breath collection was started soon enough after breakfast, and with short enough sampling times, for an increase to a peak value to be observed.

The mean of the ratio of the highest exhalation rate of radon to the lowest after breakfast was 2.08 (range 1.42 to 3.81) for five subjects (two sets of observations for one of these); after lunch it was 2.04 (range 1.78 to 2.65) for five subjects. The mean times of the maximum and minimum were at about 0.5 hour and 1.8 hours, respectively, after either breakfast or lunch. The variability in the manner in which the exhalation rate of radon decreased, evident in Fig. 1, precluded more detailed comparison of the results.

We observed a strong correlation between the exhalation rate of radon and the pulse rate in the five subjects where this was measured simultaneously with the breath collection. The most striking data (subject 03-607) are shown in Fig. 2. The correlation coefficient was +0.93 ($N = 7$), which was highly significant ($P < .001$). The correlation coefficients for the data from the four other cases ranged from +0.86 to +0.97, and these were all highly significant ($P < .001$ to $P \leq .01$). These correlations suggest that the change in the rate of exhalation of radon observed postprandially is related in some way to the change in blood flow associated with the digestive process, since the blood carries the radon to the lung. A possible explanation is that the increased blood flow in the viscera flushes out a reservoir of radon dissolved in one or more organs (for example, the liver). Whatever the cause, it is clear that a representative value of the exhalation rate of radon cannot be determined from analysis of breath collected less than about 2 hours after a meal. The relatively short duration of the phenomenon, and the longer sampling times than used by us, may be the most likely reasons that such a pronounced effect has not been observed before.

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Muscle Crossbridge Stroke and Activity Revealed by Optical Diffraction

Abstract. *Optical diffraction measurements during rapid releases of active toad muscle show that the sarcomeres contract within 1 millisecond by an amount up to but not greater than 12 nanometers. This distance is identified with the effective working stroke of a crossbridge. The crossbridges immediately start cycling to produce the normal contraction velocity in unloaded muscle.*

Recent advances in our understanding of muscle contraction rest largely on experiments in which step functions of length or tension are applied to an active muscle (*1, 2*) and the mechanical response is measured with a time resolution in the order of 1 msec. These macroscopic measurements involve a whole muscle fiber or at least a substantial part of its length. We have now obtained direct evidence of the structural response at the sarcomere level, using a redesigned form of our dynamic optical diffraction equipment (*3*) to provide a time resolution of better than 0.5 msec and a spatial resolution (*4*) of the order of 2 nm. The results show that (i) the effective crossbridge stroke in a freely contracting muscle is approximately 12 nm, and (ii) crossbridges are cycling at their normal rates within 1 msec after a large rapid release (> 1 percent).

In essence, the experimental system measures the angular spacing of the first-order diffraction beams produced by directing a He-Ne laser beam normally on a muscle specimen (*3*). The two beams are sampled by a rotating slotted disk at a rate of 2.4 kHz and each beam passing through a slot falls on a photodiode. Measurements of the time interval between the outputs of the two photodiodes, together with a knowledge of the system geometry, give the desired angular separation, from which the sarcomere length can at once be deduced. The photodiode outputs are fed into a Hewlett-Packard 2100 S computer, which provides digital data at the sampling rate of 2.4 kHz and also removes systematic noise produced by flutter of the rotating disk. A quick-release device allows specimens to contract freely by amounts up to 2.5 percent of their lengths (that is, 30 nm per half-sarcomere) in a time between 0.1 and 0.2 msec. The experiments reported here were carried out on bundles of four or five semitendinosus fibers from the toad *Bufo marinus*, mounted in Ringer solution at 10°C. Figure 1 shows the data points from two experiments in which the specimen was released abruptly while in dynamic equilibrium during an isometric tetanus.

In Fig. 1a the specimen was allowed to contract freely by 1 percent; as the initial sacromere length was 2.5 μm, this was equivalent to a contraction of 12.5 nm per half-sarcomere. In Fig. 1b the contraction was 2 percent, equivalent to 30 nm per half-sarcomere as the initial sarcomere length in this experiment was 3.0 μm.

It can be seen in Fig. 1a that the sarcomere contraction closely follows the applied displacement throughout the interval between successive sampling points; that is, in a time $\leq$ 0.4 msec. Within the scatter of approximately $\pm$ 2 nm about the mean level after the displacement, it is of course possible that there could be some exponential recovery or some undetected fluctuations.

To the accuracy of these experiments, however, the sliding filaments in a half-sarcomere undergo a relative displacement of 12.5 nm in a time $\leq$ 0.4 msec, so that their relative velocity is $\geq$ 30 μm/sec. The velocities of frog muscle fibers contracting under a very light load have been measured by Gordon *et al.* (*5*); their values, adjusted to a temperature of 10°C, indicate a maximum relative velocity (V_{max}) of 3.1 μm/sec. Thus the velocity in a half-sarcomere when the tension is released is at least ten times greater than the maximum that the muscle itself can generate.

Evidently this sarcomere contraction of 1 percent is not achieved by the crossbridge cycling action that drives normal muscle contraction. It could be produced entirely by the elastic recovery of stretched crossbridges, as in Harring-

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ton's helix-coil theory of the crossbridge (*6*), or by a combination of elastic relaxation as in Huxley and Simmons' two-component model (*7*).

Experiments with smaller quick releases (0.5 and 0.75 percent, or 6 and 9 nm per half-sarcomere) gave results that were consistent with Fig. 1a. Thus for releases up to about 1 percent the relative sarcomere length change is equal to the relative muscle length change. For the 0.75 percent release, measurements were also made at points distributed along the length of the specimen. The observed contraction was 0.75 percent at every point, even though a wide variation in sarcomere shortening (from 0.5 to 1.8 percent) had been measured at these points during activation of the muscle from its resting state. This observation demonstrates the structural homogeneity of the muscle once it has become fully active.

A large release step of 2.0 percent (30 nm per half-sarcomere, on a sarcomere length of 3.0 μm) gave the result shown in Fig. 1b. There is an almost instantaneous (< 0.4 msec) contraction $A \rightarrow B$ of 0.8 percent (12.5 nm per half-sarcomere) just as in Fig. 1a, followed by a slower contraction $B \rightarrow C$ at constant velocity until the full 2.0 percent is reached some 7 msec later at C. The transition to the slower contraction is quite sharp. It was found to occur at the same absolute value of 12 nm per half-sarcomere in two separate experiments with differing sarcomere lengths (2.5 and 3.0 μm), indicating that it is a well-defined property of the crossbridges. In the Huxley and Simmons model (*1, 7*), it is the sum of approximately 5 nm from elastic contraction of the S-2 myosin subfragment and 7 nm from rotation of the S-1 head. In the Harrington model it represents complete end-to-end retraction of the randomly coiled crossbridge. We may observe that interpretations of 300-Hz dynamic measurements on the basis of the Harrington model led independently (*8*) to an average end-to-end separation of 12 nm; the coincidence may be fortuitous, but the value is certainly consistent with these more direct optical observations. It thus appears that for toad muscle at 10°C, releases of up to 12 nm per half-sarcomere are taken up within 0.5 msec, but the remaining contraction is attained much more slowly. We therefore identify this displacement of 12 nm per half-sarcomere with the effective crossbridge stroke for the toad muscle. We also observe that this quantity should be given in the Huxley-Simmons model by the intercept of T_2 on the displacement axis, where the tension remains at zero and there is no "quick recovery phase." In the various experiments published by Huxley and Simmons zero tension is not attained, but extrapolation of three of their published curves gives values of 15 nm (*9*), 18 nm (*7*), and 13 nm (*1*), in reasonable agreement with our direct observation of 12 nm.

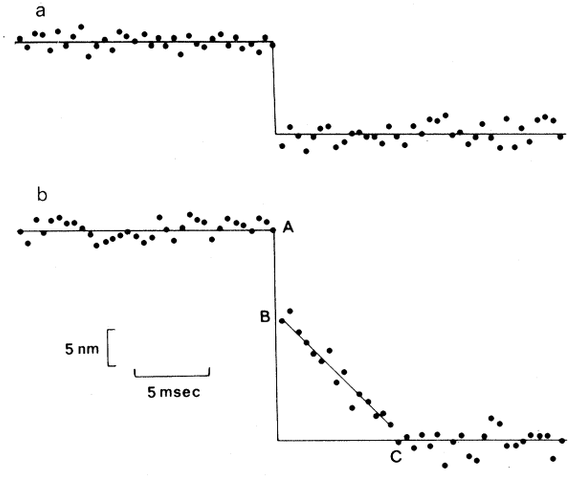

Fig. 1. Sarcomere contraction produced by rapid release of muscle in tetanus (•). Length per half-sarcomere is plotted vertically, time horizontally. Heavy full line shows the contraction step applied to the whole specimen, scaled down to units of nanometers per half-sarcomere. (a) Release of 1 percent (12.5 nm per half-sarcomere on an initial sarcomere length of 2.5 μm); (b) release of 2 percent (30 nm per half-sarcomere on an initial sarcomere length of 3.0 μm). The preparation was a bundle of fibers of toad semitendinosus muscle at 10°C. Vertical bar, 5 nm per half-sarcomere; horizontal bar, 5 msec.

In the slower stage ($B \rightarrow C$ in Fig. 1b) the contraction velocity is 2.9 μm/sec for a half-sarcomere. This is substantially the same as V_{max} within the accuracy to which that quantity is estimated. Thus after a large (> 1 percent) release the sarcomeres first contract rapidly by 12 nm, completing this contraction in less than 1 msec at 10°C; this is the period of the quick recovery phase. The normal crossbridge cycling process immediately comes into play, taking up most of the remaining contraction at the constant velocity of V_{max} because the tension is still close to zero. At the end of this period the contraction slows down abruptly because of the highly nonlinear force-extension characteristic (*10*) of the tendon, which carries the tension at each end. For values appropriate to Fig. 1b the tension in the tendon would rise from zero to the full tetanic value for an extension of 18 μm. This corresponds to a contraction in the muscle fibers of only 1.8 nm per half-sarcomere, so that the conclusion of the process is not resolvable in our experiments. Nevertheless, the observations demonstrate and explain the existence of normal crossbridge activity immediately after the elastic response to a displacement step, as predicted by Podolsky and Nolan (*2*), even though no increase in tension takes place.

It should be remarked that the equality described above between the muscle length change and the (rapid) sarcomere length change strictly requires correction because of this finite compliance of the tendon. However, the maximum correction, when the muscle is completely unloaded by a release of ~ 1 percent, would be only about 0.1 percent of the muscle length; for smaller releases it is much less than this, so the equality is valid within the experimental accuracy.

Other experiments, to be reported subsequently with fuller experimental detail, showed that (i) releases of up to 0.7 percent in resting muscles produced a rapid contraction of 0.2 percent (2.5 nm per half-sarcomere) followed by a slow exponential contraction to the level of the release and (ii) stretches up to 0.8 percent produced a rapid extension of the same amount in both resting and active muscles, the experimental data having the qualitative form of an inverted Fig. 1a.

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