Studies on the Flight Mechanism of Insects

I. The electrophysiology of fibrillar flight muscle

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Abstract Fibrillar type flight muscle powers the flight machinery of the more phylogenetically advanced groups of flying insects. A comparison of responses from single fibers in insects from various orders having fibrillar muscle reveals fundamental differences. In single fibers of flies and wasps the response to a single threshold stimulus is an all-or-none, uniformly rising, in most cases overshooting action potential. Beetles give variable responses, some of which appear similar to the type mentioned above, and others which summate and facilitate. Some of the latter responses vary with time in a cyclic manner, and some are altered by the intensity of the stimulus. Further differences appear when the two types of muscle are exposed to ether and carbon dioxide. In the wasp and fly ether produces a neuromuscular block, while CO₂ effects a rapid depolarization of the resting fiber membrane. Both reactions are completely reversible. The electrical responses of beetle muscle are somewhat affected but only by massive doses. The implications of these data are discussed relative to the existence of fibrillar muscle "types."

Introduction

On the basis of histological evidence, two major categories of insect flight muscle have been described; viz., fibrillar and non-fibrillar. The details of the structural organization of flight muscles have been studied in a variety of insects (27, 28). The distribution of these two types of muscle structure among the various orders of flying insects indicates that the flight muscles are of one type or the other, except in a few "transitional" orders in which both fibrillar and non-fibrillar types occur (24).

An asynchronous relationship between the frequency of muscle action potentials and wing-beats, a functional characteristic of the fibrillar structure,
has been demonstrated with extracellular recording techniques (25, 26, 3). This has been confirmed with intracellular microelectrodes (10), and is illustrated in Fig. 1.

![Figure 1](image_url)

**Figure 1.** The “asynchronous” neuromuscular control of fibrillar flight muscle. This simultaneous recording of intracellular muscle action potentials (upper trace) and wing-beat frequency (lower trace) in a fly (*S. bullata*, Parker) illustrates that there is no simple 1:1 relationship between the two events. Action potentials are recorded with an intracellular microelectrode. Wing-beat frequency is recorded electrostatically, i.e. an electrostatic charge, induced on a leucite plate by rubbing with a woolen cloth, is placed on the wings of the insect. As the wings move up and down, the disturbance created in the electrostatic field is recorded by external electrodes mounted close to the insect’s body. Low amplitude ripple indicates 60 cycles/sec. Artefact preceding first action potential is from an adjacent fiber.

Non-fibrillar muscle operates in a conventional synchronous manner. Thus, different insect flight muscle types can be described as *non-fibrillar-synchronous* or as *fibrillar-asynchronous*. The former type is found among the more primitive, less well developed fliers, the latter in the more versatile phylogenetically advanced flying insects.

Recently, evidence has been introduced (5–7) which suggests that muscle
from different insect groups that is morphologically fibrillar and functionally asynchronous reacts differently to rapid changes in length as indicated by length-tension curves of different types. To determine whether these differences in mechanical properties are correlated with differences in electrical properties, a comparative study is presented in which the electrical activity in single muscle fibers of insects from the order Coleoptera is compared with that of several species of Diptera, Hymenoptera, and Hemiptera.

METHODS

Although records have been obtained on a number of different species, a complete study was made only on the following insects: Sarcophaga bullata, Parker, the flesh-fly; Vespula diabolique (Hymenoptera), a wasp; Pissodes strobi, Peck (Coleoptera), the white-pine weevil; Tenebrio molitor (Coleoptera), the meal-worm; and Nezara viridula (Hemiptera), a stink-bug.

All preparations were in situ; no insect Ringer’s solutions were used. A silver chloride reference electrode was inserted directly into the body of the insect. The dorsal longitudinal flight muscles of the fly, wasp, and bug were exposed by excising a portion of the scutellum. Due to the presence of an air space immediately under the exoskeleton in this area, the muscle fibers do not adhere to its inner surface; therefore, the surgical procedure could be performed with a minimum of injury. Because of the practically isometric nature of the contraction of these muscles, there was virtually no movement to disturb the inserted electrode. Since the dorsal longitudinal flight muscles of the beetles were adherent to the exoskeleton, these muscles were severely injured during the operative procedure and thus were not used. The basalar muscle of the large beetle Oryctes, a preparation recently introduced for neuromuscular studies (14), was selected for use. The beetles were mounted laterally so as to permit the removal of the pleuron and exposure of the basalar muscle. The basalar, a direct muscle, is used not only in the down-stroke of the wing, but also assists in adjusting its airfoil characteristics (14). Thus, this muscle has a dual function, whereas the dorsal longitudinal flight muscle of the flies, wasps, and bugs serves as a power muscle, delivering its force through an indirect lever arrangement during the downstroke of the wing (8).

Due to the small size and delicate nature of the insects used, the nerves innervating the muscle fibers were not isolated for direct stimulation; two small diameter teflon insulated silver wires were inserted into the area of the central thoracic ganglion. Pulses were obtained from Grass isolation transformers connected to two Grass S4A stimulators. The stimulators were arranged so that the first of two stimuli (conditioning) could be followed at a variable interval by a second (test) stimulus, both with independently controlled duration and intensity.

Electrical activity in the individual fibers was recorded by microelectrodes of the conventional type connected to the oscilloscope through a negative capacity transistor amplifier (1).
A. Resting Potentials

Although insect flight muscle possesses little connective tissue, impalement of individual fibers is somewhat hampered by the presence of dense tracheolar networks which surround and invade the muscles. This difficulty is easily overcome by pulling back slightly on the electrode, and advancing it gently until a sudden precipitous drop of potential in the negative direction is recorded. The virtual absence of connective tissue, the isometric nature of the muscle contraction, and the ready availability of material afford a preparation easily adapted for classroom use.

The average values of the resting potentials observed in five insects representing four different orders are presented in Table I. These values include all determinations that could not be ruled out as a result of obvious injury. The over-all values are somewhat lower than those reported for vertebrate striated muscles but are similar to those observed in invertebrates. The similarity in the magnitude of the resting potential of the bug and the beetle Tenebrio points out the variations that one may encounter in insects of different orders, while the differences between the two beetles emphasize that variations of significant value may be present in insects within the same order. The variations in the

## Table I

### Summary of Average Values of Resting and Action Potentials*

<table>
<thead>
<tr>
<th>Insect (Order)</th>
<th>Diptera (fly)</th>
<th>Hymenoptera (wasp)</th>
<th>Hemiptera (stink-bug)</th>
<th>Coleoptera (Pine weevil)</th>
<th>Coleoptera (Meal worm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. fibers reported</td>
<td>348</td>
<td>150</td>
<td>115</td>
<td>150</td>
<td>178</td>
</tr>
<tr>
<td>Resting potential, mv</td>
<td>(40-77)</td>
<td>(50-68)</td>
<td>(25-55)</td>
<td>(50-65)</td>
<td>(45-58)</td>
</tr>
<tr>
<td>Action potential, mv</td>
<td>92</td>
<td>80</td>
<td>58</td>
<td>70</td>
<td>57</td>
</tr>
<tr>
<td>Overshoot, mv</td>
<td>24</td>
<td>18</td>
<td>8</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Latent period, msec.</td>
<td>(0-26)</td>
<td>(0-21)</td>
<td>(0-10)</td>
<td>(4-12)</td>
<td>(5-10)</td>
</tr>
<tr>
<td>(Stimulus to onset of rise)</td>
<td>(1.0-3.1)</td>
<td>(1.8-3.0)</td>
<td>(2.5-4.5)</td>
<td>(3.0-6.8)</td>
<td>(1.9-3.4)</td>
</tr>
<tr>
<td>Time from onset to peak, msec.</td>
<td>2.5</td>
<td>2.8</td>
<td>6.0</td>
<td>10.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Time from onset to 1/4 decline, msec.</td>
<td>(1.8-3.5)</td>
<td>(2.0-3.9)</td>
<td>(4.5-6.0)</td>
<td>(8.4-12)</td>
<td>(2.8-5.0)</td>
</tr>
<tr>
<td>Maximum rate of rise, n/sec.</td>
<td>6.5</td>
<td>6.5</td>
<td>8.4</td>
<td>22.3</td>
<td>7.0</td>
</tr>
<tr>
<td>(4.0-12.0)</td>
<td>(5.0-9.5)</td>
<td>(6.0-10.5)</td>
<td>(20.0-25.2)</td>
<td>(5.5-8.0)</td>
<td></td>
</tr>
<tr>
<td>(46-60)</td>
<td>(31-48)</td>
<td>(12-21)</td>
<td>(6-10)</td>
<td>(15-23)</td>
<td></td>
</tr>
</tbody>
</table>

* Figures in parentheses indicate range of values.
magnitude of the resting potentials are probably due to dissimilarities in the ionic content of the hemolymph as a result of differing dietary habits (18).

B. ACTION POTENTIALS

Measurements of resting potentials and individual fiber responses elicited by a single stimulus which were all-or-none and uniformly rising are presented in Table I. This type of potential response was observed in all fibers of flies and wasps, only occasionally in the bugs, and rarely in the beetles. Miniature oscillations such as have been observed in insect tymbal muscles (20, 29) were not observed in any of the insects studied here; however, on numerous occasions sporadic bursts of small (5 to 10 mv) potentials were noted, but since these usually occurred as the electrode was being positioned, it was felt that these were due to injury or the irritation induced by the electrode itself.

In response to a single stimulus, the single flight muscle fiber of the fly and of the wasp gave an all-or-none, uniformly rising, and in most cases overshooting action potential; i.e. the membrane potential underwent a brief excursion in the positive direction. This was the only type of response evoked by such a stimulus in these insects. Under these conditions, there was at no time an inflection during the rising phase indicative of a junctional type component or any other suggestion of a compound nature.

In all insects the average values of the action potentials showed the property of overshoot to some degree. Overshoot is, however, not of critical importance to the animal, for in some cases, the insect was quite capable of sustained free-flight even though no overshoot could be recorded.

Studies involving direct stimulation of muscle membrane have been completed on the fly and will be included in a later report. The physical parameters of the fly fibers are similar to those described for Cicada (16), but comparable studies have not as yet been completed for the beetle.

The action potentials of all the insects exhibit a somewhat prolonged time course. The total duration of an action potential is, however, not always a well defined parameter, since many authors do not clearly indicate the points at which their measurements are taken. It can be seen from Table I that one beetle, Pissodes, showed a prolonged latent period of 4.9 msec. while the other beetle Tenebrio had an average latent period of only 2.8 msec, indicating that sites of innervation are more numerous in the latter. This further illustrates that one is likely to encounter differences within orders of insects and the practice of extrapolating data must be exercised with caution.

While the type of action potential presented in Table I was found to the exclusion of all others in the fly and wasp, it was more the exception than the rule in the insects of the orders Coleoptera and Hemiptera. In the latter two groups the action potentials frequently exhibited an inflexion during the rising
phase indicative of a junctional type component. This suggests that either the nerve-muscle junction is modified in some unique way in these groups or that the pattern of innervation is more diffuse, the greater number of junctional areas increasing the frequency with which the microelectrode encounters these regions.

The pattern of innervation of the flight muscles has been studied for a number of insects (28). The nerves are known to terminate at multiple sites along the muscle fiber membrane (multiterminal innervation), a pattern prevalent in the arthropods. Recordings made simultaneously from two microelectrodes in the same fiber (fly), separated by a distance of about 1 mm, show no detectable latency. This indicates that the impulse is delivered simultaneously at multiple sites, thereby activating the entire muscle fiber all along its length; i.e., these muscles do not propagate electrically. This mechanism functions in the absence of a propagation system such as is found in the vertebrate fibers.

The action potentials evoked by a single stimulus in the single fibers of the basalar muscle of the two beetles displayed variations which at times occurred spontaneously and at others appeared related to the intensity of the stimulus. In the beetle fibers a junctional type potential (j.p.) was often the only response to a single stimulus, as shown in Fig. 2A. In this case, two independently controlled stimuli each evokes only a j.p. (A), and is repeated as the interval between the two stimuli is decreased (B). The interval may be decreased still further, whereupon a spike-like component arises from the summated junctional potentials (C).

The junctional potential in response to a single stimulus may or may not reach threshold for the spike in beetle fibers. In Fig. 3, the same stimulus repeated after return of the potential to the resting level may give rise to a spike or just a j.p. Either type of response may result from the first stimulus (Fig. 2).

**Figure 2.** Summation of junctional type potentials in the beetle *P. strobi*, Peck. Each of two stimuli elicits a junctional type potential (A). This event is repeated in B where the interval between stimuli has been shortened. As the interval between stimuli is shortened still further, the junctional potentials sum their effects to produce a spike-like response as in C.
This behavior suggests that there may be some long-lasting effect on excitability as part of the response of the fiber to stimulation.

The spontaneous cyclical variations of response encountered in these muscle fibers are further illustrated in Fig. 4. This phenomenon occurred during constant, repetitive (1/sec.) stimulation. The first action potential (A) rises uniformly, overshoots, and returns to the original resting level. The second response (A-1) is identical to the first (A) in magnitude and time course, but shows a slight deviation during the falling phase as its arrival back to the original resting level is delayed. The third response (A-2) follows as the previous two, but breaks slightly earlier during the falling phase thus showing a marked slowing and "humping." The fourth stimulus produces again a response identical to the previous three, but this one breaks at a slightly earlier
time than the others during the falling phase and thus reaches a critical firing level which erupts into a damped oscillation (A-3). Although stimulation is carried on under the same conditions, the subsequent stimuli produce the sequence of responses B-C-D. The total response appears exhausted in D, but as the next stimulus arrives, the potential seen in A reappears, and the cycle is repeated many times.

![Figure 4](image)

**Figure 4.** A cyclic sequence of responses from a beetle muscle fiber. A stimulus of constant intensity and duration, given at 1 sec. intervals produces action potentials which vary from a uniformly rising, overshooting type (A), through a series of intermediates (A-1, 2, 3, B, C), ending with a small residual junctional type potential (D). Following this last minimum response, the cycle of events repeats.

Either there is a cycling of excitability of the fiber, or each stimulus leaves a residual effect that remains at least 1 second. The multiple firing of spikes occurs as a transient event as the muscle fiber readjusts to prevailing physico-chemical conditions.

In addition to spontaneously occurring variation of electrical activity in the beetle fibers, a series of electrical potentials was observed which appeared graded in relation to the intensity of the stimulus. In some fibers only two amplitudes of potential could be evoked, as shown in Fig. 5 A. As the stimulus intensity is increased to the point where a response is just apparent, the
Figure 5. The influence of stimulus intensity on the electrical activity in beetle muscle fibers. The pattern most frequently seen followed that series shown in A and B. Note that in B, an oscillatory discharge is evoked as a critical level of excitation is reached. Many intermediate gradations of this occurred, but only once was a complete step series seen as in C.
smaller of the two potentials appears. As the intensity of the stimulus is increased, the larger spike evolves. In some fibers it is possible to show intermediate stages in the development of the spike (B, C), but a complete series such as the latter (C), was observed only once. In this case the position of the electrode may have been unique with respect to multiple nerve endings. The response in B is frequently seen. As the intensity of the stimulus is increased, the muscle fiber responds with an oscillation that appears damped as the potentials grow progressively smaller.

The responses from fibrillar muscle in the Hemiptera studied were generally more difficult to obtain. The reason for this remains obscure in view of the fact that the preparation seems ideally suited for such studies. The action potentials most frequently observed showed a junctional component during the rising phase and in this respect showed a greater similarity to the beetles than to the fly-wasp group.

C. THE EFFECTS OF CHEMICAL AGENTS

Ether The commonly used anesthetic agent ether attacks the neuro-muscular system of flies and wasps at two sites; viz., the central thoracic ganglion and the neuromuscular junction. Insects of the orders Hemiptera and Coleoptera are relatively unaffected except in doses which proved lethal; the reactions in these latter two groups were not reversible.

A small amount of liquid diethyl ether was placed in a blow-bottle, so that only the vapors were forced out through a small bore capillary pipette. Since the insects (flies and wasps) become rapidly anesthetized when exposed to ether vapors, the over-all effects of this agent can be more clearly observed as the animal recovers. Thus, after the administration of ether is discontinued, and recovery proceeds, spontaneous, repetitive, action potentials begin to appear. At this time, the wings remain quiescent. Potentials can occur without movements, and in this case may be explained by the simultaneous contraction of muscles mutually antagonistic (8) or by the occurrence of a block between the process of transmission and contraction. The potentials appear sporadic at first, and reduced in amplitude. Gradually, the potentials fire with increasing frequency, until a continuous, regular, sustained barrage of impulses is observed. At this point, the wings begin to beat or can be made to beat if wing movement is started by blowing on the wings. This build-up in frequency of the electrical discharge is necessary for driving the flight machine. Since a single stimulus produces little mechanical response, a series of impulses is necessary to activate the system to the point at which flight occurs. This phase of hyperexcitability, expressed by an uncontrolled beating of the wings, is regarded as “anesthetic flight.” During this period, the insect is unable to sustain itself in free flight due to a loss of control over the direct flight mus-
cles—muscles which normally set the articulations thereby enabling it to alter its angle of attack during different phases of the up and down stroke, maneuvers which are necessary to provide aerodynamic lift. Sporadic bursts of these action potentials continue after flight movements have ceased.

It can be demonstrated that the ganglionic elements supplying the flight muscles are influenced by ether vapors. If the ganglion is rapidly excised while the muscle is giving bursts of ether-induced potentials, the discharge ceases abruptly; the muscle resting potential remains unchanged. The regular discharge of ether potentials can also be recorded from the isolated ganglion as ether vapors are blown over it.

To study the effect of ether on the neuromuscular transmission process, muscle action potentials, evoked by an electrical stimulus, were recorded continuously as ether vapor was blown over the insect. The resting potential remained unchanged except for a slight depolarization which occurred as flight movements started and which probably resulted from disturbances due to movements of the abdomen. Since the recovery period is easier to study, the

![Figure 6](image)

**Figure 6.** The neuromuscular blocking effect of ether (wasp). Ether vapors reduce the total action potential to a junctional type response (A). The response grows progressively during recovery (B, C, D) until the normal original action potential appears (E), completely masking the junctional component.
electrode was inserted into a single fiber and stimulating electrodes were placed in the region of the central thoracic ganglion. The insect was then anesthetized and stimulation was started as recovery proceeded. The first response to a single stimulus was that shown in Fig. 6 A. At this time, only a small junctional type potential can be evoked. The response grows progressively and in B, the j.p. appears larger. As the j.p. grows larger, a spike component arises

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**Figure 7.** Summation of junctional potentials (wasp). After treatment with ether, two stimuli elicit only junctional potentials (A). These responses summate as the interval between stimuli is decreased (B, C). (C) which continues to enlarge as it arrives earlier out of an heightened junctional component (D). As recovery is completed, the uniformly rising, all-or-none, overshooting action potential characteristic of these fibers appears (E). There is in this last response no evidence of a junctional component. The junctional response revealed by ether treatment is capable of summation as seen in Fig. 7. These results are identical in both flies and wasps; the other insects showed only an occasional sporadic burst of ether potentials, and a general refractoriness to the influence of ether.

A similar effect of ether on nerve tissue and the neuromuscular junction has
been described for the vertebrate and generally attributed to a disturbance in the metabolism of the tissues (17). In the case of the vertebrate, this effect of ether strongly resembles that produced by curare (2, 15), and indeed, these agents act synergistically, the effectiveness of one being enhanced by the other.

The mechanism by which curare effects a neuromuscular block has been extensively studied, and it is generally held that curare competes with acetylcholine for specific receptor sites located in the end-plate regions (11); the mechanism of ether block is not understood. Curare is generally ineffective in producing neuromuscular block in insects and other invertebrates. Since ether does produce this block in the insects (flies, wasps), it may be that the mechanisms by which these two agents effect their action are not the same, but are linked together through some common factor.

It is suggested that this common factor lies in a disturbance of the ionic balance in the immediate environment of the cell. It is not justifiable to speak of the effects of ions as isolated entities, since so many reports now show that ionic interactions must be considered. Because of these interdependencies, influences, and antagonisms which exist between ions of different species, it seems more appropriate to speak in terms of ion balances. Thus, by lowering the calcium content of a system, one studies not only the lowered content of this ion, but also the excess of magnesium ion which now exists in an unantagonized form (12). Such a state of calcium-magnesium antagonism has been shown to occur at the vertebrate end-plate.

It has been demonstrated that ether is an especially effective agent for causing calcium release from muscle tissue (4). If ether is disturbing the calcium state by lowering the effective ionic concentration, a hyperexcitability of nerve with consequent spontaneous firing would be expected. This loss of calcium would leave a number of magnesium ions unantagonized and would essentially increase the effective concentration of magnesium ions. The excess magnesium could cause a neuromuscular blockade as has been shown to occur in other insect muscles (18, 30).

The synergism between ether and curare in the vertebrate may thus involve a dual attack on the transmitter substance. Since calcium (or more correctly an optimal balance between calcium-magnesium) appears necessary for the liberation of acetylcholine (13), ether, by reducing the calcium level, decreases the amount of acetylcholine production, while curare competes with the reduced amount of Ach present. The decreased production of transmitter substance, plus the complexing of the residual amount results in a neuromuscular blockade—an effect which is further enhanced by the excess of magnesium ions.

**Carbon Dioxide**  Carbon dioxide is widely used as an anesthetic agent for insects. A stage of anesthetic flight, similar to that induced by ether follows the administration of the gas, but the flight movements are notably ephemeral.
Direct recordings from isolated ganglia show this to be an effect on the ganglion of very short duration.

To study the effects of CO₂ on the muscle fiber, a small stream of 100 per cent CO₂ was directed over the insect preparation. The amount of gas was kept at a minimum; the aperture emitting the gas was of about 1 mm outside diameter. The effect of CO₂ on both resting and action potential was identical in the fly and wasp and so is presented for the wasp in Fig. 8. Stimulation was set at 1/sec., so that in 3 seconds the response changed from that of A to D.

Depolarization was almost immediate and completely reversible. The muscle action potential as it appeared before the administration of carbon dioxide is shown in Fig. 8 A. The original level of polarization and the level of zero potential are indicated. As CO₂ flows over the insect, a depolarization occurs and the action potential becomes diminished (B). Progressive depolarization is accompanied by progressive diminution of the action potential (C, D).
Exposure of the beetle and bug preparation to similar concentrations did not depolarize the membrane but rather produced a neuromuscular type block as was seen in the fly and wasp when they were treated with ether vapors. A similar effect on the neuromuscular junction of the spiracular muscle of a locust has been reported recently (19). Only a slight depolarization was noted in the latter fibers in the presence of 100 per cent CO₂ while many showed no depolarization at all under this concentration. Thus a dual effect of carbon dioxide; i.e., one on the neuromuscular junction and another directly on the muscle membrane itself is suggested.

The differential effects of carbon dioxide on the two groups of insects—the depolarization of the muscle fiber membrane in flies and wasps on the one hand, and blockage of the neuromuscular transmission process in bugs and beetles on the other, may be due to differences in the pattern of innervation of the individual fibers or to more subtle modifications in the membrane itself.

**DISCUSSION**

A comparison of the electrical activity in single fibrillar flight muscle fibers of insects from several orders reveals differences both in the neuromuscular mechanism and in the excitability of the muscle membrane itself. These differences are expressed in the response of single fibers to an electrical stimulus and to the influence of ether and carbon dioxide. On the basis of the foregoing information at least two functional types of fibrillar muscle may be described; *viz.*, one characteristic of Diptera and Hymenoptera, the other of Coleoptera. Hemiptera occupy a somewhat intermediate position.

The differentiation is based first on the fact that whereas the all-or-none, uniformly rising action potential with low latency and fast electrical change is exclusively the normal response of the muscle fibers of the flies and wasps studied, the beetle fibers give variable responses. When the latter are all-or-none, they are definitely slower in rise and fall and have a longer latency, as compared with the muscle potentials of the fly and of the wasp. Second, the variable responses, sometimes a junctional response, at other times an all-or-none spike, appear to depend on previous stimulation even when as much as 1 second has elapsed between stimuli and also on the intensity of the stimulating shock. Occasionally stimuli of constant amplitude, repeated at 1 second intervals, gave a response series cycling between a pure junctional potential and an all-or-none response. This cycling continued for long periods of time. This effect might result from some long lasting influence of the stimulus such as the persistence of the transmitter substance or from a change in the responsiveness of the junction to the nerve impulse. Alternatively some unknown factor not controlled in these experiments might be responsible.

In the same way the graded responses to graded stimuli are subject to different possible explanations. The most obvious is multiple innervation of single
fibers. However, several observations make this explanation questionable. In the beetle *Oryctes* the basalar muscle appears to be innervated by only one fiber (14). A recent study of the same muscle in *Tenebrio* by Smith (27) failed to reveal evidence of multiple innervation. Different sized nerve fibers were not found with the electron microscope and only one axon entered into a junctional region. Finally one would have to assume several fibers, as many as five, to explain the five steps in Fig. 5 C. Another possibility, in view of the fact that in these experiments stimulation was diffuse though directed to the area of the thoracic ganglion, is that with higher intensities multiple firing of the ganglion cells was induced. The number of nerve impulses would have to be more or less related to the strength of the stimulus. These stimuli were, however, of very short duration, considerably less than 1 millisecond.

Again some unknown factor may be involved. A peculiar feature of the basalar muscle of the beetle *Oryctes* is that the extent of activation of the muscle is a function of the passive muscle length. Changes in length induce changes in activation after a delay (6, 21, 22). It is not known how length exerts its effect on the contractile mechanism but certainly one of the most obvious possibilities is *via* the excitation processes of the membrane. There is no evidence that such a mechanism operates at the level of electrical activity and some evidence that any correlation between electrical change and length change is insufficient to account for the effect on the active state (14). However, if one can generalize about beetles from the experiments on *Oryctes* and the present ones on *Pissodes* and *Tenebrio*, it is perhaps significant that the type of fibrillar muscle that shows the unusual dependence of its activation mechanism on passive muscle length should also exhibit special electrical properties. Perhaps the factor accounting for the above results is muscle length which was not controlled in these experiments.

By contrast in Hymenoptera, and perhaps in Diptera, which have similar electrical behavior, the active state is not a function of passive muscle length, changing only momentarily with length changes. Such a transient effect of length change may indicate that length is acting on some intermediate stage of the excitation-coupling sequence and therefore since in these insects length change does not affect the electrical properties of the membrane, all responses are the same all-or-none spikes.

The interesting differential effects of carbon dioxide and ether on the two types of fibrillar muscle also suggest some fundamental difference either in the transmitter or in the receptive membrane of the junction. Though the effect of ether is somewhat similar in the two types, large amounts are required in the beetle and the effect is not easily reversible. The effect of carbon dioxide is qualitatively different in the two types, and the rapid, reversible depolarization of the membrane characteristic of the muscle fibers of Diptera and Hymenoptera is not found in the Coleoptera studied.
The Hemiptera present a special problem possibly by representing a more primitive condition. Referring to Table I the characteristic size and duration of the potentials of Nezara compare very well with those of the beetle Tenebrio. Likewise the ganglionic responses to electrical stimulation are variable. One might expect that if the mechanical and electrical behavior are related as suggested above, the mechanical properties of the flight muscle fibers of Nezara would resemble those of the beetle Oryctes more closely than those of the bee Bombus (5, 9).

Portions of this work were submitted by Dr. McCann to the graduate college of the University of Connecticut in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1959. A preliminary report has been published (23).

Dr. McCann's work was carried out during the tenure of a fellowship from the National Heart Institute of the National Institutes of Health.

This investigation was supported by the National Institute of Neurological Diseases and Blindness and by the Research Fund of the University of Connecticut.

Received for publication, December 28, 1960.

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