followed by thin-layer chromatography. After this treatment, each ecdysone gave only two radioactive spots, corresponding to the tri- and tetraacetates of the ecdysone. Thus, the significant finding here is that α- and β-ecdysones are formed from cholesterol in the isolated abdomen lacking the PTG.

In contrast, when labeled cholesterol was injected into larvae or isolated abdomens on day 2 of the fifth instar, a stage at which the PTG still do not show histological "activity," neither ecdysone could be detected after 24 hours. This shows that the PTG are indeed somehow related to ecdysone biosynthesis (*13*). Although it would be difficult to prove that no ecdysone is secreted from the PTG, there is no doubt that ecdysones can be synthesized outside these endocrine organs.

It should be noted that when exogenous α-ecdysone ([23,23,24,24-tetra-^{3}H]α-ecdysone) was injected into *B. mori* larvae at the same stage (day 6 of instar 5), more than half of the α-ecdysone was converted into β-ecdysone within only 15 minutes (*14*), so that the relative content of β-ecdysone is larger than that of the α form. Thus, the equal amounts of biosynthetic α- and β-ecdysones, as estimated from the comparable conversion yields (above), together with the rapid conversion of administered α-ecdysone to β-ecdysone, suggest that (i) biosynthetic α-ecdysone is not present in the free form, but rather in a bound form which is slowly hydroxylated into β-ecdysone; and (ii) the bound α-ecdysone should be such that the ecdysone is readily liberated under conditions of extraction.

Although we have thus furnished the first clear-cut evidence for ecdysone production outside the PTG, the exact role of these glands remains enigmatic. It is possible that the so-called PTG hormone secreted from PTG into the body fluid catalyzes a step in the biosynthesis of bound α-ecdysone from cholesterol.

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Length-Force Relation of Calcium Activated Muscle Fibers

Abstract. *Calcium activated skinned frog muscle fibers develop a large relative force at a sarcomere length of 1.0 micrometer. Since the normal myofilament lattice is perturbed at this length, regularity of the lattice does not appear to be an important factor in the contraction mechanism.*

Force in striated muscle fibers is developed by lateral interaction of the thick and thin myofilaments. Since the three-dimensional lattice formed by these filaments varies with fiber length, the relation between length and force provides information about the nature of the filament interaction. The most careful studies of the length-force relation have been made with electrically stimulated frog muscle fibers (*1, 2*), in which case the calcium that activates the myofilament interaction comes from the sarcoplasmic reticulum. It was discovered, however, that in electrically stimulated fibers a central core of myofibrils is not activated when sarcomeres shorten to lengths less than about 1.7 μm (*3*), which makes it difficult to interpret the length-force relation of electrically stimulated fibers at these lengths. To eliminate the effects of incomplete activation, we measured the length-force relation in skinned fibers directly activated by calcium (*4*). The main finding was that considerable force is developed at sarcomere lengths as short as 1.0 μm, even though the myofilament lattice at this length is distorted relative to that at normal sarcomere lengths.

Fibers from frog semitendinosus muscles were skinned and mounted in a force transducer according to the method of Hellam and Podolsky (*5*). Two bathing solutions were used: (i) relaxing solution containing (in millimoles per liter) KCl (140), $MgCl_2$ (1), the disodium salt of adenosine triphosphate (5), imidazole (10), and the dipotassium salt of ethyleneglycol bis(aminoethylether)tetraacetic acid (EGTA, 3) and (ii) contracting solution, in which the calcium salt of EGTA replaced the potassium salt. Both were buffered to *p*H 7. The concentration of Ca^{2+} in the relaxing solution was approximately $10^{-9}M$ and in the contracting solution it was $2.5 \times 10^{-5}M$, which fully activates the myofilaments (*5*). After the initial dissection at 20°C, the fiber and experimental solutions were kept at 4°C in a thermoelectrically cooled chamber. The skinned fiber was mounted in mineral oil between two clamps (free fiber length, 1 to 5 mm) and then transferred to relaxing solution. The fiber was adjusted to a length, l_0, which corresponded to a striation spacing of 2.0 to 2.2 μm, as determined by a laser diffraction pattern (6328 Å). The fiber was then transferred to contracting solution and force was allowed to develop. The maximum force, P_0, was reached in 5 to 15 seconds. The laser pattern showed that activation changed the striation spacing in the middle of the fiber by less than 0.1 μm. After the force had reached a steady level, the

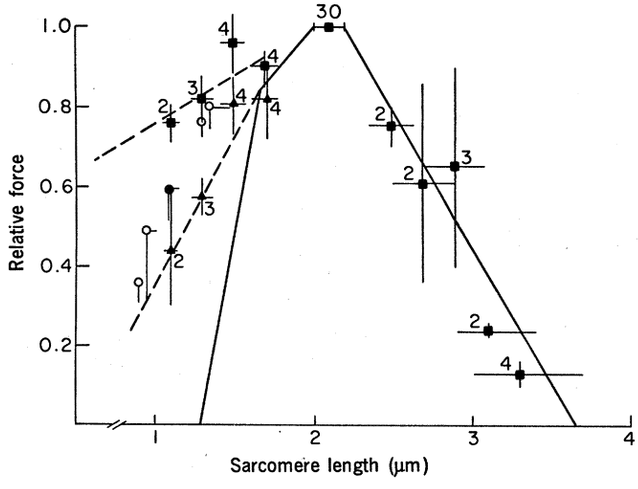
fiber was transferred back to relaxing solution. In relaxing solution its length was changed by increasing or decreasing the distance between the clamps holding the ends of the fiber. In the latter case, this caused buckling of the fiber; in the former, the fiber developed resting tension. The fiber was then put into contracting solution, and the force at this new length recorded until it reached a steady value, at which time the fiber was put back into relaxing solution. After one or two contractions at lengths different from l_0, the length was again adjusted to l_0 (while in relaxing solution) and the force in contracting solution remeasured as a control.

The length-force curve obtained from results for 14 fibers is shown in Fig. 1 (*6*). The solid line shows the curve of Gordon, Huxley, and Julian (*2*) for electrically stimulated intact fibers. For sarcomere lengths greater than 1.7 μm the relative force developed by both preparations is substantially the same. The same result was reported previously for partially activated skinned muscle fibers (*5*).

For sarcomere lengths shorter than 1.7 μm the relative force developed by intact and skinned fibers is considerably different. A typical force trace from a skinned fiber at short sarcomere length is shown in Fig. 2A. The fiber, which was initially slack, became taut very early in the record, within 1 to 2 seconds after contraction began. Unlike force development at sarcomere lengths above 1.7 μm (*5*), the initial S-shaped rise in force was generally followed by a slow upward creep that often lasted as long as 1 minute before the force became essentially steady. In Fig. 1 the squares represent the peak active force. The triangles represent the force generated before creep. This was taken as the force at which the back extrapolation of the early stages of creep intersected the upward extrapolation of the force curve during the maximum rate of force development, as shown by the dashed lines in Fig. 2A. As discussed below, the true active force developed by a skinned fiber as a function of active sarcomere length lies between these two limits, which are shown by dashed lines in Fig. 1.

One possible explanation for the upward creep of force at short sarcomere lengths is the development of nonuniformity (dispersion) of sarcomere length due to the shortening of some sarcomeres at the expense of others. However, unlike the situation at long sarcomere lengths, where the slope of the length-force curve is such as to promote the development of nonuniformity (*2*, *7*), the slope of the length-force curve at short sarcomere lengths is such as to maintain uniformity, so that any nonuniformity that developed would have to be at least partially irreversible (*8*). The large forces that developed at the short lengths might then have been due to some sarcomeres irreversibly shortening to less than the average sarcomere length, thereby causing the remainder (the active ones) to be longer than the average sarcomere length and consequently able to generate more force.

To rule out this possibility, experiments were done with 12 additional fibers. One of these experiments is shown in Fig. 2B. After the muscle was adjusted to length l_0 the fiber was placed in contracting solution. As soon as the force became steady, the fiber was allowed to shorten quickly to an average sarcomere length close to either 1.0 μm (range 0.9 to 1.2 μm, six fibers) or 1.3 μm (range 1.3 to 1.38 μm, six fibers). The force dropped to less than 0.1 P_0 and then started to redevelop, rapidly at first and then more slowly. In the six



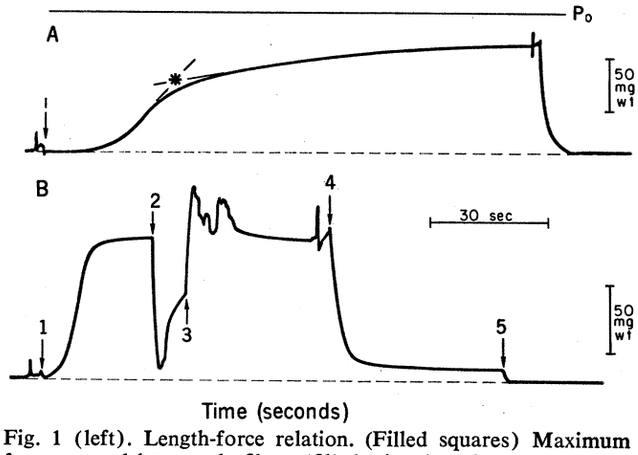


Fig. 1 (left). Length-force relation. (Filled squares) Maximum force exerted by muscle fiber; (filled triangles) force exerted by fiber before creep (see Fig. 2A and text). The number next to each point is the number of force measurements averaged. The vertical bars give the standard error of the mean, and the horizontal bars are the estimated uncertainties in measurement of sarcomere length. (Circles) Active force developed at a given sarcomere length from experiments like that in Fig. 2B. The horizontal lines extending from the circles represent the maximum possible sarcomere dispersion; the vertical lines represent the uncertainty in redeveloped active force due to the development of resting tension during the experiment. The filled circle denotes the point from the experiment of Fig. 2B. The solid curve represents the data of Gordon, Huxley, and Julian (*2*). At short sarcomere lengths the true length-force relation for fully activated sarcomeres lies between the two dashed lines, one drawn through the points for peak force, the other through the points for force before creep. Fig. 2 (right). (A) Force development at short sarcomere length. The maximum force, P_o, 129 mg wt; the average striation spacing during contraction, 1.7 μm; the apparent fiber diameter in oil, 90 μm. Note the slow approach of force to a steady level following the initial rapid force development. The intersection of two dashed lines represents the extrapolated force before creep (force at asterisk). (B) Experiment to determine reversibility after contraction at short sarcomere length. The fiber is initially in relaxing solution (l_o, 4.5 mm; sarcomere length, 2.2 μm; fiber diameter in oil, 86 μm). (1) Fiber put into contracting solution, (2) release to 2.2 mm, (3) restretch to 4.5 mm, (4) transfer to relaxing solution, (5) release for measurement of resting tension. Initial resting tension at l_o, less than 1 mg wt; initial active force at l_o, 100 mg wt; force at point 3, 59 mg wt; total force after restretch, 103 mg wt; resting tension at end of experiment, 9 mg wt; active force after restretch, 103 — 9 = 94 mg wt.

fibers allowed to shorten to 1.0 μm, an average force of 0.45 P_0 (range 0.32 to 0.59 P_0) was allowed to redevelop before the muscle was again stretched to length l_0 (*9*); the fibers shortened to 1.3 μm were allowed to redevelop an average force of 0.64 P_0 (range 0.48 to 0.80 P_0) before restretch to l_0. When the force became steady after restretch, the muscle was transferred to relaxing solution and any resting tension noted. If some sarcomeres irreversibly shorten when the muscle contracts at length l, then upon restretch to l_0 the active sarcomeres would be stretched to a length beyond the plateau of the length-force curve and the fiber would generate less force than it had previously generated at length l_0. From the amount of active force developed after restretch, the maximum sarcomere dispersion could be estimated (*10*).

The results from the five fibers that were allowed to develop enough force for their points to lie well above the lower dashed line of Fig. 1 are shown in the figure as circles (*11*). The horizontal lines extending from the circles represent the maximum uncertainty due to sarcomere dispersion. The vertical lines represent the uncertainty in redeveloped active force due to the development of resting tension during the experiment. The fact that each of the five points lies between the dashed line drawn through the points for maximum force and that drawn through the points representing the extrapolated force before creep (*12*) is evidence that the true length-force relation for fully activated sarcomeres is also between these lines.

The force developed by calcium activated skinned fibers at short sarcomere lengths is greater than that of electrically stimulated intact fibers (*13*). Even when electrical stimulation is facilitated by caffeine the force at sarcomere length 1.0 μm is close to zero (*14*), while in directly activated fibers it is between 0.3 and 0.7 P_0. At this sarcomere length the ends of the thick filaments are driven against (or possibly penetrate through) the Z disks (*15*), which decreases the length of thick filament available for forming cross bridges. The magnitude of the force developed by calcium activated skinned fibers under these conditions indicates that the force required for this process is relatively small compared to the force of contraction.

At sarcomere length 1.0 μm there is complete double overlap of the thin filaments. While it is possible that the length of thin filament that has not gone through the M line is still in its normal trigonal position relative to the adjacent thick filaments, it is more likely that the thin filaments are displaced from this position along most of their length, particularly if the stabilizing forces for the lattice are electrostatic and dispersive in nature (*16*). In this case, the observation that the force is not reduced much more than would be expected simply from the associated loss in thick filament length implies that in active muscle the thick filament projections can search azimuthally (*17*) as well as radially (*18*) in forming cross bridges with the thin filament. It also indicates that the force developed by a cross bridge is relatively insensitive to the azimuthal disposition of the bridge. These properties make it easier to understand how force can be produced by the same basic cross bridge mechanism in the smooth and striated muscles of various species in spite of wide differences in filament arrangement.

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9. Both release and stretch were done by manually turning the knob of a Prior micromanipulator that held one of the clamps. Release was done in less than 1 second, stretch in 1 to 4 seconds. The length of the preparation was measured with the eyepiece micrometer of a dissecting microscope.
10. Consider a fiber initially at length l_0 and striation spacing 2.2 μm. If some of the sarcomeres became irreversibly shortened while the muscle contracted at length l [corresponding to an average sarcomere length of $(l/l_0) \times (2.2\ \mu m)$] the active sarcomeres would be at a length, X', greater than average. When the muscle was restretched to length l_0 the active sarcomeres would be at a sarcomere length, Z', greater than 2.2 μm. The value of Z' can be determined by noting the active developed force after restretch, from the length-force relation to the right of the plateau. The active sarcomere length at length l is $X' = Z'\ (l - l_b)/(l_0 - l_b)$, where l_b is the length of the irreversibly shortened sarcomeres. Although l_b is, in general, unknown, the maximum active sarcomere length is less than $Z'l/l_0$, which may be used as the upper limit of sarcomere dispersion.
11. The fibers shortened to about 1.0 μm before restretch showed an average reversibility of active force at l_0 of 86 percent (range 72 to 100 percent). Those shortened to 1.3 μm showed an average reversibility of 91 percent (range 84 to 100 percent). There did not appear to be a correlation between percent reversibility and the amount of force allowed to redevelop before restretch. Because of this, those fibers allowed to redevelop only a small amount of force before restretch were not informative, as presumably they could have been allowed to redevelop more force without producing significantly more irreversibility.
12. The physiological significance of the extrapolated force before creep is not clear. However, the second set of experiments described in the text show that it is a measure of the lower limit of the amount of force a skinned fiber is capable of generating at a given "true" sarcomere length, that is, the sarcomere length of the active, pulling sarcomeres as opposed to those that are irreversibly shortened.
13. Further evidence that short sarcomeres have the ability to produce relatively large forces comes from experiments with ATP activated, glycerol extracted rabbit muscle fibers. Although this preparation almost always tears apart when force develops at lengths less than the equilibrium length (80 percent of the length in situ), it is able to develop a force of about 0.5 P_0 at one-half the equilibrium length before tearing. [A. Weber, *Biochim. Biophys. Acta* **7**, 214 (1951).]
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