LETTERS TO THE EDITOR


Recently, Lecarpentier et al. (1985) described in this journal the real-time kinetics of sarcomere relaxation. In this paper, the recording of the first-order maximum of the diffraction pattern is of essential importance in the interpretation of their results. In our recent paper (Karemaker et al., 1986), we have shown that the interpretation of this kind of diffraction pattern is questionable, on the basis of the recorded asynchrony and asymmetry.

Figure 2 of the paper by Lecarpentier et al. (1985) shows, exactly, these difficulties in interpretation. This figure shows, instead of symmetrical spectra, asymmetrical spectra both, in intensity and in place domain. The estimate of sarcomere length from the right diffraction pattern differs about 15% from that of the left. The differences in both intensity and place imply that the muscle is not an ideal grating. The physiological basis of this non-ideality of the sarcomere grating can be found, e.g., in discrete length populations (Judy et al., 1982), Bragg-reflection from skew planes (Ruedel and Ferenczy, 1979), and/or misalignment of neighboring planes of sarcomeres (Yeh et al., 1980). Besides these interpretation problems with regard to static diffraction patterns, the "microstructure" or subpeaks in the first-order line (Cleworth and Edman, 1969; Lieber et al., 1984; Karemaker et al., 1986) complicates the usefulness of the gravity center of this peak in dynamic studies. In our study, we have shown that the gravity center of the first-order line can be found, e.g., in discrete length populations (Judy et al., 1982), Bragg-reflection from skew planes (Ruedel and Ferenczy, 1979), and/or misalignment of neighboring planes of sarcomeres (Yeh et al., 1980). Besides these interpretation problems with regard to static diffraction patterns, the "microstructure" or subpeaks in the first-order line (Cleworth and Edman, 1969; Lieber et al., 1984; Karemaker et al., 1986) complicates the usefulness of the gravity center of this peak in dynamic studies. In our study, we have shown that the gravity center of the first-order line can be found, e.g., in discrete length populations, the mean sarcomere length does not change at all, but the gravity center (interpreted in terms of mean sarcomere length) changes substantially. So, in conclusion, the gravity center of the first-order line of the diffraction spectrum is not the result of an ideal grating. It shows highly asymmetrical diffraction patterns (both in intensity and place). These spectra do not allow exact measurement of the sarcomere length in static studies due to this asymmetrical behavior.

In dynamic studies, the change in sarcomere length cannot be derived from the change in the center of gravity of the first-order line, because a change in gravity can be the result of alterations in intensities of subpeaks in these first-order lines.

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Reply to the Preceding Letter

Sarcomere kinetics studied by laser diffraction permit a clearer understanding of mechanics at the cellular level. Our method of real-time measurement of sarcomere dynamics previously described (Lecarpentier et al., 1985) is not based on analysis of the gravity center displacement of the first-order diffraction line by means of photodiode array, lateral photodiode, and video camera (Krueger and Pollack, 1975; Manring et al., 1977; Iwazumi and Pollack, 1979; ter Keurs et al., 1980; Van Heuningen et al., 1982). Our technique consists of a densitometric wedge, whose transmission is related to the lateral shift of the whole first-order line, itself directly related to the instantaneous sarcomere length (SL) through the relationship \( SL = SL_0/(1 + \alpha \log g/h) \), where \( SL_0 \) is the resting sarcomere length at \( L_{max} \), \( \alpha \) is the constant characteristic of the optical set-up, \( g \) is the product of the variations in intensity of the first-order diffraction line and the variations in intensity due to the beam's lateral movement on the densitometric wedge, and \( h \) is the variation in intensity of the first-order line. Moreover, the accuracy of the method is not significantly altered by the broadening of the diffraction line which may occur during contraction, since we have calculated that, after the
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densitometric wedge, an upper limit increase of 50% in diffraction line breadth provides a relative error in the lateral shift determination of less than 0.2% (Lecarpentier, 1983). Finally, we have found that the lateral shift is similar on both sides of the diffraction pattern, regardless of the slight asymmetry in intensity between the two first-order lines observed both in cardiac tissue (Pollack and Krueger, 1976; Krueger and Pollack, 1975; Manring et al., 1977; Krueger et al., 1980) and in whole skeletal muscle (Cleworth and Edman, 1972; Supinski and Kelsen, 1982).

The existence of "microstructures" or subpeaks in the first-order diffraction lines has not been noticed in all striated muscle experimental models. In mammalian cardiac trabeculae, bands of diffraction pattern do not generally show microstructures in the first-order diffracted lines both at rest and during contraction (Pollack and Krueger, 1976; Gordon and Pollack, 1980; Van Heuningen et al., 1982; Hultgren and Hamrell, 1985). On the other hand, diffraction pattern of single skeletal fiber has been shown to be composed of microstructures. Interpretations of these "microstructures" have proved complex (Cleworth and Edman, 1972; Rudel and Zite-Ferency, 1979; Tameyasu et al., 1982; Lieber et al., 1984). However, our method remains valid for measurement of the lateral shift of the first-order line with microstructures observed in single skeletal fiber, providing the microstructures persist virtually unchanged throughout the contraction. This has been demonstrated to be the case by Cleworth and Edman (1972) and corroborated by Tameyasu et al. (1982).

In heart muscle, subpeaks have occasionally been observed in very thin ventricular cardiac trabeculae (Krueger and Pollack, 1975) and in thinned frog atrial strands, especially at large initial sarcomere length (>3.5 μm) (Manring et al., 1977). Moreover, in electrically excitable single cardiac cells, the similarity of first-order line behavior contrasts markedly with that observed in spontaneously beating cells, which are characterized by nonsynchronized sarcomere shortening and fragmentation of the first-order diffracted light (Krueger et al., 1980). In preparations in which contraction is characterized by a random distribution of position and displacement of subpeaks (Karemaker et al., 1986), sarcomere dynamics analyzed by the first-order diffraction line shift would obviously be complicated. This is, however, not the case with right ventricular rat cardiac trabeculae, for which our method for measuring real-time sarcomere dynamics appears to be quite valuable.

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