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# Millisecond time-resolved changes in x-ray reflections from contracting muscle during rapid mechanical transients, recorded using synchrotron radiation

(cross-bridge movement/contraction mechanism/high-intensity x-ray beams)

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**ABSTRACT** **Low-angle x-ray diffraction diagrams have been recorded from frog sartorius muscles by using synchrotron radiation as a high-intensity x-ray source. This has enabled changes in some of the principal reflections of interest to be followed with a time resolution of 1 ms, during small but very rapid length changes imposed on a contracting muscle. The 143-Å meridional reflection, which is believed to arise from a repeating pattern of myosin cross-bridges along the length of the muscle, shows large changes in intensity in these circumstances. During both rapid releases and rapid stretches, by amounts that produce a translation of actin and myosin filaments past each other by about 100 Å and that are completed in about a millisecond (i.e., before significant cross-bridge detachment would be expected), an almost synchronous decrease in 143-Å intensity occurs, by 50% or more. This is followed, in the case of quick releases, by a rapid partial recovery of intensity lasting 5–6 ms (which may represent cross-bridge release and reattachment) and then by a more gradual return to the normal isometric value. Quick stretches show only the slower return of intensity. Immediately after the length change, the initial drop in 143-Å intensity can be reversed if the release (or stretch) is reversed. These changes provide evidence of a more direct kind than has hitherto been available that the active sliding of actin filaments past myosin filaments during contraction is produced by longitudinal movement of attached cross-bridges.**

The outstanding problem in understanding the mechanism of muscular contraction is to discover the nature of the process by which the relative sliding force between actin and myosin filaments is developed. It is generally supposed that the cross-bridges, the enzymatically active heads of the myosin molecules, function in a cyclical manner to produce this force. It is thought that they attach to actin in one part of their cycle, then undergo some structural change that enables them to exert tension and, during shortening, to pull the actin along a short distance—probably 100 Å or so—towards the center of the A-band. They then detach from actin and are recharged by ATP before beginning a new cycle of attachment further out along the actin filament (1, 2). Whilst this general mechanism has been able to give a good account of many phenomena in muscle, it has proved remarkably difficult to produce direct and decisive evidence that the cross-bridges really do behave in this fashion, because of the inherent difficulty of obtaining dynamic structural information on the size and time scales involved. Moreover, because the processes take place asynchronously at all the cross-bridges, probes of their configuration will usually give only an averaged value of changes in them during the cycle.

Surviving muscles give low-angle x-ray diffraction diagrams that contain a considerable amount of information about the configuration of the cross-bridges and that alter in characteristic ways during contraction (3–7). The changes in these patterns give general support to the cross-bridge theory but have not so far provided conclusive evidence that some form of longitudinal movement of attached cross-bridges produces the relative sliding of actin and myosin filaments. Such evidence can best be sought in experiments in which one attempts to bring about a partial and temporary synchronization of the action of the cross-bridges by the application to the muscle of small but very rapid mechanical transients—in the present case, rapid length changes. Earlier mechanical experiments have shown that some of the steps in the cross-bridge cycle revealed by this technique have time constants of only a few milliseconds and that other changes in the cross-bridges appear to take place even faster (8, 9). Thus the experimental problem is to record the x-ray diagrams sufficiently rapidly to resolve these structural transients.

We have used the electron–positron storage ring DORIS at Deutsches Elektronen Synchrotron (DESY), Hamburg, as a high-intensity x-ray source, and we have already reported experiments on muscles during normal isometric contraction (10). Improved design of the mirror-monochromator type x-ray camera and of the data recording system has now enabled us to record many of the principal low-angle x-ray reflections from muscle with a time resolution of 1 ms or less. We have observed large changes in the intensity of the 143-Å meridional reflection occurring on this time scale during rapid stretches and releases of the muscle, in which adjacent actin and myosin filaments underwent a relative longitudinal displacement of the order of 100 Å or less.

## MATERIALS AND METHODS

**Muscles.** Sartorius muscles were carefully dissected from large healthy specimens of the frog *Rana esculenta*. The muscles were usually 4 cm or more in length, about 6 mm wide near the pelvis, and about 1 mm in thickness. They were mounted in Perspex cells with thin Mylar windows in oxygenated Ringer's solution at 5°C. The muscles were stimulated directly through platinum electrodes parallel to their length.

**Mechanical Apparatus.** Rapid changes of muscle length were made by using a servo-system based on an industrial moving-coil vibrator (Ling 201, Ling Dynamic Systems, Royston, Cambridgeshire, England). A photodiode detector measured the displacement of the armature of the vibrator and position control was achieved by using conventional feedback circuitry (9). The response to a step input was complete in 1–2 ms. A strain gauge tension transducer was mounted on the end of the armature and the tibial tendon of the muscle was attached to the transducer by a short length of narrow-gauge stainless steel tubing. A length step, usually 0.1–1.0 mm (about 0.25–2.5% of muscle length) could be applied at a selected time relative to

the start of stimulation and was synchronized with respect to the time channels of the counting system. Length and tension signals were processed by using voltage-to-frequency converters and were averaged over the same time intervals used for collecting intensity data.

**Experimental Protocol.** The behavior of the pattern was studied during both single twitches and short tetani a few hundred milliseconds in duration. The behavior of the pattern around the time of the rapid release or stretch was recorded in 1-ms channels. Good records of the 143-Å meridional reflection could usually be obtained in 50–100 cycles of contraction (i.e., a total recording time of 50–100 ms per frame). Records of the equatorial and off-meridional layer line reflections had a poorer signal-to-noise ratio than those of the 143-Å meridional. Specimens were moved in the beam during the course of an experiment so as to minimize the irradiation of a given area. Care was also taken to ensure that specimens were accurately perpendicular to the incident x-ray beam and that they remained so during contraction and during the length changes.

**X-Ray Camera and Recording System.** The x-ray beam from the storage ring DORIS was focused and monochromatized by an eight-segment totally reflecting quartz mirror system and a bent single-crystal germanium monochromator of triangular form (11). Under optimal conditions the flux at the specimen was of the order of $10^{11}$ counts/s, and considerable care was exercised in the operation of the beam shutters and in the experimental protocol so as to irradiate the specimen as little as possible, because damage was apparent after a few minutes of exposure. The x-ray wavelength used was 1.5 Å.

Patterns were recorded by using a delay-line type position-sensitive counter with direct time digitization to give positional information. The camera and counting system are described in greater detail elsewhere (10–12). The total flux entering the counter was usually of the order of 1–2 × $10^5$ counts/s.

## RESULTS

**Changes in 143-Å Reflection Produced by Sudden Changes in Muscle Length.** When a quick release was applied to a contracting muscle, there was a large and abrupt decrease in intensity of the 143-Å meridional reflection, followed by a slower return towards the original intensity level (Figs. 1–4). In experiments in which the total length change was of the order of 1% of muscle length, the intensity fell by 50% or more of its original value and larger releases gave progressively larger falls. This decrease took place within about 1 ms (average time for major part of change ≈ 0.8 ms). In most experiments, there appeared to be a slight delay, of about 0.6 ms, between the applied length and tension change and the drop in intensity. However, because these experiments necessarily had to be done on large muscles (to give a high enough counting rate in the x-ray pattern), it is possible that part at least of this delay was due to mechanical transmission time effects along the length of the muscle.

In most of our experiments the position-sensitive counter was oriented perpendicular to the meridian so that the intensity and the width of the 143-Å reflection could be monitored together. It was apparent that the large drop in intensity was not accompanied by any increase in width of the reflection across the meridian, and indeed in a number of cases the reflection became narrower during the release. Thus the change was not brought about by any disorientation of the muscle fibrils or by any other type of disorder that might have brought about an increase in the width of the reflection. Nor was there any measurable indication that the reflection was being replaced by a broad unsampled peak. We also checked that no change of tilt of the muscle took place, by performing the experiment over a range of initial tilt angles. The width and the axial spacing of the reflection along the meridian also remained constant.

The initial drop in intensity was followed by a fairly fast initial recovery phase in which the intensity rose two-thirds of the way or more back towards its level before the release within about 5 or 6 ms. When the initial release was moderate—say 0.7%—the initial recovery of intensity was almost back to its original level, whilst a second slower phase became more apparent after larger releases (Fig. 4), following approximately the tension recovery curve.

These changes in intensity of the 143-Å reflection during release were very consistent from one experiment to another, as can be seen by comparing the record from a single run (Figs.

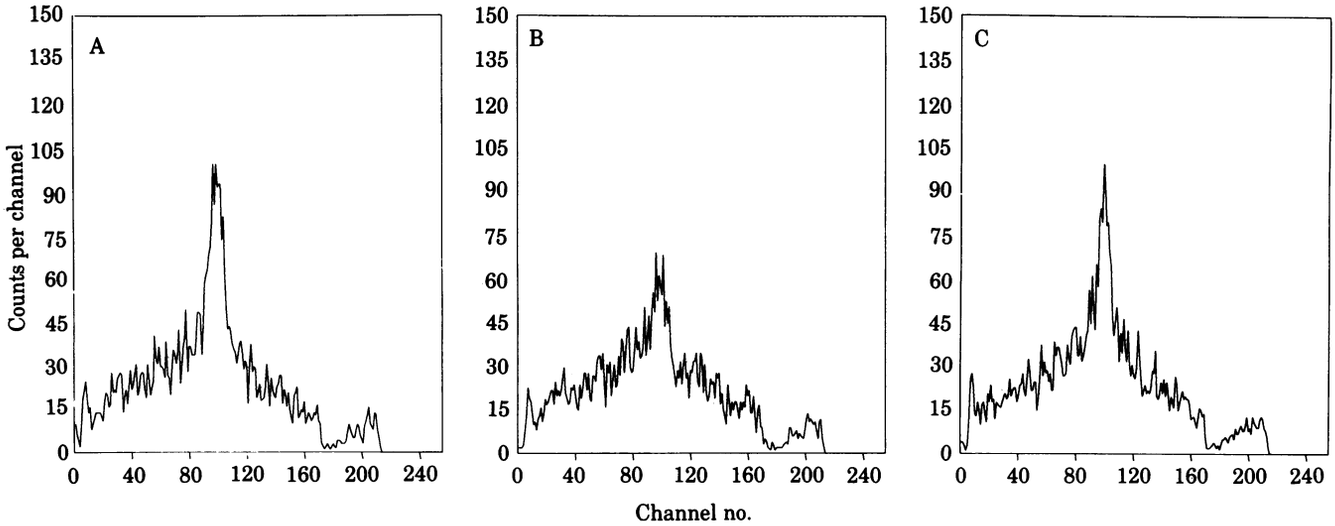


FIG. 1. Intensity traces through the 143-Å meridional reflection from a contracting frog sartorius muscle. The position-sensitive counter was perpendicular to the meridian. The curves show raw data without smoothing or background subtraction. Each trace represents an individual 1-ms interval. Channel number on the abscissa refers to position along the counter; each channel represents ≈0.3 mm along the counter, with specimen-to-counter distance ≈2.3 m. (*A* and *B*) Pattern in successive 1-ms intervals at the start of, and immediately after, a quick release. (*C*) Reflection 5 ms later. Total number of cycles was 100. Axial extent of counter was from 1/136 to 1/150 $Å^{-1}$.

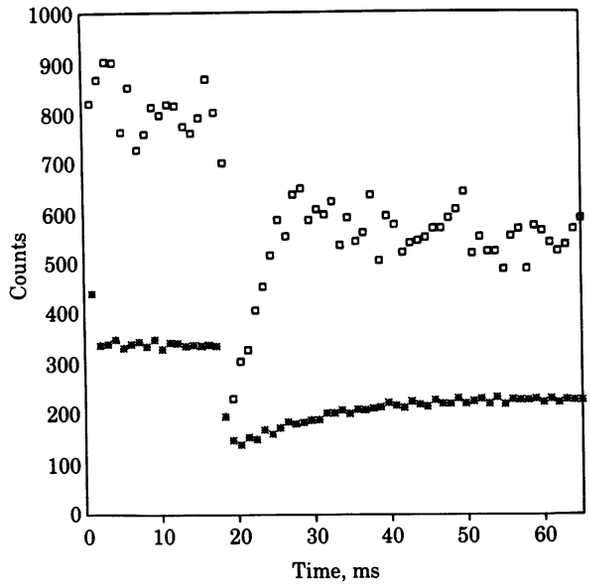


FIG. 2. Time course of changes in intensity of 143-Å meridional reflection from muscle as in Fig. 1 during a quick release. One single experimental run of 100 cycles is represented. □, Number of counts in the reflection (after background stripping) in each 1-ms interval; *, average tension during corresponding 1-ms time intervals. Extent of quick release was 0.6 mm, 1.3% of muscle length. Backgrounds were derived throughout by a least-squares filling program with polynomials of order 2–4 applied to chosen regions of the diagram outside the reflection.

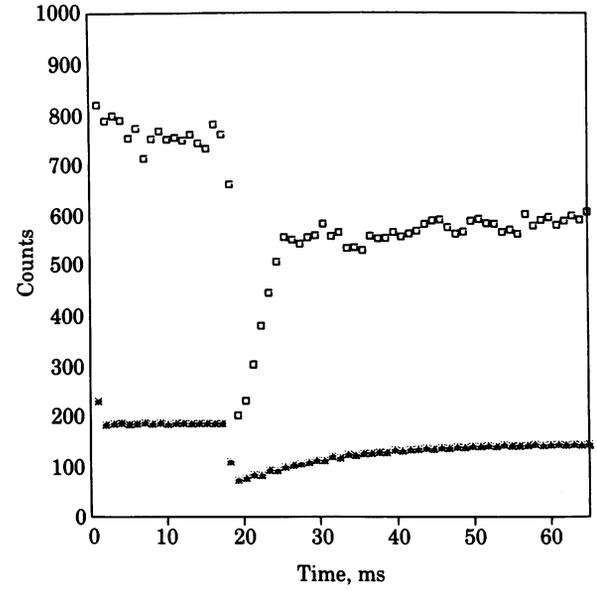


FIG. 4. Time course of change in 143-Å intensity during quick release experiments. The average of seven experiments on five different muscles, all with release of 1.3%, is shown. Note rapid recovery of intensity during 5–6 ms after relase, which shows an approximately linear time course. Symbols as in Fig. 2.

1 and 2) with the average of seven different runs on five different muscles (Figs. 3 and 4). More than 50 other experiments showed the same large decrease. No detectable changes in the 143-Å intensity were observed in resting muscles subjected to similar changes in length.

A similar drop in intensity was observed during rapid stretches of the muscle (Fig. 5). Again, a length change of about 1% brought about an intensity decrease of about 50% or more, unaccompanied by any increase in the width of the reflection. In the case of stretches, many experiments showed essentially no measurable delay between the length or tension change and the fall in intensity, and an average value of the delay was about 0.1–0.2 ms.

Another distinction between the effect of stretch and release on the 143-Å reflection was that there was no rapid recovery of intensity after stretch. Indeed, several records showed a further slight fall in intensity after the stretch was over, followed by a slow recovery similar to the slow recovery phase after release.

Different muscle specimens show considerable variation in the intensity of the 143-Å meridional reflection during contraction, which may be stronger, or weaker, than in the resting state (13). However, irrespective of this, the muscles all showed similar behavior associated with the rapid length change.

**Double Step Experiments.** We have also carried out a smaller number of experiments (not illustrated) in which a quick release is followed some milliseconds later by a quick stretch to the original length or vice versa. We found that when the muscle was restretched within 1 or 2 ms of the release, while

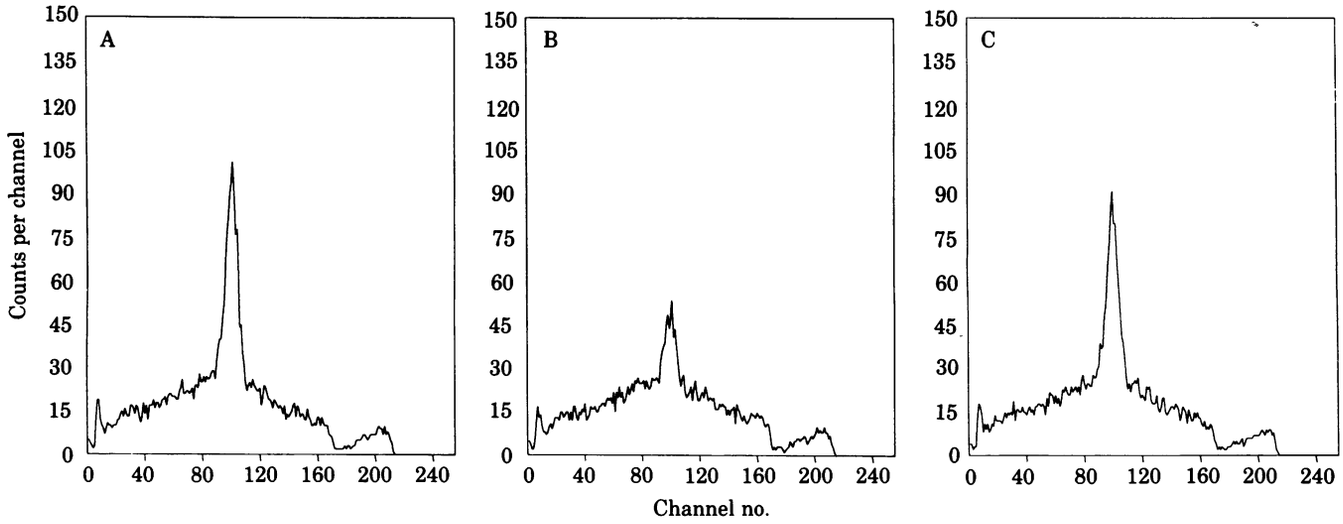


FIG. 3. Intensity traces through the 143-Å reflection. The counter was perpendicular to the meridian. Curves show averages of six experiments on four different muscles. (*A* and *B*) Patterns in successive 1-ms time intervals as in Fig. 1. (*C*) Pattern 5 ms later.

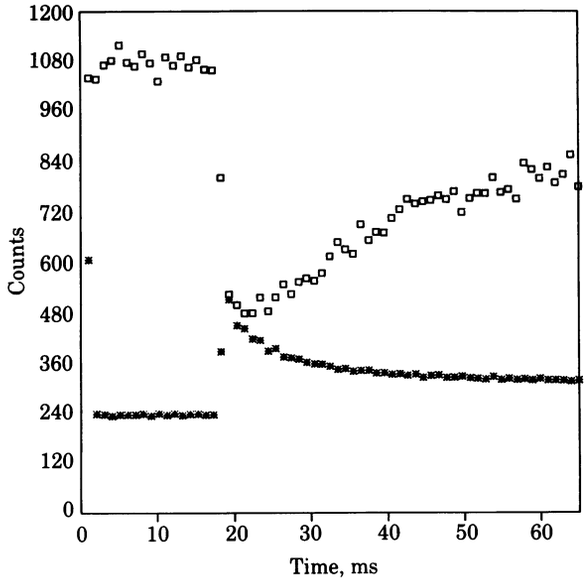


FIG. 5. Time course of 143-Å intensity, averaged over a number of experiments, during rapid stretch. All stretches were 1.3% of muscle length. Note initial fall virtually synchronous with tension change, but recovery of intensity much slower than after a release. Symbols as in Fig. 2.

the intensity was still near its minimal value, a substantial increase in intensity occurred, almost to the original value before the release. However, when the restretch occurred after the initial rapid recovery of intensity had taken place, then a further drop in intensity was produced. In experiments in which the muscle was first stretched, a subsequent release produced a recovery of intensity when the interval was between 1 and about 40 ms; thereafter the release produced a decrease in intensity. Thus for both releases and stretches the initial rapid drop in intensity can at first be reversed by reversing the length change. Then, as the intensity recovers, so the ability to show a decrease in intensity recovers too.

**Effect of Sudden Length Changes on Other Parts of X-Ray Pattern.** As we have already indicated, the effects of these rapid stretches or releases on the intensity of the 143-Å meridional reflection were large and reproducible. We also investigated the effect of such abrupt length changes on the off-meridional layer-line reflections, in particular the 429-Å reflection, and on the equatorial reflections. With these parts of the pattern, however, the results were much less clear-cut, because the changes, if they were present at all, were very much smaller and apparently more variable. In the case of the equatorial reflections, some specimens showed a transient small increase of the [11] intensity by about 10% during release followed by a fall, whereas others showed virtually no change or a small decrease. Some muscles showed a small decrease in the [10] intensity at the time of the quick release, others virtually no change at all. The only conclusion that we can draw at present is that no very large change in the equatorial pattern occurs upon quick release or quick stretch. A similar lack of change during quick release has been reported by Sugi and his colleagues (14).

A similar lack of any striking change was also apparent in the 429-Å layer-line reflection. This reflection is already quite weak in a contracting muscle, so the statistical accuracy of the measurements in 1-ms time channels during rapid length changes was considerably inferior to that achieved in the meridional reflections. Some specimens showed a small increase in intensity accompanying the release, and none of them showed a significant decrease in intensity. It was also clear that there was no transient appearance of a strong rigor-like ≈380-Å layer-line reflection at the time of the quick release, even on a 1-ms time scale.

## DISCUSSION

The very small changes in the equatorial reflections and in the 429-Å layer line upon quick release or quick stretch do not give any positive indication of the behavior of the cross-bridges, but they do enable us to rule out certain possibilities.

Thus, in releases in which the tension falls to a half or even one-quarter of the isometric value and then recovers again, it is clear from the equatorial results that half or more of the cross-bridges have *not* at any time moved away from the actin filaments and back to positions occupied in resting muscle. There is no sign of any substantial temporary shift towards a resting pattern, which would have very different equatorial intensities. Thus, if a substantial number of bridges were in a detached state at any one time, they must have remained in the vicinity of the thin filaments for times of the order of tens of milliseconds, waiting to reattach and redevelop tension. Alternatively, bridges may detach, and reattach again quite quickly, but redevelop tension at a slower rate. The equatorial part of the pattern merits further study, especially when even faster counters are available to take advantage of the high intensity of these reflections and reduce statistical noise, which at present impairs our ability to detect relatively small changes.

Similarly, the behavior of the pattern in the region of the 429-Å layer line rules out certain possibilities. There is no indication of a transient return of a strong resting pattern, nor is there evidence during release for the momentary appearance of a substantial amount of rigor-like pattern. At the end of a quick release, even though a considerable number of bridges are synchronized in the sense that they are no longer developing tension, they do not seem to form a regular helical array either attached to or detached from the actin filaments.

On the other hand, the very large changes in the intensity of the 143-Å meridional reflection—a reflection that arises from the average axial repeat of the cross-bridges—show quite decisively that there has been a major change in a longitudinal component of their structure or positioning during a quick release or stretch. This change is brought about by a relatively small (100 Å or less) longitudinal translation of the actin and myosin filaments past each other and occurs only in an actively contracting muscle. It is therefore very difficult to escape the conclusion that the reflection is generated by attached cross-bridges, that a substantial number of such bridges are attached to actin during isometric contraction, and that those bridges undergo some kind of longitudinal deflection during a small quick release and are brought to the end of their working stroke. Thus the experiments provide rather direct evidence, previously lacking, for the reality of this type of cross-bridge mechanism, involving attachment to actin and longitudinal movement of attached bridges.

The present experiments do not, however, resolve the question of the detailed nature of that movement. One explanation of the changes we see might be that they arise simply from changes in cross-bridge tilt. If, in the isometrically contracting muscle, the attached bridges tended to be perpendicularly oriented to the thin filaments (or at least centered at that orientation), then either rapid shortening or rapid lengthening could increase their average angle of tilt and spread out their density projected onto the long axis of the filaments. Thus the 143-Å meridional reflection, which arises from the planes of cross-bridges repeating at approximately 143-Å intervals along the length of the muscle (even when attached to actin) would

be reduced in intensity. Whether this is a feasible mechanism for producing the large intensity decrease depends on the shape of the cross-bridge, at present a somewhat uncertain quantity. Also, the change in tilt, if it occurs, apparently has little effect on the equatorial pattern, which places interesting restrictions on the type of movement involved.

Another possibility is that the change represents some type of interference effect between planes of attached and of unattached bridges. If this were the case, one might expect to see a large increase in the intensity of the 71.5-Å reflection accompanying the decrease in 143-Å intensity, and a saturation and reversal of the effect as the release per half sarcomere exceeded about 70 Å. Neither such effect was observed, but some combination of tilt and interference is still possible.

A further possibility is that the intensity decrease is due to a longitudinal disordering of the attached bridges, brought about by an impairment of the transverse register of the thick or the thin filaments. If the disorder introduced has the form of an axial displacement of entire filaments on either side of their original mean position, then no change in the width of the reflection need occur, but there could be a large intensity decrease. However, the intensity lost from the "sampled" peak would reappear within the confines of the broader peak given by an individual filament and its associated bridges. Because we find no detectable increase in background counts in this region of the diagram, such an effect could contribute only a small part (less than 10% on present results) of the large intensity decrease that we see. Moreover, the rapid reversibility of the change argues strongly against explanations involving disorder.

Whatever the detailed explanation of the intensity change, however, the *fact* of the change in longitudinal cross-bridge organization remains. This movement is synchronized with the length and tension changes during stretch and is only slightly delayed during a release. The details of the present analysis are affected very little by whether (intensity) or $(\text{intensity})^{1/2}$ is used as a measure of the extent of structural change. However, the interpretation is complicated by the compliance of the tendons and by the mechanical transmission properties of the muscle. Nevertheless, it is clear that the decrease in intensity must be due to an undamped or lightly damped movement of the cross-bridge heads. And the initial reversibility of the decrease indicates that it is not due to cross-bridge detachment.

The rapid early recovery of intensity after a release should not be confused with the very rapid early recovery of tension, reported by Huxley and Simmons (8), and taking place so much more rapidly as to be indistinct in many of our tension records. During most of the 5 ms or so of the initial intensity recovery, there is little further change in tension. When this phase is over, the bridges are in a state in which reextension of the muscle produces not an increase in intensity of the 143-Å reflection but a further fall. This would be consistent with the bridges having reattached in a more perpendicular orientation awaiting some further step in the reaction cycle before developing tension again.

The present experiments, therefore, besides their principal basic finding, raise a considerable number of interesting and unresolved questions. However, the fact that observable structural changes do take place on this time scale, where they can be correlated with rate processes detected biochemically and mechanically, is very encouraging. The availability of high-intensity x-ray beams from an increasing number of electron storage rings in different parts of the world, together with appropriate high-speed counters and electronic data switching and storage devices, should make feasible the further study of these dynamic aspects of structure not only in muscle but also in other systems.

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