CONTRACTION BANDS AT SHORT SARCOMERE LENGTH IN CHICK MUSCLE

MARTIN HAGOPIAN. From the Department of Pathology, New York Medical College, New York 10029

INTRODUCTION

The sliding filament model for contraction of striated muscle has received widespread acceptance (see Huxley, 1965, for review). In this model, shortening occurs by a sliding of the myosin (thick) and actin (thin) filaments relative to one another. The filaments of each type maintain a constant length during the process. The shortening of the sarcomeres (Z line to Z line) to about 1.5 μ in vertebrates may be readily explained, since the actin filaments attached to the Z lines are free to interdigitate when they meet in the middle of the sarcomere and form a contraction band (Huxley,

1961; Spiro and Sonnenblick, 1964). However, once the sarcomeres shorten to less than 1.5 μ, the myosin filaments, which are approximately 1.6 μ in length (Page and Huxley, 1963), hit the Z discs, and they are bent back. Exceptions to the crumpling of the thick filaments as they hit the Z line have been reported in barnacle muscle (Hoyle et al., 1965; Leyton and Ullrick, 1970) and in an insect muscle (Zebe et al., 1968). In barnacle muscle, spaces opened up in the Z line for the penetration of the thick filaments whereas, in the insect muscle, electron micrographs suggest penetration of the Z lines by thick filaments. The present work shows and examines the contraction bands at short sarcomere length found in the glycerinated chick pectoral muscle. The maximally shortened sarcomere in this study measures about 1.3 μ in length. Prior to the disorganization of the filaments in extreme shortening, it appears that the myosin filaments are able to penetrate the intact Z line at sarcomere lengths of 1.5–1.3 μ.

MATERIALS AND METHODS

White Leghorn chick pectoral muscle was glycerinated at −10°C in 50% glycerol for a period of 1–6 months. Pieces were removed at intervals, washed in saline (0.9% NaCl), and immersed in 6.3% glutaraldehyde buffered with 0.2 M phosphate at pH 7.6 for 3 hr. The tissue was rinsed in 0.1 M phosphate buffer for about 18 hr and then postfixed in 2% OsO_4 in Veronal-acetate buffer (pH 7.6) for 1 hr at room temperature. Dehydration in graded concentrations of acetone was followed by embedding in Araldite (Ciba Products Co., Summit, N. J.). To minimize the errors in measurements of filament length and sarcomere length, longitudinal sections were cut with the knife edge parallel to the fiber axis. Sections were double-stained with uranyl acetate and lead citrate, and were examined with the Philips 200 electron microscope.

RESULTS AND DISCUSSION

The sarcomere length (mid Z line to mid Z line) of the glycerinated chick pectoral muscle varies because of local stretching and shortening. Since the sarcomere length ranges from 2.3 to 1.1 μ, the myofibrils have sarcomeres which show all the band patterns common to vertebrate muscle. Fig. 1 is an electron micrograph of some sarcomeres which are about 2.2 μ in length. In this picture the actin or thin filaments (*IF*) are approximately 1.0 μ in length, and the myosin or thick filaments (*A*) are about 1.5 μ. At a higher magnification the myosin filaments have tapered ends. Fig. 2 depicts a region of a glycerinated muscle fiber in which there is a local shortening of sarcomeres to about 1.5 μ. Because the 1.0 μ actin filaments (*IF*) have bypassed at the mid-sarcomere zone, they have obliterated the H zone and have formed an A contraction band (*CB*) approximately 0.5 μ in width in the central region of the sarcomere. Also, since the 1.5 μ myosin filaments now extend from one end of the sarcomere to the other end, the I bands have disappeared.

The A contraction band increases in width as the sarcomere shortens. Fig. 3 shows a sarcomere approximately 1.4 μ in length with an A contraction band about 0.6 μ in width, and an additional dense zone on each side of the Z line (Z line contraction band). In Fig. 4 there is a sarcomere approximately 1.3 μ in length with an A contraction band of about 0.7 μ in width, and a dense zone on either side of the Z line slightly wider than that in Fig. 3. At a sarcomere length of less than 1.3 μ, most of the band patterns and filaments become disorganized. Fig. 5 demonstrates an example of a sarcomere approximately 1.2 μ in length. Although in this picture the twisted Z line and M band are still present, the filaments are disorganized and band patterns are no longer discernible (compare to Figs. 3 and 4). It can thus be seen that the maximum shortening of the organized sarcomeres is 1.3 μ. Such findings suggest that at a sarcomere length between 1.5 and 1.3 μ, the ends of the tapered myosin filaments penetrate the Z line into the adjacent sarcomeres and form dense zones on either side of the Z lines. If the myosin filaments were bending back or crumpling on the Z lines as has been previously stated (Huxley, 1960; Spiro, 1967), they would have a less orderly array in the dense zones around the Z lines. Figs. 3 and 4 illustrate longitudinal sections of orderly Z contraction band filaments which appear to be coming from adjacent sarcomeres, if one considers the constant known length of the myosin filaments (*A*). Transverse sections in the Z line region are, in this respect, not helpful in maximally shortened muscle, since it is difficult to determine if the myosin filaments have penetrated the Z line or if the myosin filaments have bent back. However, transverse sections of well-oriented Z lines in stretched and shortened sarcomeres in the chick muscle reveal the typical vertebrate square lattice measuring about 220 A on a side (Landon, 1970). It is intrigu-

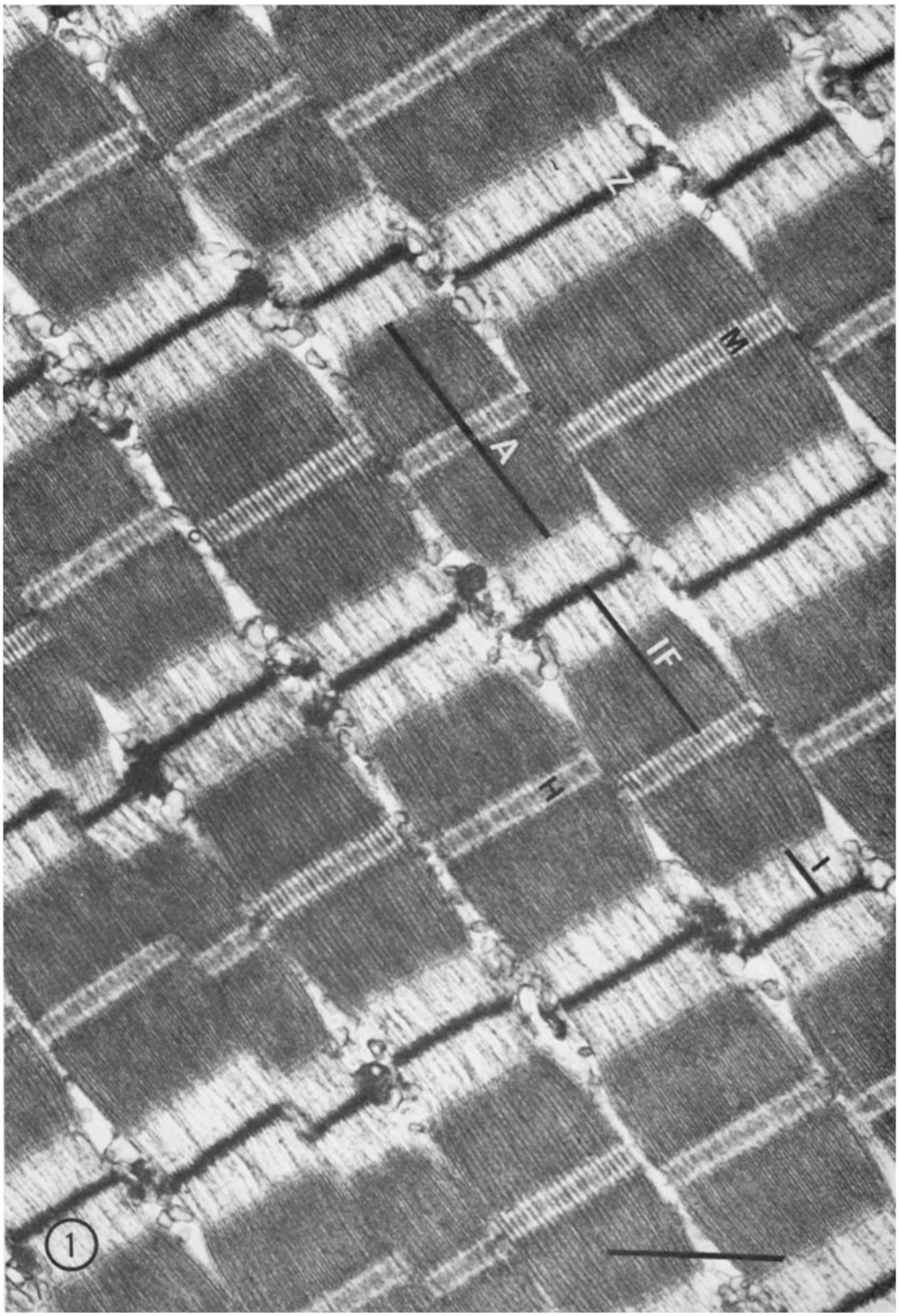

FIGURE 1 A portion of a glycerinated chick pectoral muscle fiber. Vertebrate muscle band patterns are present (*A*, *H*, *I*, *M*, *Z*). The I or actin filaments (*IF*) measure approximately 1 μ in length, the myosin filaments (*A*) are about 1.5 μ in length, and the sarcomeres (mid *Z* line to mid *Z* line) are about 2.2 μ. × 28,000. Mark: 1 μ.

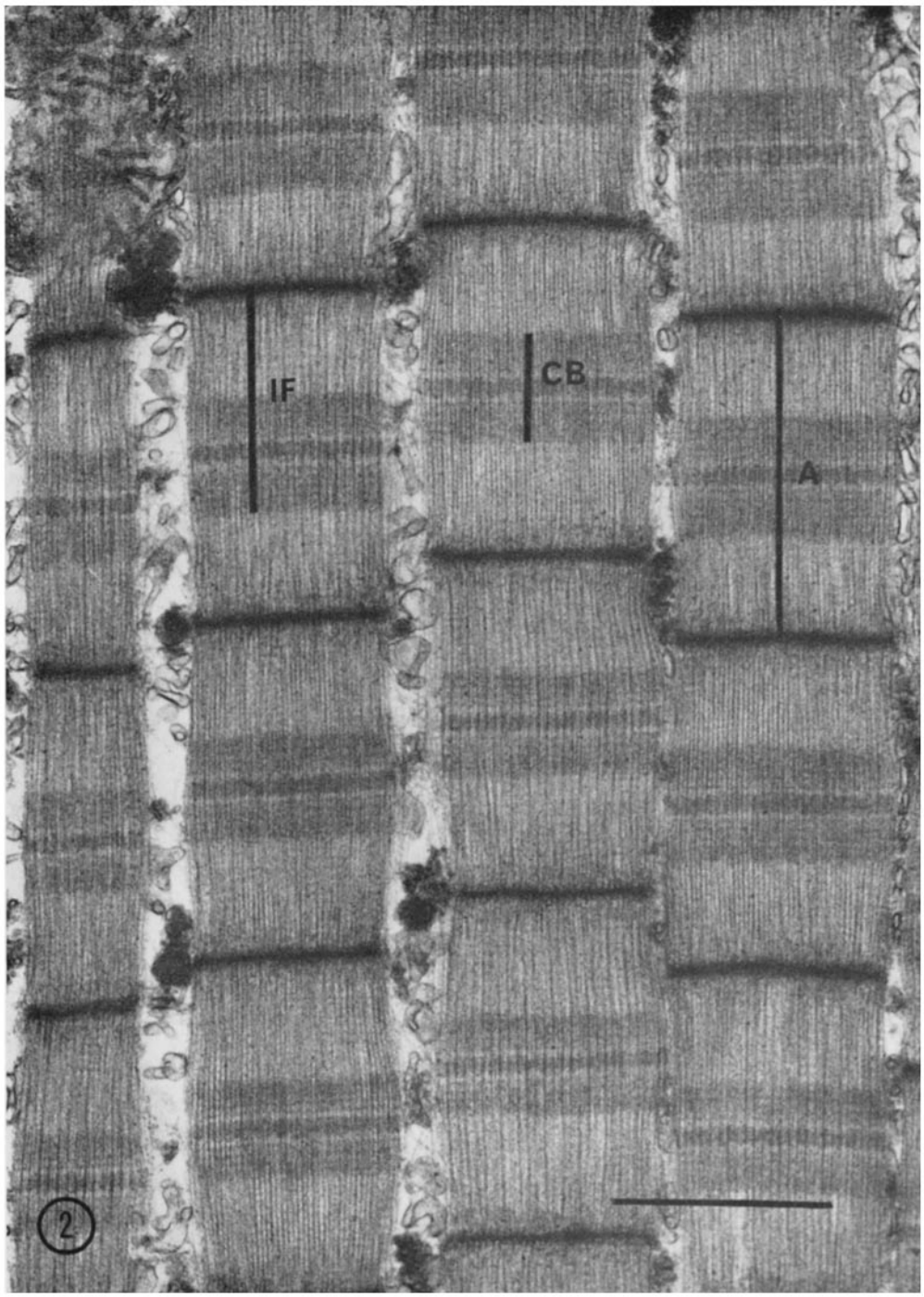

FIGURE 2 A local region of shortened glycerinated muscle myofibrils. The I filaments (*IF*) have interdigitated at the mid-sarcomere zone and have formed an A contraction band (*CB*) approximately 0.5 μ in width. The sarcomeres average about 1.5 μ in length (about the same length as the myosin filaments [*A*]). Note that the H zone and I bands have been obliterated. × 31,000. Mark: 1 μ.

ing to speculate that the tapered ends of the myosin filaments in shortened sarcomeres (1.5–1.3 μ) are able to enter the square lattice of the Z lines to a distance of 0.1 μ into the neighboring sarcomeres.

The penetration of the myosin filaments through the Z lines in sarcomeres measuring 1.5–1.3 μ explains the findings of Gordon et al. (1966 *a*, 1966 *b*). Their study revealed that sarcomeres in isolated frog muscle can shorten reversibly to about 1.30 μ, and that there is an inflection in the active length-tension curve in sarcomeres measuring 1.65 μ. It appears that this point of inflection in the length-tension curve in the present study results from the penetration of the myosin filaments into the Z lines. Z line penetration by the thick filaments was reported in barnacle muscle (Hoyle et al., 1965; Leyton and Ullrick, 1970), and in an insect muscle (Zebe et al., 1968). In the barnacle supercontracted sarcomeres, large spaces open in the Z lines. Nothing resembling these perforations in the Z lines occurs in the chick pectoral muscle. However, in the extremely shortened insect muscle the Z line penetration indicates somewhat similar findings as the present study, since there is a small penetration of Z lines by the thick filaments. Irreversible contracture at sarcomere lengths less than 1.3 μ in the study by Gordon et al. (1966 *a*) is probably attributable to structural disorganization of the myofibril.

The findings in this investigation are consistent with the model of contraction in striated muscle. The model also seems to apply when the chick pectoral muscle sarcomeres are reduced to 1.3 μ in length.

SUMMARY

Contraction bands of short sarcomeres in glycerinated chick pectoral muscle were studied. The maximally shortened sarcomere measured approximately 1.3 μ in length. In this short sarcomere it appeared that the tapered ends of the myosin filaments were able to penetrate the Z line in an orderly arrangement, and were able to form a dense zone in the adjacent sarcomere.

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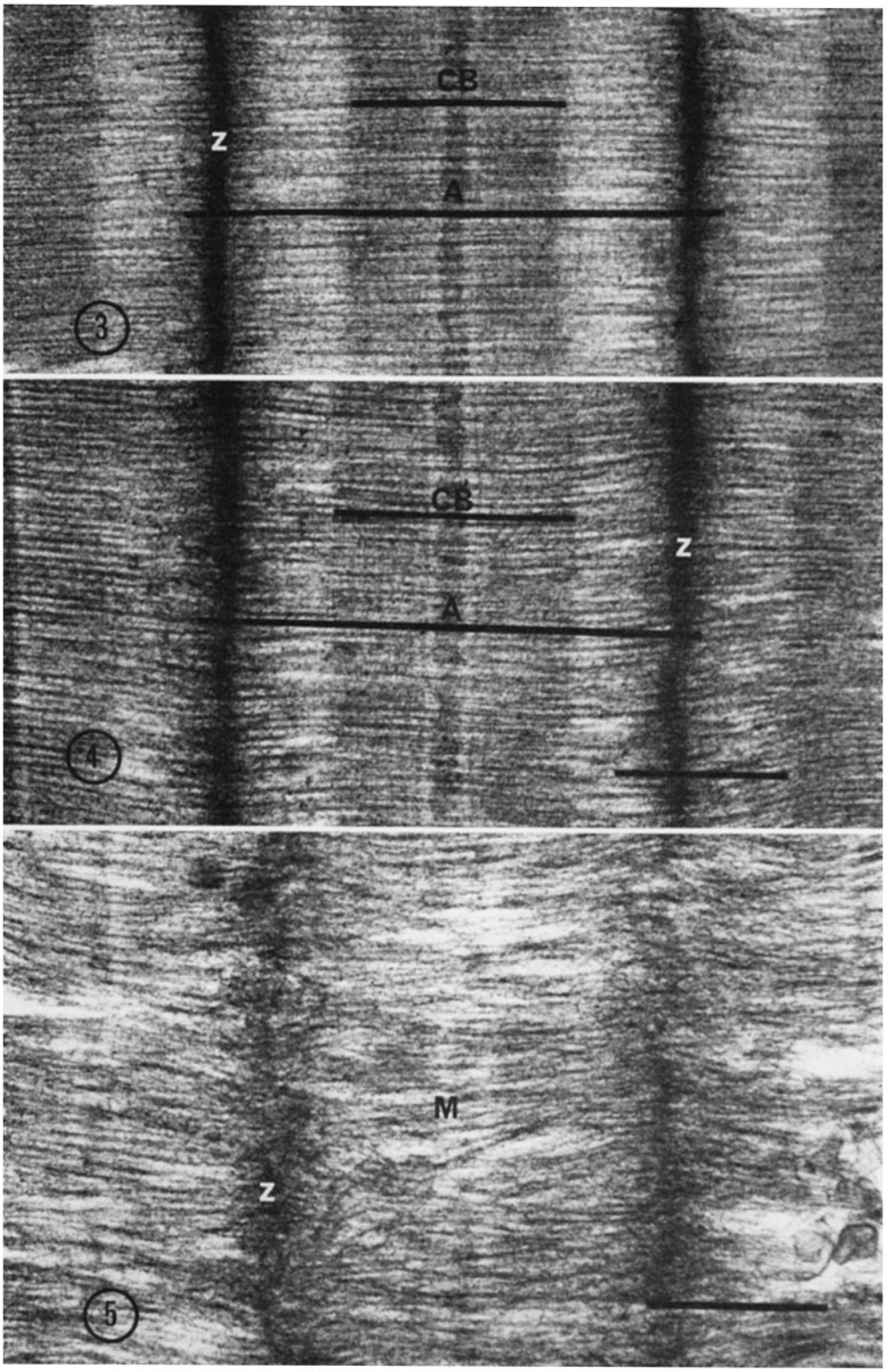
FIGURE 3 A shortened sarcomere. The sarcomere is 1.4 μ in length and the A contraction band (*CB*) is about 0.6 μ in width. It thus appears that the tapered ends of the myosin filaments (*A*) have penetrated the Z lines (*Z*) and have formed dense zones on each side of the Z lines. × 52,000.

FIGURE 4 A maximally shortened sarcomere before disorganization of the band patterns. The sarcomere is approximately 1.3 μ in length and the A contraction band (*CB*) is about 0.7 μ in width. The tapered ends of the myosin filaments (*A*) appear to penetrate the Z lines about 0.1 μ on each side. × 52,000. Mark: 0.5 μ.

FIGURE 5 A sarcomere shortened beyond its limits of organization. The sarcomere is about 1.2 μ in length. The Z line (*Z*) and M band (*M*) are still discernible, but the other band patterns and filaments have now become disorganized. × 52,000. Mark: 0.5 μ.



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