23. Lobsters were placed unrestrained in a large aquarium. The stimulus was given by suddenly introducing a wooden rod into the visual field. Each of the three groups (juvenile, adult, and clawless adult) consisted of ten animals, with five trials each; there was a minimum of 5 minutes between trials.
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25. Measurements for both crayfish and lobsters were made in the connective between the first two abdominal ganglia.
26. Supported by grants from the National Institute of Neurological and Communicative Diseases and Stroke and the Muscular Dystrophy Association to F.L. and from the National Research Council and the Muscular Dystrophy Association of Canada to C.K.G. We thank C. L. Prosser, H. L. Atwood, J. Atema, and especially M. P. Charlton for helpful comments on the manuscript, and J. She and R. Zweig for able technical assistance.

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Return of Myosin Heads to Thick Filaments After Muscle Contraction

Abstract. *The heads of myosin molecules, which move to the vicinity of the thin filaments to react with actin during muscle contraction, return to the thick filaments after contraction. The return occurs in two stages; a rapid return of the majority of the myosin heads is followed by a slow return of the rest.*

The heads of myosin molecules play a major role in producing the contractile force of muscle. They are arranged in a regular manner in the vicinity of the thick filaments in living resting muscle (*1*), and, when the muscle is activated, move promptly to the vicinity of the thin filaments (*2*) to undergo tension-generating reaction with actin. After contraction, the myosin heads return to their resting positions, but the time course of the return has not been well defined; Huxley (*3*) has concluded, from his observation on the intensities of the axial x-ray reflections of muscle after a short tetanus, that the return takes at least several seconds; whereas Podolsky *et al.* (*4*) have concluded from their observation on the intensities of the equatorial x-ray reflections that the return is almost completed within 100 msec after the fall of contractile tension. In a further study on the time-dependent intensity changes of the equatorial reflections, we obtained a result suggesting that the return occurs in two stages; a prompt change in the equatorial intensities on cessation of tetanic stimuli is followed by a gradual change lasting several seconds.

A sartorius muscle, together with the pubic bone, was isolated from the bullfrog *Rana catesbeiana*. The muscle was held isometrically in a specimen chamber by clamping the pubic bone at one end and connecting the tendon to a force transducer (Shinkoh, type UL) at the other end. The chamber had Mylar windows for passing x-rays through the middle of the muscle and was filled with oxygenated Ringer solution (4°C), which was continuously renewed with a perfusion pump. At the beginning of each experiment the sarcomere length of the muscle was adjusted to 2.2 μm by moving the force transducer; the sarcomere length was measured by passing a helium gas laser (λ = 0.6328 μm) through the same part of the muscle as to be exposed to x-rays, and observing the optical diffraction pattern. The muscle was stimulated tetanically, for 1 second at a time, with supramaximal electrical pulses (20 hertz) given through a pair of electrodes placed parallel to the muscle axis.

The equatorial x-ray diffraction pattern of the muscle was recorded by a position-sensitive counter of the type developed by Allemand and Thomas (*5*). The outputs of the counter were fed into a data collection system that was synchronized with the tetanus (see below). The recorded pattern (that is, the intensity distribution of the x-rays scattered along the equator) showed the 1,0 and the 1,1 reflections arising from the hexagonal array of the myofilaments. The intensities of these reflections were obtained by measuring the area under the peaks on the intensity distribution; the background level under each peak was drawn in by eye. This background level, relative to the peak height of each reflection, was of approximately the same magnitude as that of the densitometer traces of the equatorial patterns recorded on x-ray films.

Time-dependent changes in the in-

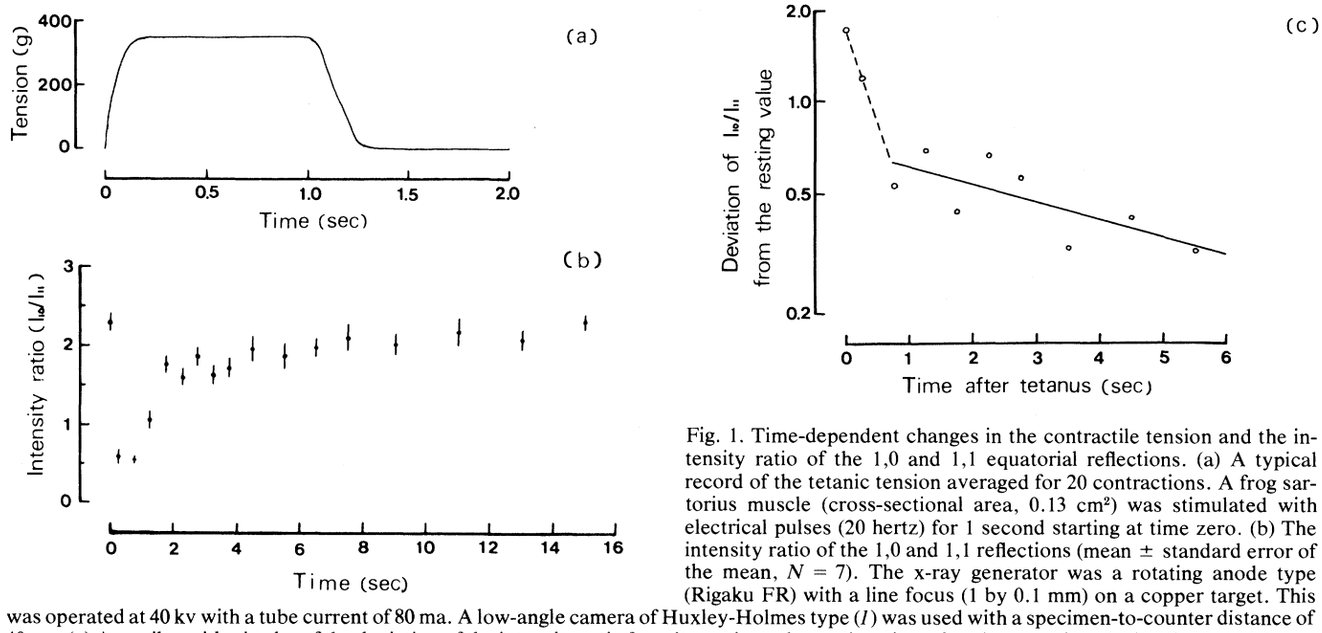

Fig. 1. Time-dependent changes in the contractile tension and the intensity ratio of the 1,0 and 1,1 equatorial reflections. (a) A typical record of the tetanic tension averaged for 20 contractions. A frog sartorius muscle (cross-sectional area, 0.13 cm^2) was stimulated with electrical pulses (20 hertz) for 1 second starting at time zero. (b) The intensity ratio of the 1,0 and 1,1 reflections (mean ± standard error of the mean, N = 7). The x-ray generator was a rotating anode type (Rigaku FR) with a line focus (1 by 0.1 mm) on a copper target. This was operated at 40 kv with a tube current of 80 ma. A low-angle camera of Huxley-Holmes type (*1*) was used with a specimen-to-counter distance of 40 cm. (c) A semilogarithmic plot of the deviation of the intensity ratio from its resting value against time after the cessation of stimuli. The solid line is the regression line for the points after the fall of tension.

tensities of the 1,0 and 1,1 reflections were studied for 16 seconds from the onset of tetanic stimulation. The 16-second period was divided into 16 phases of 0.5 to 2 seconds in duration, and the equatorial intensities from separate phases were stored in separate memory segments of the data collection system. In order to obtain reasonable photon statistics for each memory segment, the tetanic contractions (and the data collection) were repeated 20 times at 2-minute intervals. The tension records of the 20 contractions were averaged by a multichannel analyzer combined with a V-F converter. A typical averaged record is shown in Fig. 1a. The average tetanic tension was 91 ± 1 (standard error of the mean, $N = 7$) percent of the maximum value (2.97 ± 0.21 kg/cm^2, $N = 7$) measured at the beginning of each experiment. At the end of each 1-second stimulation, the tension fell to the resting level within 400 msec.

The time-dependent change in the ratio of the 1,0 and 1,1 intensities ($I_{1,0}$:$I_{1,1}$) is shown in Fig. 1b. The mean intensity ratio for the resting muscle at the sarcomere length of 2.20 μm was 2.29 ± 0.12 ($N = 7$), a value in approximate agreement with that obtained by the film method (2.33 ± 0.07, $N = 17$). The intensity ratio in the first and second 500-msec phases during tetanus were 0.58 ± 0.09 ($N = 7$) and 0.53 ± 0.05 ($N = 7$), respectively; the difference between the two values was insignificant. After tetanus, the intensity ratio returned to its resting value; the deviation from the resting value became statistically insignificant ($P > .05$) at 7 seconds after the end of tetanus. When the deviation was plotted against time on a semilogarithmic scale, it became apparent that the time course of the return could be divided into two stages; a rapid return is followed by a slow return. The initial rapid return (that is, the rapid increase in the intensity ratio) occurred almost simultaneously with the fall of tension, and ended within 1 second after the end of tetanic stimulation (*6*). The slow return (that is, the slow increase in the intensity ratio) continued after the tension had fallen to zero.

With the use of the intensity ratio, the electron density distribution in the transverse section of the filament lattice was calculated for each 0.5- to 2-second phase. From this, the relative mass of the thick to the thin filaments (thick/thin) was estimated by the method of Haselgrove and Huxley (*2*). Changes in the relative mass between the successive phases were attributed to radial movements of the myosin heads; a decrease in the relative mass, for instance, was interpreted as being caused by movements of myosin heads from the vicinity of the thick filaments to that of the thin filaments. In order to estimate the approximate number of myosin heads that had moved, we compared the changes in the relative mass with the change elicited by the shift of all the myosin heads from the thick to the thin filaments, namely the change in the relative mass elicited by putting a resting muscle into rigor (*7*). The estimated number of myosin heads in the vicinity of the thin filaments is plotted against time in Fig. 2.

For obtaining the values in Fig. 2, we considered the fact that the sarcomeres shortened during contraction although the muscle was held isometrically. Such consideration was necessary since the intensity ratio is known to vary with sarcomere length (*2, 8*). The details of the consideration, and some conclusions derived, were as follows.

1) During tetanus, the sarcomere length was about 2.05 μm (measured with the light-diffraction method). Therefore, for calculating the number of myosin heads present in the vicinity of the thin filaments during tetanus, we needed the intensity ratios for the muscles in the resting state and in rigor at the sarcomere length of 2.05 μm. These were 1.95 ± 0.15 ($N = 4$) and 0.33 ± 0.02 ($N = 4$), respectively. The calculation indicated that 74 percent of the total myosin heads were in the vicinity of the thin filaments in the first 500-msec phase, and 79 percent in the second 500-msec phase. It must be noted that the first 500-msec phase included the time of the rapid rise of tension. Then, the approximate agreement between the values for the first and second 500-msec phases indicates that the movement of the myosin heads to the thin filaments at the onset of tetanus was too prompt to be detected with the time resolution in our study.

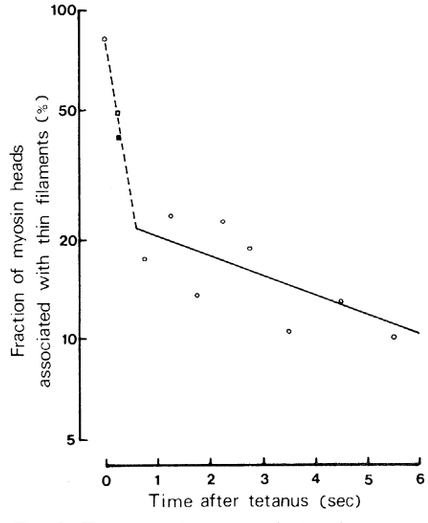

Fig. 2. Fraction of the myosin heads present in the vicinity of the thin filaments. These values were obtained by the following procedure (*2*). (i) With the use of the equatorial intensities (corrected for the Lorentz factor), the electron-density distribution in the transverse section of the filament lattice was calculated by Fourier synthesis. (ii) From this, the relative mass of the thick to the thin filaments was calculated, assuming that the lowest density in the electron-density map represented the background level. (iii) From the relative mass, the fraction of the myosin heads associated with the thin filaments was estimated (open circles). The solid line is the regression line for the points during the slow return of myosin heads. During the rapid return, the fraction could not be estimated precisely because of nonuniformity of the sarcomere lengths (see text). The open and closed squares represent the values obtained if the sarcomere lengths are assumed to be 2.20 and 2.05 μm, respectively.

2) During the rapid return of the myosin heads, which coincided with the rapid fall of tension, the number of myosin heads remaining in the vicinity of the thin filaments could not be determined precisely because of nonuniformity of the sarcomere lengths (*9*); some sarcomeres were shortening while the others were lengthening, as was indicated by the blurring of the light-diffraction pattern during the fall of tension. Therefore, as first-order approximations, we calculated the number on two different assumptions: the sarcomere length was assumed to be either 2.05 or 2.20 μm. The calculation based on the latter assumption required the intensity ratio for the muscle in rigor at the sarcomere length of 2.20 μm; this was 0.34 ± 0.03 ($N = 4$). The results from the two assumptions (the open and closed squares in Fig. 2) did not differ significantly from each other (*10*).

3) During the slow return, which took place after the tension had fallen to the resting level, the number of myosin heads in the vicinity of the thin filaments could be estimated precisely, as the sarcomere length had returned to 2.20 μm. Approximately 20 percent of the myosin heads were present in the vicinity of the thin filaments at the beginning of the slow return (Fig. 2).

Our results contradicted the conclusion of Podolsky *et al.* (*4*) that the return of myosin heads is almost completed within 100 msec after the fall of tension. However, the data they presented (their figure 3) were not in essential disagreement with ours; in the two observations from which they derived their conclusion, the intensity ratio obtained

soon after tetanus was indeed smaller than the values obtained before tetanus. They regarded this difference as insignificant and arrived at the above conclusion. The difference we detected (2.29 ± 0.12 before tetanus and 1.76 ± 0.09 at 500 to 1000 msec after tetanus, $N = 7$) might have been too small to be revealed by only two observations.

Huxley (*3*) has concluded from his observations on the axial x-ray reflections that the return of myosin heads to their resting positions takes at least several seconds; there is a discrepancy between the fall of tension and the return of myosin heads. Our results supported this conclusion and showed that the discrepancy arises from the behavior of about 20 percent of the myosin heads that do not return promptly (Fig. 2). The nature of the prolonged stay of these myosin heads in the vicinity of the thin filaments is not clear; they might be remaining there without being attached to actin, or they might be attached to actin without producing significant contractile force. At present we cannot distinguish between these possibilities. Some mechanical experiments, such as stiffness measurements of the muscle after tetanus, might solve this problem.

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10. The rapid increase in the intensity ratio upon the cessation of stimuli is likely to have been caused in part by the lengthening of the sarcomeres (*6*). However, the rapidity of the initial return of myosin heads shown in Fig. 2 cannot be attributed to the lengthening of the sarcomeres, since the lengthening has been taken into account in calculating the value plotted in Fig. 2 (that is, a correction has been made for the lengthening).
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Appetitive and Replacement Naps: EEG and Behavior

Abstract. *Consistent subjective, behavioral, and electroencephalographic sleep-stage differences were found between afternoon naps of 11 habitual appetitive nappers (who nap lightly for psychological reasons apparently unrelated to reported sleep needs) and 10 replacement nappers (who apparently nap regularly in response to temporary sleep deficits). Both types of naps were compared with naps of 12 confirmed non-nappers.*

Although the need for sleep is universal, extensive research has yet to clarify the functions of sleep, the efficiency with which it is obtained, and the mechanisms underlying the recovery from fatigue. Sleep is not a unitary phenomenon, and broad individual differences in the amount of sleep and its different stages are obtained. In addition, not all of an individual's sleep is obtained during nighttime hours; some individuals nap during the day. The functions served by daytime napping may differ widely among individuals. Like sleep in general, daytime napping is apt to serve functions other than physiological, restorative, and homeostatic ones. For many people a brief nap is reported to have restorative value far exceeding the length of time involved. In contrast, for some other individuals, the aftermath of napping seems to be sufficiently unpleasant that it is actively avoided.

To determine the frequency of napping in a young adult population and to evaluate whether there were differences among subjective experiences of napping, 430 college students were surveyed and patterns of napping and their attitudes toward napping were evaluated. Sixty percent of the students indicated they sometimes, usually, or always took catnaps during the day; only 40 percent answered they rarely or never napped. This finding is consistent with the frequency described in the literature (*1*) among a similar age group.

On the basis of criteria developed from pilot surveys, at least two different kinds of nappers could be identified from an analysis of these questionnaire descriptions of naps. (i) Replacement nappers nap to make up for previously (or soon to be) lost night sleep, and (ii) appetitive nappers nap primarily for reasons other than sleep need and apparently derive psychological benefits from the nap not directly related to the physiology of sleep. Specifically, a replacement napper was defined as a subject who had answered "no" to the criterion question "Do you nap even when you do not feel very tired?" and an appetitive napper was one who had responded either "definitely yes" or "possibly yes" to this question. A number of other questionnaire responses consistent with this distinction significantly differentiated between appetitive and replacement nappers. About 22 percent of the 261 nappers were classified as appetitive nappers, and 78 percent napped primarily for replacement reasons (*2*).

Subgroups of 11 appetitive and 10 replacement nappers (each of whom typically napped in the afternoon at least once a week), as well as 12 consistent non-nappers (who reported they rarely or never napped because napping had unpleasant mental and physical aftereffects) were asked to take a nap in a standard sleep laboratory. Subjects were included in the study only if an interviewer, who was unaware of the subjects' questionnaire responses, made the same classification as the questionnaire assignment. Thus, subgroup assignments were made by the independent classifications of both the questionnaire responses and a blind interviewer. Any subjects (particularly the non-nappers) who expressed doubts about being able to sleep in the laboratory were assured that the physiological data would be valuable even if they only rested quietly.

Standard electroencephalographic (EEG) and electrooculographic (EOG) recording electrodes were applied by an experimenter who was blind as to the subjects' napping classification. Subjects were provided with a comfortable bed in which to take a nap. Although the length of the nap was not discussed, subjects were aroused by a loud telephone buzzer 60 minutes after they were asked to begin their nap. Arousal reaction time, defined as the time taken to pick up the