Evidence from Insect Fibrillar Muscle about the
Elementary Contractile Process

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ABSTRACT Bundles of myofibrils prepared from the dorsal longitudinal flight muscles of giant water bugs show oscillatory contractile activity in solutions of low ionic strength containing ATP and \(10^{-8}-10^{-7} \text{ M Ca}^{2+}\). This is due to delay between changes of length and changes of tension under activating conditions. The peculiarities of insect fibrillar muscle which give rise to this behavior are (1) the high elasticity of relaxed myofibrils, (2) a smaller degree of \(\text{Ca}^{2+}\) activation of ATPase activity in unstretched myofibrils and extracted actomyosin, and (3) a direct effect of stretch on ATPase activity. It is shown that the cross-bridges of striated muscle are probably formed from the heads of three myosin molecules and that in insect fibrillar muscle the cycles of mechanochemical energy conversion in the cross-bridges can be synchronized by imposed changes of length. This material is more suitable than vertebrate striated muscle for a study of the nature of the elementary contractile process.

The elementary process in muscular contraction is the process by which the chemical energy released by hydrolysis of the terminal phosphate of ATP is converted into mechanical energy. In striated muscle this conversion is carried out by the organized macromolecular structure of the proteins of the myofibril. In the intact muscle cell, the rate of conversion of chemical to mechanical energy under given initial mechanical conditions is controlled by the ion movements through the plasma membrane and associated changes in the membrane potential. These operate through a sequence of events involving the internal membrane systems of the cell to cause a rise in the concentration of free \(\text{Ca}^{2+}\) ions in the immediate environment of the myofibrils, and it is this increase in \(\text{Ca}^{2+}\) concentration which changes the state of the contractile machinery from that characteristic of resting muscle to that characteristic of activity. No detailed evidence for these statements will be presented in this review. They apply equally to insect fibrillar muscle and to other types of striated muscle (1).

It will also be taken as established that, during active or slow passive changes of length in striated muscles, the actin-containing I filaments slide...
past the myosin-containing A filaments without net change in the lengths of these filaments. The evidence that this is true for vertebrate muscle has been summarized by H. E. Huxley (2) and is further strengthened by the failure of Huxley, Brown, and Holmes (3) to find any changes in the axial spacings associated with the A filaments or the I filaments, as indicated by X-ray diffraction measurements on resting and actively contracting frog muscle. It seems clear that length changes in striated muscles are produced by sliding of one set of filaments relative to the other, driven by an elementary process in the cross-bridges which operates cyclically to convert the energy of hydrolysis of ATP into a sliding motion. Proteins other than actin and myosin are involved in the control by Ca$^{2+}$ of the rate of energy conversion (4), and possibly in the actual energy conversion process (5), but they too act in association with the cross-bridges, whose configurational changes during the enzymic activity hold the key to an understanding of the mechanism of muscular contraction.

At the level of analysis which can be reached from mechanical, electron microscopical, X-ray diffraction, and biochemical studies, the most important problem which now needs to be solved is the structure of the cross-bridges and the nature of the cyclic movement which must occur during activity. Is each cross-bridge formed by the head of one myosin molecule? Is one ATP molecule, or a constant small number of ATP molecules, hydrolyzed at each cycle of movement of a cross-bridge? Are the cycles of movement of the bridges randomly timed, or do they succeed one another in regular sequence? What is the amplitude of sliding produced by one cycle of movement, and what force is generated? At what frequency do the bridges operate? These are some of the questions which it may be possible to answer from a study of insect fibrillar muscle because of special features of the organization of this tissue and the peculiar mechanical activity which it shows. Before discussing them, a concise summary will be given of some recent observations in Oxford on this type of muscle. A full review has recently appeared (1).

**RECENT OBSERVATIONS ON INSECT FIBRILLAR MUSCLE**

**Material**

The histologically and physiologically distinct type of striated muscle with which this review is concerned is found in the power-producing flight muscles of certain orders of insects and in the sound-producing muscles of certain cicadas. From its distribution within the class Insecta (6), it is clear that it has evolved more than once. Most of the insects in which it occurs are small, but examples have been found, in tropical beetles and water bugs, on which it is possible to perform all the normal types of physiological and biochemical experiment. The evidence to be discussed in this review comes mainly from...
the dorsal longitudinal muscles of giant water bugs of the genus *Lethocerus*, which contain about 6000 fibers 10–20 mm long and 50–70 μ in diameter (7). No significant qualitative differences in the structure or properties of the contractile machinery are known to exist between different fibrillar muscles.

**Structure of the Myofibrils**

The myofibrils of insect fibrillar muscle contain the usual structural elements found in all striated muscles. They have the following features.

1. The myofibrils are very compact structures, up to 3 μ in diameter. Preparations consisting of bundles of myofibrils can be made by treating with certain nonionic detergents fibers stored in 50% glycerol at −18°C, and these retain all the essential features of the contractile mechanism (8).

2. In transverse section, the hexagonal array of filaments is organized with extreme regularity across the whole diameter of the myofibril. In positively stained electron micrographs, the A filaments have a diameter of about 170 Å; X-ray diffraction gives an equatorial spacing of 530–560 Å (9). Both these dimensions are larger than in vertebrate muscle. Unlike vertebrate muscle, the I filaments occupy a position equidistant between two A filaments, so that the ratio of I to A filaments is 3:1 instead of 2:1 as in vertebrate muscle. This difference is found in all insect flight muscles, not merely in fibrillar muscles.

3. In longitudinal section of fibers fixed at their normal length, the A filaments usually occupy about 90% of the sarcomere (length, 2.4 μ). At their ends they taper and, in *Calliphora* flight muscle, appear to be continued by a filament of lower density into the structure of the Z line (10). The H zone is of normal appearance, and there is a well-marked M line, with fine striations, formed by material making a network of connections between A filaments (9).

4. The I filaments have approximately the same thickness and length as in frog muscle.

**Mechanical Properties of Myofibrils**

Bundles of glycerinated fibers have been used by White to investigate the passive mechanical properties of the myofibrils. The fibers were immersed in solutions of low ionic strength at pH 7.1, either without ATP (“rigor” solution) or with 4–6 mM ATP and 2 mM EGTA [ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid] (“relaxing” solution; Ca<sup>2+</sup> < 10<sup>−9</sup> M).

The tension/length relationship of such fiber bundles after 4 min equilibration at constant length is shown in Fig. 1. In relaxing solution, tension

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increases with extension up to about 10% of slack length and then levels off to a plateau of about 15 mg/fiber. If the bundles are stretched beyond this length and then slowly released, tension reaches zero at a greater length than at the start of stretch; it is evident that some material in the fiber has reached its elastic limit. In rigor solution, tension increases more steeply, and a plateau of about 19 mg/fiber is reached after only 3% stretch. The slopes of the two curves give values of 10 and 40 kg/cm² myofibril as the elastic moduli in relaxing and rigor solutions.

It is reasonable to suppose that these two chemical solutions produce the extreme conditions where the connections formed by the cross-bridges between the A and I filaments are minimally and maximally effective. If that is the case, the rise of tension in relaxing solution must be due to strain in some structure not involving the cross-bridges, which is nevertheless continuous through the sarcomere. Slow extension of fibers in relaxing solution produces little or no extension of the A filaments but increases the width of the H zone as expected if sliding of the I filaments is taking place; extension in rigor solution extends the A filaments in proportion to the extension of the sarcomere.

Reedy, Holmes, and Tregear (9) showed by X-ray diffraction and electron microscopy of glycerinated fibers that in relaxing solution the cross-bridges are oriented mainly at right angles to the filaments at the myosin period of 146 A; in rigor they are attached to the I filaments with the periodicity of 388 A characteristic of the actin structure and are angled in the direction away from the Z line. These observations show that, at least under the unnatural conditions of complete absence of ATP, the cross-bridges are
capable of moving through an angle of 45° and that, in this condition, they are sufficiently rigid to produce a considerable change in the mechanical properties of the myofibril.

The Amount of Myosin per Cross-bridge

The extreme structural regularity of the filament array of insect fibrillar muscle makes it possible to estimate with some certainty from histological and electron microscopical sections the number of A-filaments per fiber. X-ray diffraction shows that cross-bridges repeat every 146 Å along the A filaments. Using two different and consistent biochemical estimates of the amount of myosin in a known length of fiber and assuming that there are two cross-bridges at every longitudinal repeat along the A filaments, Chaplain and Tregear (11) calculated that three myosin molecules of mol wt 500,000 are associated with each cross-bridge; the statistical probability of there being two or four is less than 1%.

Extension and ATPase Activity

In vertebrate muscle, there is now good evidence that active isometric tension and ATPase activity are proportional to the degree of overlap between the A and I filaments and therefore to the number of actin/myosin interactions which can form at the cross-bridges (12, 13). Extension of insect fibrillar muscle also reduces the extent of overlap but, in contrast to vertebrate muscle, these fibers, when stretched, show a marked increase in Ca²⁺-activated ATPase activity (14). The increase in enzymic activity is found whether the fibers are first stretched in relaxing solution and then activated or are stretched under conditions of Ca²⁺ activation. Further studies show that, after comparable periods of stress relaxation, the range of extensions over which activation occurs is the same as that over which the fibers show reversible mechanical behavior in relaxing solution (Fig. 2); there is a parallel increase in the amount of additional tension developed on activation. These measurements were made on fibers which had been allowed to remain at their extended length in relaxing solution for 3–10 min before transfer to solutions containing an increasing concentration of Ca²⁺ ions. Extension beyond the “elastic limit” (Fig. 1) causes no further increase in ATPase activity and leads to the expected decline in ATPase activity when it produces a significant reduction in the degree of overlap between A and I filaments (Fig. 3).

Rüegg and Tregear (14) showed that, in addition to the increase of Ca²⁺-activated ATPase activity produced by maintained extension, sinusoidal oscillation in length by up to 3% peak-to-peak produced a further increase in enzymic activity, provided that the frequency of oscillation was in the

Chaplain, R. A. Unpublished observations.
appropriate range. This further increase was approximately proportional to the oscillatory mechanical power output (see below), with a mechanochemical coefficient of 1.0–2.4 kcal/mole. They established that the effect was not due to a change in the access of ATP by diffusion to the active enzymic sites and compared it to the increase in total energy output which accompanies the performance of work by a vertebrate muscle (the Fenn effect) (15).

Relevant to these studies are measurements which have been made of the absolute values of ATPase activity and of the extent of Ca²⁺ activation of actomyosin prepared from insect fibrillar muscle. Vom Brocke (16) first showed that the ATPase activity of actomyosin from water bug flight muscle was increased by only a factor of 2 when Ca²⁺ concentration was increased from 10⁻⁹ to 10⁻⁶ M. More extensive measurements on actomyosin and myofibrils⁴ confirm that there is a marked difference in this respect between

fibrillar and nonfibrillar muscles from the same insect (Fig. 4). As with vertebrate striated muscle, treatment of actomyosin with trypsin removes the Ca\(^{2+}\) sensitivity; ATPase activity in the absence of Ca\(^{2+}\) now has the same value as is found for untreated actomyosin in the presence of Ca\(^{2+}\). The effect of tryptic digestion can be at least partially reversed by the addition of “native tropomyosin” prepared from rabbit muscle.

Combination of these results with the measurements made by Chaplain and Tregear (11) of the amount of myosin present in a standard length of fiber (3.9 pmoles/cm fiber, if the molecular weight of myosin is taken as 500,000) shows that the average maximal rate of ATPase activity of statically
stretched fibers at 20°C (750 pmoles/cm fiber/min) corresponds to a turnover of about 4 moles of ATP/mole myosin/sec if all the myosin is enzymically active. The maximum Ca\(^{2+}\)-activated ATPase activity of water bug actomyosin at low ionic strength (0.2 μmole/mg/min) gives a similar turnover value of about 3 moles ATP/mole myosin/sec, if 50% of the protein extracted as actomyosin from the muscles is myosin of mol wt 500,000. These calculations can be only approximate, but they show the order of absolute magnitude of the enzymic activity in this tissue.

**Mechanical Properties of Activated Fibers**

The most striking physiological characteristic of the myofibrils of insect fibrillar muscles is their ability to sustain continued oscillation in a mechanically resonant system (7) when they are immersed in “activating” solution containing ATP and 10^-6-10^-7 M Ca\(^{2+}\). The property of the activated fibrils which is responsible for this behavior can be investigated by subjecting bundles of glycerinated fibers to controlled length changes and measuring the resulting tension changes in an accurately calibrated servomechanical apparatus.

Under isometric conditions, increasing concentrations of Ca\(^{2+}\) produce a progressive increase in tension which reaches a plateau at about 10–15 mg/fiber (0.27–0.4 kg/cm\(^2\) fiber), depending on the degree of initial extension. Similar values were found for the tension produced by intact beetle fibrillar muscle under maximal excitation (17). In glycerinated fiber bundles under conditions that lead to a rise in the internal concentration of ADP (and in intact muscle under conditions of anoxia), there is a considerable further rise of tension. Such fibers are said to be in the “high tension state” and are unable to sustain oscillatory activity (7). The nature of the high tension state will not be further discussed in this review (see reference 1).

Fibers activated up to the first plateau of isometric tension show characteristic delayed changes of tension if subjected to small-amplitude quick stretch or quick release. Small-amplitude sinusoidal length changes produce sinusoidal tension changes lagging behind the length changes when the frequency of imposed oscillation is in the appropriate range (7, 17). At higher amplitudes of imposed length change, the resulting changes of tension become asymmetrical.\(^5\) There is now a much larger delayed rise of tension on quick stretch than the delayed fall of tension after a quick release (Fig. 5). With sinusoidal length changes, the anticlockwise rotating loop on an instantaneous tension/length display grows asymmetrically (Fig. 6). The ascending limb of the loop remains fixed in position on the oscilloscope, passing close to the point indicating the nonoscillatory state of the preparation, and, at the lower

lengths, following closely the tension/length plot of relaxed fibers; the descending limb moves upward. The area of the loop (measuring the work per cycle) increases up to a maximum and then decreases sharply with further increase in amplitude.

These results show that, to a first approximation, the extra active tension generated as a result of the imposed stretch adds to the passive tension pro-

![Figure 5](image1)

**Figure 5.** Changes of tension in glycerinated water bug flight muscle fibers following quick stretch and quick release of 2.2% in activating solution with $5 \times 10^{-3}$ M Ca$^{2+}$. Time interval between length changes, 1 sec; tension scale, 20 mg/fiber. From Pringle and Tregear.

![Figure 6](image2)

**Figure 6.** Tension/length loops of glycerinated water bug flight muscle fibers during driven sinusoidal oscillation at increasing amplitudes from 0.4 to 3.5% by steps of 0.8%. Activating solution with $3 \times 10^{-8}$ M Ca$^{2+}$; oscillation frequency, 2/sec; extension scale, 1%; tension scale, 10 mg/fiber. From Pringle and Tregear.
duced by the stretch. The active tension produced by Ca$^{2+}$ activation is canceled, after a delay, by quick release; the magnitude of the delayed fall of tension after quick release cannot exceed the isometric tension present initially. Extension leads, again after a delay, to the generation of tension additional to that produced under isometric conditions. It is the delayed change of tension which enables activated fibers to sustain oscillation in a mechanically resonant system.

**DISCUSSION**

Cyclic motion at the cross-bridges would be most effectively translated into a uniform linear movement of the filaments if the motion of the bridges were desynchronized. It is obviously attractive to think that the oscillatory behavior of activated insect fibrillar muscle arises because in this tissue the cyclic conversion of chemical to mechanical energy in the cross-bridges occurs with some measure of synchronization. We may first examine the extent to which such synchronization is physically possible.

**Properties of a Model**

Fig. 7 shows a simplified model of the arrangement of structural elements in a half-sarcomere in striated muscle. A and I filaments are taken to be uniform throughout the overlap region. The bridges generate a shear force between the filaments, and their cycle of operation is represented by a change of angle. In the most general case, the angle may be thought to represent the relative position of points on the A and I filament during the cycle of movement. When the bridges move, the tension generated as the result of a given amplitude of relative movement between the filaments will depend on their stiffness. The more inextensible are the filaments, the greater is the tension generated by a given amplitude of movement. If mechanical energy is generated simultaneously in all the bridges, gradients of tension are established.
in the filaments; tension is greatest in the A filament near to the H zone and in the I filament nearest to the Z line. For any given relative stiffness of A and I filaments and given amplitude and timing of movement of the bridges, the distribution of tension can be calculated by considering the structure as a girder.

Inspection shows that only with the condition that both A and I filaments are completely rigid can the bridges remain parallel and exactly synchronized, if each of them is a source of mechanical energy. If there is compliance in either filament and the bridges remain synchronized and parallel, only the first and last bridge in the overlap region can impart any energy to the system. This is clearly an unrealistic condition. Since the stiffness of the filaments is finite, it can be concluded either that (1) if the amplitudes of movement of all the bridges are exactly the same, then their motions cannot be exactly synchronized, or that (2) if the bridges move simultaneously, then their amplitudes of movement cannot all be the same; depending on the relative compliance of A and I filaments, the greater amplitude of movement will occur at one end or other of the overlap region.

These deductions from a simplified model of the structure have little importance in the normal contractions of vertebrate muscle, where the elementary events are not synchronized. They become important if synchronization tends to occur, for it can then be stated with some certainty that perfect synchronization rules out all postulated mechanisms for the nature of the elementary event which demand equal stroke amplitudes in all the contractile elements; if the elementary event is of this nature, then simultaneous movement of bridges cannot occur with compliant filaments. If, on the other hand, mechanical energy is generated at each bridge by a mechanism which is in series with a local compliance in the bridge, then perfect synchronization is possible and the stroke amplitudes of the elementary events will adjust accordingly.

Evidence for Synchronization in Activated Fibrillar Muscle

Bearing these considerations in mind, we may now consider the evidence that a measure of synchronization of the elementary contractile cycles occurs in the oscillation of insect fibrillar muscle. The discontinuous generation of mechanical energy can only be due either to a near synchronization of elementary events occurring once per cycle of oscillation or to a modulation of the number of events occurring at a higher frequency. Each elementary event must be accompanied by the hydrolysis of at least one ATP molecule, and a measure of the amount of ATP hydrolyzed per cycle of oscillation might therefore distinguish between the two possibilities.

Rüegg and Tregear (14) measured the extra ATP hydrolyzed when lightly stretched glycerinated fibers were subjected to sinusoidal length oscillations.
They found that 0.4–1.1 pmoles of extra ATP/cm fiber/cycle were hydrolyzed during maximum-amplitude power-producing oscillations. Using the more accurate figure of $7.9 \times 10^{14}$ (1.3 pmoles) cross-bridges/cm of fiber (11), this gives 0.3–0.85 molecule of extra ATP hydrolyzed/cycle/cross-bridge; in these experiments the maximum figure is only slightly greater than 1 if the total ATPase activity (instead of the extra ATPase) is taken as the basis for calculation. If it is assumed that a high proportion of the bridges was active during these maximum-amplitude oscillations, it can be concluded that each active bridge hydrolyzes only a few ATP molecules in each cycle of activity. Since each bridge represents three myosin molecules, it follows that the frequency of cyclic operation of the enzyme system responsible for the generation of mechanical energy is not markedly greater than the frequency of oscillation, and is probably the same.

The Mechanism of Synchronization

If the elementary contractile events are indeed occurring at the frequency of oscillation in insect fibrillar muscle, there must be a mechanism peculiar to (or at least specially well developed in) this type of muscle which achieves this synchronization. The significant peculiarities are the following: (1) the high elasticity of relaxed fibers, (2) the smaller degree of Ca$^{2+}$ activation of ATPase activity found in unstretched fibers and extracted actomyosin, and (3) the increased ATPase activity of fibers which have been subjected to preliminary stretch in relaxing solution; it has also to be borne in mind that (4) during oscillation the extra tension does not appear simultaneously with applied stretch but after a delay.

The high resting elasticity implies that there is elastic material in the sarcomeres which is continuous from one Z line to the next. This could have one or more of the following locations: (1) there might be material connecting the A filaments to the Z line, such as the connecting filaments described by Auber and Couteaux (10); (2) there might be material connecting the ends of the I filaments in the middle of the sarcomere; (3) there might be continuous material, distinct from the A and I filaments, which is not easily visualized by electron microscopy. Each of these suggests a different mechanism for stretch activation. If the A filaments can be strained through connecting filaments, then extension might alter the internal structure of the A filaments so as to increase the possibility of interaction between myosin and actin molecules. If the I filaments can be strained, the possibility of interaction might be increased by improving the register between interacting sites. If the hypothetical continuous material exerted an inhibitory action on enzymic activity, extension might decrease the extent of this inhibition. It is still not possible to decide with certainty between these alternative possibilities, but the evidence favors a model based on the presence of connecting filaments (1).
There is no evidence that the increased ATPase activity of stretched fibers involves synchronization of the elementary cycles in the absence of imposed oscillation; under isometric conditions, tension is steady and synchronization is excluded for the same reasons as in vertebrate muscle. The effect of stretch must be to increase either the number of enzyme molecules which are active or their mean turnover frequency, and some further stimulus is needed to effect synchronization.

The best evidence that the stretch increases the number of active enzyme sites rather than their turnover frequency comes from the observation (14) that ATPase activity increases as the oscillation amplitude is increased. Under these conditions, the frequency of molecular turnover is locked to the oscillation frequency, and the number of active enzyme sites is the only parameter which can increase. It is also probably significant that absolute measurements of the ATPase activity of extracted actomyosin and of stretched fibers under isometric conditions give a turnover frequency of only 3–4 ATP molecules per myosin per sec. Although it cannot be excluded that steady stretch produces some increase in the frequency of molecular turnover, it seems likely that the main effect of stretch is on the number of sites of actin-myosin interaction.

When length changes are imposed on Ca²⁺-activated fibers, they can have a further effect in addition to those which occur under relaxing conditions; they can affect the timing of elementary contractile cycles which involve attachment of the cross-bridges to the I filaments. Bridges which are in the energy-releasing phase of their cycle of movement and are therefore attached may be slowed in their motion by extension and accelerated by shortening of the fibers. The reattachment of detached bridges may be delayed by extension. In either case, length change at velocities similar to or greater than the speed of bridge movement will tend to bring into synchronization all those bridges which are attached during the period of the length change or, in the case of extension, those bridges which would have attached if the movement had not occurred. These additional effects occurring only during activation might be expected to occur in any type of muscle, since they do not depend on any of the properties which are characteristic of insect fibrillar muscle.

Transient effects of quick release and quick stretch have recently been reported in frog muscle. Armstrong, Huxley, and Julian (18), working with isolated fibers during tetanic stimulation, described damped oscillations in length following sudden small (5–10%) changes in the load and delayed changes of tension following sudden small changes of length (of about 50 Å/half-sarcomere). Civan and Podolsky (19) have given a more complete analysis of the highly damped oscillation in velocity of shortening which occurs after sudden changes of load. These phenomena bear a strong resem-
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blance to those observed in insect fibrillar muscle, but differ in that the
effects are small and transient. Experiments in which maintained oscillations
have been reported from glycerinated vertebrate muscle fibers (20, 21) have
not been analyzed in sufficient detail to decide whether they are manifesta-
tions of the same phenomenon; an alternative explanation of these oscillations
is possible in terms of chemical diffusion gradients within the fibers (7).

It is possible to suppose that the difference in oscillatory performance be-
tween vertebrate and insect fibrillar muscles arises from the absence in ver-
tebrate muscle of any influence of extension on the number of elementary
contractile events which are occurring. In the absence of such an effect, the
most that small length changes can do is to synchronize those contractile
elements which are already in action. No extra output of mechanical energy
is then stimulated by oscillation. In insect fibrillar muscle, oscillatory changes
in length, in addition to bringing into synchronization those contractile
elements which are already in action, bring more elements into activity,
and these too are synchronized; as a result, the extra output of energy is
sufficient to overcome the additional resistance to motion, and maintained
oscillations become possible.

It has still to be settled by experiment whether more contractile elements
are, in fact, brought into action by the direct influence of mechanical events
in vertebrate muscle. It is well known that the total energy released by ver-
tebrate muscle depends on the work done (the Fenn effect), and it is possible
to demonstrate a dependence of creatine phosphate utilization on work,
when the amount of shortening is constant (15). This could, however, arise
from an influence of the load on the frequency of operation of the contractile
elements without any increase in their number. It may be difficult from ex-
periments on vertebrate muscle to distinguish between these two ways in
which ATPase activity can increase.

The Large-Small Movement Ambiguity

As was pointed out by H. E. Huxley (2), there are two possible ways in which
the cross-bridges might act to impart a sliding motion to the filaments. For
a given output of mechanical energy, (1) they might move with a weak force
over a relatively large distance, or (2) they might move with a strong force
over a short distance; to these must be added a third possibility: (3) their
force/distance relationship might be variable, depending on the mechanical
load against which they operate. This would demand that there be a local
compliance in series with the source of mechanical energy.

For vertebrate muscle, where the operation of the bridges is desynchro-
nized, a short-distance mechanism is possible because of the “vernier” be-
tween the longitudinal repeat distances on the A and I filaments, respectively
(2). The question is further discussed by Civan and Podolsky (19), who have
pointed out that the mathematically defined model of A. F. Huxley (22) is based essentially on a long distance, weak force mechanism. We must examine next whether there is any evidence from insect fibrillar muscle which may help to resolve the ambiguity.

Measurements of ATPase activity in oscillating fibrillar muscle suggest that a small number of ATP molecules are hydrolyzed for each cycle of operation of a cross-bridge, that each active contractile element performs one cycle of movement per cycle of oscillation, and that the relationship between amplitude of oscillatory length change and the magnitude of oscillatory work results from an increase in the number of contractile elements which are active. The alternatives listed above may be restated as follows in the context of active oscillation: (1) Do the individual bridges remain attached for a large fraction of the half-cycle and therefore move over relatively long distances, delivering their mechanical energy slowly with a weak force? (2) Do the individual bridges, although performing only one stroke per cycle of oscillation, deliver their energy over a small fraction of the half-cycle with a large force and then wait before commencing their next stroke? (3) Do the bridges deliver a constant amount of mechanical energy through a local compliance in the bridge itself so that their stroke amplitude, and possibly their stroke duration, depends on the local conditions?

The only direct evidence bearing on these questions comes from the observations (9) that, in relaxing solution, the bridges are detached and primarily at right angles to the A filaments, while in rigor they are attached and at an angle of about 45°. This showed that it is possible, under the extreme and unnatural conditions of complete absence of ATP, for the bridges to perform a large angular movement.

Electron micrographs suggest that the lateral distance between the outsides of the A and I filaments of water bug flight muscle is about 150 A. If the 45° angled position is taken to be the extreme end of the active stroke and the bridges first attach at right angles, one cycle of bridge movement produces a relative sliding of 150 A. There is, however, no evidence about the angular position on first attachment; if the bridges attach at 45° in the other direction, a relative sliding of 300 A could result from one stroke. These approximate figures are the maximum movements that could occur if a change of angle is the mechanism by which mechanical energy is imparted to the filaments.

Preliminary experiments suggest that maximum oscillatory work is done when the amplitude of sinusoidal length change is 3–5% peak-to-peak. If there is no series compliance in the filaments or elsewhere in the fibers and all movement is occurring as sliding between the filaments, this corresponds to an interfilament displacement of 360–600 A (sarcomere length, 2.4 μ), which is definitely too large to be produced by synchronized angle changes.
in the bridges. At the other extreme, if the A and I filaments were very compliant, little relative sliding movement would occur at the bridges. The truth must lie somewhere between these two extremes, and it is not difficult to suggest reasonable values for the compliance of the different elements of the structure which will allow the mechanical work during oscillation to be explained by simultaneous bridge movement over the possible distance. For the reasons given earlier from inspection of the simplified model of Fig. 7, the stroke amplitudes of the bridges cannot be the same throughout the half-sarcomere if synchronization is to be perfect, and there would have to be some compliance in the bridges. If the mechanism of mechanochemical energy conversion is such that the stroke amplitudes have to be equal, the bridges can still act nearly simultaneously during the half-cycle of shortening without upsetting the validity of the large distance, weak force model.

Further implications of this model are considered in more detail elsewhere (1). The delay between length changes and tension changes is most readily explained by the time which it takes for movement at the bridges to build up strain in the filaments and so generate tension in the fibers. The velocity of bridge movement and the compliance of the filaments are now the main factors which determine the magnitude of the delay. It is interesting to note that, in different insects with fibrillar flight muscles, the frequency of wing beat varies over the range of 20–1000/sec; the optimum frequency for oscillatory power output and, therefore, the magnitude of the delay between length changes and tension changes must vary over a similar range in different species. Comparative studies might provide a good test of the long distance, weak force model, since there should be large differences in either the filament compliance or the molecular turnover rate of different types of fibrillar muscle.

It can, however, be maintained that the observations (9) of the large bridge angle change in rigor have no relevance to the events occurring during activation, and we must therefore examine how a short distance, strong force model could provide an explanation of the oscillatory activity. With this model, it must be supposed that different bridges deliver their mechanical energy in succession over only a small fraction of the total distance that the filaments slide during the shortening phase of the oscillation; they must then become detached and remain so during the rest of the shortening phase and the whole of the lengthening phase. To borrow some analogous terminology, their "mark-to-space ratio" must be very small, since we know that their frequency of operation is that of the oscillation. The chief difficulty in this model is to see what determines their moment of attachment, since some bridges must wait until nearly the end of the shortening phase, in spite of the fact that the signal which determines the timing of their cycles is the immediately previous extension. One possibility, following the reasoning
given by Huxley (2), is to suppose that attachment is impossible unless and until sites on the two filaments come into very close register; as shortening proceeds this will happen successively along the half-sarcomere, and a wave of short distance bridge motion will move along the filaments as bridges which are already in an activated condition attach and deliver their energy as mechanical work. The previous extension phase of the cycle must have put these bridges into a state of activation, and the phosphorylmyosin intermediate postulated by Tokiwa and Tonomura (23) is a possible candidate for this energy-rich but metastable state of the contractile element. The delay between length changes and tension changes depends on the rate at which the wave of short distance contractile events travels over the half-sarcomere, which is influenced, as in the other model, by the rate at which the short distance movement occurs.

An advantage of this model is that it does not impose a structural limit on the amplitude of sliding which can occur during power-producing oscillation, but it becomes necessary to seek a different explanation for the observation that there is an amplitude limit at about 3–5% peak-to-peak length change, above which the magnitude of oscillatory work declines (Fig. 6).

The largest output of mechanical work so far obtained in these experiments (measured from the area of the tension/length loop on the oscilloscope) is 0.7 erg/cycle/cm fiber, or 4.3 kcal of mechanical energy/cycle/mole myosin. Tregear (24) points out that this means that, if one ATP molecule is hydrolyzed per myosin molecule per cycle, the efficiency of mechanochemical energy conversion is about 30% if all the myosin molecules are activated. This may be approaching the limit of work output of which the fibers are capable. There is thus the possibility that the limit is not structural, but occurs when the oscillation is of an amplitude that activates fully all the elementary contractile sites.

In conclusion, it must be said that study of the oscillatory activity of insect fibrillar muscle has not yet produced conclusive answers to many of the questions which can be asked about the nature of the elementary contractile process. There are, however, a large number of possible experiments which can be performed on this tissue which may give clearer answers than can be obtained from vertebrate muscle.

REFERENCES

**Note**: A discussion of the papers by Doctors Pringle and Twarog begins on page 168.