SEVERAL forces have combined to reveal a phenomenon of striking proportions. What may prove to be the quantum element underlying muscle contraction was discovered by chance, following the development of a high-resolution optical diffractometer designed to follow the dynamics of shortening in isolated cardiac muscle. Records showed a most unusual feature: periods of sarcomere shortening were punctuated by periods of pause during which there was little or no length change, conferring a staircase-like character on the shortening waveform.

Could shortening really occur in discrete quanta? To find precedent for quantal behavior one need not look far. Nearby, at the myoneural junction, for example, a detailed understanding of the mechanism of intercellular communication followed the discovery that acetylcholine was discharged in quantal packets. These packets sum to form the end plate potential in much the same way that the steps apparently sum to give the shortening waveform. In the latter case, however, an unfathomable implication has impeded acceptance.

The difficulty is that stepwise shortening implies a colossal degree of synchrony. Why so? The optical diffraction pattern reflects the behavior of a sizable fraction of sarcomeres ranging over the field illuminated by the laser. This ensemble could pause in either of two ways. In the complicated way, some sarcomeres would shorten while others lengthened in such a way that the average velocity remained zero for a period; this situation would then recur fortuitously time and again as contraction progressed to give the observed cascade of pauses and steps. In fact, this possibility is now ruled out; even when the sample volume is diminished extraordinarily, the pauses remain in evidence (see below).

The alternative way is simpler. It implies that members of the ensemble pause and shorten synchronously. To those seriously engaged in thinking about molecular contractile mechanisms, this is disturbing. It implies that the elements underlying shortening can somehow act in concert to produce the observed quantal behavior. The synchronous domain is vast. In the experiments cited above, for example, the incident beam encompassed some 100 million myofilaments, a region which by molecular standards is essentially infinite.

The synchrony implied by stepwise shortening, then, questions the very foundation underlying current ideas of how muscles shorten. The present theory, for example, contains no provision to synchronize the action of bridges. Needless to say, the suspect of Maxwell's demon calling the strokes has not served to instill confidence in the phenomenon itself. On the other hand, if steps do not arise out of some instrument artifact, their discovery could prove seminal: Synchronized, quantal behavior implies a fundamental rethinking of how muscles shorten.

The remainder of this article will present the evidence that has convinced me that muscle shortening does, indeed, occur in synchronized steps. I will go on to discuss some implications of the phenomenon, and finally, I will briefly mention a possible molecular mechanism that is, in fact, unrelated to cross-bridge cycling. To those brought up on a steady diet of sliding filaments, the proposal may prove distinctly unpalatable.

Segment Length Dynamics

Following the pioneering work of Gordon, Huxley, and Julian, a number of investigators have perfected methods of measuring the dynamics of fiber segments. Figure 1 shows an example. In this case each segment was demarcated by a pair of thin hairs from the ear of a black cat; segment length was tracked optically. The steps apparent in Figure 1 were not a chance observation. Of 21 fibers studied, stepwise shortening was absent in only 5; in the remaining 16, shortening steps were punctuated either by brief hesitations or by more prolonged pauses such as those of Figure 1. The patterns were clearly repeatable from cycle to cycle.

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The prototype apparatus originated in Prof. K. A. P. Edman's laboratory in Sweden. Black labrador dog hairs were used. Although the apparatus was designed for different types of experiment and the published records are on a compressed time scale, several of the traces (eg, Edman et al, Figure 8) show distinct pauses.

Glass microelectrode tips have also been used as segment markers. Housmans plunged these into the belly of a papillary muscle and used an optical scanner to follow the inter-marker spacing. Surprisingly noise-free, the records showed both smooth and stepwise patterns. The stepwise patterns were particularly apparent when the load was kept modest, and they were repeatable from contraction to contraction.

Finally, in a more detailed study, Tameyasu et al used a high-speed video system to follow shortening in single isolated frog heart cells. Segments were demarcated primarily by natural markers such as glycogen granules. Stepwise shortening was found regularly. The number of steps, however, was limited, as the steps became progressively less distinct with shortening. Nevertheless, the step pattern was repeatable from contraction to contraction as well as from preparation to preparation. Catecholamines decreased the pause duration, accounting for their positive inotropic action.

The results are consistent: Fiber segments shorten in steps. The studies include cardiac and skeletal muscle, natural and artificial markers, and oriental and occidental investigators. They imply that segments as long as 1 mm may shorten and pause synchronously.

A great advantage of the surface marker approach is its ability to track a fixed region; the markers adhere to the fiber surface and the surface adheres to the contractile proteins. This is not true of the various striation-based methods (see below), where fiber translation across the optical axis during contraction results in a progressive shift in the sampled population, a shift that could have the potential to wreak havoc (but see rebuttal by Pollack, followed by Huxley's rebuttal of the rebuttal). In the segment method the tracking of a consistent region averts such potential. Indeed, results have been compiled and submitted for publication that confirm the method to be fully immune to the effects of translation, not only in the axial direction but in the plane normal to the fiber axis as well (Granzier et al, unpublished data).

A disadvantage, on the other hand, is that the sampled region is perhaps too global to reveal shortening dynamics on the molecular scale. For this, it is necessary to turn to the sarcomere length detection methods.

**Sarcomere Length Dynamics**

These approaches are based on the muscle's striation pattern. They fall into two classes — those in which sarcomere length is inferred from the optical diffraction pattern and those in which sarcomere length is measured directly from the striation pattern.

The first to note that a transilluminated muscle fiber produced a diffraction pattern was Ranvier. By virtue of the regularity of the pattern of striations, an incident beam is bent (ie, diffracted) at an angle that is uniquely dependent on the wavelength of the light and the spatial frequency of the striations. This permits the inference of sarcomere length. Because the sharpness of the diffraction pattern improves with the purity of the incident beam, the advent of the laser has made the use of diffraction as common, almost, as the use of the tension transducer.

It is no surprise, therefore, that stepwise shortening was first identified by the laser diffraction method. Indeed, the very first mention of step-like phenomena came a decade earlier from a Russian group who...
reported “sawtooth-like” shortening patterns. In the absence of modern optical technology, however, their diffractions records had limited resolution and their observations went unnoticed.

The widespread use of laser diffraction notwithstanding, this method has come under attack. It is up to the task of discerning features as fine as steps? The basis for doubt stems from the question of the extent to which a muscle fiber may be treated as “thin” diffraction grating. When treated as a thick grating, the influence of striation skew over the fiber thickness enters. If the skew angle happens to lie at the critical “Bragg angle,” an exceptionally strong reflection will arise and will dominate the diffraction pattern. If clusters of striations alternately satisfy and fail to satisfy the Bragg condition, it has been argued that apparent steps and pauses could arise.

This hypothesis runs into several obstacles. First, direct tests of the Bragg mechanism have proved unsupportive. Since the critical angle is wavelength dependent, each wavelength should reveal a different set of Bragg planes. But they do not. The pattern of reflections remains wavelength insensitive.

A second argument is that diffraction provides a consistent result. When segment length and diffraction methods are employed on the same fiber during the same contraction, the records are generally superimposable. Steps and pauses occur with good consistency at the same position on each of the two shortening waveforms. All and all, diffraction works!

The above-mentioned consistency extends also to the striation imaging methods considered next. These methods are independent of the thick grating/thin grating controversy; yet the shortening waveforms they produce contain steps and pauses whose magnitudes are similar to those found with optical diffraction.

In the first of these two methods a cine camera was used to photograph the striation pattern at 4000 frames/sec. The optical diffraction pattern was registered simultaneously. Frame-by-frame analysis of the film gave shortening patterns that were noisy but clearly stepwise, and they closely resembled the patterns obtained simultaneously by diffraction.

Intrigued by the question of whether shortening was stepwise, Prof. H. Sugi launched a reanalysis of his own high-speed cine records obtained in a study of the spread of activation, published well over a decade ago. A micropipette placed near the fiber edge had been used to inject depolarizing currents. Because contraction was thereby localized, the fiber did not translate and the cine recordings were able to track the shortening of an unchanging cluster of striations. Although some shortening patterns were smooth, many showed clear stepwise shortening (Sugi and Toride, unpublished data).

The seemingly endless number of look-alike frames required for processing in the cine method prompted the development of less cumbersome approaches. Following an idea put forth by Claes et al, an electronic sensor was developed from which sarcomere length could be computed on-line with high resolution. The striation pattern is projected onto a linear photodiode array. A scan, requiring only several hundred microseconds, produces a periodic signal corresponding to the A- and I-bands. The frequency of this signal is computed on the fly using a phase-locked loop, a circuit exquisitely suited to extract the frequency from a periodic signal that may also contain substantial noise. The method works well; when used properly, the effect of fiber translation is insignificant and sarcomere length resolution is better than 0.1%. Records obtained using this method show stepwise shortening at least as clearly as any of the other methods. An example is shown in Figure 2. Under conditions in which the load remained modest, records showing cascades of steps similar to those of Figure 2 were found consistently.

An advantage of this method is that it samples a highly restricted volume. In Figure 2, for example, the sampled volume was 30 μ in length and several microns either way in breadth. By contrast, the segment method samples a region some 10,000 times more vast. The presence of pauses and steps in regions so disparate in size implies that discrete behavior observed in large regions comprises similarly discrete behavior in small regions, synchronized over space. In other words, local behavior is stepwise.

To recapitulate: Four classes of methods have been
brought to bear on the question of whether sarcomeres shorten in synchronized steps. Stepwise shortening was observed by all. Three of the four have been repeated and confirmed in more than one laboratory. On the other hand, an insidious artifact may yet lurk in the background, awaiting revelation. While such a possibility cannot be ruled out, it is not at all obvious how the artifact might affect four so diverse methods equally, especially when pairs of methods used simultaneously give the same result.

Is Stepwise Shortening Obligatory?

This question has two parts. Is stepwise shortening found in all kinds of muscle; and in a given muscle, is contraction always stepwise?

To the first question, the answer is as yet unknown. Because steps are found in cardiac and various types of skeletal muscle, it is tempting to speculate that the phenomenon may be universal. However, the mechanism underlying stepwise shortening has not yet been elucidated, and until it is, it will not be possible to establish which features of the shortening mechanism are the critical ones for the appearance of steps and which are not. Thus, the generality of the phenomenon is difficult to predict.

On the other hand, stepwise phenomena have been found in an altogether different kind of contractile system, implying that the features giving rise to steps may indeed be fundamental. Using high-speed cinemicroscopy, Baba studied the time course of bending in the giant abfrontal cilia of the mussel, *Mytilus edulis.*

The bending motion was not continuous; rapid phases were punctuated by periods of little or no bending. Each bending step was attributed to a stroke of the dynein arms, causing a discrete sliding of the axonemal microtubules. The parallelism of this phenomenon to stepwise sarcomere shortening seems self-evident. It lends credence to the possibility that the kind of quantal action considered here may quite possibly constitute a fundamental basis underlying biological motion.

The other side of the question is whether stepwise shortening is an obligatory feature in a given specimen. The alternative is that it is not. The question arises because records obtained under the very same experimental conditions sometimes show steps and sometimes do not. Can the stepping mechanism be turned on and off?

Probably not. Under certain conditions stepwise shortening is practically ubiquitous. For example, in releases of unstimulated fibers and in lightly loaded isotonic contractions (Tirosh et al, unpublished observations), stepwise shortening is observed in more than 95% of fiber regions. This implies that the failure to observe steps in the few remaining regions is not likely to be due to a vagary of the muscle’s shortening mechanism but, perhaps, to some technicality. A likely possibility is incomplete synchrony.

Synchrony is clearly less than infinite. If synchrony extended the full length of the fiber, the steps would be as distinct on the muscle shortening trace as on the sarcomere shortening trace. They are not. Following release from isometric-to-isotonic contraction, muscle-shortening waveforms exhibit up to several inflections. But these are rounded and less distinct than the quantitatively comparable inflections (ie, steps) seen on the sarcomere length traces. Synchrony is substantial — but incomplete.

Synchrony will be impaired by end effects. Clamping the specimen inevitably causes the load distribution over the fiber cross section to be nonuniform, an end-effect that may penetrate well into the fiber. This is especially true in cardiac specimens, where tendons are in short supply and clamping transforms adjacent tissue into what is perhaps best described as hamburger. Such nonuniformities impair step detectability. Since the local stepping pattern is a function of load, variations in load over the cross section will cause the steps to go out of phase with one another, thereby smoothing the detected waveform. When the load is low or zero, the load variation is small in absolute terms and the impairment of synchrony will be minimal; but when the load is high, the effect will grow in significance. This may explain why steps are regularly detected when the load is low but less regularly detected when the load is high.

All of this implies that the failure to detect steps on occasion probably stems from purely technical considerations. The phenomenon may be locally obligatory, but its detectability will necessarily depend on the extent to which synchrony is retained within the sampled volume.

Steps and Sounds

A provocative implication of the discontinuous nature of shortening is that associated events may also occur discontinuously. Consider the consumption of fuel. If the ATP hydrolytic cycle is as intimately tied to shortening as presently conceived, splitting may occur as a linked series of explosions, not as a slow, continuous burn. If, so, shortening heat may also evolve discontinuously. The challenge is to develop sensors with enough resolution to test for such areas of potential discreteness.

Discreteness has already been confirmed in the generation of sound. Sound? That contracting muscles generate sound is easily demonstrated by stuffing your thumbs into your ears and moving your fingers slowly, as though waving. Or, for a more dramatic demonstration, place the business end of an electronic stethoscope (in which the frequency components of interest are amplified) over the first dorsal interosseous muscle between your thumb and forefinger and move your forefinger to and from your middle finger. Steel yourself for a deafening roar!

Although it has long been known that muscles generate sound, the origin of the sound has remained obscure. Clearly, sound is associated with movement. If movement ceases during a pause, the fiber should remain silent. In testing such an hypothesis, measurements must be confined to a region over which synchrony of steps and pauses is assured. This may have
been so in the experiments of Gordon and Holbourn, \(^\text{39}\) where sounds were detected from the orbicularis oculi, an eyelid muscle. During a blink, all motor units of this muscle contract in unison. The sounds came as a series of distinct clicks, not as a continuous tone.

The discrete clicks have been confirmed in isolated muscles. \(^\text{40}\) Spike-like sounds were observed when isolated frog sartorii were allowed to shorten under light load, confirming that the clicks come from the muscle itself, not the nerve. The discreteness of the sounds is consistent with the discreteness of the contractile process; it remains to be demonstrated, however, that the sounds are coincident with the steps.

Do Cross Bridges Cycle Synchronously?

The critical question regarding the steps must certainly be: What is the underlying mechanism? For what reason has nature chosen to effect biological motion in discrete, synchronous steps instead of in a smooth, continuous manner? Is there some advantage, or is synchrony merely an incidental by-product of the shortening mechanism?

A logical starting point is the presently accepted theory, \(^\text{2, 4}\) in which contraction is mediated by the action of cross bridges. The theory presupposes that bridges act independently, but one may imagine a molecular coxswain calling the strokes. A step could then arise as cross bridges rotate in synchrony. The period between synchronous rotations would correspond to the pause. Could this be the underlying mechanism?

Probably not. Steps of similar size and character appear as the fiber relengthens. \(^\text{41}\) If steps arise out of synchronous bridge rotation, the bridges would have to be endowed with the ability to cycle in reverse.

A more conclusive point is that the steps are observed in sarcomeres stretched to the point where thick and thin filaments no longer overlap. Their presence has been confirmed with both the laser diffraction and phase-locked loop methods. \(^\text{43, 44}\) Unless the mechanism underlying steps beyond overlap is qualitatively different from the one operating with overlap, the source of stepwise shortening cannot lie in synchronous cross-bridge rotation.

On the other hand, if cross bridges do rotate, any proposed mechanism must build in this feature. If bridge action is stochastic, the task increases in formidability: How can discrete, synchronous behavior be derived from a process that is random?

The point may be moot. The question of whether cross bridges rotate is under intensive investigation in several quarters, and the negativity of early results is causing a stir (cf Goody\(^\text{45}\)). Among the new probes, the electron-spin-resonance method provides a "snapshot" of the distribution of cross-bridge angles. \(^\text{43}\) The angles were found to be broadly distributed in relaxed fibers but restricted to a unique angle in rigor. During contraction, the signal comprised two components indistinguishable from relaxed and rigor. The authors quite naturally assigned the relaxed component to the unattached bridges and the rigor component to the attached bridges. The difficulty is that the attached bridges should not have been caught at a unique angle; if they had been rotating, the snapshot would have revealed a species with a new angular distribution.

A more direct approach was taken by Güth, \(^\text{44}\) who took advantage of the fact that the fluorescence of the tryptophan contained within the S-1 subunit of the cross bridge was polarized. Güth examined rabbit psoas fibers in relaxed, rigor, and contracting states. Provided the level of substrate was adequate (a problem Güth identified in earlier studies of this type), no difference of cross-bridge attitude could be found between relaxed and contracting fibers. This result has recently been confirmed using an extrinsic fluorescence probe, eADP. \(^\text{45, 46}\)

The observations are in concurrence: The expected pattern of variation of cross-bridge angle is not found. Similarly, when contracting or rigor fibers are quick-stretched or quick-released \(^\text{44, 47}\) the cross-bridge angle remains stubbornly unchanged. All this notwithstanding, it is possible that bridge rotation does occur as theorized but is restricted to a small, proximal segment of the bridge that remains unsampled by any of the several probes. An altogether different approach, fluctuation analysis, suggests not.

If a cross bridge cycles, its stroke will be reflected as a miniscule fluctuation on an otherwise steady tension signal. If the number of sampled bridges can be restricted, such fluctuations will become increasingly prominent. (To prove the point, consider an extreme condition with only two or three bridges: Fluctuations will dominate the tension signal.) By studying single myofibrils with diminished overlap and employing a transducer that was sensitive enough to measure the tension of a single cross-bridge, Iwazumi was able to test for the presence of such tension fluctuations. \(^\text{48}\) Except for the noise induced by building vibrations and other ambient sources, he found none; the tension signal was flat. Though enough ATP was present to preclude rigor, Iwazumi concluded that cross bridges could not be cycling.

The inexorable consistency of these results is unsettling. It raises serious doubt about the most fundamental attribute of the accepted contractile mechanism. Though cross-bridge rotation has been implicit in our thinking for years, evidence to the contrary is now accumulating rapidly. The implication for stepwise shortening is profound: The mechanism need not rest on asynchronous cross-bridge rotation.

How then might the steps originate?

Toward a Mechanism

An important clue is that the steps appear whether or not the fiber has been activated. When unstimulated fibers are stretched or released by a servo-motor, the resultant sarcomere length changes are stepwise. \(^\text{44}\) The stepping mechanism is therefore independent of the kinetics of activation; it is evidently tied up with the basic structure.

The structural element responsible for the steps could reside inside or outside the myofibril. If the
element were outside the myofibril, it might be irrelevant to the contractile mechanism per se, whereas if it were inside the myofibril, it could be associated with an element that is integrally involved in contraction.

Outside the myofibril are four potential structures: connective tissue, cell membrane, sarcoplasmic reticulum, and the recently identified intermediate filament network. None of these elements, however, possess the regularity that would be necessary to account for synchrony. Irregular, random structures could not explain the synchronous occurrence of steps and pauses among huge numbers of myofibrillar sarcomeres. Further, when these elements have been more or less stripped away by skinning or by paring the preparation to a small bundle of myofibrils, the steps persist.

Inside the myofibril, the on the other hand, the degree of structural regularity is high; crystallinity is the hallmark of myofibrillar architecture, as any well-prepared electron micrograph or x-ray diffraction pattern demonstrates. A shortening step could arise if some axial dimension within the lattice changed discretely. How might such a quantal change of length arise?

The obvious first choice is relative sliding of thin over thick filaments. Regularly positioned resistance points could give rise to brief shortening pauses. Simplicity notwithstanding, this hypothesis is probably inadequate, for the steps found in fibers stretched beyond overlap would need to be explained by an entirely different class of mechanism.

An alternative possibility within the myofibril is a stepwise change of filament length. Which filament? When a fiber is stretched beyond overlap, continuity between Z-lines is not lost. It is retained through a set of filaments that interconnect each end of the thick filament with the nearest Z-line. When the fiber is stretched, these “connecting filaments” are likewise stretched. Probably they support much of the resting tension.

Connecting filaments are not well-known; they are ignored in most textbooks. Yet they are well-studied and apparently uncontroversial. Connecting filaments were identified long ago by Huxley and Peachey, confirmed by Sjostrand and by Carisen et al., examined for structural detail by Locker and Leet, and characterized biochemically in a series of experiments from the laboratories of K. Maruyama and K. Wang (cf. Locker for review).

If the length of the thick filament remains constant in an unactivated fiber, imposed length changes must be conferred fully on the connecting filaments. This is an inescapable feature of the filament arrangement. If the sarcomere length change occurs stepwise, the change of connecting filament length must also be stepwise.

We see, therefore, that the stepping mechanism likely resides in the connecting filaments, at least in the unstimulated fiber. No other element changes length. This inference is consistent with the structure. Isolated connecting filament proteins are threadlike but often contain isolated nodules distributed along their length, where the threadlike material appears to have gathered up. These nodules may represent discrete, localized regions of shortening.

All well and good. But this cannot be the complete answer. Stepwise behavior remains in evidence at sarcomere lengths far below those at which resting tension is present — lengths at which the connecting filaments would presumably be slack. Another element must be responsible for these steps.

Since experiments have consistently shown that thin filaments do not shorten, the remaining element is the thick filament. The suggestion that thick filaments shorten in steps, or for that matter shorten at all, is obviously at odds with accepted views. However, the classical observations of Huxley and Hanson and Huxley and Niedergerke have not been broadly confirmed. On the contrary, a surprisingly large number of papers published since the mid-fifties have reported A-band shortening or thick-filament shortening of substantial magnitude (for review, cf. Pollack). The issue is far from settled, and I feel it would be premature to discount this possibility out of hand.

Strongly against the thick filament shortening hypothesis has been the observation that the axial reflections on the x-ray diffraction pattern do not shift as the sarcomere shortens. Thick filaments cannot “shrink” longitudinally. However, segmental shortening is by no means excluded. If each shortening step were restricted to a small, distinct segment along the filament and if the number of shortened segments remained modest, this phenomenon could well escape detection by x-ray methods. The hypothesis remains plausible.

By elimination, we have arrived at the hypothesis that stepwise length changes are mediated by length changes of two elements, connecting filaments and thick filaments. Connecting filaments would account for the steps when the load is increased or decreased in fibers that are unactivated; in activated fibers, with thin filaments bound to cross bridges, steps would be generated by the thick filaments. The division of labor is thus based solely on whether the sarcomere has or has not been activated.

Could steps of similar nature plausibly arise out of length changes of two separate structures? Does this hypothesis seem, perhaps, too complicated? Intuition says yes. But modern electron micrographs lend credence to this proposition. The thick filament—connecting filament combination appears not as two distinct filaments but as one long filament running from Z-line to Z-line, a filament whose mid-region (thick filament) is slightly thicker than its flanking regions (connecting filaments). From this perspective, it is easy to envision discrete length changes arising from any region along the length of this compound filament. Two apparent sources merge effectively into one — variations on a single theme.

The proposed hypothesis obviously represents a departure from accepted views, one that is perhaps more radical than may be apparent on the surface. If thick filaments shorten and do work, is it necessary to invoke cross-bridge cycling as the sole work-producing
mechanism? And in light of recent negative evidence for angle changes of S-1 during contraction (cited above), is it reasonable to invoke cross-bridge cycling at all? Could thick filament shortening accomplish the entire task?

These are questions for the future; they have not been settled here. What has been settled here, I hope, is the question of whether the shortening process is quantal. Stepwise shortening has now been confirmed in several laboratories using four different classes of methods. The phenomenon, unlike the mechanism proposed to account for it, is therefore out of the speculative arena. The steps are waiting to be scaled.

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G H Pollack

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