~ones~~ have been developed for storing the respective fuels, so that it can efficiently utilise both. It is indicated that they differ in their enzyme systems, particularly those involved in the building up and subsequent utilisation of the two types of metabolites. This assumption finds support in the fact that the two types of fibres markedly differ in their mitochondrial content.

Due to their bigger size, the surface area per unit volume of broad fibre is considerably less than that in narrow fibres. All the more, when the number of capillaries surrounding a broad fibre is about the same as those around the narrow one, it can be concluded that the narrow fibre (compared to the broad one) is supplied with oxygen at higher rate.

From all these observations it is evident that the pigeon breast muscle is made up of two distinct components, ~~the~~ whose morphological characteristics ~~of which~~ seem to correlate with function. With poor mitochondrial content associated with high glycogen load, lack of myoglobin and smaller surface area, the

white broad fibres seem to be largely for functioning anaerobically. On the other hand, high mitochondrial content, together with a large store of fat, presence of myoglobin and greater surface area afforded in the narrow fibres suggest that they possess a well organised oxidative mechanism.

SUMMARY

Two types of fibres— the red narrow and white broad in the pectoralis major muscle of the pigeon are described with respect to their microscopic structure, fat, glycogen and mitochondrial inclusions and blood supply.