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# The physiology of insect fibrillar muscle

## III. The effect of sinusoidal changes of length on a beetle flight muscle

BY K. E. MACHIN AND J. W. S. PRINGLE, F.R.S.

(with an appendix written with the assistance of F. W. DARWIN)

The mechanism of the rhythmic activity of insect fibrillar muscle is best investigated by a method which eliminates the effect of load parameters. Because of the peculiar properties of this type of muscle, sinusoidal analysis is more convenient than transient analysis; the validity of this method is established by demonstrating that this muscle has no significant discontinuities in its tension/velocity relationship. Small sinusoidal length changes are imposed on the muscle with a high-impedance mechanical drive, and the resulting sinusoidal changes of tension give information about the properties of the resting and active muscle. It is shown that the resting muscle can be represented by a visco-elastic model with a time-constant of 1.2 ms. At oscillation frequencies in the range 0 to 55 c/s the stimulated muscle behaves as if there were a time delay of about 7 ms between length changes and tension changes. The muscle has been studied at various lengths, with various frequencies of stimulation and under anoxic conditions. It is not possible to describe its active properties in terms of a simple model; it is suggested instead that an explanation of the effects must be sought in the fine structure of the myofibrils. Oscillatory length changes are not accompanied by synchronous potential changes in the external surface membrane of the muscle fibres.

### INTRODUCTION

The oscillatory contraction which is characteristic of insect fibrillar muscle with an inertial load (Boettiger 1957*a*, *b*; Machin & Pringle 1959) involves cyclical changes in the length and in the tension of the muscle. The earlier paper described the influence of the load parameters (mean tension, mass, stiffness and damping) on the amplitude, frequency and power of the oscillation, and also the effect of the frequency of nervous excitation. The dynamic tension/length display used in the experiment (figure 1) showed the oscillation as a loop rotating anticlockwise when work was being done by the muscle on the load, and it was found that not only did the area of the loop (indicating work done per cycle of oscillation) vary with the experimental conditions, but also that its shape depended on certain load and stimulation parameters.

The shape of the loop on a cathode-ray tube display cannot readily be described quantitatively. Furthermore, the loop is determined as much by the external conditions as by the properties of the muscle. It nevertheless contains information about the relationship between tension change and length change in the active muscle; this is important in any consideration of the mechanism responsible for the rhythmical activity. This paper describes experiments carried out to obtain a quantitative measure of the properties of the muscle only. Sinusoidal length changes were imposed on the muscle by an apparatus of sufficient power to ensure that the muscle's own energy output (or damping) had a negligible influence on the form of the motion. The resulting tension changes were determined over a range of oscillation frequencies, the difference between the tension/length relationships in the resting and the active muscle giving some guide to the mechanism of the rhythmic source of energy.

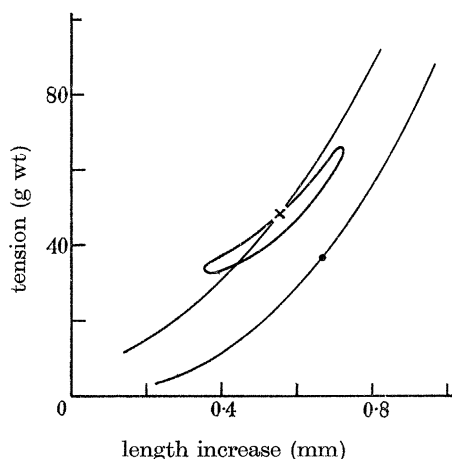


FIGURE 1. A typical oscillatory contraction of *Oryctes* basalar muscle. ●, unstimulated working point (lying on the tension/length curve for the unstimulated muscle); ×, stimulated but non-oscillatory working point (lying on the tension/length curve for the stimulated muscle).

#### THE BASIS OF SINUSOIDAL ANALYSIS

Two principal methods are available for the experimental analysis of electrical or mechanical systems. In 'transient analysis' the response of the system to a sudden change in the external conditions is studied. In a 'frequency-response' analysis, the effect on the system of sinusoidal changes of conditions is measured for a large number of frequencies. It has long been implicit in electrical network theory that these two methods yield identical information, and that the results of one type of analysis can be predicted from those of the other.

Both transient and sinusoidal methods have been used in the study of visco-elastic materials; Alfrey & Doty (1945) have demonstrated the equivalence of the two types of analysis in this application also. The relations which they derive are only valid for materials whose behaviour is linear. Both types of analysis have also been used in the study of muscle; the terminology used is different from that of Alfrey & Doty, and varies between authors. Thus experiments measuring the change of length produced by a sudden change of force (the 'creep curve' of visco-elasticity studies) are called 'after-load' experiments by Hill (1938) and 'isotonic transient' experiments by Buchthal & Kaiser (1951). The measurement of the change of tension after a sudden change of length ('stress relaxation curve') is called a 'quick-stretch' experiment by Gasser & Hill (1924) and an 'isometric transient' experiment by Buchthal & Kaiser (1951). Sinusoidal analyses of varying degrees of refinement are described by Winton (1937), Pryor (1950) and Buchthal & Kaiser (1951). The latter authors also show that results obtained from sinusoidal and transient experiments are in reasonable agreement.

In view of the theoretical equivalence of the transient and sinusoidal analyses, the choice of method in any particular case is dictated mainly by experimental convenience. In general the sinusoidal analysis is capable of the higher accuracy.

For example, in the present series of experiments frequencies between  $2 \sim^*$  and  $250 \sim$  are used; to obtain the same information from a transient experiment it would be necessary to study the response of the muscle over a period of  $\frac{1}{4}$  s with a time resolution of better than 2 ms. However, if the properties of the preparation vary during the somewhat long time needed for the sinusoidal analysis, the increase of accuracy may be illusory. A further advantage of sinusoidal analysis is that many types of frequency response have been detailed by engineers (e.g. Macmillan 1951) and associated with various configurations of circuit elements. If the results of a sinusoidal analysis of a muscle are suitably displayed, it may be possible to recognize them as combinations of well-known functions. Some guide to the nature of the contractile process may thereby be given.

With non-linear materials, transient analysis seems at first sight likely to yield more useful information. It is, however, usually possible to represent the properties of such materials by a series of linear approximations; sinusoidal analysis can then yield meaningful results.

#### SINUSOIDAL ANALYSIS APPLIED TO NON-LINEAR MATERIALS

A linear material is one whose behaviour can be described in terms of a combination of Hookean springs and Newtonian viscous elements. It must be noted that the 'model' formed by this combination of elements is merely descriptive, and does not necessarily imply the existence of corresponding structures in the material. Furthermore, the elements need not necessarily be physically realizable. Springs of negative stiffness or dashpots of negative viscosity are not excluded, but the presence of such elements will imply a source of energy within the material. When a linear material is stretched infinitely slowly the resulting force is accurately proportional to the change of length. Similarly the viscous force arising from a given rate of change of length (velocity) is proportional to that velocity. The force resulting from any arbitrary deformation can be derived by summing the two components due to change of length and to velocity.

In some materials the relation between stress and strain is not linear, but follows a smooth curve without discontinuous change of slope. This curve can be represented approximately by a series of straight segments. The properties of such a material can be completely evaluated by sinusoidal analysis provided that a measurement is made on each straight segment, and provided that the sinusoidal changes are confined within a segment. In a practical case (figure 2) sinusoidal changes of length of small amplitude can be used to derive the elastic modulus at each point on the curve. The ratio

$$\frac{(\text{amplitude of oscillatory component of force})}{(\text{amplitude of oscillatory component of length})}$$

will tend towards a limit as the amplitude is reduced; this limit is related to the elastic modulus. Furthermore, as the amplitude is reduced, the variation of force tends towards a truly sinusoidal one; in other words, the proportion of harmonics

\* As in the earlier paper, the symbol  $\sim$  is used for frequency of mechanical oscillation, the stimulus frequency being given as  $\text{---/s}$ .

in the force wave-form tends towards zero. If the shape of the stress-strain curve is known it is possible to predict how the ratio (force)/(length) will tend to its limit, and how the harmonic content of the force wave-form will depend on amplitude.

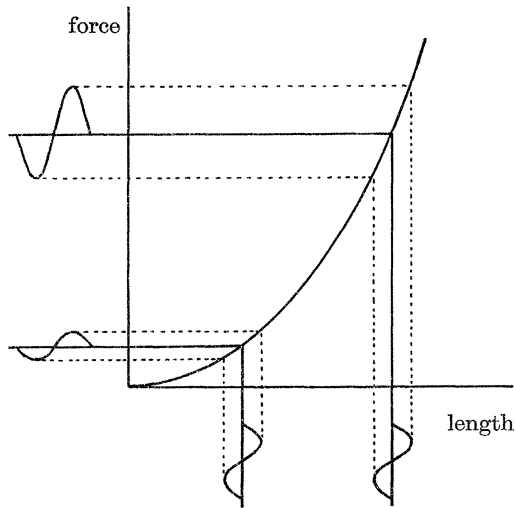


FIGURE 2. Sinusoidal analysis of a non-linear material.

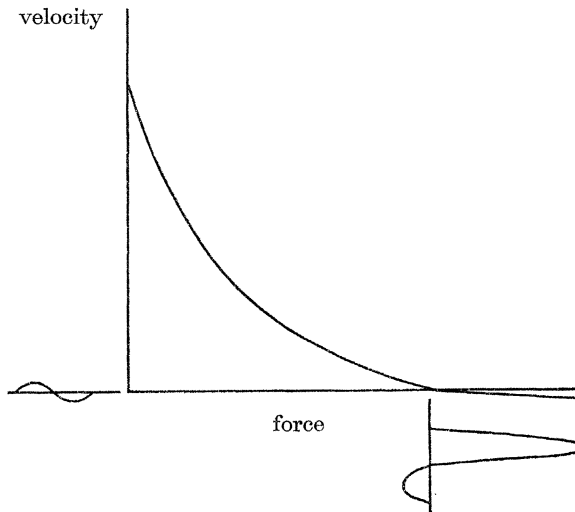


FIGURE 3. A sinusoidal variation of velocity applied to Katz's force/velocity relation for frog sartorius.

With certain types of non-linearity little useful information can be obtained by sinusoidal analysis. For example, the force-velocity relationship for a vertebrate muscle (figure 3, after Katz 1939; see also Aubert 1956) has a discontinuity at zero velocity. The force arising from a sinusoidal variation of velocity would never itself be sinusoidal, and little meaning could be attached to the ratio of force to velocity. Again, if sinusoidal analysis were carried out on a non-linear elastic element with 'click' properties (Pringle 1954) no useful results would be obtained.

A particularly subtle form of non-linearity, depending on the interaction of viscous and elastic elements, has been described by Buchthal & Kaiser (1951) in single fibres of frog semitendinosus muscle. They find that the stiffness of the fibre determined by a sinusoidal method depends markedly on the amplitude of vibration, and does not appear to tend towards a limit. The amplitude dependence is much greater than can be predicted from the curvature of the static tension-length curve. They explain it by regarding the muscle as a series of parallel visco-elastic chains, some of which may become slack in parts of the vibration cycle.

Hill (1953) has adduced a number of objections to the use of sinusoidal analysis to investigate muscle. He points out, first, that non-linearities in elastic elements introduce complications; in the present experiments the amplitude of vibration is sufficiently small for these effects to be negligible. The discontinuity in the force-velocity relationship is then cited as another complicating factor; experimental results given later indicate that with the present muscle negligible distortion arises for this reason. Finally, Hill maintains that the results of sinusoidal analysis may only give information about the 'parallel elastic components' and the 'series elastic element'. For reasons advanced by Pringle (1960) and discussed later, we consider that the separation into 'contractile component' and 'elastic elements' is artificial and not at present useful in the analysis of the properties of insect fibrillar muscle.

#### MATERIAL AND METHODS

All experiments have been made on the basalar muscle of males of the Indian rhinoceros beetle, *Oryctes rhinoceros* (L.), at 25 to 30 °C. The preparation of the basalar muscle is described in the first paper of this series (Darwin & Pringle 1959, hereafter referred to as paper I) and the mechanical and electrical apparatus in the second (Machin & Pringle 1959, referred to as paper II: see also Machin 1959). Figure 4 shows a simplified block diagram of the arrangement of component units.

A sinusoidal voltage of known amplitude and frequency from a Solartron Type OS.103 Oscillator is fed to a moving-coil vibrator whose movement is monitored by a photo-electric displacement transducer. The output from this transducer provides negative feedback to the vibrator, linearizing its voltage/displacement relationship and raising its mechanical impedance; it is also fed to the X-plates of a cathode-ray tube. Tension is measured with a piezo-electric transducer connected to the frame carrying the dissected beetle, and a fine wire tied to the basalar apodeme joins the muscle to the moving element of the vibrator. The tension transducer and the beetle mounting have a resonant frequency of the order of 5000 ~. The whole apparatus is carefully designed to exclude other resonances at lower frequencies, but a slight resonance remains at about 110 ~ due to lateral oscillation of the moving element of the vibrator when the pull of the wire from the muscle is not truly vertical. The tension measurement is extended into the range 0 to 10 ~ (to which the piezo-electric transducer is insensitive) by mixing in, with a suitable electrical network, an output proportional to vibrator current. The final output gives a linear indication of tension over the range 0 to 1000 ~ with a negligible phase error.



The tension output is used in three separate ways. First, it is fed to the Y-plates of the cathode-ray tube so that the elliptical tension/length diagram is displayed. Secondly, the harmonic content of the tension output is measured on a meter preceded by a rejection filter which removes the fundamental component. Finally, the tension output is fed to a Solartron Type VP.253 Resolved Component Indicator. This instrument compares the phase of its input wave-form with that of the oscillator, and displays on two meters the components of its input which are in phase and in quadrature with the oscillator voltage. Furthermore, it discriminates against all frequencies other than that of the oscillator, and therefore gives

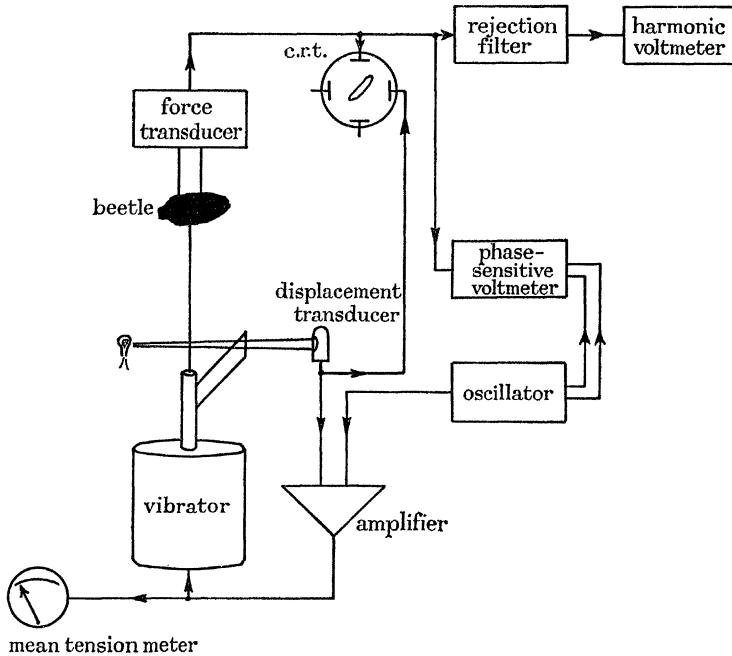


FIGURE 4. Block diagram of the apparatus.

no indication on hum, noise or harmonics. The readings of the instrument can be interpreted as follows. One meter gives that component of the tension which is in phase with the sinusoidal changes of length; this component can be attributed to Hooke's Law and called 'elastic force'. The other meter gives the component of force which is in quadrature with the length change, i.e. in phase with the velocity. This component can be termed 'viscous force'.

In each experiment both meters are read for an ascending series of vibration frequencies from 2 to 100 ~ or 2 to 200 ~. Because of the long time-constant of the meter display and the transient rise of tension at the moment of stimulation, it was found most satisfactory to cover the whole range of frequencies first for the resting muscle and then for the stimulated muscle. During the time (1 to 2 min) needed to make a complete run, the performance of the muscle did not change significantly under constant stimulation at the stimulus frequencies used (usually 40/s, see paper II). A second run with the resting muscle was sometimes made as a control.

## HARMONIC DISTORTION

It was clear from the free-oscillation results (figure 1) that the length or tension changes could not be truly sinusoidal, since the oscillatory loop was not an ellipse. Much of the distortion clearly arose from the curvature of the tension/length relationship for the muscle (paper II), but under some conditions (e.g. figure 14*A* and *C* of that paper) additional distortion was apparent, probably due to the variation of the 'viscous' properties of the muscle along its tension/length curve. However, some non-linear process with a discontinuity might be involved (cf. Katz 1939 and Buchthal & Kaiser 1951); in this case the force variation would not be sinusoidal, however small the amplitude of vibration. The proportion of harmonics

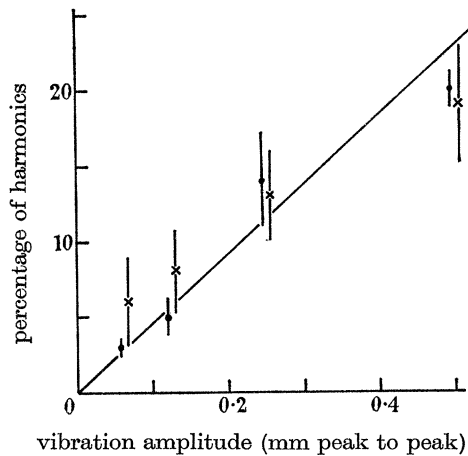


FIGURE 5. Harmonic content of the tension wave-form for various amplitudes of vibration. Mean of five experiments; 'wings' drawn to twice the standard error. ●, unstimulated; ×, stimulated.

in the tension wave-form was therefore determined for various amplitudes of sinusoidal length change. If the percentage harmonic content steadily approaches zero as the amplitude is reduced, and the form of this variation agrees with that calculated from the non-linearity of the tension/length curve, it is reasonable to assume that no additional non-linear processes are occurring.

The results are shown in figure 5. The relation between tension and length (figure 1) is nearly exponential for both resting and active muscle; for such a relationship the percentage harmonic content should be almost linearly related to the amplitude. The straight line on figure 5 is calculated from the exponential approximation.

It is clear that the observed harmonic distortion is not significantly different from that predicted from the non-linear tension/length relationship. Certainly the discrepancy of 300 to 700 times found by Buchthal & Kaiser (1951) is absent. It may be concluded, then, that the processes involved in the rhythmical contraction of fibrillar muscle are essentially linear. There is no evidence for processes occurring preferentially in one half of the cycle ('de-activation by release' only; see Pringle 1954).



Amplitudes of less than 0.05 mm peak-to-peak were used in the experiments to be described. From figure 5 the harmonic content of the tension wave-form is likely to be less than 2%; under these circumstances useful meaning can be attached to measurements of 'elastic force' and 'viscous force'.

#### PRESENTATION OF RESULTS

Each experiment yields the following data:

(1) The constant voltage delivered by the driving oscillator. From the properties of the apparatus this can be converted into vibration amplitude ( $\Delta L$ : millimetres).

(2) The in-phase and quadrature components of the voltage representing tension at each frequency used. These can be converted into the two alternating components of tension ( $\Delta F_e$  and  $\Delta F_v$ : grams weight).

At any frequency where the quadrature component of force is zero (in particular at a very low frequency), the relation between in-phase force and vibration amplitude describes the elastic properties of the muscle. Thus the elastic modulus  $E_e$  will be given by

$$E_e = \frac{\Delta F_e \cdot L}{A \cdot \Delta L},$$

where  $\Delta L$ ,  $\Delta F_e$  are, respectively, the vibration amplitude and the in-phase alternating component of tension.

$L$ ,  $A$  are the length and area of the muscle.

In an analogous way it is possible to define a 'viscous modulus'  $E_v$  given by

$$E_v = \frac{\Delta F_v \cdot L}{A \cdot \Delta L},$$

where  $\Delta F_v$  is now that component of force which is in quadrature with the length changes.

The experimental results consist therefore of pairs of values of  $E_e$  and  $E_v$ , one pair for each frequency used. These are conveniently presented as follows. On a set of Cartesian axes insert a point with co-ordinates ( $E_e$ ,  $E_v$ ) labelled with the corresponding frequency. The curve representing the behaviour of the muscle is obtained by joining up the points for various frequencies in ascending order of frequency.

This type of presentation, which will be called a 'vector modulus plot', has the following properties:

(1) If a line is drawn from the origin to a point on the curve, its length represents the dynamic modulus of the muscle at that frequency, and the angle between the line and the  $E_e$  axis is the phase angle between force and length.

(2) The curve describing a purely passive material must lie entirely within the first quadrant; a curve which penetrates into any other quadrant is characteristic of a material containing a source of energy. Examples of common visco-elastic elements and their vector modulus plots are given in figure 6. This type of plot has been extensively used by electrical engineers (e.g. Macmillan 1951) to describe combinations of circuit elements; their results are directly comparable with vector modulus plots provided that the correct analogies are used.

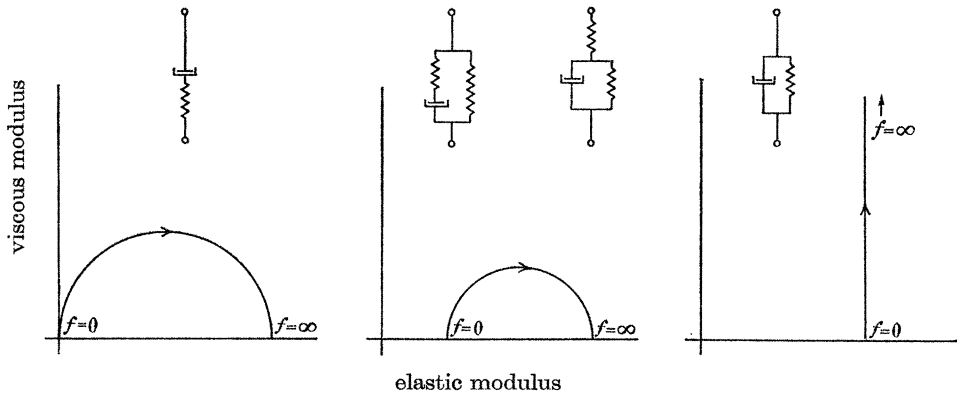


FIGURE 6. Vector modulus plots for three types of visco-elastic element represented by the combinations of springs and dash-pots shown.

#### THE GENERAL FORM OF THE EXPERIMENTAL RESULTS

A typical vector modulus plot for an unstimulated basalar muscle is shown dotted in figure 7(a). With increasing frequency of vibration both elastic and viscous moduli increase. When the muscle is stimulated the full-line curve is obtained. The elastic modulus at low frequencies is rather higher than that of the unstimulated muscle; as the frequency is increased the elastic modulus at first decreases and the viscous modulus becomes negative. This negative component, which as noted earlier cannot occur with a passive system, shows that the muscle is doing work on the apparatus. At a certain passive frequency the viscous modulus becomes zero, and above this frequency rises rapidly.

While all experiments give results conforming to this general pattern, the detailed shape of the curves varies with the experimental parameters and with the condition of the preparation. With preparations capable of delivering the highest

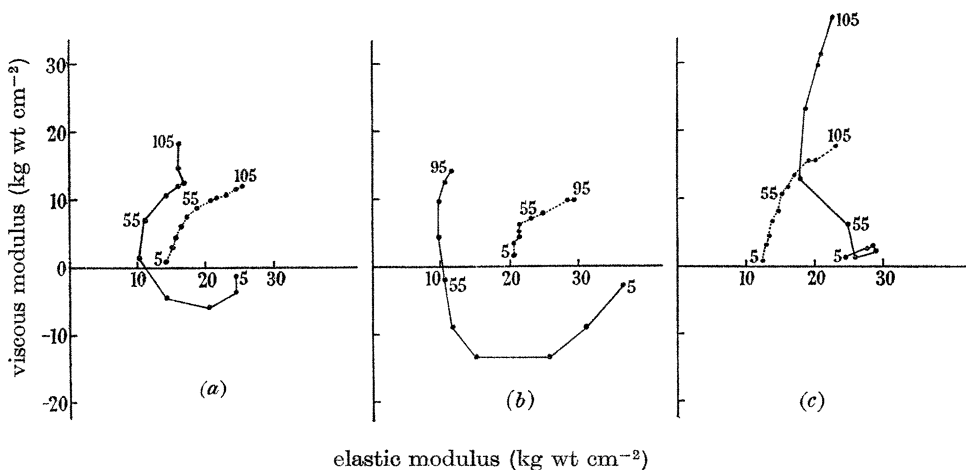


FIGURE 7. Typical vector modulus plots for beetle basalar muscle. . . ., unstimulated; —, stimulated. Points are shown at frequency increments of 10 ~, starting at 5 ~.

power, results similar to figure 7(b) are obtained; figure 7(c) is typical of a moribund preparation.

In the experimental results which follow, the frequency of stimulation was 40/s unless otherwise stated.

#### THE EFFECT OF MUSCLE LENGTH

It has been shown in paper II that the power output of an oscillating muscle varies with muscle length, reaching a maximum at about the length obtaining in the live beetle. The effect of length on the vector modulus plot has been investigated, typical results being shown in figure 8.

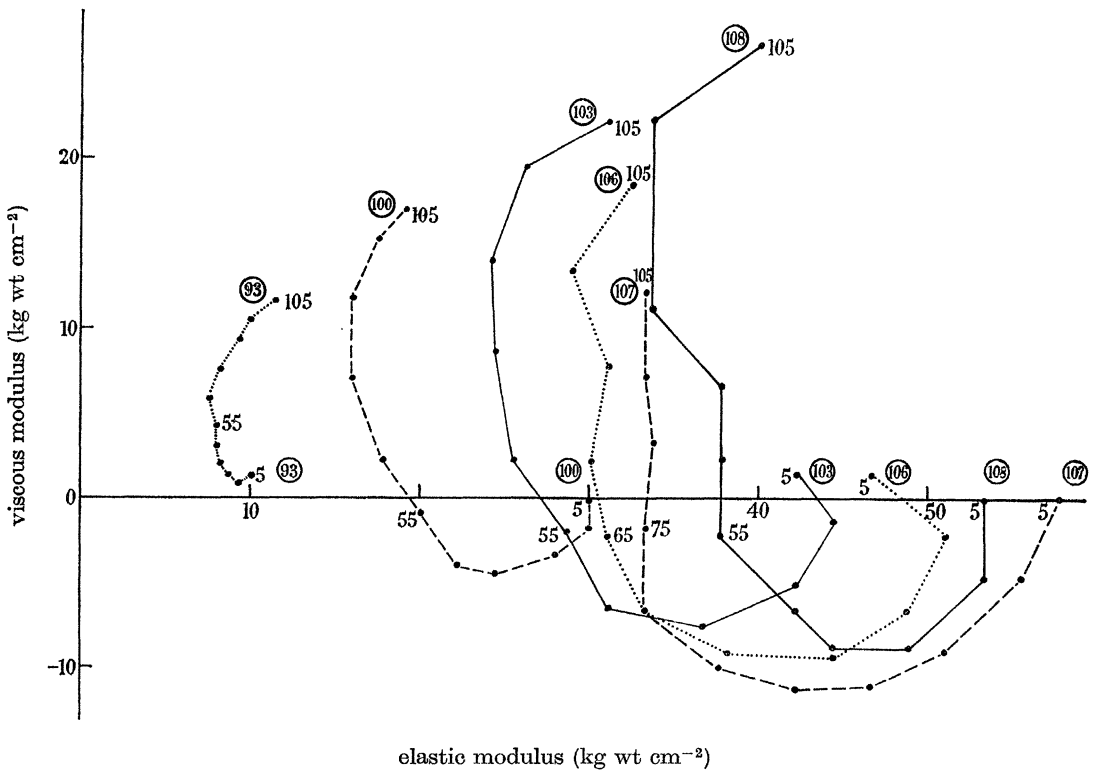


FIGURE 8. The effect of muscle length on the vector modulus plot of a stimulated muscle. The encircled figures give the muscle length as a percentage of its length in the intact insect.

At low frequencies the elastic modulus of the muscle increases with length. Since the tension/length curve for the muscle is non-linear, having greater slope at greater extension (figure 1), this is not unexpected. The extent of the negative excursion of the curve also increases with length. Below 95%  $L_0$ \* the negative region disappears; there is also some evidence that above 107%  $L_0$  it becomes relatively smaller, although the measurements are unreliable due to the irreversible

\*  $L_0$ , the 'reference length', is the length of the muscle in the intact insect (see paper II, p. 208).

changes which may take place in the muscle at such high loads. Between 100 %  $L_0$  and 107 %  $L_0$  the maximum negative phase angle between tension and length remains substantially constant, but falls off outside these limits. This is in reasonable accordance with previous findings (paper II, figure 14) that the tension/length diagram for large amplitude oscillations develops sharply pointed ends outside the range of 99 %  $L_0$  to 104 %  $L_0$ .

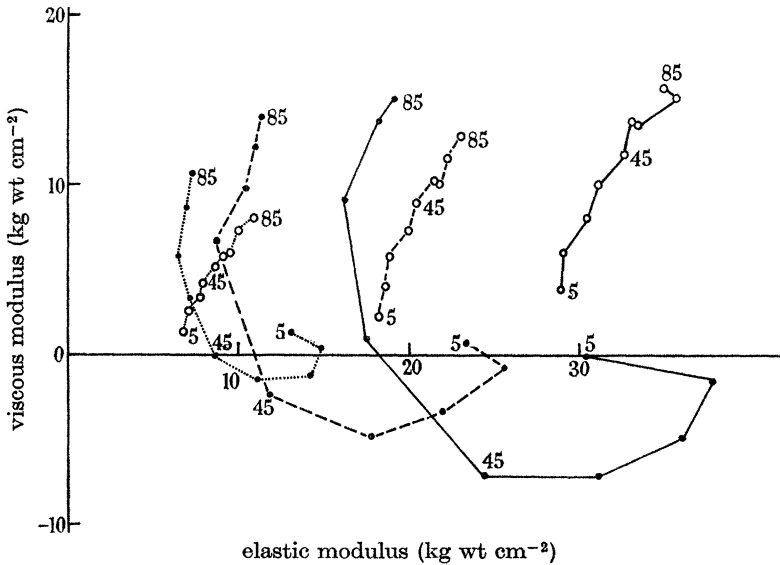


FIGURE 9. As figure 8: different preparation. . . ., 94 %  $L_0$ ; ----, 100 %  $L_0$ ; —, 103 %  $L_0$ .  
○, unstimulated; ●, stimulated.

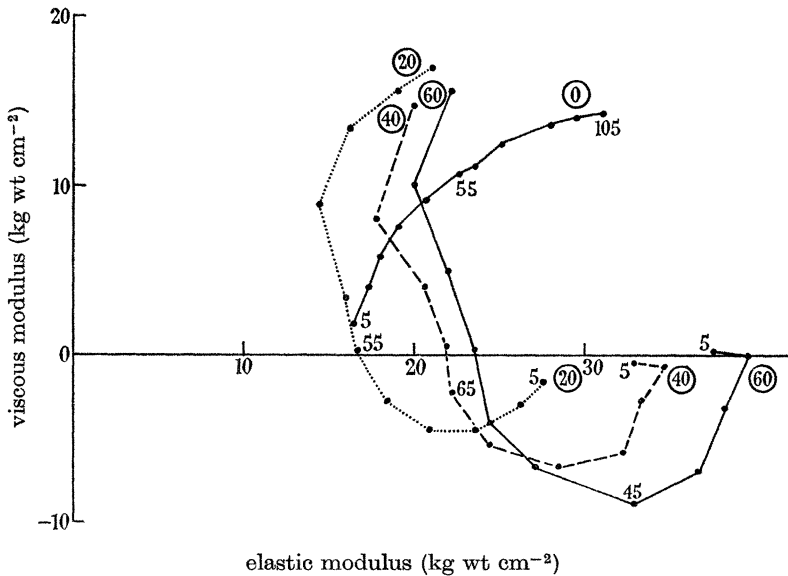


FIGURE 10. The effect of stimulus frequency on the vector modulus plot. The encircled figures give the number of stimuli/sec. Measurements taken at 100 %  $L_0$ .

The viscous modulus at high frequencies increases with length, but not to the same extent as the low-frequency elastic modulus. This point is illustrated more clearly by figure 9 (taken with a different preparation) in which the vector modulus plots are shown for the unstimulated and stimulated muscle at three different lengths. While the low-frequency elastic modulus of the stimulated muscle increases by 150 % as the length is changed, the high-frequency viscous modulus increases only by about 40 %. A similar increase occurs with the unstimulated muscle.

#### THE EFFECT OF STIMULUS FREQUENCY

As the frequency of the stimuli applied to the muscle is increased, the isometric tension increment at first increases; above 40/s it remains effectively constant (paper II). A similar transition from the unstimulated to the fully stimulated condition is also evident in the vector modulus plot. Figure 10 shows how the characteristic shape of plot for the active muscle develops gradually at first as the stimulus frequency is increased.

#### THE EFFECT OF ANOXIC FATIGUE

The general effects of fatigue have been described in paper II, and their onset attributed to anoxia.

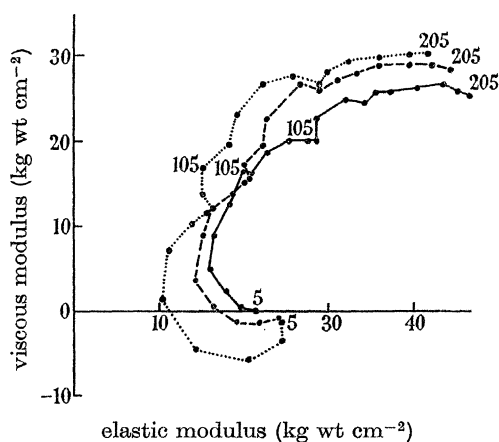


FIGURE 11. The effect of anoxia on the vector modulus plot. . . . , normal; ----, 1 hour without oxygen; —, 1½ h without oxygen, plus ½ h of nitrogen perfusion. Measurements taken at 100 %  $L_0$ .

It was found possible to keep preparations in good condition for some hours by supplying oxygen at a little over atmospheric pressure through a fine tube inserted through a spiracle. The effect on the vector modulus plot of cutting off the supply of oxygen, and eventually of replacing it by nitrogen, is illustrated in figure 11. The principal result of anoxia is the progressive disappearance of the negative region of the curve. This agrees with previous findings (paper II) that the ability of the muscle to produce oscillations, although not necessarily its ability to produce tension, is impaired by anoxia.

# DISCUSSION

In this section an attempt will be made to fit the results described above into a unifying 'model'. The dangers involved in the use of muscle models have been emphasized elsewhere (Pringle 1960); provided, however, that it is realized that the model may be no more than a summary of experimental results, it may serve to guide further investigation into the processes occurring in the contractile material.

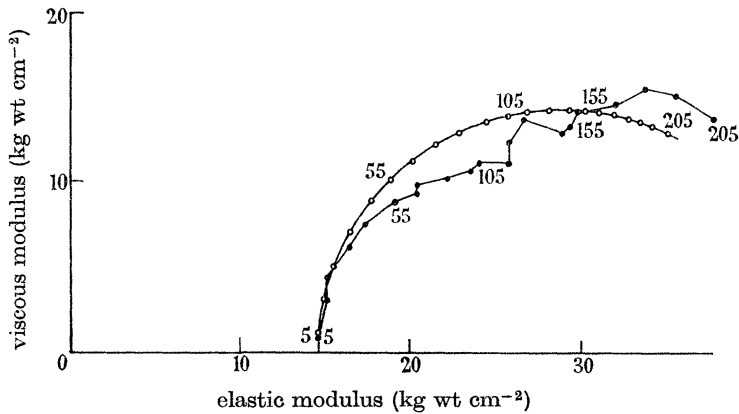


FIGURE 12. Vector modulus plot for unstimulated muscle and for 3-element visco-elastic model.

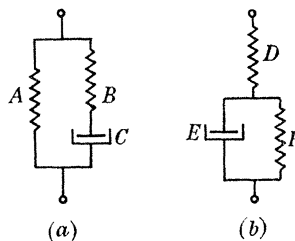


FIGURE 13. Three-element visco-elastic models of unstimulated muscle.  $A = 1.9 \text{ kg wt cm}^{-1}$ ;  $B = 3.5 \text{ kg wt cm}^{-1}$ ;  $C = 4.2 \text{ g wt cm}^{-1} \text{ s}$ ;  $D = 5.4 \text{ kg wt cm}^{-1}$ ;  $E = 3.5 \text{ g wt cm}^{-1} \text{ s}$ ;  $F = 2.9 \text{ kg wt cm}^{-1}$ . Time constant =  $1.2 \text{ ms}$ .

The unstimulated muscle will be considered first. The experimental results can be fitted approximately to a 3-element visco-elastic model, which can be of either the Maxwell or Voigt type, since the two models are formally indistinguishable (Buchthal & Kaiser 1951; Pringle 1960). Figure 12 shows a vector modulus plot for an unstimulated muscle, together with the corresponding plot for the 3-element models shown in figure 13. Without further information it is not possible to decide on the relative merits of the Maxwell and Voigt models. However, the properties of unstimulated muscle vary with length as shown in figure 9. This behaviour could be reproduced by a model in which all three elements are non-linear, but the merit of descriptive simplicity would be lost. It is found, however, that the behaviour illustrated in figure 9 can be stimulated by a Voigt model in which only the element  $D$  is non-linear, having an elastic modulus which increases with tension.



The most striking characteristic of the vector modulus plot for a stimulated muscle is the roughly semicircular shape of the curve between about 5 and 55  $\sim$ . This is shown more clearly by plotting the difference between the dynamic modulus for the stimulated muscle and that for the unstimulated muscle at the same length; this type of presentation will be called a 'difference plot'. A typical result

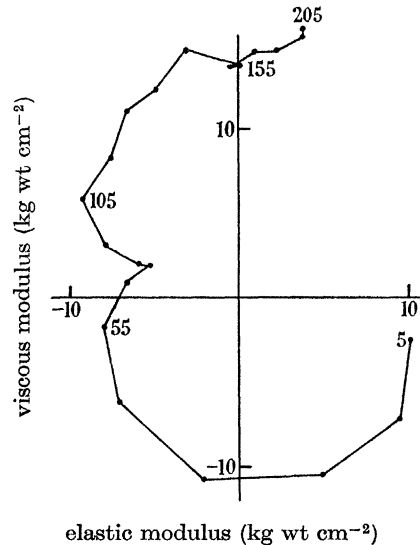


FIGURE 14. Vector modulus plot of the difference between the properties of the muscle when stimulated and unstimulated.

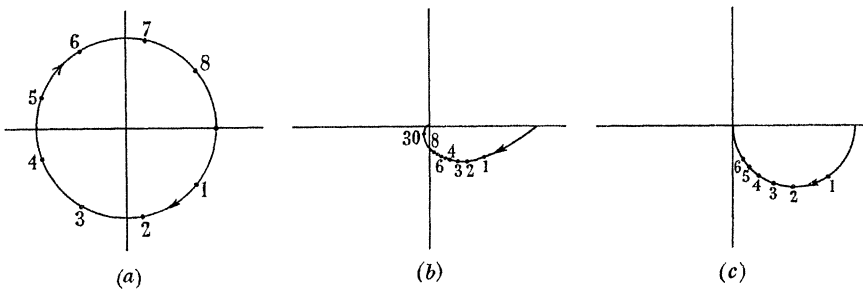


FIGURE 15. Vector modulus plot of (a) finite delay, (b) distributed delay, (c) exponential delay. Points are marked at equal frequency increments.

is shown in figure 14. This represents the vector modulus plot of the *change* in the muscle properties brought about by stimulation; it is not implied that an entirely new structural component with these characteristics is activated by stimulation.

A curve similar to the low-frequency part of figure 14 characterizes the transfer function of an element with a time delay. This transfer function has constant amplitude but phase-shift proportional to frequency; the vector modulus plot is thus a circle centred at the origin, with the frequency points equally spaced around its circumference (figure 15(a)). This 'finite delay', 'propagation delay' or 'transmission delay', as it is variously called, must be distinguished from the

'exponential delay' (figure 15(b)) (which arises from the combination of a storage element and a dissipative element) and from a 'distributed delay' (figure 15(c)) (which arises, for example, from a diffusion process). In both the latter cases the phase does not vary linearly with frequency, and the amplitude steadily decreases.

The low-frequency part of the vector modulus plot for a stimulated muscle can thus be tentatively attributed to the introduction of a finite time delay of about 7 ms between changes of length and changes of tension. Above 55 ~ the rate of change of phase in figure 14 is reduced, corresponding to a time delay of about 2 ms. Some reduction of amplitude also occurs near 75 ~. It has not been possible to match the curve both above and below 55 ~ by any simple combination of elastic and viscous elements with elements of constant delay. Furthermore, the equal spacing of the points on the low-frequency part of the curve cannot be matched by a model involving instead exponential or distributed delays.

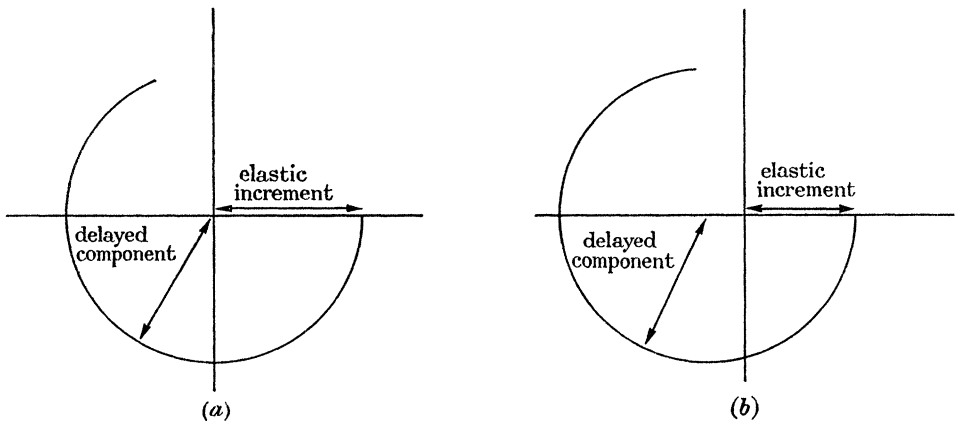


FIGURE 16. Idealized difference plots illustrating 'elastic increment' and 'delayed component'.

The effect of stimulation, then, is threefold. First, the tension increases. Secondly, the elastic modulus of the muscle at low frequencies is increased; this will be termed the 'elastic increment'. Thirdly, there arises a 'delayed component' in which changes of length produce proportional changes of tension, but only after a finite delay. The two terms are defined in the idealized different plots of figure 16. If the elastic increment and the delayed component were equal (figure 16(a)) and of a magnitude consistent with the change in slope of the isometric tension/length curve, all three effects could arise from a single element which was brought into action by stimulation. On the other hand, if the delayed component exceeds the elastic increment (figure 16(b)) it must be supposed that part at least of the delayed component arises from already-existing elastic elements in the unstimulated muscle. In the final steady state resulting from stimulation, the elastic increment has approximately the magnitude predicted from the change in slope of the isometric tension/length curve, but under some experimental circumstances the delayed component can exceed the elastic increment with the muscles studied. Some of the elastic properties of the unstimulated muscle must therefore be situated in the contractile material.

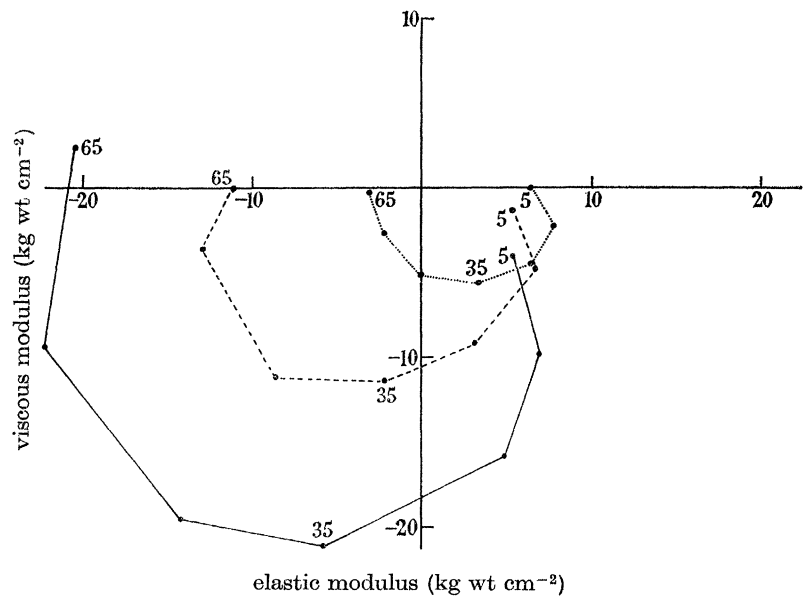


FIGURE 17. The effect of muscle length on the difference plot.  
....., 94 %  $L_0$ ; ----, 100 %  $L_0$ ; —, 106 %  $L_0$ .

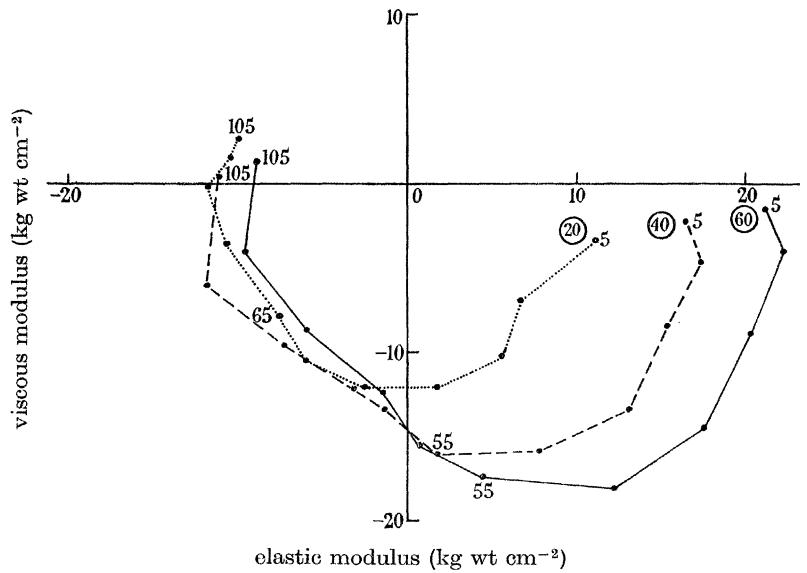


FIGURE 18. The effect on the difference plot of the frequency of stimulation.  
The encircled figures give the number of stimuli/sec.

The effect of experimental conditions on the ratio (elastic increment)  $\div$  (delayed component) is illustrated in the next three figures. As the muscle length is increased (figure 17) the ratio changes from about 1.4 to about 0.5. With increasing stimulus frequency (figure 18) the ratio increases from about 0.9 to about 1.3. During the decay of muscle activity due to anoxia, however (figure 19), the ratio remains approximately constant at 1.1.

In the transition from the passive to the active condition at the start of stimulation and from the active to the passive condition when stimulation ceases the three effects of stimulation may be still further dissociated. It was noted in paper II

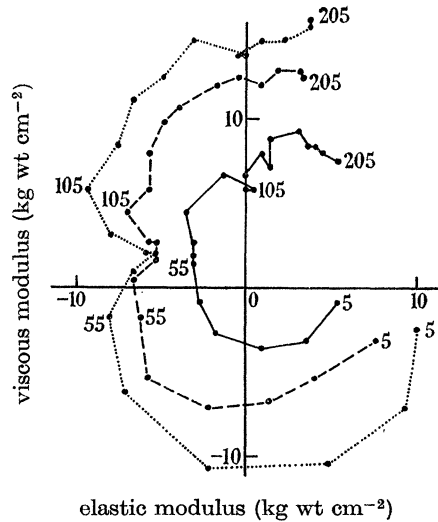


FIGURE 19. The effect of anoxia on the difference plot. ...., normal oxygen perfusion; ----, 1 hour after ceasing oxygen perfusion; —, oxygen perfusion ceased for 1 h, followed by  $\frac{1}{2}$  h perfusion with nitrogen.

(p. 218) that an increase of tension may occur before oscillation commences and that the rate of decay of the oscillation may not be that expected from the damping of the load. A detailed study of these phenomena will form the subject of a subsequent paper.

The delayed influence of length on tension could arise in at least three ways. (1) Changes of muscle length might cause corresponding changes in its membrane potential (by 'transducer action'); these potential changes would then effect the tension by the usual coupling process which must be present in all striated muscles. The delay might lie at any point in the causal sequence. (2) Changes of muscle length might modify the transfer of information between membrane and myofibrils (the coupling process). (3) The effect might arise from the properties of the contractile material, i.e. of the molecular processes involved in contraction. It is found experimentally (see Appendix) that changes of length have little effect on the potential of the accessible part of the membrane on the outside of the muscle. However, Tiegs (1955) describes deep clefts in the surface of another fibrillar muscle, into which the tracheae and probably the nerve fibres penetrate. If the

surface membrane extends intact into these clefts, its potential at the base of the clefts might be affected by changes of length in the way described, without any changes of potentials being detectable in the more accessible regions of the membrane. It is accordingly not possible to rule out completely the operation of a mechanism of type (1). However, if changes of length affect the membrane in the clefts or directly modify the coupling process as in (2), it becomes difficult to explain how the delayed component can sometimes exceed the elastic component. It would then be necessary to postulate that the length-sensing element must be functioning even in the unstimulated muscle, and for this there is no evidence. Mechanism (3), which attributes the observed effects to the properties of the contractile material, seems at present the most likely one.

It is now not possible to reconcile the properties of both unstimulated and stimulated muscle with one simple model. The Maxwell model of the unstimulated muscle (figure 13(a)) might be used to describe the properties of the stimulated muscle if it were permissible to change element *A* from a pure elasticity to one with complex properties including a time delay between length changes and tension changes. However, as noted earlier, the variation of properties with length would be explicable only if at least two of the elements were non-linear. Conversely, although the Voigt model (figure 13(b)) avoids this difficulty, the changes in stimulation will have to appear in at least two elements. The use of such models as an aid to the elucidation of the mechanisms involved therefore seems unlikely to be fruitful. Again it seems probable that all the properties observed involve the contractile material and that any separation into series and parallel components is quite artificial. Analysis of complex mechanical properties of a muscle should therefore be related to known aspects of its fine structure.

The supply of living *Oryctes rhinoceros* was obtained from the Central Coconut Research Station, Kayangulam, Kerala State, India, through the kind offices of Mr Abdulali, Honorary Secretary of the Bombay Natural History Society. The Transfer Function Analyzer was purchased with the assistance of the Government Grants Committee of the Royal Society.

#### APPENDIX. THE INFLUENCE OF CHANGE OF LENGTH ON MEMBRANE POTENTIAL

(With F. W. DARWIN)

An experiment has been carried out to discover whether changes of length affect the potential of the muscle surface membrane. The experimental method is virtually a combination of the mechanical measurement described earlier in this paper with the electrophysiological techniques of paper I. A muscle is subjected to mechanical vibration, and simultaneously its membrane potential is recorded with an intracellular capillary micro-electrode inserted near the stationary end of the muscle. The mechanical vibration frequency used was 20 ~ and the stimulus 40/s. Any component of membrane potential at 20 ~ should thus cause alternate action

potentials to be of different amplitude, the difference being the peak-to-peak amplitude of the vibration-frequency component.

An experimental result is shown in figure 20. The upper trace represents muscle tension, the lower one membrane potential. The resting tension is 20 g wt with a superimposed vibration of 5 g wt peak. On stimulation the tension rises to 30 g wt, the alternating component increasing to 7.5 g wt. The component of membrane potential at the vibration frequency is less than 1 mV peak, while the mean depolarization is about 18 mV. The increase of alternating component of tension due to stimulation is thus about 25 % of the rise in mean tension, while the vibration-frequency component of potential is less than 5 % of the mean depolarization.

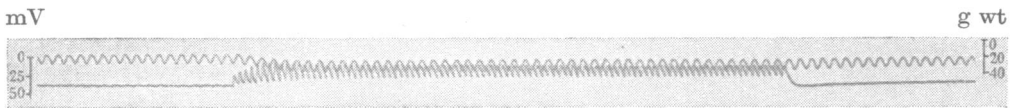


FIGURE 20. The effect of sinusoidal changes of length on the muscle membrane potential. Upper trace, muscle tension. Lower trace, membrane potential.

In an experiment of this type it is easy to obtain movement artifacts which cause a vibration-frequency component of membrane potential. If the hole in the membrane is larger than the tip of the micro-electrode, some local 'short-circuiting' of the potential will occur; if now the membrane moves relative to the electrode tip the degree of short-circuiting will vary at vibration frequency. An effect of this type could be induced deliberately by penetrating near the moving end of the muscle instead of near the stationary end. During a few of the experiments a vibration-frequency component of potential was observed; this component was usually present even when the muscle was unstimulated. The resting potential in these cases was low, and often dropped to zero after a short time, which accords with the idea that the membrane round the electrode tip was extensively damaged.

It is concluded that the interaction between length and tension does not take place by a path which includes the external muscle membrane.

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