HETEROGENEITY IN THE FIBRE COMPOSITION OF THE FLIGHT MUSCLES OF DRAGONFLIES (ODONATA)

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I HAVE read the letter, "Occurrence of giant muscle fibres in the flight muscle of *Pantala flavescens*" by Bhat[1] which appeared recently in *Current Science*, with the interest it deserves. The work reported was carried out under my direction in my former laboratory at Baroda, India, during his tenure as a research student. It is unfortunate that he chose to get it published without my knowledge. In doing so, he has presented wrong and misleading interpretations of the observations made. I should therefore like to present to your readers a discussion on the problem of heterogeneity in the fibre composition of the flight muscles of dragonflies.

Bhat[1] reports the occurrence in the dragonfly flight muscle some "giant fibres" ranging in diameter from 40 to 70 μ among the more numerous "normal fibres" of 20–30 μ in diameter. In explaining the origin of these so-called "giant fibres" he states thus: "Muscle hypertrophy generally results from continuous and prolonged exercise of the organ to cope up with high energy demand. *Pantala flavescens* is an efficient flier and remains in the air for long periods while swarming, thus giving continuous exercise to its flight muscle. This could probably account for the presence of giant fibres in some species while they are absent in other species of dragonflies."

At the outset, it must be said that it is incorrect and misleading to call these large fibres "giant fibres" and regard them as abnormal or hypertrophied. They are distinctly different from the classical giant fibres that constitute the fibrillar (asynchronous) type of flight muscle of Diptera, which may be, for instance, as large as 1800 μ in diameter (*e.g.*, *Rutilia potina*).[2] The dragonfly flight muscle, on the other hand, is of the primitive tubular (synchronous) type. If the large fibres in the flight muscle of *Pantala flavescens* did really originate as a result of muscular hypertrophy due to continuous exercise, then, all the fibres in the muscle should be large and should occur only in the actively flying adult. But these fibres are considerably fewer in number than the small fibres and we have no information as to exactly when they get differentiated. In this context, it should be mentioned that two types of fibre, Type 1: red, narrow (30 μ in diameter) and Type 2: white, broad (70 μ in diameter) of the pigeon pectoralis muscle, are known to be already differentiated *in ovo* and not during the process of the young attaining the ability to fly.[3,4] The occurrence of the two types of fibre is therefore to be regarded as a case of cellular specialization which may reflect hypertrophy of certain structures within the cell which are less evident in other cells and also represent different metabolic and functional adaptations.

The fibrillar (asynchronous) type flight muscle occurring in Hymenoptera, Hemiptera (except Cicadidæ), Ephemoroptera, Tysanoptera, Coleoptera and Diptera, has been characterized structurally as one consisting of relatively large fibres with the mitochondria (sarcosomes) distributed in between the myofibrils, the extensive invagination of the tracheoles into the fibre and a marked reduction of the endoplasmic reticulum (SR system). The primitive synchronous type found in the Orthoptera, Lepidoptera, Odonata and Hemiptera (Cicadidæ) is distinctly different from the asynchronous one in that the SR system is well developed. However, a certain extent of tracheolar penetration may occur in the flight muscles of some Orthoptera and Lepidoptera as also large mitochondria are present in the flight muscles of the Odonata.[5-10]

Functionally, insects with lower rates of wing beat possess synchronous flight muscles. For instance the swallow butterfly has a wing beat of 5 per second and the dragonfly 35 per second. These frequencies tally with the range of performance of vertebrate skeletal muscles. Insects with asynchronous flight muscles, on the other hand, have wing beats ranging from 55 to as many as 1046 per second.[9]

In correlating structure with function, it may be suggested that the special features such as small fibre diameter, presence of well-developed SR system and large mitochondria are conducive to low-frequency wing beat as well as sustained flight. On the other hand, features, such as large fibre diameter, presence of well-developed tracheolar system and large mitochondria as they occur in bees, are favourable for high frequency wing beat for considerable length of time. In the evolution of insect flight muscles, it is clear that, either the diameter of the fibres has to be drastically reduced or the indenting tracheoles have to be increased. According to Weis-Fogh the fibre diameter cannot exceed about 20 μ unless the tracheoles indent the surface and become internal.[11] In the Odonata and the Blattidæ where the fibres are supplied with tracheoles only from the surface,[9] the radiating lamellar arrangements of fibrils, with the intervening large mitochondria, are an adaptation for enhancing diffusion of oxygen inside the fibre.[11] It would be of interest to know if the tracheoles do penetrate the large fibres of the flight muscle of *Pantala flavescens*. If they do not, which is probably the case, it means that there is less diffusion of oxygen inside the large fibres. It has been shown that the flight muscles of *Pantala flavescens* have a high level of lipase activity, about six times that of the flight muscles of the locust.[12] It is also known that the locust utilizes fat as the chief fuel during flight.[13] The significance of the high concentrations of lipase in muscles indulging in sustained activity and utilizing fat as the chief fuel, has been revealed.[14] The red, fat-utilizing fibres in such muscles of vertebrates are known to contain high concentrations of fat and oxidative enzymes and to be adapted for an aerobic metabolism unlike the white glycogen-utilizing ones which are adapted for anaerobic metabolism.[4] The fact that the large fibres in the flight muscles of *Pantala flavescens* contain less fat and probably lower concentrations of lipase and oxidative enzymes than the small fibres, suggest that these large fibres have a lower capacity for aerobic metabolism. In this context the observations of Ogata and Mori[15] on the flight muscles of the Japanese dragonfly (*Anotogaster sieboldi*) are of special interest. They found two types of fibre which they compared with the red and white fibres of the vertebrate skeletal muscle. The former was found to contain considerably higher concentrations of the several oxidative enzymes studied than the latter. However, these authors have not mentioned the diameter of the two fibre types. Nevertheless, from their photomicrographs the Type 2 fibres which line the periphery of the fasciculus seem to be slightly larger than the inner ones. Similarly, the peripheral fibres in a muscle lobe of the flight muscle of another dragonfly, *Aeshna* spp. also seem to be larger than the inner ones (Weis-Fogh, Fig. 4).[11] In the pigeon pectoralis muscle too, the Type 2 (white) fibres line the periphery of the fasciculi. In the flight muscle of *Pantala flavescens*, however, the large fibres are not confined to the periphery and they range from 40–70 μ in diameter. So at least with respect to diameter, some of them seem to be intermediate like the intermediate type of fibre seen in vertebrate skeletal muscles.[4,16] One important difference between the Type 2 fibres of vertebrate skeletal muscle and that of the dragonfly flight muscle, is that the mitochondria in the latter are considerably larger than those in the Type 1 fibres of the same muscle. This is so because the insect muscle, with the acquisition of the tracheolar system, is better adapted for an aerobic metabolism.

It is known that the flight muscles of flying insects contain very high concentration of α-glycerophosphate dehydrogenase and no lactic dehydrogenase whereas the leg muscles of some insects (e.g., *Locusta* and *Belostoma*) contain equally high concentrations of the latter enzyme and of not the former.[17-19] It is therefore clear that there are two different mechanisms operating in insect muscles. In the light of this, we may speculate that the large fibres in the flight muscle of *Pantala flavescens* and probably the peripheral fibres in the flight muscles of other dragonflies too, contain the same mechanism involving the reduction of pyruvate to lactate, as in the leg muscle of the locust or for that matter in the white fibres of the pigeon breast muscle. This would mean that the larger, Type 2 fibres in the dragonfly flight muscle should have a higher concentration of lactic dehydrogenase. But Ogata and Mori[15] in their histochemical preparations observed a lower activity of this enzyme in these fibres. It should be pointed out here that by the method employed by them for the demonstration of lactic dehydrogenase activity, a higher enzyme activity would be obtained where there is higher diaphorase activity. In the pigeon pectoralis muscle too similar observations were made in earlier studies. Later works have shown that there is, indeed, higher activity of this enzyme in the

Type 2 fibres of the vertebrate skeletal muscles.[20] Similar histochemical studies on the flight muscles of dragonflies should be revealing.

From the available information on the flight muscles of dragonflies, two diverse types may be distinguished : - one as in *Aeshna* spp and the other as in *Pantala flavescens*. These two types represent two lines of evolution. In an extensive study of the pectoralis muscle in birds and bats, certain parallel lines of evolution have been traced.[4,21] If the fibre composition of the two types of flight muscles in dragonflies is compared with that of the bird and bat pectoralis muscles, the muscles of *Pantala flavescens* in having the large Type 2 fibres and small Type 1 fibres, tally with the pectoralis of the pigeon and that of the bat, *Hipposideros*. The flight muscles of *Aeshna* spp. and *Anotogaster sieboldi* correspond to the pectoralis of the sparrow and the bat, *Pipistrellus* in which there are only Type 1 fibres. In the muscles of these two dragonflies, however, the peripheral fibers in a fasciculus tend to be slightly larger and also contain lower levels of oxidative enzymes[15] (except lactic dehydrogenase ?) than the inner fibres. In the pectoralis muscle of the sparrow, though no difference in the size of the fibres has been noted, the peripheral region was shown to be different from the inner region at the biochemical level. This region was found to have a higher level of glycolytic metabolism.[22] An extensive survey of the flight muscles of dragonflies may well reveal more patterns in their cellular organization.

Structure is the complement of function. In the vertebrate muscle, the ability for fast contractions of short duration is attributed to the Type 2 fibres (phasic) and that for slower and sustained contractions to the Type 1 fibres (tonic).[4] In the light of this, it may be suggested that the occurrence of Type 2 fibres amidst the Type 1 in the same muscle, as seen in *Pantala flavescens*, is a case of structural and functional adaptation for manœuvrable flight. Dragonflies indulge in hovering flight, and the housefly and the honey-bee in manœuvrable flight.[9] It could therefore be argued that *Pantala flavescens* in having the Type 2 fibres in their flight muscles is capable of a certain amount of manœuvrability in flight.

Finally, if the present discussion has brought to light the complexity of the problem of cellular heterogeneity in the organization of insect flight muscles and the possibilities for further investigation, it has served its purpose.

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