J. Physiol. (1954) 124, 269–291

THE MECHANISM OF THE MYOGENIC RHYTHM OF CERTAIN INSECT STRIATED MUSCLES

By J. W. S. PRINGLE

From the Department of Zoology, Cambridge, and the Department of Physiology, University of Ceylon, Colombo

(*Received* 22 *October* 1953)

The twitch of striated muscle is normally initiated by the arrival of an impulse in the motor nerve. Excitation is transmitted across the neuromuscular junction, and its arrival in the muscle fibre can be recognized by the appearance of a transient depolarization of the surface membrane in the region of the nerve ending (the end-plate potential). In vertebrate striated muscle this depolarization is then propagated over the surface of the muscle fibre, but in insects and Crustacea summation of the local potentials at a large number of nerve endings is often responsible for the observed electrical spike (Roeder & Weiant, 1950), though propagated muscle action potentials have been observed (Fatt & Katz, 1953; Wilson, 1954). A twitch occurs for each propagated impulse in the muscle fibre (vertebrate) with tetanic fusion if the frequency is sufficiently high, and a contraction of some sort follows the end-plate potential when there is no propagation.

In the indirect wing muscles of Diptera and Hymenoptera (Pringle, 1949; Roeder, 1951) this sequence of events does not appear to occur. During the rapid rhythmic contraction of these muscles in the flight of the insect, the electromyogram shows a series of spikes at low frequency (about 3/sec for a wing-beat frequency of 120/sec in *Calliphora*) and little or no electrical activity synchronous with the contractions. To explain this observation the suggestion was put forward (Pringle, 1949) that the large potentials represent the normal surface depolarizations following the arrival of motor nerve impulses, but that contraction of the muscle fibres requires also the stimulus of stretch, which is produced by the contraction of the antagonist. This hypothesis, in terms of which it is possible to explain many of the results obtained by other workers on the variations of wing-beat frequency under different conditions (Chadwick, 1953), was supported by Roeder (1951), who made the further suggestion that the first contraction in a period of activity

might be a normal twitch superimposed on the resting tension in the muscles due to the elasticity of the thoracic box.

Further elucidation of the nature of the novel excitatory mechanism of the indirect wing muscles of insects has been rendered difficult by the fact (confirmed by all workers on this subject) that they become completely inactive after any operative procedure which destroys the integrity of the thorax. It was partly on the basis of this observation that Roeder suggested that the resting tension might be important, since this would be affected by operations on the exoskeleton. Unfortunately it is not possible to expose to view either the muscles or their motor nerves without producing this disturbance of their functioning, and the usual types of physiological experiment cannot therefore be made with this preparation.

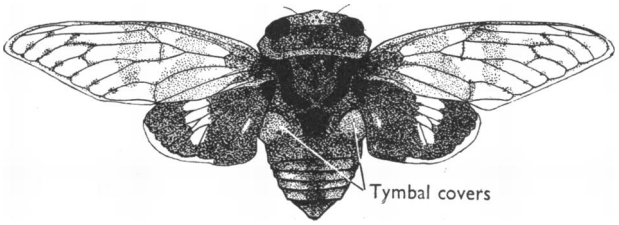


Fig. 1. *P. capitata* ♂ (natural size) showing the position of the tymbals.

In an effort to find another striated muscle showing similar rhythmic properties the sound-producing organs of cicadas were investigated, and the main muscle of this organ, known as the tymbal muscle, was found to be behaving in a similar manner (Pringle, 1953). The skeletal attachments of this muscle are such that it can be functionally isolated from the rest of the insect body together with a considerable length of its motor nerve, rendering possible a series of experiments designed to show its mode of operation.

Cicadas produce their sound by means of a pair of convex drums or tymbals situated on the dorso-lateral surface of what appears to be the first abdominal segment (Fig. 1) and covered by a fold of the exoskeleton. The mechanism, which is described in full elsewhere (Pringle, 1954), is here summarized in essentials.

The motive power for the movement of the tymbals is provided by a pair of large muscles (the tymbal muscles) which are inserted basally on a median ventral skeletal ridge and distally on a free circular disc from the centre of which a short apodeme runs to one corner of the tymbal (Fig. 2). The sound consists of a series of damped pulses produced by the 'ring' of the tymbal at its natural period of vibration when it clicks suddenly into either of its two positions on contraction or relaxation of the tymbal muscle. The mechanism has been compared to that of the lid of a tin can. The curvature of the tymbal, and therefore the critical tension on the apodeme of the tymbal muscle

required to produce its change of shape, is increased by tonic contraction of an accessory muscle, the tensor muscle; the volume and to some extent the frequency of the emitted sound are thereby altered. The volume of sound depends largely, however, on the degree of resonance of the large air sacs which fill the metathorax and abdomen, and which closely envelop the tymbal muscle and are adpressed to the inner surface of the tymbal.

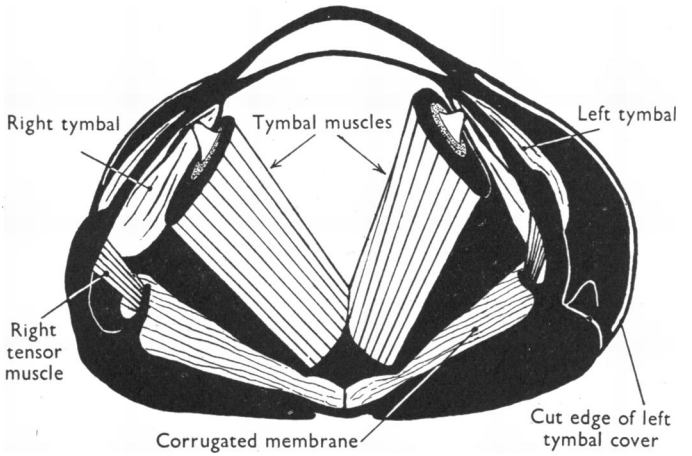


Fig. 2. Semi-diagrammatic view of the tymbals and associated musculature of *P. capitata*; anterior aspect of a transverse slab, viewed slightly obliquely.

MATERIAL AND METHODS

Two species of cicada have been used in this investigation, *Platypleura capitata* (Oliv.) and *P. octoguttata* (Fabr.). They differ little in size or structure, but certain physiological differences make one or the other species more suitable for particular experiments. The males (which sex alone possesses the organs of sound production) are about 3 cm long (excluding wings). The insects were caught in the field and kept in cages in the laboratory. They do not live long and seem to require a constant food supply; in any form of captivity they fly wildly and damage themselves against the walls of the cage. All experiments were therefore made on specimens captured not more than 48 hr previously.

Three types of preparation have been used.

Preparation A. The whole of the exoskeletal ring supporting the tymbals and their muscles is dissected free from the rest of the insect (Fig. 2). The nerves to the tymbal muscles are usually torn by this dissection, and stimuli were therefore applied direct to the muscle. As has been stressed by Chadwick (1953), however, such stimuli are also to be regarded as indirect, since freshly-prepared insect muscles, even when deprived of their motor nerves, still carry the fine feltwork of interfibral branches and endings. The existence of a precise threshold of excitation also showed that it was the nerve fibre which was being stimulated.

Preparation B. The insect is bisected by a vertical cut with a safety-razor blade passing just to one side of the mid-line, so that the ganglion and other median structures are left intact (Fig. 3). The tymbal nerve can then be followed throughout its length. This dissection slightly distorts the skeletal ring supporting the tymbal muscle, but does not impair its functioning.

Preparation C. In a fresh insect with wings and legs removed the bundle of nerves leaving the ganglion in the mid-ventral line is cut behind the ganglion with a fine pair of scissors, the

points of which are inserted just behind the second pair of legs; this isolates the tymbal, tensor and abdominal muscles. One tymbal is destroyed and electrodes are then inserted into the thorax laterally on the side of the intact tymbal in order to stimulate the tymbal nerve. This preparation has the advantage that there is a minimum of disturbance of the musculature and exoskeleton; the air sacs are also intact, so that the volume of sound emitted is greater than in the other two preparations. The functional tymbal muscle may be exposed if desired through the tympanum after removal of the ventral operculum and a second pair of electrodes inserted in it for direct stimulation or for recording the muscle potentials.

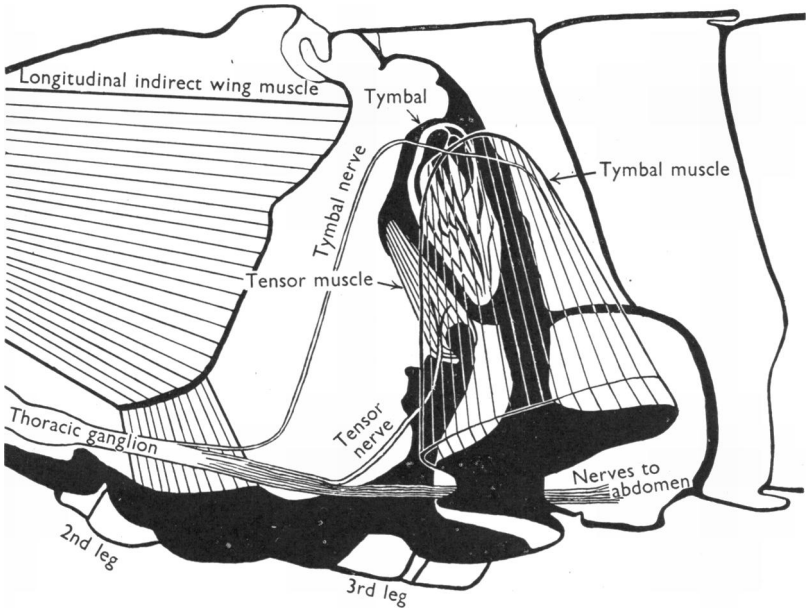


Fig. 3. Semi-diagrammatic view of the right tymbal and associated musculature of *P. capitata*, as seen from within in a specimen bisected by a vertical cut passing just to the left of the mid-line. The tymbal muscle is drawn as if transparent to show the underlying structures.

All these preparations survive very well, probably due to the fact that the tymbal muscle is completely invested by the tracheal air sac; it therefore does not dry up and is provided with its full oxygen supply. Without further precautions many of the preparations were still functioning normally after 4 hr of intermittent stimulation. When necessary the nerves were moistened with insect Ringer's solution made up to the formula of Pringle (1938) but without glucose. All experiments were done at the temperature of the laboratory (about 30° C).

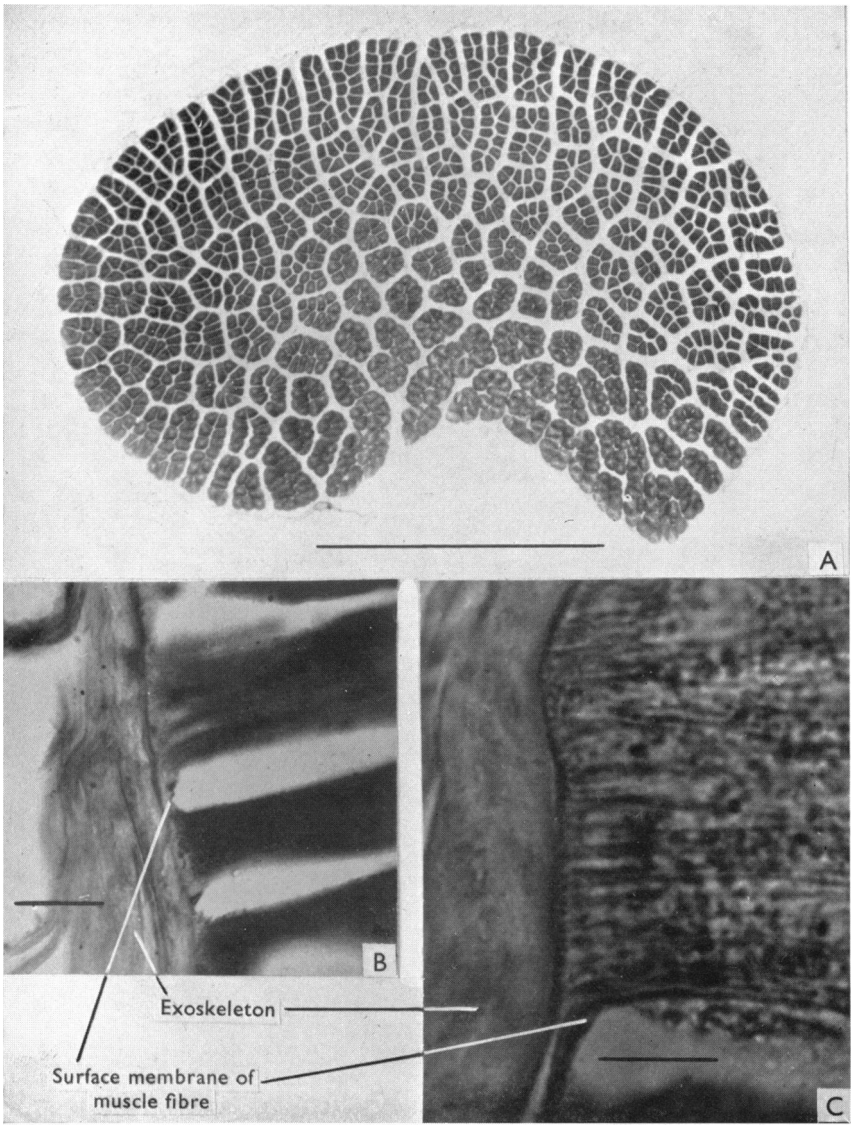
The following apparatus was used:

Ritchie-Sneath stimulator, or laboratory-built electronic stimulator, both delivering pulses of controllable frequency, intensity and duration.

Grass P4 pre-amplifier (condenser coupled) or d.c. cathode follower (Nastuk & Hodgkin, 1950), with the built-in d.c. amplifiers of the Cossor Type 1049 oscilloscope.

M.S.S. Type PMR/1 magnetic tape recorder with Grampion electromagnetic microphone, the high impedance output terminal of the audio amplifiers being connected direct to the oscilloscope for immediate photographic recording. These amplifiers have a flat characteristic from 60 to 10,000 c/s.

Laboratory-built mechanical transducer converting the movements of a light, spring-loaded



Photomicrographs from *Platypleura octoguttata*.

A. Transverse section of the tymbal muscle. The spaces between muscle fibres are filled by tracheae. Scale 1 mm.

B. Longitudinal section of the tymbal muscle at the attachment to the circular disk, showing the fibre cell membrane overlying the hypodermal cell layer. Scale 20 μ.

C. Longitudinal section of a single muscle fibre from the tymbal muscle, showing the direct insertion of myofibrils in the exoskeleton of the circular disk. Scale 10 μ.

To face p. 273

lever into a variable audiofrequency, with an analyser delivering a potential proportional to the audio-frequency for connexion to the oscilloscope. The overall characteristic of this apparatus is essentially linear over the range of movements used.

RESULTS

Histology

Each tymbal muscle is innervated by a single nerve fibre, 15 μ in diameter, which leaves the auditory nerve trunk near the ganglion and runs, in company with a smaller fibre which is not concerned in sound production, dorsad in the mid-line between the two air sacs. Turning caudad near the dorsal surface of the metathorax it runs round the distal end of the tymbal muscle and disappears between the fibres of this muscle on its posterior surface. Its finer branchings can sometimes be seen in sections of the tymbal muscle; there is no sign of a double innervation.

The fibres of the tymbal muscle extend throughout its length and are from 4·5 to 5·5 mm long in both species used. Each muscle contains about 1450 fibres, polygonal in section and with a mean cross-sectional area of 2×10^{-3} mm^2. In the distinctness of their myofibrils and in the arrangement of the nuclei (Pl. 1) the fibres resemble those of the indirect wing muscles (fibrillar) more than those of the leg and abdominal musculature of insects; they also have a high density of large sarcosomes (Watanabe & Williams, 1951). Electron micrographs of teased myofibrils show the bandings typical of insect fibrillar muscle; the sarcomere length is 2·6 μ, which compares with a figure of 2·5 μ for the indirect wing muscles of the worker bee (Morison, 1928) and 2·47 μ for the sartorius muscle of the frog at resting length (Sandow, 1936). There is a very well-developed tracheal network between the muscle fibres.

A feature which now appears to be significant in the structural organization of these insect fibrillar muscles is that the myofibrils within the fibre make direct connexion at both ends with the exoskeleton through a greatly reduced hypodermal layer (Pl. 1). There is thus little or no series elastic component (Hill, 1951*a*) and mechanical events are transmitted direct from contractile substance to skeleton without necessarily affecting the sarcolemma. This arrangement contrasts with that found in vertebrate striated muscle, where it is the sarcolemma sheath which continues into the tendon.

Physiology

The contractions of the tymbal muscle, producing movement of the tymbal from its normal position (OUT) to its other position (IN), are conveniently recorded by placing a microphone near the preparation and photographing the oscilloscope tracing of the emitted sound, the separate pulses produced by the two movements being easily separable on fast records (Fig. 4). This method has been used exclusively for recording normal muscular activity,

since any additional inertia in a direct recording system affects the muscular performance. The cut-off of the audio amplifiers at 10,000 c/s produces some distortion of the beginning of the sound pulse (whose fundamental frequency is 4400–4700/sec in *P. capitata* and 5400–7000/sec in *P. octoguttata*) (Fig. 5), and the apparent build-up of the sound wave-form in Fig. 4 is partly an artifact, but since successive pulses are recorded with the same distortion time intervals can be measured accurately.

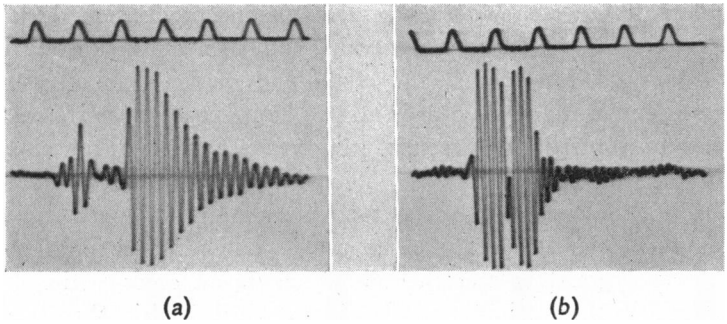


Fig. 4. Sound emitted during a single IN–OUT pulse; preparation C with direct stimulation of the tymbal muscle through the tympanum. *a*, *P. capitata*; *b*, *P. octoguttata*. Time marker, 1 msec.

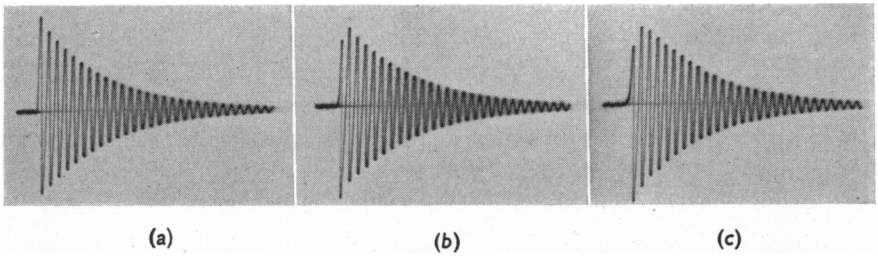


Fig. 5. Distortion of a damped 5000 c/s pulse by M.S.S. magnetic tape recorder. *a*, undistorted pulse; *b*, distortion by amplifiers; *c*, distortion of recorded and replayed record.

Stimulation of the motor nerve fibre

Platypleura capitata

Low frequency shocks applied to the tymbal muscle or to its motor nerve produce a response as shown in Fig. 6*a*. Each stimulus evokes four contractions of the muscle. The latent period between the stimulus and the first IN click of the tymbal is 7·5 msec, part of which is no doubt neuromuscular delay and conduction time in the intramuscular portion of the nerve. At a higher frequency of stimulation the number of contractions per stimulus

increases (Fig. 6*b*) until at about 50 stimuli/sec the muscle is in continuous rhythmic activity with only slight frequency modulation in time with the stimuli (Fig. 6*c*). Finally at about 100 stimuli/sec the muscular rhythm is exactly regular at 350 pulses/sec (Fig. 6*d*). This pattern resembles that of the normal free song (Fig. 6*e*), in which the pulse frequency (in the insect from which the record was made) is 390 pulses/sec.

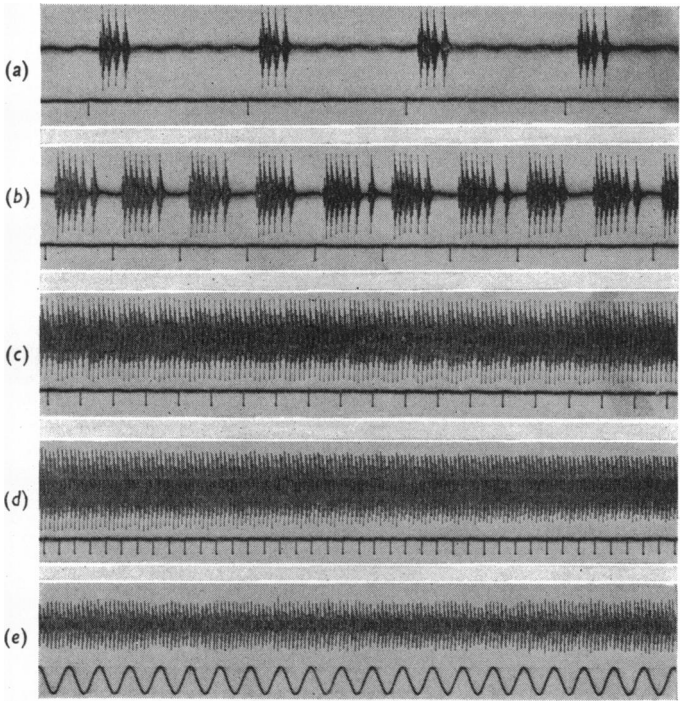


Fig. 6. *P. capitata*; preparation A. Response of the tymbal muscle to stimulation at various frequencies. *a*, 9·5/sec; *b*, 22·5/sec; *c*, 47/sec; *d*, 97/sec; *e*, *P. capitata* free song, from a magnetic tape recording. Time, 50 c/s.

Platypleura octoguttata

A similar result is obtained with this species (Fig. 7) but single stimuli produce a more rapid rhythmic activity in the muscle, the number of contractions per stimulus being, normally, 8 or 9 (Fig. 7*a*, *d*). Stimulation at about 50/sec produces a nearly steady muscular rhythm (Fig. 7*c*) and stimulation at 200/sec a regular succession of 360 contractions/sec. The muscle contraction frequency in the free song of this species is either 220/sec or 240/sec; the song has a definite pattern produced by periodic contractions of the tensor muscle (Pringle, 1954). The intensity of sound emitted at the IN and OUT clicks is much more nearly equal than in *P. capitata* (Fig. 4*a*, *b*).

After-effects of stimulation

At the end of a period of stimulation at high frequency the rhythm of muscular activity dies away gradually (Fig. 8) and has usually stopped within 0·1 sec of the last stimulus. A similar effect was noticed with the indirect wing muscles of Diptera (Pringle, 1949; owing to a printer's error Pl. 1*d* of that paper failed to show the phenomenon).

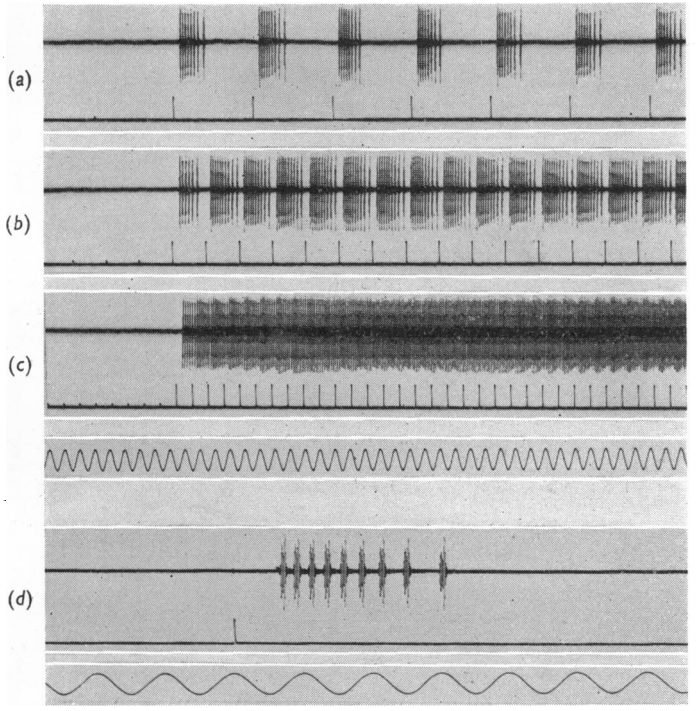


Fig. 7. *P. octoguttata*; preparation C. Direct stimulation of the tymbal muscle through the tympanum. *a*, 9·5/sec; *b*, 22·5/sec; *c*, 47/sec; *d*, response to a single stimulus on a faster time scale. Time, 50 c/s.

Prolonged stimulation at high frequency induces fatigue, which manifests itself as a reduction in frequency of the muscular rhythm (Fig. 9*a*). With such a fatigued preparation single shocks may fail to evoke any response from the muscle, or there may be only one or two contractions per stimulus with a longer latent period (Fig. 9*b*). A facilitation effect now appears, a certain minimum frequency of stimulation being necessary to produce a contraction, and the number of contractions per stimulus increasing as stimulation is continued. The facilitation lasts longer than the muscular activity. If a single or a few low frequency stimuli are given shortly after a period of high frequency stimulation the response is always a greater number of contractions

than normal (Fig. 9*c*). Note that the response to the second stimulus contains more contractions than that to the first, and that the build-up of rhythmic activity is much faster than it was when the stimuli were not preceded by high frequency activity (Fig. 9*b*). The facilitation declines slowly and is still detectable after 15 sec.

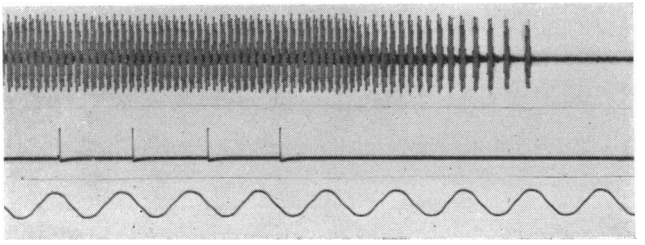


Fig. 8. *P. octoguttata*; preparation C. Direct stimulation of the tymbal muscle through the tympanum. Gradual decline in muscular rhythm after stimuli have ceased. Stimulus frequency, 47/sec. Time, 50 c/s.

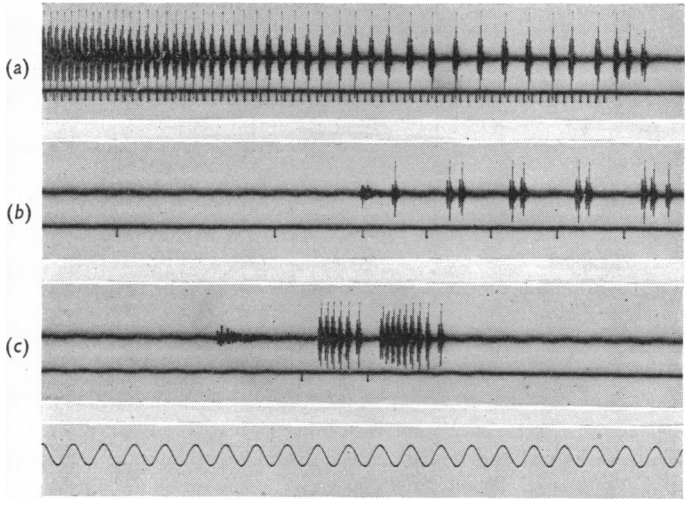


Fig. 9. *P. capitata*; preparation A. *a*, decline in frequency of rhythmic activity in the tymbal muscle during stimulation at 192/sec. (Note the very short after-activity.) *b*, fatigued preparation; failure to respond to stimulation at 9·5/sec and reduced response at 23·5/sec. (The mark at the third stimulus is an artifact.) *c*, fatigued preparation; enhanced response to two stimuli given 0·5 sec after the end of a period of stimulation at 300/sec. Time, 50 c/s.

A similar failure of the first stimulus to elicit a click of the tymbal is sometimes found in fresh preparations made by method B (Fig. 10*a*, *b*) in which bisection of the insect has destroyed the integrity of the skeletal ring supporting the tymbal muscle; here again an apparent facilitation is present. But for reasons given later this is not regarded as the same effect.

If a fresh preparation is stimulated for some time at high frequency, a different phenomenon sometimes occurs. The rhythm of muscular activity continues for some time at a high rate and then suddenly ceases with the tymbal locked in its IN position (Figs. 10*c*, 11). Only when excitation has stopped does it click back to its OUT position, emitting a single sound pulse. This phenomenon also will be discussed later.

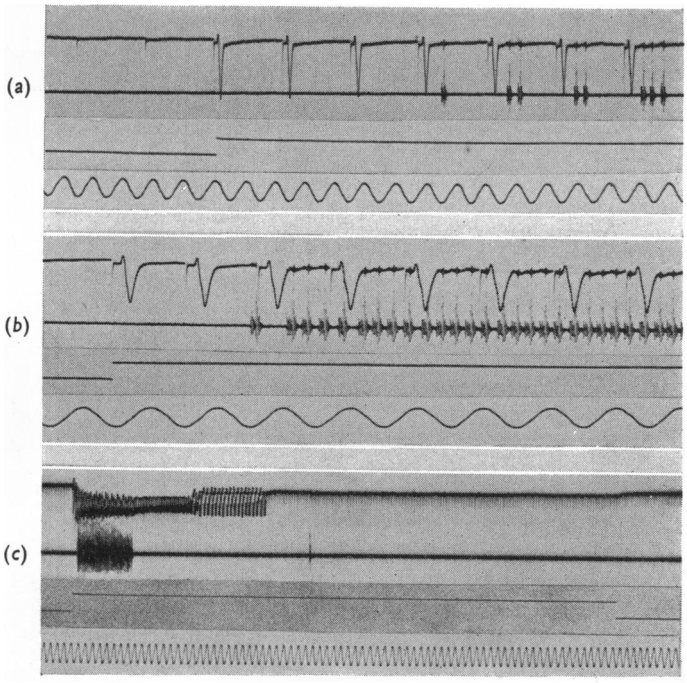


Fig. 10. *P. capitata.* First trace: electrical record from the tymbal muscle. Second trace: sound record of contractions of the tymbal muscle. Third trace: upward deflexion marks the beginning of stimulation. Fourth trace: 50 c/s. The nerve was excited by increasing the intensity of the stimulus ten times; stimulus escape on the top trace shows the individual stimuli. *a*, 22 stimuli/sec; *b*, 46 stimuli/sec; *c*, 200 stimuli/sec.

Electrical activity in the tymbal muscle

The experiments described in this and the two following sections were made with *P. capitata.*

Using preparation B, simultaneous records were made of the electrical activity in the tymbal muscle and the movements of the tymbal (recorded with the microphone) when stimuli were applied to the tymbal nerve at a distance of about 5 mm from the point where it enters the muscle. The

records of Figs. 10 and 11 were obtained with small Ag/AgCl capillary electrodes 0·1 mm in diameter at the tip and filled with insect Ringer, direct-coupled through a cathode follower to the d.c. amplifiers of the oscilloscope. One electrode tip was inserted between the fibres of the tymbal muscle 2 mm from its base, the other being at a distance on the dorsal part of the abdomen. 50 μsec stimuli were applied to the nerve through fine platinum wire electrodes.

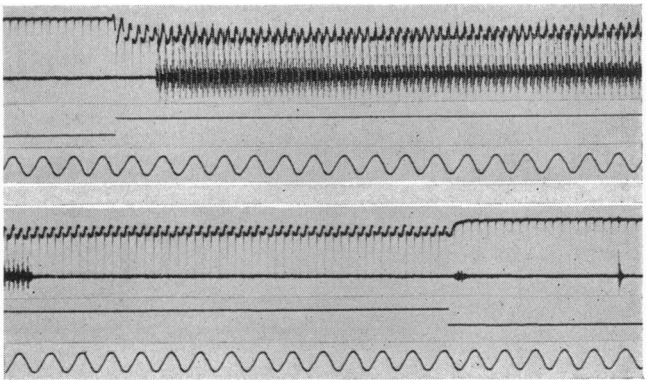


Fig. 11. *P. capitata.* Electrical and sound records from the tymbal muscle; traces as in Fig. 10. Beginning and end of stimulation at 190/sec. The tymbal locks IN before the end of stimulation and clicks OUT shortly after stimulation stops.

Fig. 10*a* shows the effect of low frequency stimulation. The first three stimuli fail to produce a click of the tymbal and then the number of clicks increases to three after the seventh stimulus. The muscle potential, however, is maximal from the start showing that neuromuscular facilitation is not responsible for the increase: there is, in fact, a slight decline in the amplitude of the potentials during the first four stimuli. Fig. 10*b* shows the beginning of stimulation at 46/sec on a faster time scale; the muscle comes into rhythmic activity after the third impulse at a frequency which reached 165/sec after 0·5 sec of stimulation. In both these records the clicks of the tymbal IN and OUT produce a small deflexion on the electrical trace due to cross-modulation on the double beam cathode-ray tube, and there is an even smaller rhythmic potential fluctuation at the frequency of movement of the tymbal, com parable to the small potential change at wing-beat frequency found by Pringle (1949) in the indirect wing muscles of Diptera, and probably due to movement of the tissue under the electrodes.

At higher frequencies of stimulation the muscle potentials show appreciable summation (Figs. 10*c*, 11). In both these experiments the tymbal failed to click OUT after a short period of high frequency rhythmic activity and

returned to its resting position only after excitation ceased. In Fig. 10*c* excitation evidently became irregular and then ceased altogether, due to nerve refractoriness or neuromuscular block; in Fig. 11 the muscle potentials are normal up to the end of stimulation, but the rhythmic activity stopped shortly after the beginning of the second portion of the record, with the tymbal locked IN. The mark on the sound trace of Fig. 11 just after the end of stimulation is an artifact due to the noise from the switch on the stimulator; there is no movement of the tymbal until it clicks OUT again, giving the typical damped sound pulse.

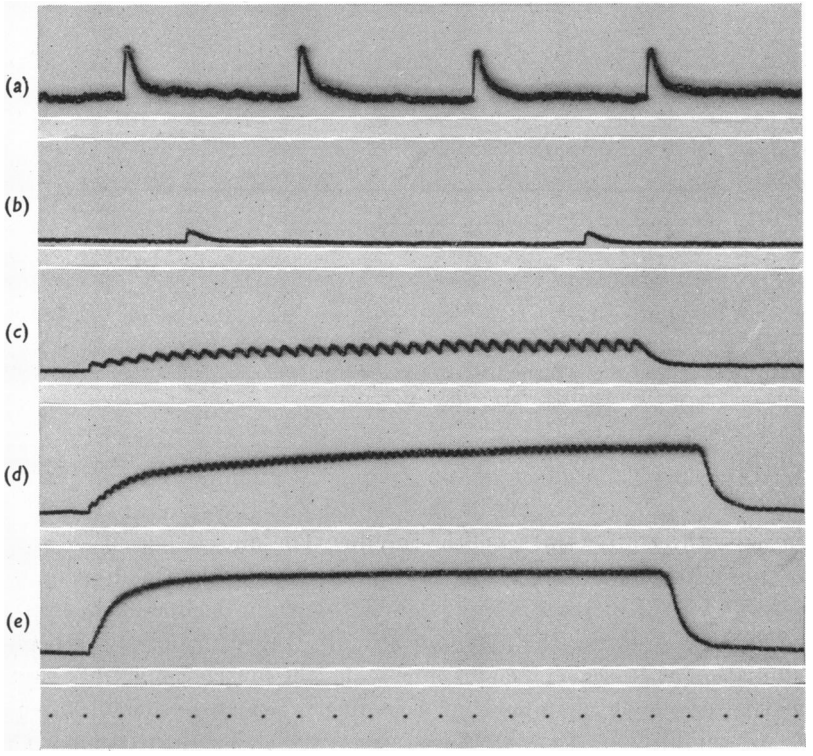


Fig. 12. *P. capitata.* Contraction of the isolated tymbal muscle. *a*, 2 stimuli/sec; *b*, single stimuli; *c*, 22 stimuli/sec; *d*, 46 stimuli/sec; *e*, 100 stimuli/sec. Records *b*–*e* are at a lower magnification than record *a*. Time marker 0·1 sec.

Contraction of the isolated tymbal muscle

The tymbal muscle, detached distally from the tymbal and connected to a light, spring-loaded lever by a short (3 mm) length of cotton thread tied to its apodeme, behaves in a very different way from the intact preparation. Low frequency stimuli now evoke a normal twitch (Fig. 12*a*). The rising phase of

the contraction lasts 10 msec and relaxation is complete in about 0·15 sec. With higher frequencies of stimulation there is mechanical summation and finally a smooth tetanus at 100 stimuli/sec. With the lever used in this experiment the extent of shortening in a tetanus was 0·5 mm (10% of the resting length) and the maximum tension was 10 g. The lever had a natural period of 6 msec.

Movement of the tymbal

The mechanical properties of the tymbal were measured with its muscle detached. The tension on the apodeme immediately after the IN click was, in one insect, 18 g, and when it had just clicked OUT again 0·5 g. The range of movement on the apodeme was 70 μ (same measurement in two determinations on different insects). The tension was measured by tying a fine wire to the apodeme, after removing the muscle and its circular attachment disk, and pulling it vertically with a spring whose extension under load could be calibrated. The movement on the apodeme was measured by waxing the tip of a fine glass tube to the cleaned circular disk and moving the tube slowly with a micromanipulator until the tymbal clicked. Considerable dissection from the inside of the tymbal is necessary in order to perform these operations and it is possible that the exoskeleton was slightly distorted, thereby changing the critical tension for the IN and OUT clicks, but the figures are certainly within the range in which the system functions normally. As has been stated, the curvature of the tymbal is variable in life by means of the tensor muscle. Attempts were made to change the critical tension by stimulation of this muscle, but after the amount of dissection necessary in order to attach the wire to the apodeme it fatigued too rapidly to allow accurate measurements to be made.

Both the IN and OUT movements of the tymbal as observed under the microscope are sudden clicks. As tension is slowly increased the whole of the exoskeleton round the tymbal comes under strain, and when the tymbal reaches 'top dead centre' this strain is partially removed by the IN click movement. The actual displacement of the apodeme at the click is small and the energy derives directly from the elasticity of the exoskeleton. As tension is slowly decreased a similar sequence of events occurs in reverse. The top dead centre position of the tymbal is nearer to the OUT position than to the IN, and the final OUT click is accompanied by a short but powerful movement of the strained tymbal rim.

DISCUSSION

The rhythmic mechanism

It is clear, first of all, that the rhythmic properties of this effector system do not reside exclusively in the tymbal muscle, since when it is removed from the tymbal and connected to a lever its response to a single stimulus is a single

twitch as in normal striated muscle. As recorded in Fig. 12 the contractions are comparable to those of a frog sartorius muscle under the same conditions and are not very rapid for an insect muscle. Roeder has obtained an isometric mechanogram from the tergal remotor muscle of *Periplaneta* (published by Chadwick, 1953) showing a rising phase of the contraction twice as rapid as that of Fig. 12 and a falling phase many times as fast. Under the more nearly isometric conditions in which the tymbal muscle functions in the intact insect, it probably performs at a higher rate of development and disappearance of tension, but even so the high frequency of rhythmic activity suggests that the movement of the tymbal does not result from a normal twitch. There is also the evidence from the electromyogram (Figs. 10, 11) which shows that the clicks bear no simple relationship to the muscle potentials.

A hypothesis which covers all the observations is that the development of tension in the tymbal muscle follows its excitation in the normal manner, but that the sudden release of tension caused by the IN click destroys the activation of the myofibrils so that the muscle is pulled out again to its initial length by the elasticity of the exoskeleton. At a certain point in this movement the tymbal clicks OUT again, restoring the system to its initial state. Provided that the muscle is still in the active state, the myofibrils then re-develop tension until the critical value is again reached, when the cycle repeats itself. On this interpretation the interval between the IN and OUT clicks is a measure of the time taken for the myofibrils to be re-extended after a quick-release, and the interval between successive IN clicks is a measure of the rate of redevelopment of tension. In a fresh preparation the arrival of a single nerve impulse evidently maintains the muscle in the active state sufficiently long for several clicks to occur.

Hill (1949) has shown that in normal striated muscle the transition from the inactive to the active state following excitation is very rapid—much more rapid than is indicated by the curve of tension in a twitch, the beginning of which is slowed by the presence of a series elastic component; but if allowance is made for the effect of this component the isometric mechanogram normally shows the time course of decay of this active state. In the tymbal muscle the tension drops rapidly, nearly to zero, after each quick-release but then redevelops if nerve impulses are arriving, and the rate of redevelopment of tension may be taken as an indication of the intensity of the active state. Fig. 13 shows a plot of the reciprocal of the intervals between IN clicks made for the tymbal muscle response to a single stimulus shown in Fig. 7*d*. The curve is similar in shape to that of the intensity of the active state of the frog sartorius muscle derived by Hill (1949), but is much faster. Hill's experiments were made at 0° C while the experiment of Fig. 7*d* was performed at 30° C; the decay of activity is known to have a high temperature coefficient (Hill, 1951*b*).

It is now possible to interpret the results of Fig. 10, made with preparation B, in which, even in a fresh specimen, the first few stimuli failed to elicit clicks of the tymbal, though excitation was reaching the muscle. As already pointed out, this dissection breaks the continuity of the skeletal ring supporting the tymbal muscle and probably alters the elastic properties of the exoskeleton in that region. In Fig. 10*a* the stimulus frequency was 22/sec, and Fig. 12*c* shows that at this frequency summation is just apparent. It is evidently only at the fourth stimulus that the muscle has shortened enough to strain the skeleton to the point where tension has built up to the critical level for the

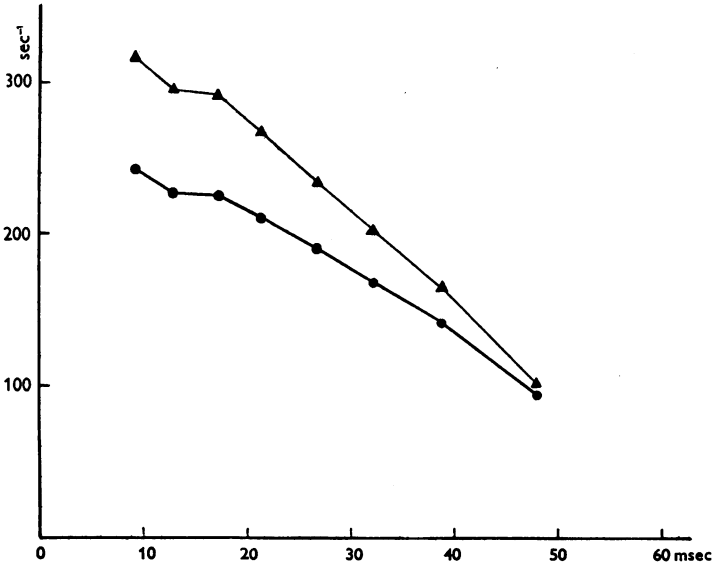


Fig. 13. *P. octoguttata.* Time course of the active state of the muscle fibres after a single excitation. Lower plot: reciprocals of the intervals between IN clicks. Upper plot: reciprocals of the OUT–IN intervals. Each measurement is referred to the instant of time mid-way between IN clicks.

tymbal to click; even so, the necessary level of tension is only developed for sufficiently long for one IN–OUT click to occur. By the time the effect of the quick-release is over the active state has decayed too far to allow tension to build up again to the critical value. At the fifth stimulus summation has proceeded further and there is time for two clicks; after the seventh there is time for three. In Fig. 10*b* the stimulus frequency is 46/sec; Fig. 12*d* shows that at this frequency summation is more rapid. In Fig. 10*b* a single IN–OUT click follows the second stimulus and the third stimulus elicits four; by the fifth stimulus summation is sufficient to maintain the myogenic rhythm and the relationship between stimuli and contractions is lost.

At this stage in the discussion it is necessary to deal with one possible criticism of the hypothesis. In discussion of the earlier results with the indirect wing muscle it was pointed out to the author by Prof. Katz that the absence of correlation between the potentials of the electromyogram and the contractions of the muscle might not be as significant as had been supposed, since the method of electrical recording with extracellular electrodes might not show a change of polarization occurring simultaneously all over the surface of the muscle fibres. The excitation by stretch which was then postulated, or the deactivation by release which is now thought to be the mechanism of rhythmic activity, might therefore be occurring by virtue of the influence of the mechanical event on the surface membrane of the muscle fibre. It has not been possible to record the membrane potentials of the tymbal muscle with intracellular electrodes and thus directly to refute this criticism. The experiment has, however, been made with the indirect wing muscles (Boettiger & McCann, 1953). These authors, in a brief note, give figures for the resting and action potentials of the fibres of the indirect wing muscles and comment on the presence of overshoot of the action potential and on the unusually long negative after-potential. They make no mention of any potential changes at wing-beat frequency, which must have been strikingly obvious if contraction and relaxation are accompanied by potential changes in the membrane. It is also noteworthy that at no time in many experiments with either the indirect wing muscles or with the tymbal muscle have any large potential changes been detected synchronous with the rhythm of mechanical activity. In some cases the fibres of the tymbal muscle were purposely damaged at the location of the intercellular electrode, a procedure which should destroy the symmetry of the electrode arrangement and reveal all changes of surface polarization occurring in intact regions of the fibre. The only electrical changes recorded corresponding to mechanical activity have been of such small magnitude that they can be accounted for in terms of movement of the tissue under the electrodes.

It is therefore concluded that the influence of the quick-release on the state of the muscle is a direct effect on the myofibrillar contractile mechanism and not an effect transmitted to the myofibrils through changes in the surface membrane. The histology of these fibrillar muscles supports the conclusion, for, as shown in Pl. 1, the myofibrils make a very direct connexion with the exoskeleton, whereas the sarcolemma is tenuous and attaches to the hypodermal cell layer at an acute angle. In some insect wing muscles, particularly those of Hymenoptera, the sarcolemma appears to have a reticular structure and not even to be a continuous membrane (Morison, 1928); the whole evolutionary tendency in these fibrillar muscles seems to be to emphasize the independence of the myofibrils, which in themselves contain the whole mechanism of the rhythmic activity.

This being so, it is necessary to make a distinction which is not apparent in normal striated muscle between the intensity of the active state of the fibre as a whole and the state of activity of the contractile mechanism, which, following Buchthal (1951), will be referred to as the *activation*. A quick-release *deactivates* the contractile mechanism but does not destroy the active state of the muscle, since activation redevelops once the influence of the quick-release is over. Deactivation is a process occurring in the myofibrils which is prevented from affecting the electromyogram by some irreversible step in the link between excitation and activation.

The disappearance of tension in an excited muscle on sudden release is a phenomenon which has been known for some time. It was first adequately described by Gasser & Hill (1924) in the sartorius muscle of the frog, and has since then been studied by Hill on a number of occasions, most recently in 1953. Rarely, however, has the experiment been made of pulling the muscle out again to its resting length immediately after the release. Gasser & Hill give the result of one such experiment (their fig. 10, record 2) which appears to show that the isometric tension at the stretched length is immediately restored by the re-stretch. These investigators purposely reduced the speed of re-stretch 'so that the tension attained on stretching would be maintained'. They had previously observed that a quick stretch not preceded by a release produced an overshoot above the isometric tension for the stretched length. It is not clear whether this phenomenon was actually observed when the stretch followed closely on the quick-release or whether they merely assumed that it would occur and wished to avoid it. Their record as published has a close time-scale and it is difficult to measure the interval between the quick-release and the full restoration of length, but it appears to be about 10 msec. If the sartorius muscle shows the same effect as has been described in the cicada tymbal muscle, it might be that it would not have been observed in Gasser & Hill's experiment, because activation would be fully restored by the time the stretch was complete.

Hill (1953) again applied quick-releases to a stimulated frog sartorius muscle in order to measure the 'instantaneous heat' liberated on the disappearance of tension. He records a suggestion by D. R. Wilkie that a quick-release followed at once by a quick stretch should be tried, but the experiment was not performed. Hill clearly anticipated that the result would be a re-absorption of the 'instantaneous heat', which would imply that activation had not been destroyed by the release. It cannot therefore be decided at present whether the cicada effect is a general property of striated muscles, or is a specialization associated with the peculiar mechanical system to which the tymbal muscle is coupled.

Re-interpretation of the results with indirect wing muscles

It can, however, be concluded with some confidence that the mechanism found in the cicada tymbal muscle is at work in the indirect wing muscles of higher insects, and with the clearer picture which is now available it is necessary to re-interpret the results obtained by Pringle (1949) and Roeder (1951). The hypothesis then advanced was that stretch of the muscle by its antagonist was the adequate stimulus for contraction, provided that the muscle was in an excited condition due to the arrival of motor nerve impulses. For this 'excitation by stretch' must now be substituted 'deactivation by release'. As an explanation of the myogenic nature of the rhythm of activity, either hypothesis is equally adequate, but the new concept has important implications.

Comparison of the IN click of the cicada tymbal with the wing-beat of a fly reveals an apparent large difference in the time scale. The time occupied by the release in the tymbal muscle can be estimated as 0·05 msec (see next section). In *Calliphora* with a wing-beat frequency of 120/sec the duration of the stroke is of the order of 4 msec. It seems at first sight that the explanation advanced for the rhythmic mechanism in the tymbal system can hardly apply to the wings. Boettiger & Furshpan (1952) have shown, however, that in the higher Diptera there is by no means a linear relationship between the movements of the thorax and the movements of the wings. They describe a 'click' mechanism in the wing articulation by which energy stored in the elasticity of parts of the exoskeleton during the almost isometric contraction of the indirect wing muscles is released suddenly and dissipated in wing movement; the articulation is such, moreover, that this is achieved in both directions of stroke. Such a mechanism must produce a much more rapid drop in tension in the muscles than the straightforward lever system figured in most text-books, and with it the activation and deactivation cycle suggested for the tymbal muscle can be applied to the indirect wing muscles.

It will be interesting to see if a similar type of articulation is always associated with the myogenic rhythmic type of flight motor, which also occurs in Hymenoptera (Roeder, 1951) and in some Coleoptera (Roeder, personal communication). If so, it will be necessary to revise completely current views about the factors determining wing-beat frequency (Chadwick, 1953). Theoretical treatment of the wings and their articulations as a mechanically resonant system (Sotavalta, 1952) becomes misleading; attention must be focused separately on the factors affecting the rates of development and disappearance of tension in the indirect wing muscles. The development of tension is presumably affected by such factors as the frequency of motor nerve impulses, temperature, availability of metabolites, etc., while the rate of disappearance should be controlled largely by mechanical factors such as

the inertia and loading of the wings and the form of the articulation (adjusted perhaps by the direct wing muscles). All these factors are known to affect wing-beat frequency, but experiments must in future aim at an analysis of their influence on the separate phases of the cycle of activation and deactivation, rather than merely on the wing-beat frequency as a composite whole.

The nature of deactivation by release

The alteration in the properties of the myofibrils when the tension in them is released by the IN click of the tymbal has been termed deactivation. Since the effect is a hitherto undescribed property of a striated muscle, probably occurs also in the indirect wing muscles and is not excluded as a property of all striated muscle, it is worth examining carefully all the evidence which can be obtained from these experiments about its behaviour under various conditions.

The observed phenomenon is that the sudden release of the muscle while it is developing a tension greater than 18 g produces in it a change of state so that 1–2 msec later it is pulled out again to its initial length and the tension has fallen to 0·5 g; and that tension is then redeveloped without any further excitation from the motor nerve. The extent of muscular movement is 70 μ in fibres 5 mm long—a shortening of 1·5%.

The actual click of the tymbal is very rapid. Since it produces a powerful ring in a structure which is mechanically resonant at about 5000 c/s the movement must be fast compared to a single cycle of this oscillation and a figure of 0·05 msec for its duration is probably a maximum value. Further indication of the high speed of tymbal movement is provided by the sound wave-form from *Platypleura octoguttata* (Fig. 4*b*) where the onset of the IN click shows a build-up nearly as rapid as can be expected from the characteristics of the recording system and where the transition from the IN to OUT sounds occurs apparently instantaneously.

The tymbal movement during the click, however, does not result entirely from shortening of the tymbal muscle, but partly from the elastic recoil of the exoskeleton which has been strained during the portion of the cycle during which the tymbal is moving from OUT to top dead centre. The figure of 18 g is the tension in the apodeme immediately after the IN click; at top dead centre it must be slightly greater than this, but a measurement could not be made with the available apparatus. At the moment of the IN click there must be a slight shortening of the muscle due to the partial removal of strain. Measurements of the stress and strain changes at the IN click would be valuable since they should give a direct estimate of the elastic modulus of the active contractile tissue, an estimate which cannot be obtained from vertebrate muscle owing to the presence of a series elastic component (Hill, 1953). In

the tymbal system the measurements could be made at the actual point of insertion of the myofibrils.

The delay before the OUT click may occur for one of two reasons. Either the release does not produce instantaneous deactivation and there is a gradual transition to the deactivated state with a progressive fall in tension from 18 g to 0·5 g, or deactivation is virtually instantaneous and the delay is due to the 'viscosity' of the deactivated muscle as it is extended under the elastic load of the strained exoskeleton. No way has been found of deciding between these two possibilities. The velocity of muscular extension between the IN and OUT clicks in *P. capitata* is about 1% of the fibre length per msec, which is a comparable speed of elongation to that found in relaxed vertebrate muscle. Buchthal (1951) has a figure showing a velocity of about 0·2% per msec under a load of 0·11 times the isometric tension. In the tymbal movement the elastic restoring load must be considerable since the OUT click contributes at least half of the sound energy output. The IN–OUT delay could therefore be explained in terms of instantaneous deactivation, but more experimental data are needed.

The IN–OUT interval is normally 1·4–1·7 msec in *P. capitata* and 1·0–1·2 msec in *P. octoguttata*; it changes very little in a particular insect either with the level of excitation or with fatigue. In *P. octoguttata* there is reduction of about 5% during the first 30 msec of rhythmic activity, followed by an increase to about 5% above the initial value if excitation continues, but the changes are too small to be measured accurately from records made with the present apparatus. A larger change is produced by varying the mechanical properties of the tymbal, as is shown by the following experiment. In *P. octoguttata*, preparation C, the tymbal muscle was stimulated directly through the tympanum at 22 stimuli/sec and pressure was applied with the tip of a needle to the point of insertion of the tensor muscle, thereby increasing the curvature of the tymbal. The results are shown in Table 1. There was some

TABLE 1. *Platypleura octoguttata*, preparation C. Effect of increasing the curvature of the tymbal by pressure on the point of insertion of the tensor muscle. Direct stimulation of the tymbal muscle at 22/sec.

	Initial values	Pressure applied	Pressure removed
No. of clicks per stimulus	14–15	10–11	13–14
IN–OUT interval (msec)	1·13±0·022*	0·91±0·031*	1·10±0·015*

* Standard error of the mean; seven measurements.

residual facilitation due to a previous high frequency stimulation and the number of clicks per stimulus is greater than normal. Pressure with the needle reduces the number of clicks per stimulus, showing that the critical tension for the IN click was increased; there is also a reduction in the duration of the IN–OUT interval. Evidently the increased curvature of the tymbal also increases the force tending to restore it to its OUT position after it has

clicked IN, and the OUT click occurs sooner. This result suggests again that the delay is due to 'viscous' resistance to elongation in the deactivated myofibrils, but it could be accompanied by a progressive deactivation.

It has been mentioned already that after high frequency activity of the muscle the tymbal sometimes fails to click OUT, and then remains IN for the rest of the period of excitation, returning only when excitation ceases (Figs. 10*c*, 11). This represents either a failure of the deactivation mechanism or a failure of the elasticity of the exoskeleton to pull the muscle out again to its initial length before tension has redeveloped. When it occurs the muscle goes into a normal tetanus and there is no possibility of restarting the cycle. Unfortunately the sound-trace during high-frequency activity is too confused to allow an estimate to be made of the IN–OUT interval immediately prior to the IN failure, so that again there is no evidence about the actual speed of deactivation. In life, jamming of the rhythmic cycle is probably prevented by a simultaneous contraction of the tensor muscle whenever the tymbal muscle is strongly excited.

The IN failure of the tymbal recalls a similar phenomenon in the haltere muscle of Diptera, which is a modified wing muscle and whose myogenic rhythm can, like that of the indirect wing muscles, be explained in terms of deactivation by release. Pringle (1949) noted that, although this muscle is capable of producing high-frequency oscillation of the haltere even when its motor nerve is cut, it sometimes responds to electrical stimulation at 10/sec by a tetanus. If the quick-release mechanism in the haltere articulation fails for any reason to operate, or, if it does operate but does not produce deactivation sufficient to allow the muscle to be extended again to its initial length, a tetanic contraction is to be expected by analogy with the results with the cicada tymbal muscle. It is interesting to note that the haltere muscle appears to be inherently slower than the tymbal muscle for at 10 stimuli/sec the latter, when isolated, still shows discrete twitches. Since the indirect wing muscles of *Calliphora* generate a regular rhythm of activity with motor nerve impulses arriving at as low a frequency as 3/sec they may be inherently even slower still, although by the deactivation cycle they can move the wings 120 times a second.

Nothing in the present series of experiments eliminates the possibility that the re-stretch of the tymbal muscle during the IN-OUT interval or the OUT click may affect the reactivation of the myofibrils. Thus it cannot be decided whether reactivation starts immediately after the IN click but has not developed far enough in the next 1–2 msec to impose any severe restraint on the elongation of the myofibrils, or whether it occurs only after the OUT click when a small but rapid increase in tension must occur in the apodeme. The shape of the curve of Fig. 13 is not greatly altered by plotting the reciprocals of the OUT–IN intervals instead of the intervals between IN

clicks. Deactivation by release is certainly the most significant feature of the cycle of activity in insect fibrillar muscle, but activation by stretch may serve to accelerate the rhythm once the first deactivation has occurred.

SUMMARY

1. In the song of cicadas the sound is produced by the 'ring' of the tymbal when it clicks IN or OUT into either of its two positions on contraction or relaxation of the tymbal muscle. This muscle is histologically and physiologically similar to the indirect wing muscles of Diptera, but, unlike them, may be functionally isolated together with its single motor nerve fibre.

2. Single shocks applied to the nerve evoke a series of clicks from the tymbal, and above 50 stimuli/sec the muscle goes into rhythmic activity at a high frequency which is not proportional to that of the nerve impulses.

3. The electromyogram shows potentials corresponding to the arrival of nerve impulses but little or no electrical changes synchronous with the rhythm of mechanical activity. At high frequencies of stimulation the potentials summate.

4. The detached tymbal muscle gives normal twitches little faster than those of frog sartorius muscle at the same temperature, with mechanical summation and tetanus if the frequency of stimulation is sufficiently high.

5. The apodeme of the tymbal muscle moves 70 μ ($1\frac{1}{2}$% of the length of the muscle) during the cycle of activity. With the accessory tensor muscle relaxed, the IN click of the tymbal occurs at a tension of about 18 g and the OUT click at about 0·5 g.

6. Myogenic rhythmic activity is maintained by *deactivation* of the myofibrils by the quick-release at the instant of the IN click, the muscle being restored to its initial length in the next 1–2 msec by the elasticity of the exoskeleton and then redeveloping tension at a rate dependent on the intensity of the active state of the muscle. A single nerve impulse maintains the active state for sufficiently long to allow several clicks to occur.

7. The myogenic rhythm of the indirect wing muscles of certain higher orders of insects derives from a similar mechanism and not from excitation by stretch as suggested by Pringle (1949).

8. Deactivation by release is a new property of a striated muscle and is not excluded as a property of all striated muscle.

I am grateful to Prof. Koch and the staff and assistants in the Department of Physiology, Colombo, for the help which they gave me during my stay in Ceylon. The visit was made possible by the award of a Leverhulme Research Fellowship and by financial assistance from the H. E. Durham Fund of King's College, Cambridge, and from the British Council. Part of the apparatus used was purchased with the aid of the Government Grants Committee of the Royal Society. Prof. A. V. Hill kindly read the first draft of the manuscript and made several helpful suggestions, and I have had useful discussions with Dr M. G. M. Pryor and Dr K. E. Machin.

REFERENCES

BOETTIGER, E. G. & FURSHPAN, E. (1952). The mechanics of flight movements in Diptera. *Biol. Bull., Woods Hole*, **102**, 200–211.

BOETTIGER, E. G. & MCCANN, F. (1953). Single fibre action potentials in insect fibrillar muscle. *Fed. Proc.* **12**, 17.

BUCHTHAL, F. (1951). The rheology of the cross striated muscle fibre and its minute structural interpretation. *Pubbl. Staz. zool. Napoli*, **23**, suppl. 115–146.

CHADWICK, L. E. (1953). The motion of the wings. The flight muscles and their control. In *Insect Physiology*, ed. Roeder, K. D. New York: John Wiley.

FATT, P. & KATZ, B. (1953). The electrical properties of crustacean muscle fibres. *J. Physiol.* **120**, 171–204.

GASSER, H. S. & HILL, A. V. (1924). The dynamics of muscular contraction. *Proc. Roy. Soc.* B, **96**, 398–437.

HILL, A. V. (1949). The abrupt transition from rest to activity in muscle. *Proc. Roy. Soc.* B, **136**, 399–420.

HILL, A. V. (1951*a*). The effect of series compliance on the tension developed in a muscle twitch. *Proc. Roy. Soc.* B, **138**, 325–329.

HILL, A. V. (1951*b*). The influence of temperature on the tension developed in an isometric twitch. *Proc. Roy. Soc.* B, **138**, 349–354.

HILL, A. V. (1953). The 'instantaneous' elasticity of active muscle. *Proc. Roy. Soc.* B, **141**, 161–178.

MORISON, G. D. (1928). The muscles of the adult honey-bee. *Quart. J. micr. Sci.* **71**, 563–651.

NASTUK, W. L. & HODGKIN, A. L. (1950). The electrical activity of single muscle fibres. *J. cell. comp. Physiol.* **35**, 39–73.

PRINGLE, J. W. S. (1938). Proprioception in insects. I. A new type of mechanical receptor from the palps of the cockroach. *J. exp. Biol.* **15**, 101–113.

PRINGLE, J. W. S. (1949). The excitation and contraction of the flight muscles of insects. *J. Physiol.* **108**, 226–232.

PRINGLE, J. W. S. (1953). Physiology of song in cicadas. *Nature, Lond.*, **172**, 248–249.

PRINGLE, J. W. S. (1954). A physiological analysis of cicada song. *J. exp. Biol.* (in the Press).

ROEDER, K. D. (1951). Movements of the thorax and potential changes in the thoracic muscles of insects during flight. *Biol. Bull., Woods Hole*, **100**, 95–106.

ROEDER, K. D. & WEIANT, E. A. (1950). The electrical and mechanical events of neuro-muscular transmission in the cockroach, *Periplaneta americana* (L.). *J. exp. Biol.* **27**, 1–13.

SANDOW, A. (1936). Diffraction patterns of the frog sartorius and sarcomere behaviour under stretch. *J. cell. comp. Physiol.* **9**, 37–54.

SOTAVALTA, O. (1952). The essential factor regulating the wing-stroke frequency of insects in wing mutilation and loading experiments and in experiments at subatmospheric pressure. *Ann. (zool.) Soc. zool.-bot. fenn. Vanamo*, **15**, no. 2.

WILSON, V. J. (1954). Slow and fast responses in cockroach leg muscles. *J. exp. Biol.* (in the Press).

WATANABE, M. I. & WILLIAMS, C. M. (1951). Mitochondria in the flight muscles of insects. I. Chemical composition and enzyme content. *J. gen. Physiol.* **34**, 675–689.