

THE EXCITATION AND CONTRACTION OF THE FLIGHT MUSCLES OF INSECTS

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The beating of the wings of all the higher orders of insects except the dragonflies (Odonata) is produced by indirect muscles which are attached not directly to the bases of the wings, but longitudinally and vertically across the box-like thoracic cavity (Text-fig. 1). By the alternate contraction of these two sets of muscles the thorax is distorted in such a way that the wings are moved on their basal articulation. In addition to the indirect musculature, which in the Diptera and Hymenoptera occupies the greater part of the volume of the thorax, several smaller muscles are attached directly to the wing bases; these alter the position and incidence of the wings and are usually considered to be the means of control of flight, the indirect muscles providing the energy for the wing beats.

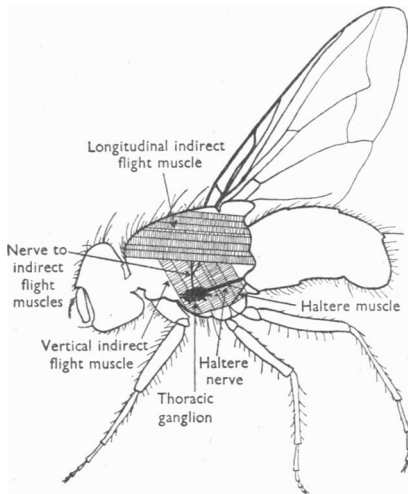
It has been known for some time (Keilich, 1918) that the indirect muscles differ considerably in their histological appearance from other insect muscles. The large, striated muscle fibres, which stretch from one side of the thorax to the other, are more or less completely divided longitudinally into well-defined myofibrils, which may be only loosely bound together into fibre units. In the muscid Diptera a sarcolemma and some sarcoplasm are present, but the fibrils may be teased out even in the unfixed tissue, and are readily seen after fixation in hot alcohol, which appears to dissolve away the boundary membrane of the fibre.

There has been no modern physiological investigation of these indirect muscles. This paper describes some observations on the blue-bottle, *Calliphora erythrocephala* Mg., which suggest that they have a number of peculiar properties.

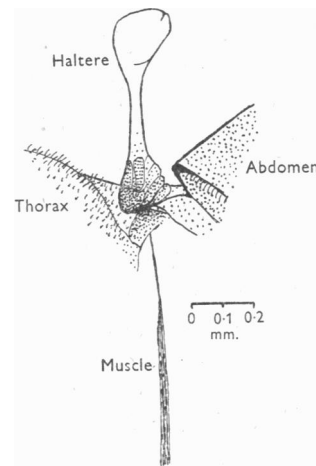
The frequency of the wing beat. Recent observations (Sotavalta, 1947) show that in certain small midges (*Forcipomyia*) the frequency of wing beat is above 1000 beats/sec. Frequencies in the region of 150-300 beats/sec. are commonly found in the larger Diptera (*Calliphora*, *Musca*, *Eristalis*). Even the lower

figures represent a rate of muscular action far higher than is found elsewhere in the animal kingdom; a frequency of 1000 beats/sec. involves a complete cycle of contraction and relaxation in 1 msec.

Sotavalta, after a series of experiments on the effect on the frequency of wing beat of altering the air pressure and the loading on the wings, concludes that the inertia of the wing is the main factor controlling the beat frequency. Sellke (1936) and Pringle (1948) describe experiments on the halteres of Diptera (Text-fig. 2) which lead to the same conclusion in the case of these organs, which are modified hind wings. These observations pose an interesting question.



Text-fig. 1.



Text-fig. 2.

Text-fig. 1. Anatomy and innervation of the indirect flight and haltere muscles of *Calliphora*: diagrammatic view of a fly bisected by a vertical median cut.

Text-fig. 2. Ventral view of the left haltere of *Calliphora*. The organ oscillates during flight through an angle of 150° in a vertical plane at a frequency of 100–150/sec.

If the contraction of the muscles is produced, as in all known somatic striated muscles, by the arrival of a nerve impulse at the neuromuscular junction, the frequency must be determined centrally in the thoracic ganglion, and the change in frequency as the loading is altered must be a reflex from sense organs in the wings or in the wing muscles. No sensory endings can be observed histologically in the wing or haltere muscles, and it is known (Pringle, 1938) that the main proprioceptive sense organs in the legs of insects are the campaniform sensilla embedded in the chitinous skeleton. Campaniform sensilla are always found in considerable numbers at the base of the wings and halteres (Vogel, 1911; Pflugstaedt, 1912), and in the halteres they have been shown to be sensitive to the strains produced by the oscillation of the organ (Pringle,

1948). It is highly probable that they are the proprioceptors for the wings. The following experiments, however, suggest that the dependance of wing and haltere frequencies on loading is not a reflex phenomenon.

EXPERIMENTAL

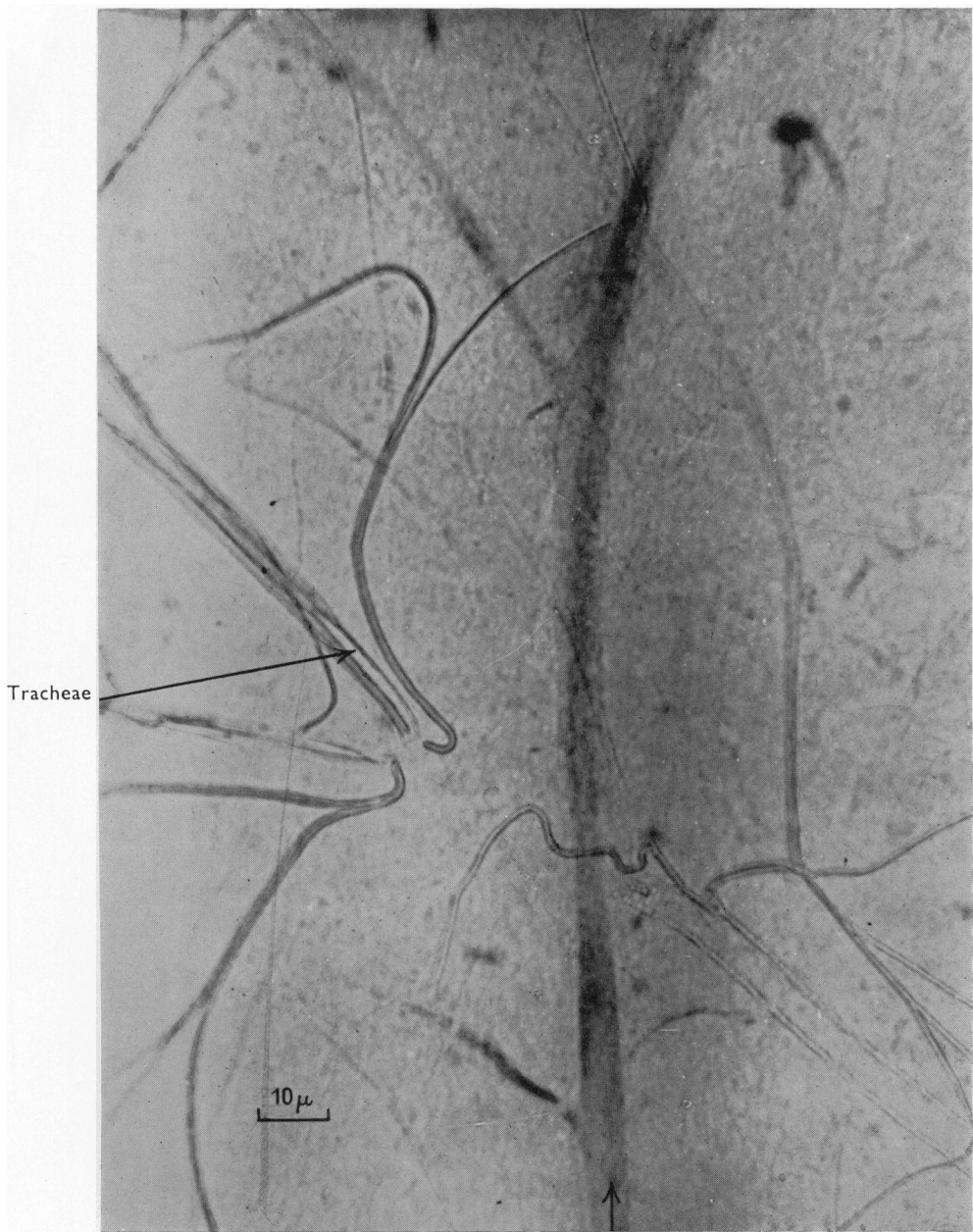
Oscillation of the isolated haltere. The halteres of the higher Diptera are moved by means of a single muscle attached to the cuticle near the base of the organ (Text-figs. 1, 2). Contraction of this muscle moves the haltere upwards, and it returns to a ventral position, when the muscle relaxes, by virtue of the elasticity of the hinge. The other end of the muscle is inserted on the pleuron of the metathoracic segment, so that by dissection outwards in a fly bisected by a vertical median cut it may be exposed without distortion of the skeleton and consequent alteration of its natural length.

Some preparations made in this way show an active oscillation of the haltere, even when the thoracic ganglion is completely removed and all nerves to the muscle are cut. When this occurs, the oscillation may be stopped by touching the haltere with the point of a needle and gently releasing it. If it is now given a slight flick with the needle, the oscillation restarts and builds up to full amplitude in the course of a few cycles. This starting and stopping may be repeated many times. Since the whole of the organ is isolated from the central nervous system, no reflex mechanism can in this case be responsible for the oscillation.

'Anaesthetic flight.' Flies recovering from ether anaesthesia pass through a stage when they fly steadily for several minutes before any obvious reflexes have returned. For example, in an unanaesthetized fly flight is regularly inhibited by contact of the legs with a solid object; this reflex is absent in the 'anaesthetic flight' condition. The dependance of wing-beat frequency on loading is present in this 'anaesthetic flight' as in the normal fly.

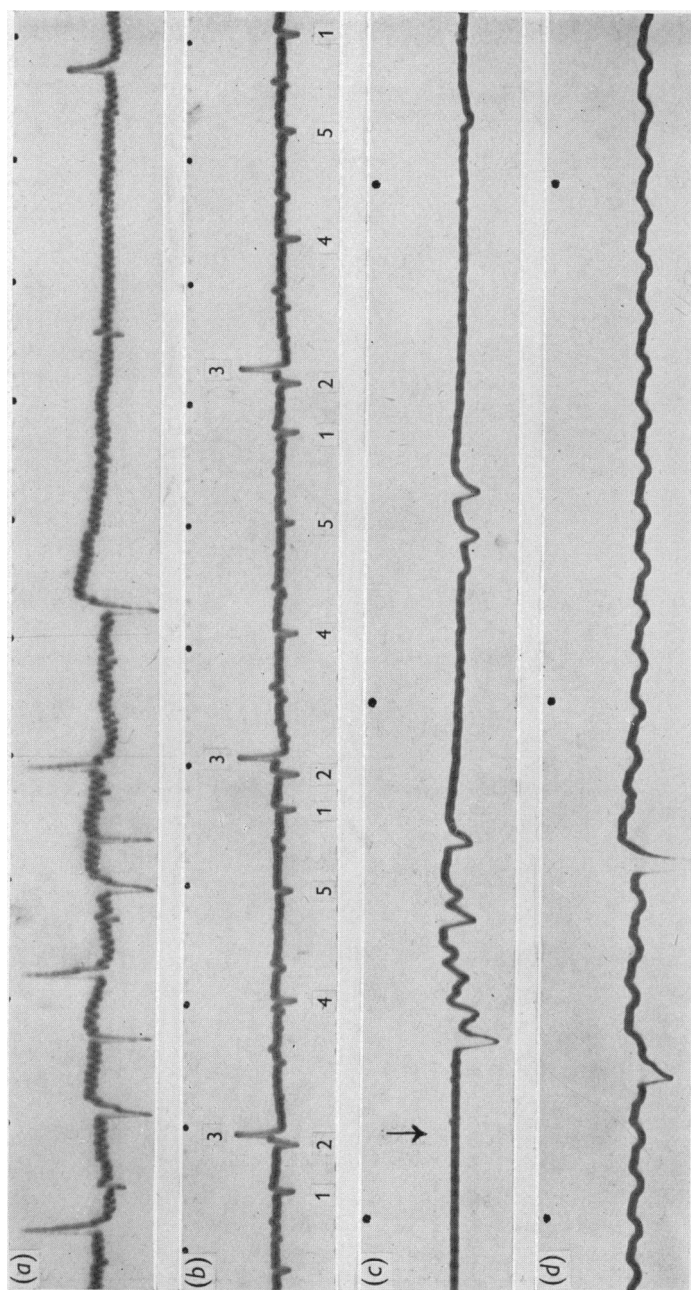
Elimination of proprioceptive sense organs. The sense organs on the wings may all be removed by section of the wings at the extreme base. A fly so treated will still make the muscular movements of flight during recovery from ether anaesthesia, the distortion of the thorax being sufficient to produce an audible buzz, in this case at a high frequency, since the inertia of the wings has been reduced almost to zero. If the distortion of the thorax is resisted by pressure dorso-ventrally between two solid objects, the frequency of the buzz is reduced in proportion to the pressure applied. In this case not only are normal reflexes absent due to the anaesthetic, but the probable proprioceptors of the wings have been completely removed.

The conclusion from these experiments is that the frequency of contraction of the muscles is not determined centrally in the ganglion, but is controlled directly by the loading upon them.



Photomicrograph of the branching of a single nerve fibre on the surface of a portion of the vertical indirect flight muscle of *Calliphora*: from a fresh preparation injected with Rongalit methylene blue.

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Electrical records from the longitudinal indirect flight musculature of *Calliphora*. Time marks $\frac{1}{10}$ sec. (a) During a short period of wing movements after full recovery from anaesthesia. (b) During the steady 'anaesthetic flight'; the distinctive spikes are numbered. (c) At the start of wing movements; the arrow marks the approximate instant of removal of contact stimuli to the legs. (d) At the end of a period of flying; higher amplification.

Effects of electrical stimulation

Wing muscles. If a fly is bisected vertically with a sharp razor blade, no gross damage is done to the musculature of the thorax, and the two halves may be used for physiological experiments. That there is little upset to the nervous system is shown by the fact that the half containing the thoracic ganglion shows the normal leg reflexes (Pringle, 1940).

In such a preparation the indirect flight musculature cannot be made to contract by electrical stimulation. Direct current or condenser discharges ($CR \approx 2 \times 10^{-4}$ sec.) applied through platinum wire electrodes placed in a variety of positions on the longitudinal muscles fail to elicit any movement visible under a high-power dissecting microscope, at any frequency of stimulation from 1 to 400 shocks/sec., and up to intensities sufficient to produce electrolysis.

Haltere muscle. Similar experiments have been made with the haltere muscle. In this case the result is usually the same, but in one or two preparations a slight movement of the haltere has been observed at low frequencies of stimulation, the individual twitches fusing into a smooth tetanus at about 10 shocks/sec. The small size of the haltere (0.7 mm. long) has precluded recording of the contractions, and the results have been observed visually. The movement produced in this way is slow and quite different from the rapid flick characteristic of the normal oscillation.

If condenser discharge shocks are applied to a haltere muscle showing its normal rhythmic activity, the contractions bear no relation to the shocks at any frequency of stimulation between 40 and 400/sec. The movement of the haltere has been observed stroboscopically during stimulation, and the frequency of oscillation is unaffected by the electrical stimuli.

Innervation

The nerve supply to the indirect flight muscles of *Calliphora* is readily seen in a specimen injected 1–2 hr. previously with a small quantity of Rongalit methylene blue (prepared as described by Pantin, 1946). Each portion of the musculature is supplied by a single nerve fibre (Pl. 1), which branches freely over the surface of the muscle. There is no sign of the double or triple innervation characteristic of the leg musculature of Crustacea (Wiersma, 1941) and insects (Pringle, 1939, and unpublished histological data). The nerve fibres supplying both the longitudinal and vertical indirect flight muscles leave the ganglion in a single common trunk (Text-fig. 1) which passes between the two sets of muscles and sends branches into each of them. There are not more than five or six nerve fibres to the whole of the indirect musculature of one side.

Electrical response

Electrical records from the flight muscles of *Calliphora* have been obtained with a concentric electrode made from a no. 19 hypodermic needle and a piece of enamelled copper wire. The electrodes were connected through a $0.05 \mu\text{F}$. condenser (to avoid movement artefacts due to grid current) to a condenser-coupled amplifier and oscilloscope.

Pl. 2 shows the type of record obtained with the electrode inserted from the top of the thorax into the longitudinal indirect flight muscle of one side. The intact fly is supported on a piece of wire waxed to the centre of the top of the thorax (technique of Hollick, 1940), and has a stream of air directed at it from in front. Movements of the wings are readily elicited in a fly mounted in this way by removal of a piece of paper from the grasp of the legs.

At each cycle of wing beat there is a small electrical effect, $200\text{--}500 \mu\text{V}$. in amplitude (Pl. 2*a*, *d*). When the electrode is placed accurately in the centre of the longitudinal muscle the deflexion of the trace occurs once per cycle (Pl. 2*d*); if it is between the two muscle sets the combined effect produces a nearly sinusoidal trace (Pl. 2*a*). This regular effect is accompanied by individual "spikes" of much larger amplitude (approx. $5\text{--}10 \text{ mV}$.), which bear no relation to the cycles of wing beat. At the beginning of the flight (i.e. immediately the contact stimulus to the legs is removed) there is a rapid burst of these large spikes (Pl. 2*c*), and their frequency then falls to a level which is maintained during the wing movements. During 'anaesthetic flight' there is a continuous discharge (Pl. 2*b*).

The records show that at least five different types of spike are distinguishable by their polarity, amplitude and rhythm (Pl. 2*b*). The form of the electrical record is typical of insect muscle action potentials (cp. the leg muscle records in Pringle, 1939). The different spikes are probably due to different portions of the musculature being innervated by different single fibres; the constancy of the rhythm in each unit is remarkable (Pl. 2*b*). At the end of the flight activity the electrical effect at wing-beat frequency dies away in a gradual manner over several cycles (Pl. 2*d*). The last large spike occurs, in this experiment, $\frac{1}{5}$ sec. before the wing beats cease.

DISCUSSION

It is clearly impossible to explain the above results in terms of a neuromuscular system similar to anything so far described. The most probable interpretation is as follows:

(*a*) The arrival of an impulse in the nerve fibre supplying a portion of the indirect musculature sets up an excitation process in the muscle fibres which produces an electrical spike. Whether this is a true propagated action potential or an exaggerated end-plate potential remains undetermined (cp. Katz & Kuffler (1946) on the Crustacean leg neuromuscular junction).

(b) Excitation of the muscle fibres alters the state of the contractile elements in such a way that they become sensitive to the stimulus of stretching. On being stretched, the myofibrils respond with a twitch-like contraction whose duration is controlled by the loading, and then relax.

(c) Contraction of one set of muscles stretches the antagonist, which in turn responds with a twitch-like contraction. In the special case of the haltere, the contraction of the single muscle sets in motion a mechanically resonant system and the muscle is re-excited by the elastic rebound.

(d) This myogenic rhythm continues for so long as the excitation of the muscle fibres is maintained by the arrival of nerve impulses above a critical frequency. When the nerve impulses stop the muscular contractions die out owing to the failure of one or other set of muscles to supply enough energy to maintain the rhythm.

The independence of the excitation process (as measured by its electrical spike) and the contraction process in the myofibrils is the most interesting feature of these experiments, and is in contrast to the properties of the other type of insect muscle studied by Pringle (1939) in the legs of the cockroach and by Heidermanns (1931) in the flight muscles of dragonflies. In both those preparations a single electrical stimulus to the muscle fibres was accompanied by a single twitch, as in vertebrate striated muscle.

The extreme sensitivity of the contraction process to the mechanical conditions also represents a specialization to the peculiar function of these indirect flight muscles. To produce an active contraction of the myofibrils it is evidently necessary both that the system should be in an excited state and that the mechanical conditions should be correct. When the thorax is vertically bisected, the loading on the muscles is altered by an upset of the antagonism between the longitudinal and vertical bundles and, although the muscles may be excited by electrical stimulation, no contraction occurs.

The fact that a high frequency of wing beat can be produced by this system implies that the latency between the stimulus of stretching and the response of the myofibrils is very short. This may point to the processes linking the surface excitation phenomena with the contractile process as the main cause of the latent period of normal muscles.

The small electrical effect of the actual contraction is significant for the physiology of the fly as a whole. If conducted action potentials were produced in the thoracic musculature at each cycle of wing beat, it is difficult to see how the large currents generated could fail to spread to the other muscles and to the thoracic ganglion, all of which lie in the restricted space of the insect thorax. At the frequency at which the spikes actually occur (about 3 per sec. in each unit in Pl. 2b) the direct flight and the other tonically contracting muscles of the thorax will be almost unaffected.

SUMMARY

1. The effect of loading on the frequency of the wing beat and haltere oscillation of Diptera is a property of the indirect flight muscles, and is not a reflex phenomenon.

2. The indirect flight muscles of *Calliphora* cannot be made to contract by electrical stimulation after exposure by vertical bisection of the thorax.

3. Electrical records from the indirect flight muscles show a small effect at wing-beat frequency accompanied by spikes of large amplitude.

4. The results suggest that an impulse in a nerve fibre supplying the indirect flight muscles sets up an electrical spike in the muscle fibres, and as a result of this the myofibrils are brought into a state of excitation in which they contract actively when stretched.

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