CHAPTER 6

SOME HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON THE STRUCTURE OF THE PECTORALIS MAJOR MUSCLE OF BIRDS

In the earlier part of this work I have recorded some observations on the structure of the pectoralis muscle in a few birds and suggested the possibility of some relationship between the structure of the muscle and the mode of flight. Following this line of investigation, a comparative study of the diameter of the fibres of this muscle in birds exhibiting different modes of flight was made and the present discourse deals with certain structural peculiarities of the pectoralis in relation to function in some birds.

Material and Methods

This work was undertaken simultaneously with the work on the diameter of the muscle fibres in the pectoralis, presented in the previous chapter, and the observations presented here are based on birds used in the former work.

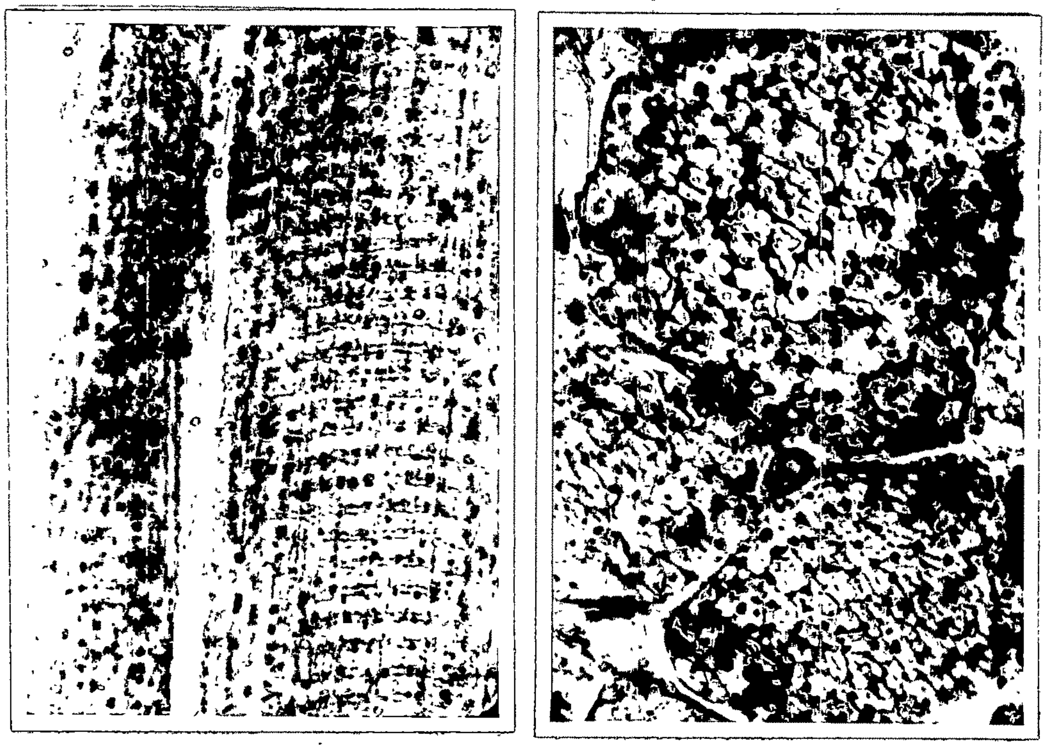
General observations on the structure of the muscle were made from fresh frozen sections as well as from frozen sections of muscle pieces fixed in 10% neutralised formalin.

Studies on the distribution of fat, glycogen and mitochondria in the pectoralis were confined ~~only~~ to the dove, parakeet, kingfisher, kite, and fowl. (not needed: which were procured alive.) The animals were decapitated and small pieces of muscle were cut out and fixed in appropriate fixatives. For the demonstration of fat, the method of Baker (1946), described in the chapter 2, was employed, using Sudan Black B. For the demonstration of glycogen, thin strips of muscle fixed in Rossman's fluid (at -10°C) were embedded in paraffin and sections were stained with Best Carmine. Some sections were also stained by PAS technique, as described by Pearse (1954). The control sections were incubated in saliva. Prior to staining all sections, control as well as sample, were coated with celloidin. Altmann's method was adopted for the demonstration of mitochondria.

Observations

Histological observations showed that in all actively flying birds the pectoralis major is mainly composed of red fibres. In most of the birds exhibiting shooting or flapping type of flight the pectoralis contains some fibres with a lighter shade of red, and in between the light and dark fibres, all the intermediate types of fibres are found. The notable exception to this is the dove, which, like the pigeon, has red and white fibres quite distinct and without any intermediate type of fibres. In the mixed type of pectoralis of these birds the lighter fibres are usually confined to the small superficial layer of the muscle; the main bulk of the muscle in the deeper region is totally devoid of them. But in some birds, like the cattle egret, kingfisher, and dove, the pectoralis contains lighter fibres distributed throughout the substance of the muscle, though in the superficial layer they are comparatively more in number. The distribution pattern of white fibres in the dove is most striking and resembles essentially that of the pigeon (fig.6.1). Except in the dove, where the difference in diameter between the red and white fibres is sharp, in the mixed type of pectoralis an absolute relation between size and colour of fibres is difficult to establish. Observations on the pectoralis of several birds give the impression that broader fibers tend to be lighter in colour, but occasionally in transverse section some of the narrow fibres show lighter colour. In contrast to the birds with mixed type of pectoralis, in some birds, like the bee-eater and swallow, the muscle fibres are uniformly dark throughout. ~~the muscle~~.

Fig.6.1 T.S. of the dove pectoralis. Haematoxylin. (The broad and narrow fibres, like those ~~of~~ in the pigeon pectoralis, are stained differentially with Haematoxylin) x430



Figs. 6.2 & 6.3 L.S. and T.S. of the kite pectoralis (superficial layer)
Sudan Black B
x1700

In the kite and vulture, the <u>pectoralis major</u> can be divided into superficial and deeper parts according to the nature of the muscle fibres. The superficial part which forms the main bulk of the muscle contains only dark fibres, whereas the deeper layer contains only light fibres, but the diameter of the fibres in the two regions does not show any significant difference. The separation between these two parts is complete in the vulture but incomplete in the kite. The pectoralis of the hawk differs from that of the kite and the vulture in that it is the mixed type, with light and dark fibres distributed throughout the substance of the muscle.

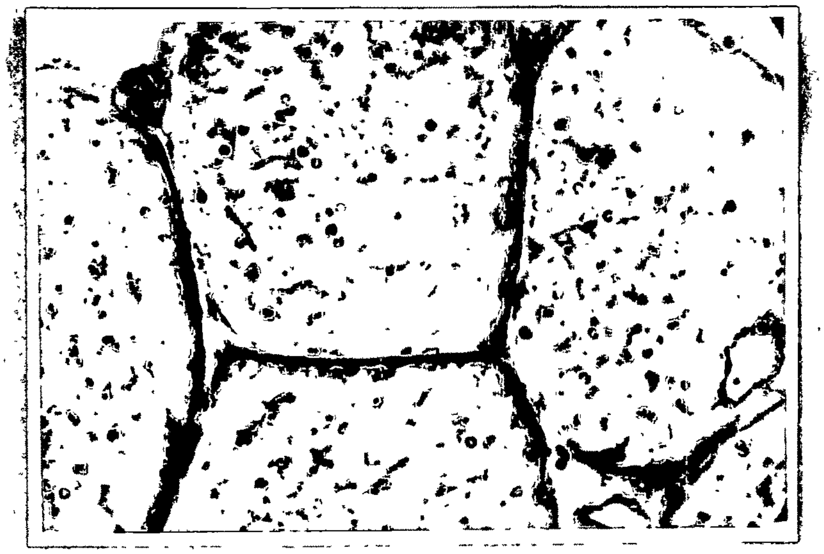


Fig. 6.4 T.S. of the kite pectoralis (deep layer)
Sudan Black B.

In the fowl and partridge, all muscle fibres are pale in colour, even though there is a great variation in the diameter of the fibres. ~~Out~~ of two specimens of partridge dissected, ~~in~~ one ~~specimen~~, in the deeper layer of the pectoralis a small patch towards the anterior region showed a tinge of red, the micro-scopic examination of which showed the presence of narrow dark fibres and the light ones.

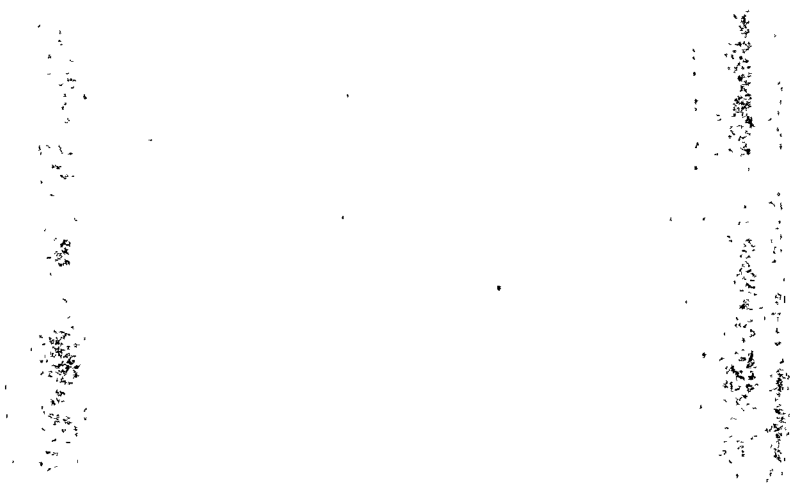


Fig.6.5 T.S. of the parakeet pectoralis-Sudan Black B-
(superficial layer)

Fig.6.6 T.S. of the dove pectoralis-Sudan Black B- x1700

The colour of the muscle fibres is mainly due to the presence of the respiratory pigment myoglobin and granular inclusions (mitochondria and fat globules). When stained with Sudan Black B, the fat globules appear dark whereas the mitochondria are stained lightly. All the dark fibres in the pectoralis of birds contain heavy granular inclusions arranged in longitudinal streaks in between (Kolliker's) muscle columns(fig. 6.2), thus rendering the latter very conspicuous (figs.6.3,6.5 and 6.6), and the fibres in the pectoralis of the kite are no exception to this (figs. 6.2 and 6.3). The contradicting observations by George and Jyoti (1955a) and George and Naik(1956), namely, that the fibres in the pectoralis of kite are devoid of heavy granular inclusions, were primarily due to the fact that their observations on the granular inclusions were confined to the teased out muscle fibres, stained with Aceto-Carbol Sudan III. The latter does not reveal all the fat, and, moreover, large amounts of interstitial connective tissue and fat in the pector[redacted] kite, renders the observation of sarcoplasmic inclusions in teased out preparations practically impossible. The muscle columns are less conspicuous in the light fibres of the mixed type of pectoralis. Due to decrease in the granular deposition, the treatment of Sudan Black B leaves the fibres of the fowl (fig.6.7) and partridge and the white fibres of the dove (fig.6.6), practically unstained.

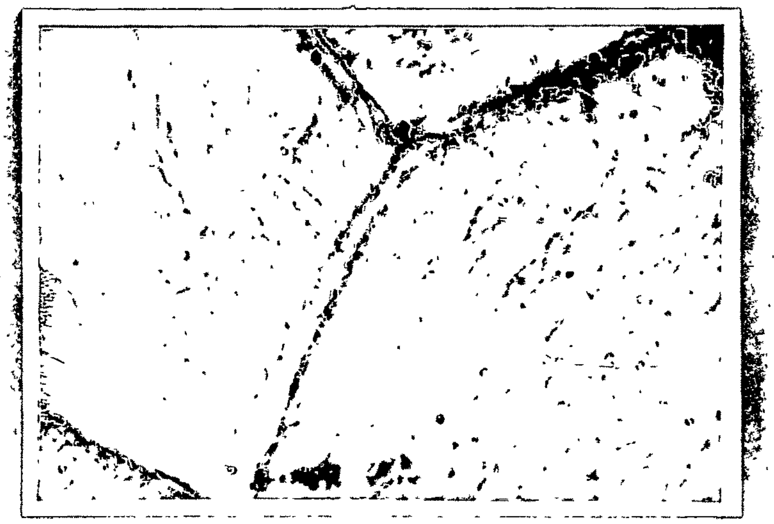


Fig.6.7 T.S. of the fowl pectoralis - Sudan Black B - x1700

Observations on the sections stained with Sudan Black B and the preparation made by Altmann's method show that the dark fibres of the pectoralis of birds contain a larger number of mitochondria while they are comparatively less in the light fibres. In The fibres of the fowl and partridge pectoralis and the white fibres in the pectoralis of the dove, mitochondria are hardly detectable under the microscope. Paul and Sperling (1952) in the cyclophorase preparation of the fowl breast muscle observed few or no mitochondria. The electron microscope photograph of this muscle by Bennett and Porter (1953), however, shows some sarcosomes quite clearly.

Fig.6.8 T.S.of the parakeet pectoralis - Best Carmine and Haematoxylin - X430

Histological study of glycogen shows that in the superficial part of the pectoralis of the kite, glycogen is uniformly distributed in all the fibres. Similarly, in the pectoralis of parakeet, where few light fibres which are present in the superficial layer, differ from the dark fibres only slightly, glycogen being uniformly distributed in all the fibres (fig.6.8). In the pectoralis of kingfisher the light and dark fibres are better differentiated and the fibres contain varying amounts of glycogen (fig.6.9). In this bird, though glycogen tends to accumulate more in broad fibres, the relation between the size of the fibres and glycogen content is uncertain.

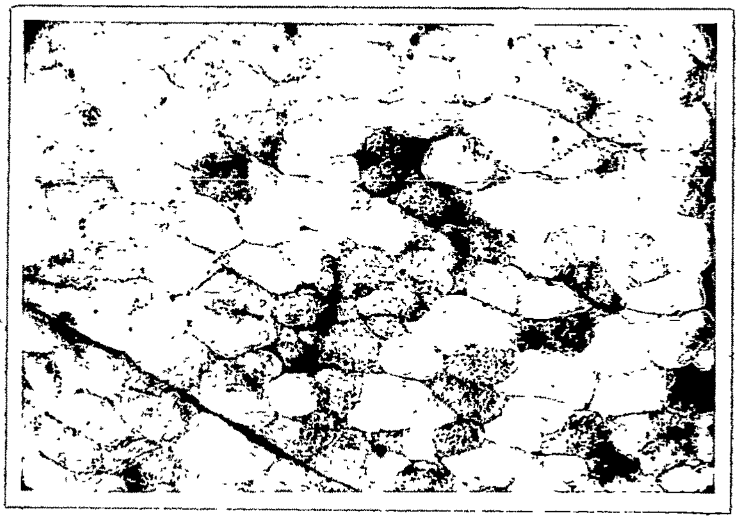


Fig. 6.9 T.S. of the kingfisher pectoralis
Periodic acid - Schiff, Haemalum x 180

In the dove pectoralis the glycogen is concentrated in large amounts in the broad fibres whereas in the narrow fibres it is comparatively much less (fig. 6.10).

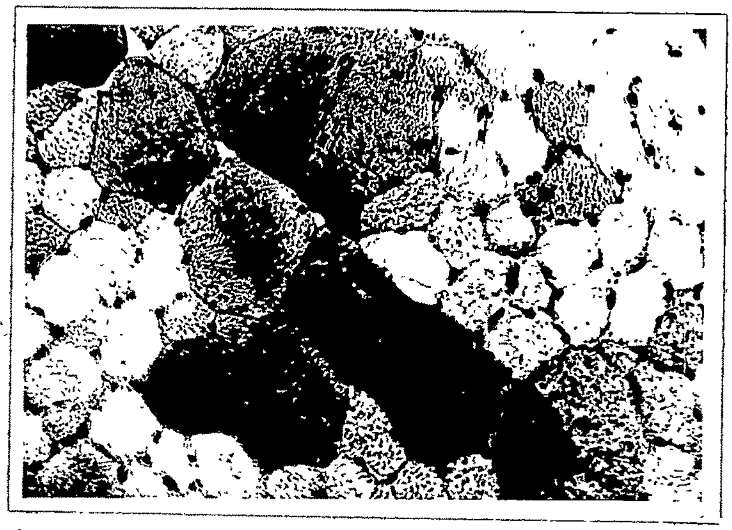


Fig. 6.10 T.S. of the dove pectoralis
Periodic acid - Schiff, Haemalum x 430

Discussion

In mixed type of muscle, the main differences between the light and dark fibres are of the mitochondrial density and the fibre diameter. Paul and Sperling (1952) have shown that the mitochondrial density bears a direct relation with ' cyclophorase activity'. The reduction in the activity of succinic dehydrogenase with increase in fibre diameter in the mixed muscles of the rat (Nachmias and Padykula, 1958), the bat (George, Shusheela and Scaria,1958) and certain muscles of reptiles (Shah,1958) has been noted. It appears that as differences in diameter between the dark and light fibres increase, the associated differences in their structural and physiological make up, also increases. The broad white fibres of the pectoralis of the dove contain negligible amounts of mitochondria, whereas the narrow fibres are loaded with them. Similar observations have been made on the pectoralis of the pigeon. It has also been shown that in the narrow red fibres of the pigeon and dove the dehydrogenases activity is very high compared to the broad white fibres, in which it is almost negligible, (George and Scaria, 1958).

With increase in the diameter of the fibres the rate of diffusion of gases between blood capillaries and the middle part of the fibre should decrease. This could be compensated for, were the blood capillaries surrounding the broad fibres more numerous than those surrounding the narrow fibres. But even in the pectoralis of pigeon, the

the number of blood capillaries surrounding the broad fibres is about the same as those around the narrow fibres.

From the above, it can be concluded that the narrow fibre is adapted for increased oxidative processes. It has also been shown that depending on the size of the bird and the mode of flight the average diameter of the pectoralis muscle fibres of the birds has to be of some optimum value. So, it is suggested that decrease in the diameter of some fibres has to be compensated for by increase in the diameter of others. But whether this system gives an advantage to mixed muscles over others having only one type of fibres is questionable. Unless the inherent oxygen requirement in light fibres is less due to some additional mechanical advantage associated with increase in diameter, it is difficult to understand how these fibres can cope up with the dark fibres in which the oxidative processes are better developed.

The works of Green (1951) and George and Jyoti (1955 a and b) have shown that in the pectoralis of birds, fat is oxidised directly during long and sustained activity and that the fat in muscle is now to be looked upon as a reserve store which can be utilised for the production of energy. George and Scaria (1958) histochemically demonstrated higher lipase activity in the narrow fibres than in the broad fibres in the pectoralis of the pigeon and dove. It appears that the processes leading to the synthesis and oxidation of fat are better developed in the narrow fibres than the broad ones. On the other hand it has been shown that there is a higher concentration of glycogen in the broad white fibres than the narrow red fibres of the pectoralis of the pigeon. ~~Same is~~ the condition in the pectoralis of the dove. In other birds with mixed type of pectoralis no definite relation between the size of the fibres and glycogen content could be found. Nachmias and Padycula (1958) reported that in biceps of albino rats the glycogen tends to be more abundant in the large fibres but they could not find a positive consistent correlation between the size of the fibres and the glycogen content. In most of the mixed muscles there is a great deal of overlapping between the dark and light fibres and thus apart from the labile nature of the muscle glycogen, the difficulty in identifying the dark and light fibres in sections treated with various reagents during the process of staining, makes the correlation between the distribution of glycogen and fibre diameter, difficult to establish.

This work brings forward once again the general relationship that exists between structure and function in animal tissues. In fowl and partridge the pectoralis which is little used, contains fibres poor in mitochondria. On the other hand, in all the active birds the pectoralis is primarily made up of dark fibres rich in mitochondria.

The division of the pectoralis in the kite and vulture presents an interesting problem. The complete or incomplete separation of the pectoralis major into superficial and deeper layers has been noted in several birds (Fisher, 1946; Fisher and Goodman, 1955; Jollie, 1957). It is not known whether, histologically, these layers (described by the latter workers) resemble those in the pectoralis of kite. Fisher (1946), from his work on the locomotor apparatus of vultures, suggested the possibility that this layer was a factor in the forward motion of the wing, to raise the anterior end of the wing and increase the angle of incidence when the bird is gaining altitude. Since the superficial layer is physiologically more active than the deeper layer, it is quite possible that these two layers of the pectoralis of the kite and vulture differ in their mode of action.

Summary

Structural differences based on fat, glycogen and mitochondrial content in the muscle fibres of the *pectoralis major* of some birds have been reported and an attempt has been made to correlate these differences with fibre diameter.