Zeitschrift für vergleichende Physiologie 58, 429—440 (1968)

Mechanical and Electrical Activity in the Indirect Flight Muscles of the Honey Bee

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Received January 20th, 1968

Summary. 1. Recordings were made from single elements in the indirect flight muscles of the honey bee with the usual insulated metal electrodes uninsulated at the tip. With the "indifferent" electrode in the head few neighboring elements showed up. All recorded elements had random phase differences.

2. In the dorsoventral (DV) muscles about 80% of the spikes are not followed by striking junctional components, 20% are. Practically all spikes from the dorsolongitudinal (DL) muscles have a junctional component following.

3. The DV-muscles in the intact animal shorten linearily with increasing action potential frequency (Fig. 6). The DL-muscles are stretched slightly.

4. Approximately 50 to 100 msec before the onset of oscillations the action potential frequency in both groups of muscles (DV and DL) increases to 30—60 Hz for 2 to 4 action potentials. The DV-muscles shorten (evidently isotonically) about 20 to 40 μ and start oscillations with a lengthening. An experiment performed by BOETTIGER (p. 438) might explain the events that start the oscillations. The oscillations end about 180 to 200 msec after the ending of the action potentials. The DV-muscles return to their resting length in the next 200 to 1,000 msec.

5. The duration of the action potentials depends strongly on the temperature (Fig. 4).

6. The wingbeat frequency increases with the action potential frequency (Fig. 10).

Zusammenfassung. 1. Bei Ableitung von Einzelelementen aus den indirekten Flugmuskeln der Honigbiene mit der indifferenten Elektrode im Kopf, erfaßt man mit den üblichen, bis zur Spitze isolierten Metallelektroden nur wenige Nachbarelemente. Alle erfaßten Elemente haben offensichtlich zufällige Phasenverschiebungen gegeneinander.

2. In den dorsoventralen (DV) Muskeln folgen bei ca. 80% der Elemente keine auffälligen e.p.p. Komponenten, bei ca. 20% findet man eine e.p.p. Komponente. In den dorsolongitudinalen (DL) Muskeln hatten praktisch alle Elemente eine auffällige e.p.p. Komponente.

3. Mit ansteigender Aktionspotential-Frequenz verkürzten sich die DV-Muskeln im intakten Tier linear mit der Frequenz (Abb. 6). Der DL-Muskel wurde etwas gedehnt.

4. Etwa 50—100 msec vor dem Beginn von Oszillationen steigt die Aktionspotential-Frequenz in beiden Muskelgruppen (DV und DL) für 2—4 Aktionspotentiale auf 30—60 Hz. Der DV-Muskel verkürzt sich (offensichtlich isotonisch) um 20—40 μ und beginnt die Oszillationen mit einer Verlängerung. Ein Experiment

* Supported by the National Science Foundation, grant GB 6528.

von Boettiger (S. 438) kann zur Erklärung der Vorgänge dienen, die die Oszillationen einleiten. Die Oszillationen enden 180—200 msec nach Aufhören der Aktionspotentiale. Die DV-Muskeln gehen in den nächsten 200—1000 msec auf die Ruhelänge zurück.

5. Die Länge der Aktionspotentiale hängt stark von der Temperatur ab (Abb. 4).
6. Die Flügelschlagfrequenz steigt mit der Aktionspotential-Frequenz (Abb. 10).

Introduction

Following an analysis of sounds produced by flies and bees (Esch and Wilson, 1967) we suggested a formal model which describes the nature of the various sounds and their relationship to possible skeletal and muscular mechanisms. Reviewing the sounds produced in the communication of bees (Esch, 1961, 1967) we found that the theoretical model is insufficient for an understanding of the behavioral meaning of these sounds. Numerous publications describe the functioning of asynchronous (or fibrillar) flight muscles (literature in Pringle, 1967) of the type found in the bee. But most of these studies were made on preparations quite different from the intact animal. Therefore one can only guess about the coordination of the different muscle groups during flight or sound production. A first study on the coordination of the indirect flight muscles in an intact fly was made by Wilson and Wyman (1963). It brought the fragmentary data together from the literature along with new material. But many important questions remained unanswered. No comparative studies on other aysnchronous fliers were attempted.

We repeated Wilson's experiments using honey bees and tried at the same time to monitor the slow contractions of the dorsoventral muscles (72 in Snodgrass, 1956) and of the dorsolongitudinal muscles (71 in Snodgrass, 1956) before and after flight. We hoped to get some clue as to what might start and stop the oscillations. At the same time we studied the influence of the temperature on the electrical activity in these muscle groups.

Methods

The experiments were carried out with individuals of *Apis mellifera* from our apiary.

Mechanical Recordings. The animals were cemented to a holder at the attachment area of the dorsoventral muscles to the scutum. The ventral attachment area of the dorsoventral muscles was connected either to a Grass Ft 0.03 (displacement) transducer or to a simply constructed photo (displacement) transducer. Provisions were made so that the animal's legs did not interfere with the measurements. A second Grass Ft 0.03 transducer was fixed to the tergum of the propodeum just below the scutellum at a certain angle to the long axis of the animal. Before we fixed the transducers in this fashion we had checked, with a three dimensional transducer setup, in which directions movements could be expected to occur. We then attached the transducers so that they measured movements in these directions.

Thus we excluded unwanted lever effects as far as possible. Fig. 1 gives a diagram of one of the experimental setups used. We were able to move the displacement transducer with a power transducer and thus, with a rigid connection between transducer and animal, could estimate the forces required to move the displacement transducers themselves. It takes a force of 30 milligrams to displace the Grass transducer 1 μ, however, this force evidently did not interfere with the slow contractions. The Grass transducer's resonant frequency, being near 100 Hz, distorted the amplitudes of the oscillations during flight. Therefore we based the results of the measurements made during flight only on data from photo-transducers. This type of transducer consisted of a small vane of a few milligrams weight which was cemented to the animal and which transmitted the movements of the animal into a light beam which was focused on a photo-resistor (DONALDSON, 1958). Its resonant frequency was in the kHz range.

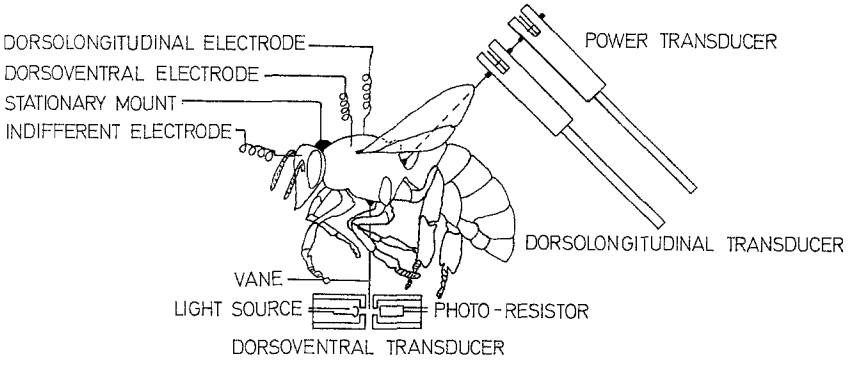

Fig. 1. A diagram of the experimental setup

Electrical Recordings. Platinum wire electrodes were used in all critical experiments. A piece of wire, 5 mm long with a diameter of 0.1 mm, was sharpened to a tip of approximately 50 μ and completely insulated with the exception of the very tip. The electrode was soldered to a copper wire with a diameter of 0.07 mm which completed the connection to the preamplifier. The "indifferent" electrode was placed in the head and the two "different" electrodes were pushed through predrilled holes dorsally into the dorsoventral and the dorsolongitudinal muscles. We used either Tektronix Type 122 preamplifiers (coupling time constant 1 sec) or Medistor Model A-35 negative capacity electrometer amplifiers (DC coupling). The preamplifiers were connected to a Beckman 4 channel Dynograph recorder for direct recording of the action potentials together with the transducer responses. At the same time tape recordings of all four channels were made with a Presicion-Instrument Pl-6100 tape recorder (FM mode, response flat from DC to 10 kHz). The tapes could be replayed with 1/100 of the original speed and thus undistorted recordings of fast responses could be made with a Varian ink recorder. Taped recordings of the events studied were also analyzed with a Data Retrieval Computer (Nuclear Chicago Model 7100).

Temperature Recordings. The body temperature of the animals was measured with a micro-thermistor (Fenwal GB 41 J 1) which was cemented dorsally to the thorax and thus allowed an accurate measurement of the muscle temperature (ESCH, 1960).

Narcosis. In a large number of experiments we used a brief ether narcosis (3—5 min) to fix the animals in the apparatus. The animals did not perform normally for at least 1 hour. Therefore we used animals which we had put into a refrigerator at about 8° C for 15 min prior to an experiment. The animals came back to room temperature during the mounting process and performed well immediately. In all experiments in which we studied the start of flight we used cold-anesthetized animals.

Results

1. Electrical Activity in the Dorsoventral (DV) Muscles

Single units in the DV-muscles could easily be discriminated on the basis of electrical activity. The unit nearest to the electrode appeared as a spike of from 10 to 30 millivolts, depending on the electrode material. More distant units appeared maximally with 25% of this value. With a single electrode one can see that the different units discharge with approximately the same frequency but with phase independence as in the fly (Wilson and Wyman, 1963). A second electrode in the same muscle, placed approximately 200 μ to the side of the first, showed

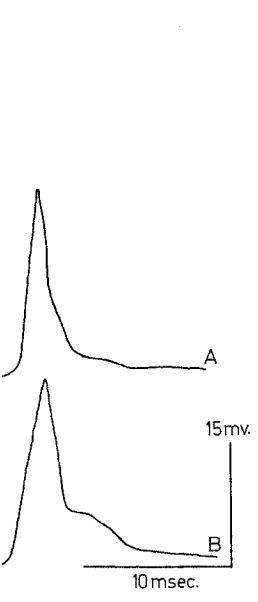

Fig. 2

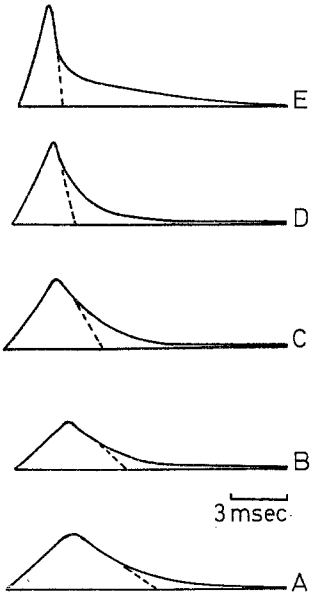

Fig. 3

Fig. 2. *A* An action potential from the dorsoventral muscles with a small junctional component following. *B* An action potential from the same muscles with a considerable junctional component

Fig. 3. Action potentials from the dorsoventral muscles at different temperatures. Each potential is an average of 500 single potentials. *A* 20° C, *B* 22° C, *C* 24° C, *D* 31° C, *E* 33° C. The dotted line indicates how the spike was measured

discharges of elements which could not be detected with the first electrode. A monitoring of the electrical activity of the whole muscle, as is necessary in flight manoeuvre studies, would therefore require more than one electrode. This is especially true if the muscle is served by more than one nerve or nerve branch.

We recorded two types of action potentials: (1) In 80% of our recordings we measured a spike with very little evidence of a junctional component following it (Fig. 2A), in the remaining 20% the spike had a considerabel junctional component following (Fig. 2B). The length

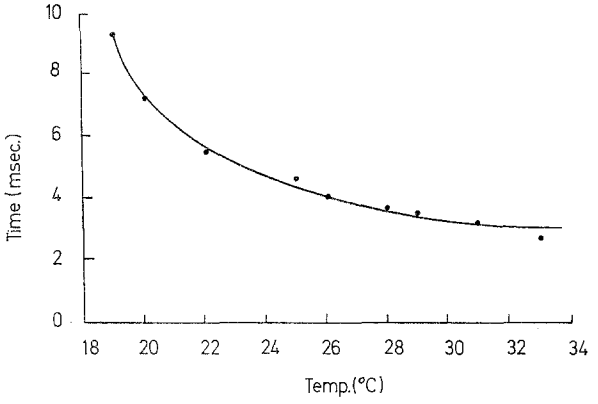

Fig. 4. The length of action potentials from the dorsoventral muscles versus the temperature at which they were measured

of both types of spikes is heavily dependent on the muscle temperature. Fig. 3 shows action potentials at different temperatures. Each action potential represents the average of 500 single potentials which were added in the data retrieval computer in transient averaging mode. The whole series was made from one bee which was preparing for flight. Fig. 4 gives a graphic representation of this series. The length of a spike increases rapidly as the temperature drops into the range of 20° C. The lowest temperature at which our animals produced action potentials was 18° C. This observation is based on 50 animals at various times of the year.

From time to time we found an element which showed a striking junctional component. This element tended to produce double discharges (Fig. 5).

2. Electrical Activity in the Dorsolongitudinal (DL) Muscles

Single units from the DL-muscles could be recorded as easily as those from the DV-muscles. All action potentials have, in contrast to those of the DV-muscles, long lasting junctional components. Most

units tend toward double or triple discharges. The spikes showed the same dependence of length on temperature as did the spikes from the DV-muscles. Different elements fired with approximately the same frequency but with all possible phase relationships. The electrical activity of the DL-muscles is apparently not fundamentally different from the activity in the DV-muscles.

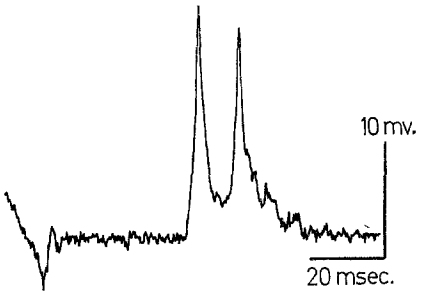

Fig. 5. Double discharge in a dorsoventral element

3. Coordination of Electrical and Mechanical Activity in Both Groups of Muscles

Dorsoventral Muscles. Both ends of the muscles (72 in Snodgrass, 1956) are directly attached to the thorax capsule. Therefore contractions of these muscles decrease the distance between the dorsal and ventral attachment areas. A check with a three dimensional transducer setup showed that with the onset of electrical activity in the muscle the ventral attachment area moves upward in the direction of the muscle fibers. This is about 90° to the long axis of the animal. With the appearance of action potentials the muscles do shorten. The degree of contraction relates linearily to the action potential frequency, up to about 10 Hz. The amplitude of the contraction at this frequency is about 15 μ (Fig. 6). All observable elements fire with phase differences. Sometimes an apparently accidental synchronization occurs; all recorded elements fire within 30 milliseconds. Under these circumstances the muscle responds with a small twitch of 2 to 3 μ in excess of the state of contraction characteristic of this stimulation level. Along with the contraction one observes a considerable heat production. At a spike frequency of 10 Hz the temperature rises at a rate of 2.3° C per minute. It reaches values of about 10° C above environmental temperature, between 20 and 30° C, a value which was also found in an earlier study. Using an estimation from this investigation (Esch, 1960) about 0.3 cal/min are required to keep the thorax temperature 10° C above that of the environment.

Dorsolongitudinal Muscles. These muscles (71 in SNODGRASS, 1956) have their anterior ends attached directly to the scutum. This attachment area was taken as a reference point, the animals were fixed here. The posterior ends of these muscles are connected to the postphragma of the mesothorax, which upon contraction of the DL-muscles, moves

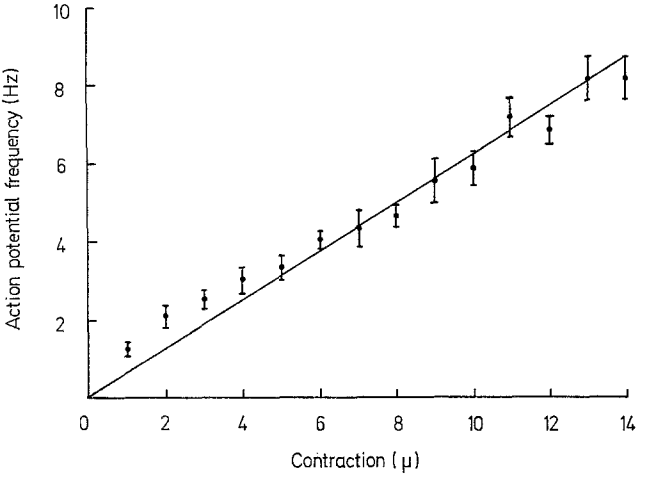

Fig. 6. The relationship between action potential frequency and contraction in the dorsoventral muscles in an intact animal

the scutellum and the parts behind the scutellum around a hinge in front of the scutellum. A transducer, connected to the area just behind the scutellum and at an angle that allows the transducer arm to follow the movement around the hinge, was used to measure the contraction of the DL-muscles. At the beginning of electrical activity we recorded a movement of the transducer that indicated that the DL-muscles were not contracting but were actually being stretched. An observation of the dorsoventral muscles at the same time showed clearly that this muscle stretches the dorsolongitudinal muscle. A contraction of 15 μ by the DV-muscle stretched the DL-muscle between 1.5 and 3.0 μ.

4. The Events at the Beginning and at the End of a Flight

Observations were made on animals fixed to a pair of transducers (Fig. 1) to measure contractions of DL and DV-muscles simultaneously. One of each of the "different" electrodes was inserted into a DL and a DV-muscle.

Many animals showed an action potential frequency of about 10 Hz several minutes before flight. The DV-muscles contracted and the

DL-muscles were stretched as previously described. Heat production in the muscles caused a temperature increase of about 10° C above

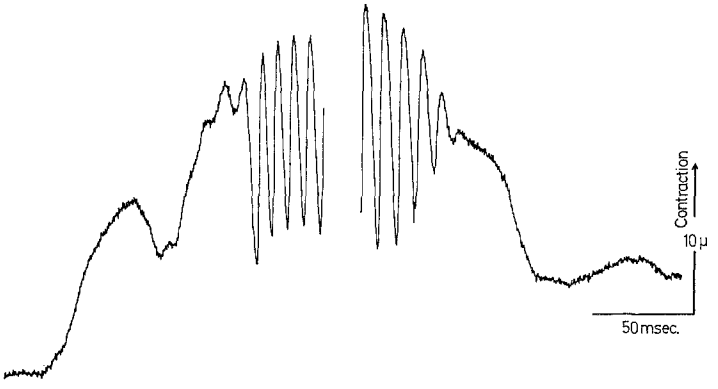

Fig. 7. Contraction of the dorsoventral muscles immediately before the start of oscillations and the relaxation after the end of the oscillations

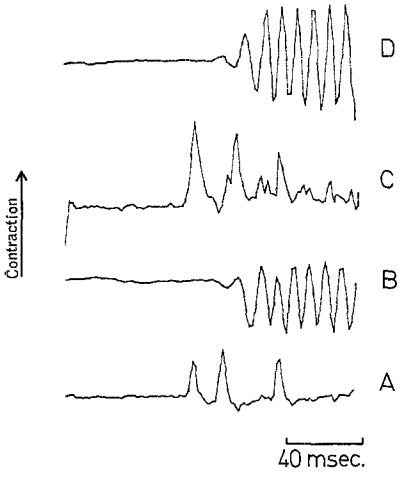

Fig. 8. The events at the beginning of flight: *A* Action potentials from the dorsoventral muscles; *B* Length change in the dorsoventral muscles; *C* Action potentials from the dorsolongitudinal muscles; *D* Length change in the dorsolongitudinal muscles

that of the environment. When an animal went into flight the frequency of the action potentials in both groups of muscles rose to 30—60 Hz 50 to 120 msec before one could record oscillations. Two to four high frequency action potentials could be observed before the frequency fell back to 10 Hz. In the DV-muscles 2 to 4 independent action potentials could be seen, while in the DL-muscles 2 to 4 discharges took place in the course of a long junctional component (multiple discharge). With the beginning of the multiple firing the dorsoventral muscles shorten between 20 and 40 µ and after reaching a plateau start to oscillate (Fig. 7). Oscillations are performed around the level of contraction reached immediately before the beginning of the oscillations. The dorsoventral muscles actually start and lead the oscillations (Fig. 8). We could not observe a significant lengthening or shortening of the

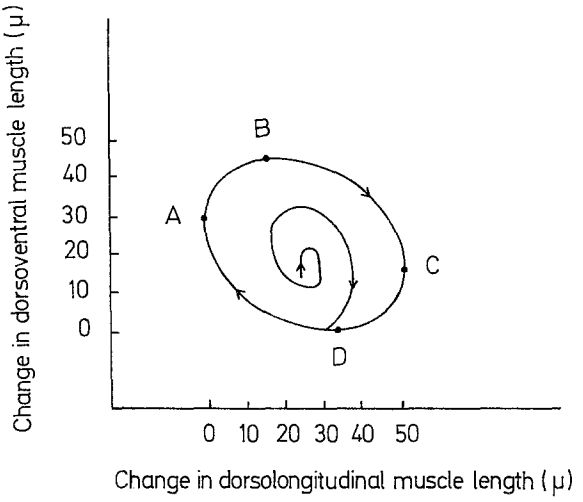

Fig. 9. An x—y plot of the length changes in the dorsoventral and the dorsolongitudinal muscles during oscillations. Oscillations start in the middle of the circle. The point runs in the direction indicated by the arrows

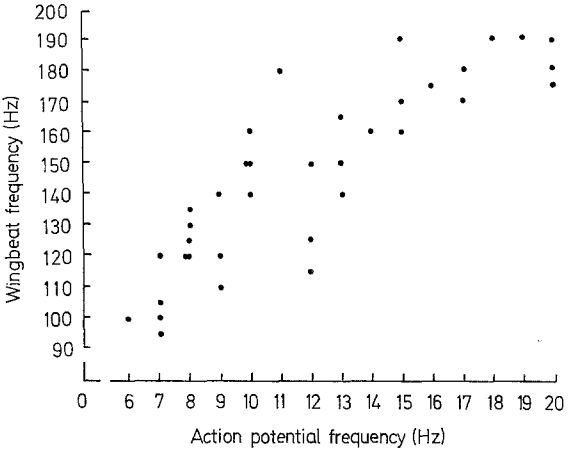

Fig. 10. Relationship between wingbeat frequency and action potential frequency. For details see text

DL-muscles prior to the oscillations. During stationary flight the oscillations of both groups of muscles had a phase difference of about 130°. An x—y recording of the length changes illustrates this fact (Fig. 9).

The shape and the duration of action potentials during flight was not different from what we observed in animals preparing for flight.

The amplitude of the oscillations in both muscle groups was between 30 and 60 μ. About 180 to 200 msec before the end of a flight the electrical activity stopped completely and the amplitude of the oscillations decreased to zero. The length of the DV-muscles increased in the next 200 to 1,000 msec to the length that they had before flight (Fig. 7).

The frequency of the oscillations depended on the frequency of the action potentials in a way similar to that described by Wilson and Wyman (1963). Each point in Fig. 10 represents averages of action potentials and wingbeat frequency measured over 1 second intervals. The measurements were taken from many different flights and different animals.

Discussion

The Flight Command. At the beginning of flight a bee unfolds the wings using the direct flight muscles and starts the wing oscillations with the indirect flight muscles. The flight command, therefore, consists of at least two components. We did not record the command to unfold the wings because it seemed to have no direct bearing on the start of the oscillations. Animals frequently unfolded the wings without any detectable oscillations following, or started oscillations with folded wings.

The action potential frequency in the indirect flight muscles was in most cases the same before and during flight. We could not find any changes in the shape or the length of the potentials which could explain why muscles did not oscillate at one time (flight preparation) but oscillated at another time (flight). The action potential frequency could not in itself be the reason for the oscillations. The command to start the oscillations seems to be the momentary high action potential frequency of practically all units in both groups of muscles (71 and 72) immediately preceeding the onset of the oscillations. An experiment performed by Boettiger (1957), with the partially detached dorso-longitudinal muscle of the bumble bee, offers an explanation of the events which could start the oscillations: Fig. 11 shows a reproduction of Boettiger's figure explaining that experiment. The detached post-phragma was connected to a mechano-electrical displacement transducer by a short chain. A second transducer was used to measure the tension that the muscles exerted. The muscles were loaded with a weight to the tension T. Stimulation with a sufficiently high frequency shortened the muscles in a normal isotonic manner to the point a. Here they started to oscillate and the amplitude increased to a steady state curve $b\ b'$ through which the muscles ran in a counterclockwise direction.

The results of our experiments suggest that in the bee both muscle groups become tensed with the beginning of the electrical activity. They do not show a drastic change in length. As one can see from the record-

ings as well as from the high metabolic activity (temperature) both muscle groups must act against each other. They move from B to T in Fig. 11. At this point the momentary high action potential frequency can bring the dorsoventral muscle from T to a in an isotonic length change. One can observe this length change (Fig. 7) which has no effect on the length of the dorsolongitudinal muscles, contrary to the changes that occured earlier. At this point the oscillations of the dorsoventral muscles start. Since the muscles run counterclockwise through b b'

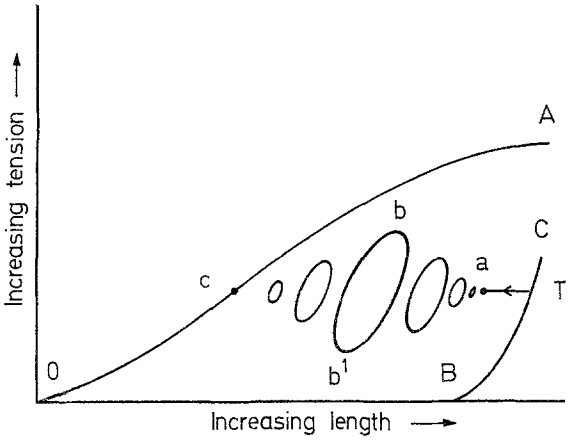

Fig. 11. A length-tension diagram from the dorsolongitudinal muscles of the bumble bee (redrawn from BOETTIGER, 1957)

the oscillations have to start with a lengthening of the dorsoventral muscles. This can be observed (Figs. 8 and 9). Once started, the oscillations can be maintained by the antagonistic action of the two muscle groups (71 and 72). A stop of the electrical activity releases the tension and the oscillations have to die off.

The Temperature Dependence of Action Potentials. DEL CASTILLO (1953) reported the dependence of the duration of action potentials on temperature in the leg muscles of a locust. While the magnitude of the changes seems to be similar to what we observed in the indirect flight muscles of the bee, the range in which these changes occur is shifted to higher temperatures in the bee[1]). The very fast increase in the length of an action potential as one approaches 18° C from higher temperatures indicates that the chemical reactions responsible for the action potentials slow down quickly at this temperature. From Fig. 4 one can see that

[1] Experiments that we performed after this manuscript went into print showed that the wingbeat frequency of the bee changes with temperature in a very similar fashion. These results will be published elsewhere.

it is very unlikely that flight or normal muscle activity can occur at temperatures below 18° C. It becomes understandable that bees always maintain a center of high temperature in the winter cluster (Esch, 1960). Since the indirect wing muscles are the main source of heat and a proper functioning of these muscles is only guaranteed at temperatures higher than 18° C an area must be available to which bees from the outer layers of the cluster can go and take part in social heat production.

Reported differences in the length of action potentials might easily depend on the temperature.

It is interesting to note that the length of a twitch in isolated flight muscles of locusts seems to follow a similar pattern of dependence on temperature. The length of a twitch rises nearly logarithmically with decreasing temperature (Buchthal, Weis-Fogh and Rosenfalk, 1957).

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