CHAPTER 12

SOME GENERAL CONSIDERATIONS.

Insect flight has been the subject of extensive studies such as those concerned with energy production, the mechanics and aerodymics of flight. A few useful reviews of the work done have appeared in the last few years, of which Chadwick's chapters (Roeder,1953) of the flight muscle metabolism and the related subjects, the biochemistry of insect muscle (Gilmour, 1953), the anatomy and histology of insect flight muscle (Tiegs, 1955), intermediary metabolism in insects (Rochestin, 1957), insect flight (Pringle, 1957), and the basic physiology of insect flight muscles (Boettiger, 1960), are outstanding. The flight muscles of insects unlike those of vertebrates show more differences among themselves in the structure of their fibres. It appears from the facts we know about the structure of the insect muscle that there are more than three lines of histological evolution in them. Tiegs (1955) Pringle (1957) and Boettiger (1960) have reviewed these structural diversities in the insect muscle but nevertheless, a brief outline of the same needs to be reconsidered here.

The leg and body muscles in the primitive forms are termed as 'tubular' muscles because of a central core of fluid sarcoplasm containing the string of nuclei. Owing to the absence of myofibrillar proteins, this core has a lower optical density than the rest of the fibre. Around this core, the myofibrils are arranged in a radial pattern and so this type of fibres is also known as the lamellar type. Such muscles have fibres with smaller diameter, 10 - 30 µ.

The sarcosomes are small and are arranged mostly irregularly between the myofibrils. It is believed that the muscles used in flight in the most primitive insects were the normal trunk and leg muscles of the thoracic segments and in some orthoptera (Pringle, 1957) there is very little difference between the leg and flight muscles in their histological features. In Odonata, Blattidae and Mantidae the tubular structure is retained in all the flight muscles and according to Tiegs (1955), Thysanura as well. The main difference between the flight and leg muscles in these insects, is the presence of large sarcosomes regularly arranged between the myofibrils. Their large size and regular arrangement in the flight muscles of Odonata can be correlated with the need for a high rate of metabolism to provide the energy for prolonged flight.

The "closely packed" type of histological structure is found in the higher Orthoptera (locusts) and Lepidoptera. The radial pattern of the fibrils is absent and the nuclei lie scattered in the sarcoplasm. The intracellular tracheoles which are absent in the tubular type appear in this type of muscles, which forms a close net work formation inside the individual fibres. The myofibrils are still smaller but the diameter of the fibres increases. The sarcosomes are bigger and the amount is more.

The fibrillar type of muscles are found in the flight muscles of the higher orders, reaching its peak of specialization in Coleoptera, Hymenoptera and Diptera. The muscles of the fibrillar type are distinguished by an unusual organization of the individual muscle fibres. Each fibre consists of a mass of sarcoplasm containing relatively numerous cross striated longitudinal fibrils. The adjacent fibrils are separated by a row of sarcosomes, The sarcosomes range in diameter from 1 to 4 µ. The muscle organized in this manner and characterized by the presence of giant fibrils and sarcosomes seems to have an extremely limited distribution in animals. It is found only in insects and even here, is encountered only in the highly specialized indirect flight muscles of the most highly evolved orders. Though adult Hemiptera and Coleoptera possess thoracic muscles which approach the fibrillar type. The full development of fibrillar muscle however is observed only in the Diptera and Hymenoptera. In Diptera, which have been extensively studied by Tiegs (1955), the fibres are of very large diameter reaching 1800 µ in the tachinid fly <u>Rutilia</u> <u>potina</u>. A particularly interesting series of histological types of flight muscles has been discribed by Tiegs (1955) from the Homoptera. The tracheation is intracellular. The fibres are arranged in small bundles in a nucleated sheath and fibrils in complex lamellae, but the structural dimensions are not significantly different from those of the close-packed type. This type of muscle has been styled as 'pseudo-fibrillar'. Tiegs (1955) has suggested an intermediate position between the close-packed and true fibrillar type, for this type of muscles in the evolutionary status of insect muscle. The classification on a histological basis is not a rigid one but rather approximate.

The Activity of the muscles:

The flight as well as leg muscles of all the non-flying insects are of the primitive tubular type with little histological difference between these muscles. Dragon-flies which belong to the primitive order, Odonata has the tubular type of muscle fibres, smaller in diameter, without any intracellular tracheoles. But the sarcosomes are large in size compared with that of the leg muscles. This structural variation is well correlated with sustained flight of this insect. Dragonflies are one of the most speediest of insects in the air. Most of the orthopteran insects are poor fliers. Locusts are exceptions. In locusts flight muscles are of the closely packed type with intracellular treacheoles and large number of sarcosomes. Most of the lepidopteran insects are good fliers keeping a steady degree of flight in a sustained manner. Migration of insects is best understood in locusts and some lepidopteran insects. Sustained flight upto 10 hours has been observed for the desert locust by Weis Fogh (1952), Butterflies and moths the chief representatives of Lepidoptera are the common flying insects observed by Williams (1958) in their migratory flights. He observed that most of the hawk moths indulge in migratory flights. The maximum speed for flight is observed in the large dragonflies and Hawkmoths

(Hokings, 1958). According to him these insects should be able to cruise at speeds upto 24 m.p.h. and to attain 36 m.p.h in short flights. Such speeds are impressive with respect to the insects' small size. Travelling at a comparable multiple of the bodylength a man should jog along at 800 m.p.h. and sprint 1200 m.p.h. The speed of insect flight and the ability of insects to sustain themselves on wings suggests a type of high muscular efficiency not encountered elsewhere in the animal world. The most famous long range flier is the monarch butterfly and the best known migration is the Swarming of the locusts in millions. To achieve such efficiency their fuel consumption and the types of the fuel utilized is of considerable importance.

Fuel and Energy problem in flight:

Though the available biochemical and physiological data suggest that fibrillar type of muscle utilizes carbohydrates as the source for energy, there appears to be no correlation between the type of the fibre and the type of fuel. The processes involved in the energy production are quite uniform for different species. In the past few years the mechanisms which provide and control energy for the intense activity of the flight muscles have received considerable attention and several excellent reviews dealing with the biochemistry and physiology of insects are available. (WigglesWorth 1953, Roeder 1953, Pringle 1957, Rochestein 1957, Boettiger 1960).

With respect to the energy problem and the fuel consumption, insects could be divided into two groups, those which utilize fat and those which utilize carbohydrates. Chadwick (1947) observed that the R.Q. in Drosophila is 1 and concluded that carbohydrate is the chief fuel. This has been confirmed by Williams (1949) and Wigglesworth (1949). Wigglesworth (1949) showed that glycogen disappeared from the flight muscles of Drosophila flown to exhaustion while fat storage was not reduced. Carbohydrate is also used in the blow fly Lucilia Sericata (Davis and Frankel, 1940; Williams et,al, 1943) and the bee, Apis mellifera (Jongbloed and Wiersma, 1935; Beutler, 1937). Sacktor (1955) isolated the glycolytic enzymes from the flight muscle homogenates of housefly, Musca domestica and suggested that carbohydrate is the chief fuel for energy during flight in this insect. Studies on the oxygen consumption of flight muscle extracts in Periplaneta (Barron and Tahmisian, 1948) and locusta (Rees, 1954) have shown an inability to metabolize butyric and octanoic acids but acetate was found to be used up presumably by means of Co A and the citric acid cycle. A low fatty acid oxidase system was obtained in the thoracic muscles of the woodroach (McShan, Kramer and Schlegel,1954).

The utilization of fat for energy in the insect muscle was first demonstrated by Krogh and Weis-Fogh (1951) who noted that the R.Q of the desert locust Schistocerca gregaria during flight was 0.75. Later on Weis-Fogh (1952) established that fat was the principal fuel during flight, though glycogen was used simultaneously during the first hour. The duration of sustained flight was proportional to the fat reserve. The monarch butterfly <u>Danaus plexippus</u> which is a migratory form, similarly appears to utilize fat as chief fuel (Beall, 1948). Like migratory birds this insect lays down large fat depots during the premigratory period. Zebe (1954) concluded on the basis of the R.Q. values that a large number of lepidopteran species utilize fat during rest and during flight.

In the light of this knowledge the results obtained the present series of investigations on the histophysiological aspects of the insect flight muscles, are of some interest. The muscles which indulge in sustained activity have been shown to utilize fat and the presence of a fat spliting enzyme in the dragonflies, locust and the bumble bee flight muscles have been shown quantitatively as well as histochemically (George, Vallyathan and Scaria, 1958). They also observed a higher lipase value for the dragonfly flight muscles, than that of the desert locust. Both these insects are sustained fliers and the difference in the concentration has been correlated with the latter being in captivity throughout the observation while the dragonflies were fresh from the air. The high concentration of lipase activity observed for the flight and leg muscles of the cockroach suggests that this insect is potentially capable to breakdown fat into fatty acids and glycerol due to the higher lipase activity. The highest value obtained among ~~the~~

the insects, is for the thoracic muscles of the male cockroach (Chapter 6).

In support to Zebe's observations (1954) a number of lepidopteran insects examined (Chapter 1 and 2) gave a considerable amount of lipase activity for their flight muscles. The highest values among these insects were obtained for the migratory forms. It was also observed that the lipase activity varied according to the size of the muscle fibres and the muscles with smaller diameter for fibres, possess greater quantity of this enzyme. The amount of the lipase activity can be considered as an index of the extent of fat being utilized for energy during activity. All these insects (Orthoptera and Lepidoptera) have the closely packed type of muscle fibres and they vary from 75 to 178 u in diameter. In the beetle muscles, the fibrillar flight muscle was three times more active for lipase activity than the leg muscles. The intercellular spaces of the flight muscles are filled with fat globules and the intracellular distribution pattern of lipase in this muscle (Chapter 5) revealed that lipase is distributed in a diffused patern i.e., active in all the fractions and maximum in the supernatent fraction. In the hymenopteran insects, bumble bee has been shown to possess lipase in its flight muscles. The lipase activity in the honey bee flight muscles was also estimated and found that these muscles also possess lipase in a good amount (Chapter 11). Further it was observed that the honey bee flight muscle homogenate is capable of oxidizing butyrate during activity especially in the peak hours of activity in the afternoon. In the resting condition the butyrate oxidation was not high (Chapter 11). These observations confirm the aforesaid contention that there is no corelation between the type of muscle and the type of fuel utilized. The fuel consumption can be better correlated to the sustained and continuous activity of the muscles irrespective of any specific histological difference. Nevertheless, some of the structural differences could be correlated generally to the functional diversity.

The information concerning the mechanisms whereby lipids can be converted to yield energy at rates adequate to support flight in insects is still rather limited. Sacktor (1955) suggested that fats may be converted into acetate in the fat body which would then be transported to the flight muscle and oxidized via., the TCA cycle. This suggestion is supported by the fact that houseflies and cockroaches could utilize acetate more rapidly than other higher fatty acids. In the flight muscle mitochondria of the honey bee, an acetate activating enzyme has been detected (Hoskins et al, 1956). The present investigation on the fatty acid oxidation in the honey bees flight muscle homogenate (Chapter 11) showing that butyrate is oxidised in the presence of malate during the most active part of the day, suggests that even if an insect is adapted to the utilization of a specific fuel there could be a provision for the utilization of other fuels also. The oxidation of fatty acids in the particulate fractions of the desert locusts' thoracic muscles has shown that short as well as long chain fatty acids are oxidized in the particulate fraction. This confirms Weis-Fogh's view on the utilization of fat in locust flight muscles. An elaborate study on a similar line in insects which are believed to be capable of fat utilization in the muscle for energy should throw some light on the pathways of fat utilization in insect muscle.

Correlation between structure and function:

In the earlier chapters it was emphasized that muscles which are metabolically more active will have fibres usually small in diameter, more of the sarcosomes which contain all the oxidative enzymes as well as play the major role in oxidative phosphorylation of the substrates. This has been shown to be more or less true for the vertebrate skeletal muscles especially the flight muscles of birds and bats. In insects which possess the same histological type of muscle fibres in the same group, this relationship has been shown to be well correlated. In the slow and fast contracting leg muscles of the cockroach where the slow muscle fibres are found to contain high concentration of all the enzymes as well as the fuel store (Glycogen) (Chapter 8) and fat (Smit, 1958) than that in the fast muscle fibres. It was also noted that the fast muscle fibres are larger than the slow ones. The slow muscle contracts at a lower frequency and for a long period while the fast muscle contracts rapidly and for a short duration (Becht and Dresdon, 1956). This also favours the suggestion that structure can be related to the functional activity. The observations pertaining to the flight and leg muscles of the beetle (Heliocopris bucephalus) shows some divergence in the above contention. The fibrillar flight muscles contain all the enzymes tested for (Chapter 3 and 4) in considerably higher concentrations than those in the prothoracic leg muscles but the fibre structure in these two fibres are different and the flight muscle fibres are considerably larger than those of the leg muscles. This difference could be due to the structural difference between the two types . Fibrillar muscle in general possesses fibres of very large diameter. The large size of the fibres for its metabolic and mechanical efficiency has been compensated by the intense tracheation of the fibres. No oxygen debt can be possible during high activity due to the chitinous coating of the tracheoles which keep them in shape and do not allow it to be squeezed as in the blood vessels of the vertebrates during high activity. Also there was negligible amount of lactic dehydrogenase detected in these muscles (Zebe and McShan, 1957). These authors suggested that on the whole the insect muscle is adapted for aerobic metabolism. And the variation in the fibre diameter is so drastic that what exactly can be the relation between structure and function of the fibrillar muscles is difficult to suggest.

Phylogenetic relations:

In general, the structural differences are mostly correlated to the functional diversity of a muscle. Thus it was shown that the flight muscles of insects are metabolically more active, possess more metabolites, more sarcosomes and oxidative enzymes than the leg muscles. It is true that fibrillar muscles contract at a speed of 1000/ sc. (Sotavalta, 1947) and a million of continuous contractions have been recorded. This is also true for insects of the higher orders which fly continuously for a sufficiently long period. But the presence of the fibrillar flight muscles in all the insects belonging to the orders Hymenoptera and Diptera irrespective of their mode of flight is suggestive of the fact that the evolution of these muscles may have some causes other than the structural correlation with the functional activity. Examples are also found in Hemiptera which live most of the time in water and very seldom use their wings for movements but still possess fibrillar flight muscles. The waterbeetles too which are good swimmers than fliers possess fibrillar muscles. The aplids and thrips also possess fibrillar flight muscles. On the other hand insects which possess only closely packed type of muscles fly continuously for considerable length of time and the locusts and butter-flies, those indulge in long range migatory flights have only the closely packed system of fibres in the muscles. If the evolution of fibriller muscles was due to the constant stimulus of the high energy output and to cope up with the activity of the insect in the air, the locusts and the lepidopterans and other insects which indulge in sustained flights would have been the right insects to possess them. And very little is known about insects possessing fibrillar flight muscles which utilize glycogen for energy, indulging in migratory flights. These observations suggest that though the fibrillar muscles with their higher mechanical efficiency are useful in flight where the wings vibrate at a considerable frequency especially in Hymenoptera and Diptera, the absence of these types of muscles in other insects also which indulge in sustained muscles activity suggest that there should be some genetic factor also which controls the occurrence of the fibrillar muscles. Lepidoptera and Diptera stand at the extreme ends of specialization of the closely packed type and the fibrillar type of muscles respectively. Some lepidopteran flight muscles contract at a rate that is found only in the fibrillar type of muscle (Pringle, 1957). This must have been attained due to the high activity of these muscles and the rate of contractions as mentioned in the case of some lepidopteran insects cannot be expalined by the laws that govern the normal contractibity of the muscles. On the other hand, the abscence of fibrillar muscles in the wingless beetle (Scarabid) while its associates which have wings and possess fibrillar muscles suggest that phylogenetical features alone are not responsible of the development of fibrillar muscles in insects.

Convergence in evolution:

In the light of what has been stressed above regarding the features of insect flight, it is evident that the evolution of flight in insects is not completely based on the type of muscle nor on the type of fuel burnt in the muscle. The tracheal mode of resperation have had a major role in the evolution of muscle function. How exactly the tracheal system is able to compensate for the lack of a well developed circulatory system as of vertebrates by which oxygen is carried and fixed in muscle with the aid of haemoglobin is interesting to assess. In birds and bats which keep themselves aloft on their wings, possess considerable amounts of myoglobin. The muscle fibres of sustained fliers are also small in size when compared with the poor fliers. The flight muscles of the migratory birds, rosypastor, contains more of the oxidative enzymes and the capacity to utilize fat in this muscle is small when compared with the flight muscle of the fowl which possess large fibres, white in colour and contains lesser amounts of enzymes. The ability for sustained flight in the fowl is therefore practically nil. But thereare birds eg. pigeon having mixed type of breast muscle where the narrow and broad fibres exist side by side in the same muscle. The bat breast muscle also has the same pattern of arrangement.of fibres as mentioned above. The breast muscle is unique in pigeon, in its structural pattern and in the arrangement of the narrow and broad fibres. The kite seems to be an exception where the flight muscle fibres are comparatively larger and still possess the capacity to maintain itself on the wings for a very long time. It is whown that the flight muscle of the kite is red and possess a good amount of lipase, oxidative enzymes and fat to be utilized for energy.

The circulatory system is quite efficient in vertebrates in transporting fuel to various sites. In insects the tracheal system is also efficient in that even at high activity of the muscle the tracheoles can directly bring the oxygen right upto the mitochondria which is the site of oxidative metabolism. If the tracheal system in insects had not compensated for the absence of a similar circulatory system with oxygen as well as fuel transporting function as found in vertebrates, the insects would also have developed a respiratory pigment like myoglobin in the muscle. The possibility is that the tracheal system aver shot itself in efficiency over circulatory system and did not allow it to develop any further.