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ISOLATION OF SINGLE SARCOMERE AND ITS CONTRACTION ON ADDITION OF ADENOSINE TRIPHOSPHATE

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SUMMARY

Single sarcomeres were isolated from pectoral muscle of chicken in considerable yield by a method essentially similar to that for isolation of myofibrils from rabbit skeletal muscle described by PERRY. On addition of adenosine triphosphate, the H-zone and the I-band of a single sarcomere disappeared and the length became equal to that of the original A-band. The Z-line was shown to be indispensable for the formation of the contraction band.

INTRODUCTION

The periodic pattern in striated myofibrils is shown by HUXLEY AND HANSON[1] to be attributable to the regular distribution of actin and myosin. In the reconstituted actomyosin threads the distribution of actin and myosin has no regularity, though HAYASHI[2] and PORTZEHL[3,4] have succeeded in orienting both myosin and actin unidirectionaliy along the thread axis. Also, some properties of contraction of the thread on the addition of ATP differ from those of living muscle. SZENT-GYÖRGYI'S[5] glycerol-treated muscle and PERRY'S[6] isolated myofibrils are muscle models standing intermediately between actomyosin threads and living muscle and contract on adding ATP in a way similar to living muscle[7]. The morphological unit in striated myofibrils is designated as a "sarcomere". Therefore, a study of the contraction mechanism of an isolated sarcomere would seem to be of fundamental importance. A method for the isolation of a single sarcomere is described in this report along with a description of some of the changes in its morphological pattern on addition of ATP.

EXPERIMENTAL

The use of fresh rabbit skeletal muscle as a starting material for isolation of single sarcomeres was abandoned after several unsuccessful trials. When pectoral muscle of young chicken, so called "broiler", was used, single sarcomeres were isolated in considerable yield by the following procedures.

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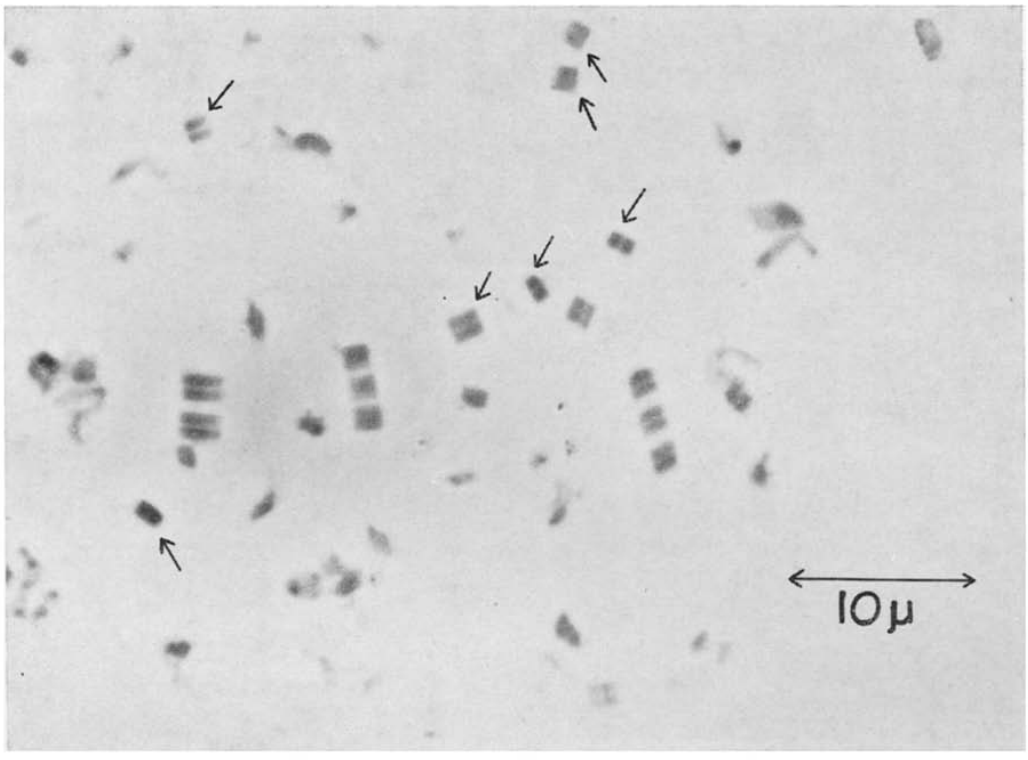

Fig. 1. Microphotograph of isolated sarcomeres. Arrow indicates isolated single sarcomere.

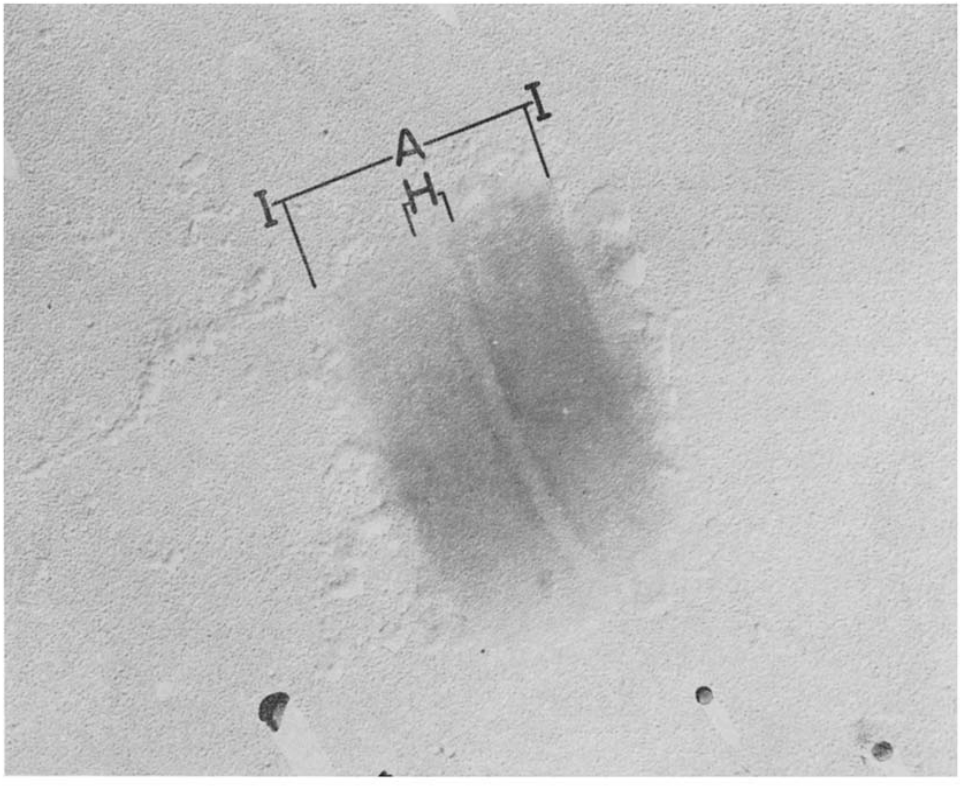

Fig. 2. Electronmicrophotograph of isolated single sarcomere (× 32625).

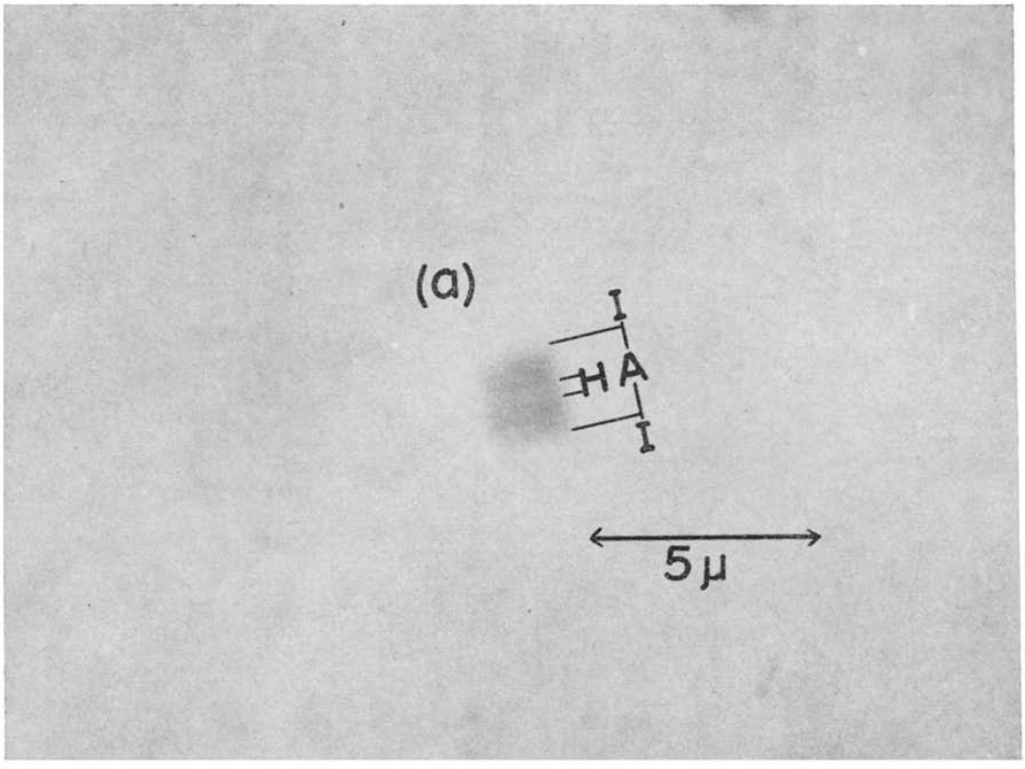

Fig. 3.

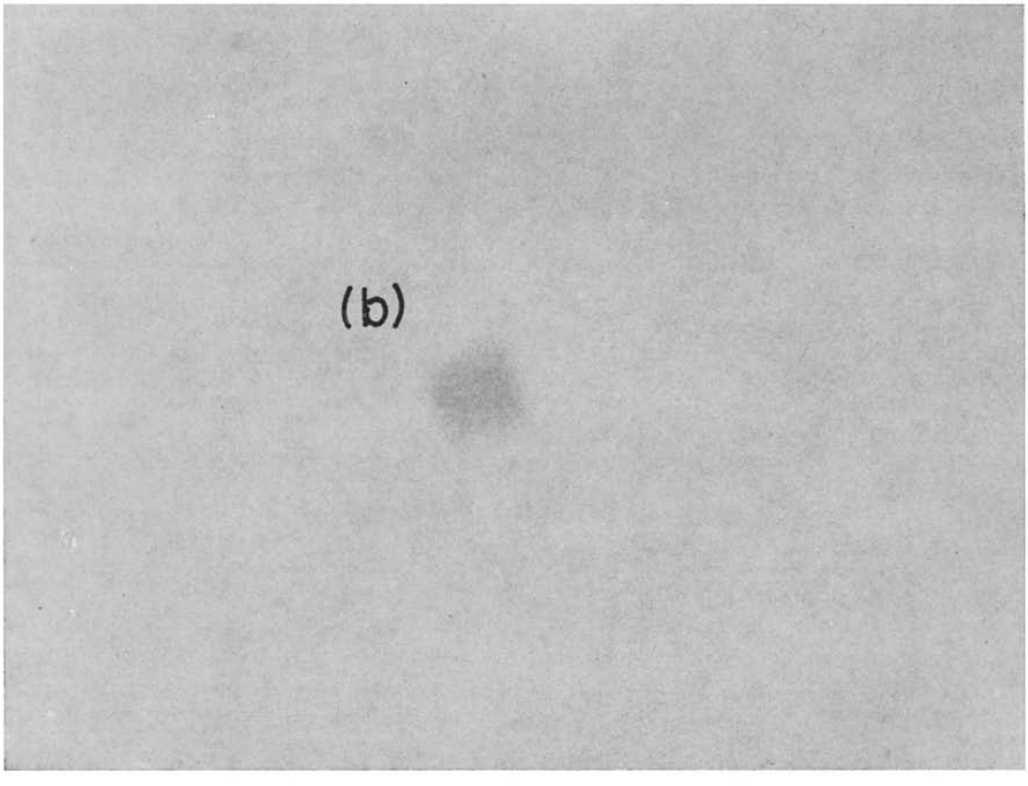

Fig. 3.

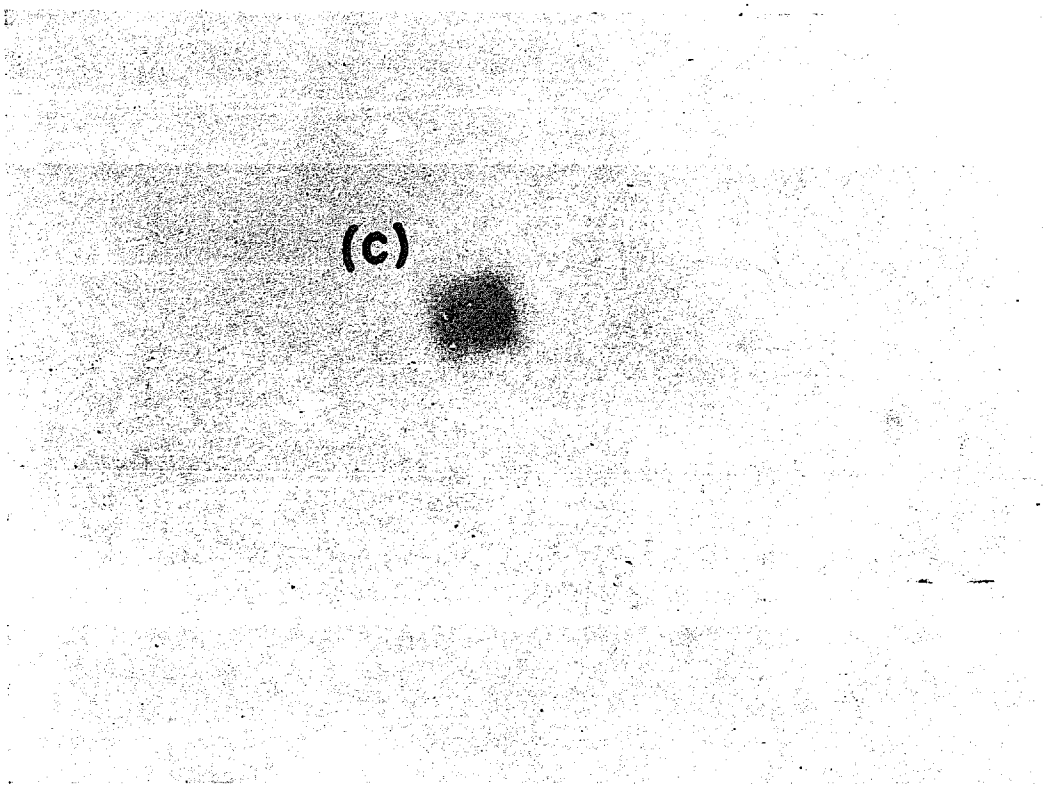

Fig. 3. Contraction of single sarcomere on addition of ATP; (a) before, (b) 70 sec after, and (c) 210 sec after addition of Mg^{2+}–ATP. (0.1 M KCl, 25 mM phosphate buffer (pH 7.0), 14°.)

10-week-old chickens (sort of Cornish ♂ × New Hampshire ♀ and White Rock) were killed and stored at —20° to —25° for 2–6 months. Sarcomeres were isolated by a method essentially similar to that for isolation of myofibrils established by PERRY[6]. All manipulations were performed at 1°. 5 vol. of 0.025 M KCl, 5 mM EDTA and 0.039 M borate buffer solution (pH 7.1) were added to 50 g of minced pectoral muscle, homogenized for 3 min and centrifuged at 600 × *g* for 15 min. The sediment was suspended with 5 vol. of the buffer solution (0.1 M KCl, 5 mM EDTA and 0.039 M borate buffer (pH 7.1)) and homogenized for 2 min. One-half of the upper layer of the precipitate prepared by centrifugation at 600 × *g* for 15 min was resuspended with 5 vol. of the buffer solution and homogenized for 3 sec. After centrifugation at 600 × *g* for 15 min, only the upper layer of the sediment was isolated, suspended with 5 vol. of the buffer solution and homogenized for 3 sec. After standing for 5 min, the floating materials were discarded. The sediment was removed by centrifugation at 450 × *g* for 3 min, and the precipitate obtained by centrifugation at 600 × *g* for 20 min was suspended with 5 vol. of the buffer solution and homogenized for 1 sec. The centrifuagtion at 600 × *g* for 20 min and homogenization for 1 sec was repeated 3 or 4 times.

A 25-ml portion of the final suspension, which contained 6–8 mg/ml of protein, was carefully layered onto 150 ml of the buffer solution in a glass cylinder (5.4 × 90 cm). After standing at 0° for 12 h, a 50-ml portion of the upper fraction was isolated and layered onto 50 ml of the buffer solution in a glass cylinder (1.5 × 54 cm). A 10-ml portion of the lower fraction was isolated after 12 h standing at 0° and used for observing contraction of sarcomeres.

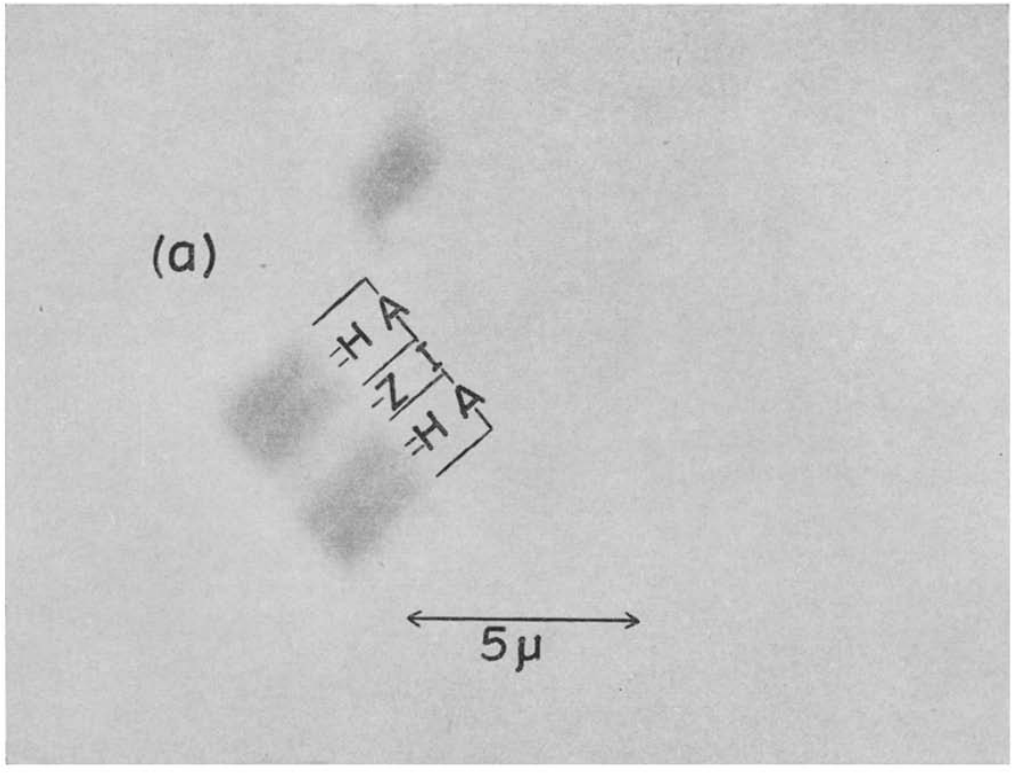

Fig. 4.

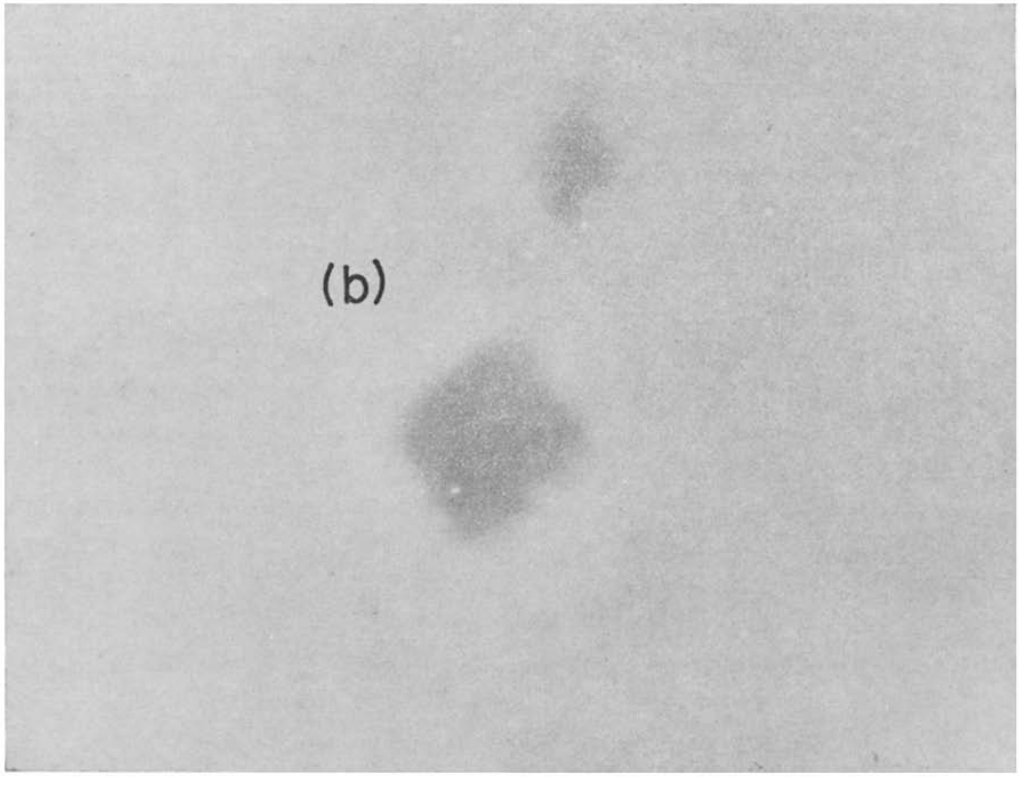

Fig. 4.

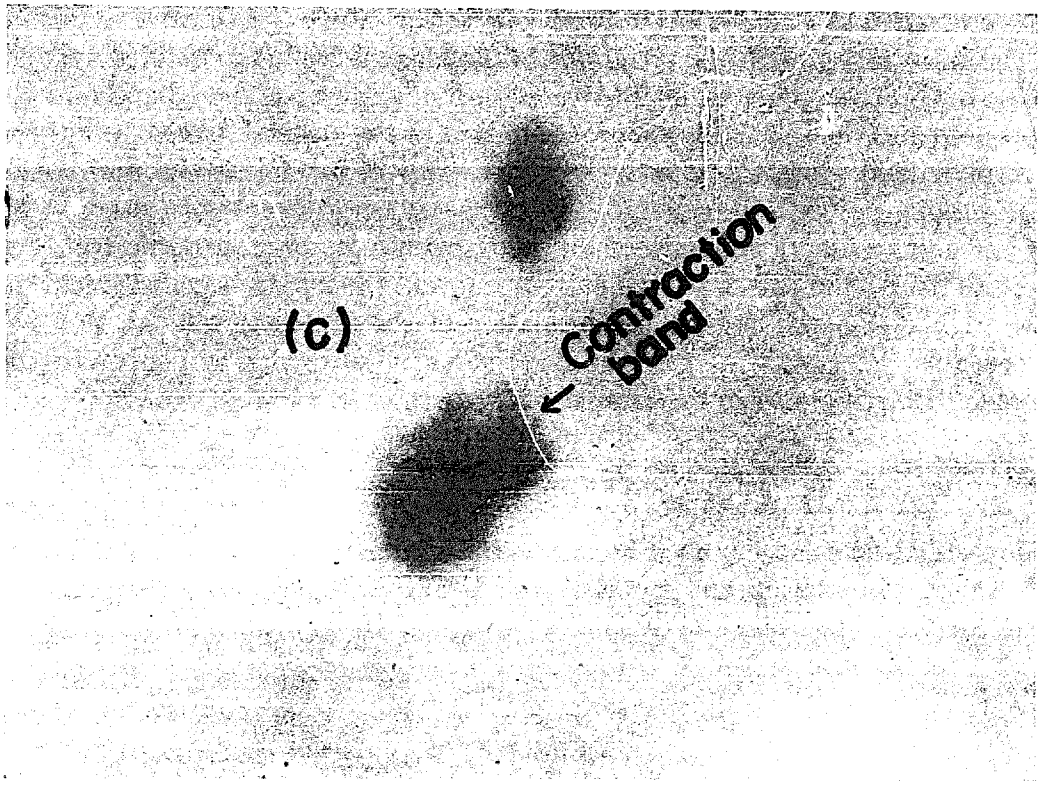

Fig. 4. Contraction of double sarcomere on addition of ATP; (a) before, (b) 70 sec after, and (c) 110 sec after addition of Mg^{2+}–ATP. (0.1 M KCl, 25 mM phosphate buffer (pH 7.0), 17°.)

RESULTS AND DISCUSSION

Fig. 1 shows a typical photograph of the material taken by an Olympus model ECETR-2 phase-contrast microscope (Chiyoda Optics Co.). As indicated by arrows in the figure, 40–50% of the myofibril fragments were single sarcomeres and the remaining amount consisted mostly of double and triple sarcomeres. In most of the isolated single sarcomeres, only the A-band and H-zone were clearly observed with the microscope (see Fig. 3). Portions of the I-band, cut irregularly at both sides of the A-band, were, however, recognized by electron microscopy, as shown in Fig. 2. This picture was taken by means of a Type JEM-5Y electron microscope (Japan Electron Optics Co.) after chromium shadowing. Some isolated single sarcomeres showed Z-lines at the ends, but single sarcomeres, with I-bands but without Z-lines, were rarely seen. Fragments cut at the A-band were never observed.

After centrifugation of the suspension obtained above at 600 × *g* for 15 min, the sediment was resuspended with 10 vol. of 0.1 M KCl and 25 mM phosphate buffer (pH 7.0). The contraction of the sarcomeres was initiated by instillating one drop of 5 mM Mg^{2+}–2 mM ATP solution* at one edge of a cover glass while observing with the microscope. The presence of the Z-line exerted remarkable influence on appearance of contraction of the sarcomeres. In the contraction of single sarcomeres without the Z-line, the H-zone and I-band, if initially visible, disappeared and the contraction stopped at this stage, as shown in Fig. 3. The contraction of single sarcomeres with

* Swelling of sacromeres was observed on addition of 5 mM Mg^{2+} and 5 mM ATP.

Z-lines proceeded to a further extent and contraction bands were formed at both sides.

The essential features of contraction of double and triple sarcomeres were similar to those of single sarcomeres, as illustrated in Fig. 4. At first, the I-band and H-zone disappeared and the length became equal to that of the original A-band and the width was increased by 30–40 %. Then the contraction band was formed around the Z-line.

In summary, it may be concluded that the sarcomere is not only a morphological unit but also a functional unit of striated myofibrils; on contraction of a single sarcomere the I-band and the H-zone disappear and the length becomes equal to that of the original A-band, and for the formation of the contraction band, the Z-line is indispensable. HUXLEY AND TAYLOR[8] have recently succeeded in contracting single sarcomeres in living muscle by stimulation with microelectrodes.

ACKNOWLEDGMENTS

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