MORPHOLOGY OF RIGOR—SHORTENED BOVINE MUSCLE AND THE EFFECT OF TRYPSIN ON PRE— AND POSTRIGOR MYOFIBRILS

MARVIN H. STROMER, D. E. GOLL, and L. E. ROTH

From the Departments of Biochemistry and Biophysics and of Animal Science, the Iowa State University, Ames, Iowa 50010. Dr. Stromer's present address is the Mellon Institute, Pittsburgh, Pennsylvania 15213. Dr. Goll's present address is the Cardiovascular Research Laboratory, the University of California Medical Center, Los Angeles, California 90024

ABSTRACT

Bovine semitendinosus muscles were sampled immediately after death, after 24 hr postmortem with storage at 2°, 16°, or 37°C, and after 312 hr postmortem with storage at 2° and 16°C. A biopsy technique was used to prevent shortening during glutaraldehyde fixation. Postfixation in osmium tetroxide was followed by embedding in an Epon–Araldite mixture. Bovine muscle was supercontracted after 24 hr storage at 2° but was only slightly contracted after storage at 16° for 24 hr. Muscle held at 37° for 24 hr was slightly less supercontracted than the 2° muscle. Striking similarities existed between muscles stored at 16° and at 2°C for 312 hr. Both were slightly shortened with narrowed I bands and an area of increased density, probably due to overlap of thin filaments in the middle of the A band. Postmortem shortening was accompanied by banding-pattern changes similar to those predicted for contracting muscle by Huxley and Hanson's sliding filament model. Treatment of myofibrils with 0.05% trypsin resulted in a rapid loss of Z lines and, in supercontracted myofibrils, caused a return of the banding pattern of resting muscle.

INTRODUCTION

Gross changes in striated muscle undergoing rigor mortis have been extensively studied. Bate-Smith (1939) reported a 10-fold increase in modulus of elasticity as rigor began. The onset of rigor was correlated with a decrease in ATP by Bate-Smith and Bendall (1947) and, in a later paper, these same authors (1949) studied the effects of glycogen reserves and temperature on the time course of rigor. Bendall (1951, 1960) has also made extensive studies of the effect of rigor on rabbit muscle. These and later reports characterized many of the observable changes accompanying rigor mortis.

Few ultrastructural observations have been reported in connection with the investigation of changes in rigor. Locker (1959) has categorized four successive patterns of contraction occurring during rigor mortis of bovine muscle and has suggested that rigor shortening and muscle contraction are morphologically the same. Locker's study, however, did not include the effects of temperature and time on postmortem shortening. Other studies of "rigor" muscle (Elliott et al., 1963) have been done on glycerol-extracted muscle which was not exposed to different temperatures and which was not free to shorten.

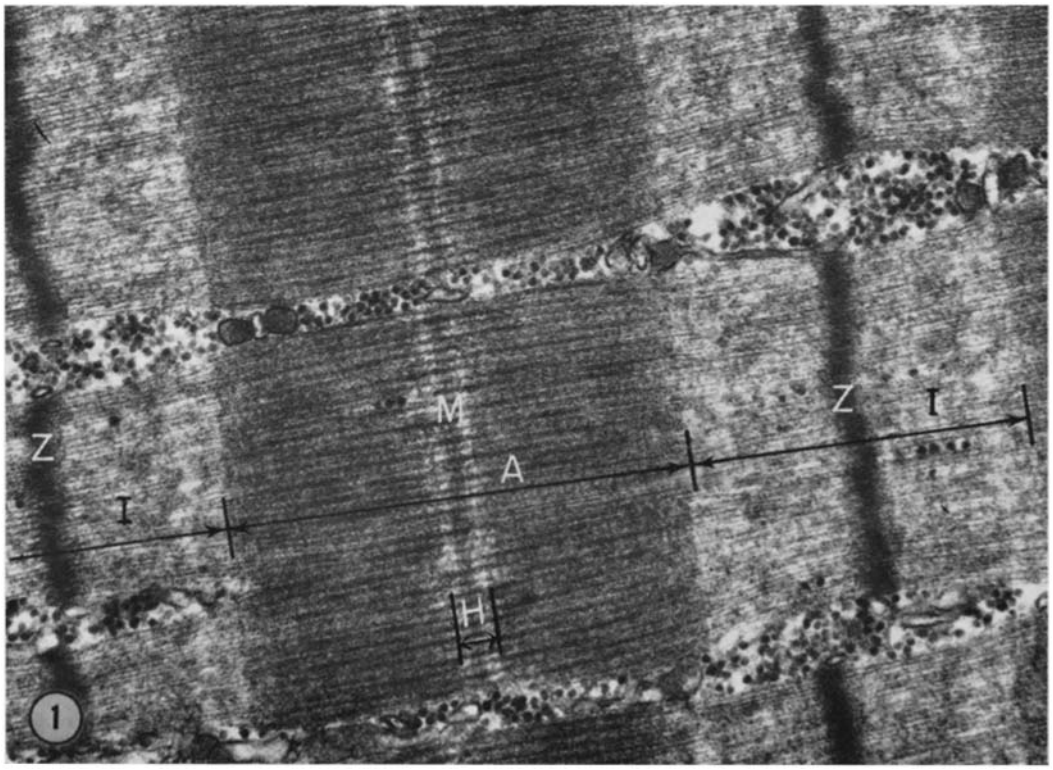

FIGURE 1 Bovine semitendinosus muscle sampled at death with a biopsy technique. This and subsequent samples were fixed with glutaraldehyde and postfixed with osmium tetroxide. Section stained with uranyl acetate and lead citrate. *A*, A band; *I*, I band; *H*, H zone; *M*, M line; *Z*, Z line. × 45,500.

The sliding filament model of muscle contraction, independently proposed by Huxley and Niedergerke (1954) and Huxley and Hanson (1954), has gained wide acceptance. Subsequent fine structural studies aided by the use of the thin sectioning technique (Huxley, 1957; Locker, 1959) together with X-ray diffraction studies (Elliott, et al., 1963) have clearly shown the structural basis for this model. Although most changes in banding patterns observed when striated muscle contracts are explained by this sliding of interdigitating filaments, the nature of the forces responsible or the chemical bonds formed and broken remains enigmatic. Evidence implicating movement of the actin-myosin cross-bridges during contraction has been supplied by Huxley et al. (1965) and Elliott et al. (1965) through the use of an X-ray camera with high resolving power in two directions. Reedy et al. (1965), by using insect fibrillar flight muscle, have also shown that actin-myosin cross-bridges are slanted at an angle of 45° to the filament axis in rigor fibers that have had their ATP washed out. Cross-bridges in fibers containing ATP were oriented almost perpendicular to the filament axis.

Clearly, it would be important to have an ultrastructural characterization of the changes during rigor mortis. On the one hand, this would contribute to a better description of postmortem alteration. On the other hand, such work might yield information of interest to interpret the properties of living muscle and its activities. Therefore, the primary purpose of this study was to follow morphological changes in banding patterns while the muscle entered rigor mortis.

The study reported here involves the use of thin sectioning and electron microscopy to observe the changes occurring in bovine striated muscle as it enters rigor at various temperatures. For this study, the condition of rigor mortis is defined in terms of shortening or stiffening rather than in terms of ATP loss. Many of the changes in banding

pattern accompanying rigor shortening are identical to those observed by Hanson and Huxley (1955) for physiologically contracting muscle. However, certain other changes may be due to the temperature effects on the contractile apparatus. During the course of this study, it was observed that trypsin had slightly different effects on prerigor and rigor myofibrils. Thus, some results of the effects of trypsin treatment on myofibril structure are included. The possible use of the slow shortening of muscle that is entering rigor as a contraction model is discussed.

MATERIALS AND METHODS

The right and left semitendinosus muscles of seven heifers were excised as soon as possible after bleeding and samples were removed immediately after excision. One muscle was then placed in a cold room at 2° ± 1°C and its homologue at either 16° ± 1°C or 37° ± 1°C. To prevent desiccation during storage, the muscles were placed in a cellophane bag which was then evacuated, sealed, and quickly dipped in warm water to form a tight air- and moisture-impermeable cover. Every animal in this study was sampled immediately after death and after 24 hr at 2°C and either 16° or 37°C. For four animals, additional samples from these same muscles were taken after 312 hr at 2° and at 16°C. The other three animals were further sampled only after 24 hr storage at 2° and 37°C. Other studies (Busch, Parrish, and Goll, unpublished results) showed that ATP level in these muscles was less than 0.2 mM after 12 hr at any of the temperatures studied. Thus, muscle excitability was a problem only in the at-death samples and use of a modification of the biopsy technique reported by Price et al. (1965) prevented shortening in these samples. Two parallel, 1–2 mm cuts were made on either side of a strip of muscle 3–5 mm wide and 40 mm long; the corner of a razor blade was used to loosen the strip from its deep attachments. A dissecting needle was employed to slip surgical threads under the strip near its ends, a 55 mm glass rod was placed on the strip, the threads were securely tied to the rod, and the strip was freed from its attach-

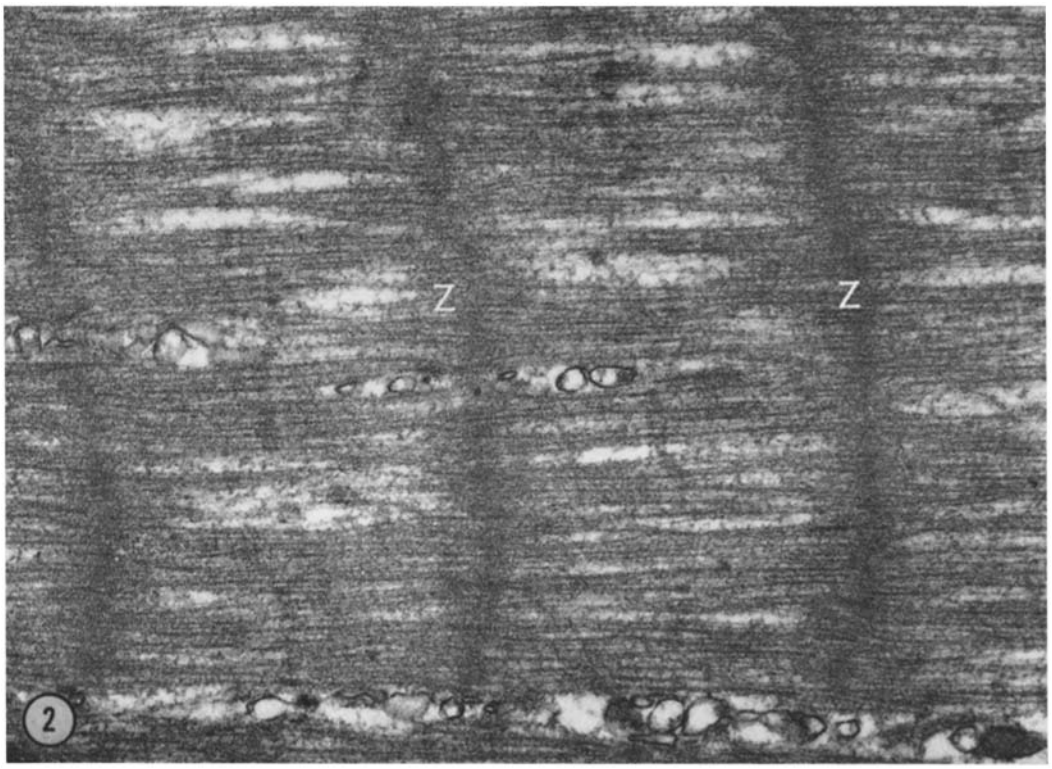

FIGURE 2 Micrograph of muscle sampled after 24 hr storage at 2°C, showing the typical supercontracted appearance seen in most samples with this treatment. Note the lateral displacement of fragments of the M line. *Z*, Z line. Section stained with uranyl acetate and lead citrate. × 45,500.

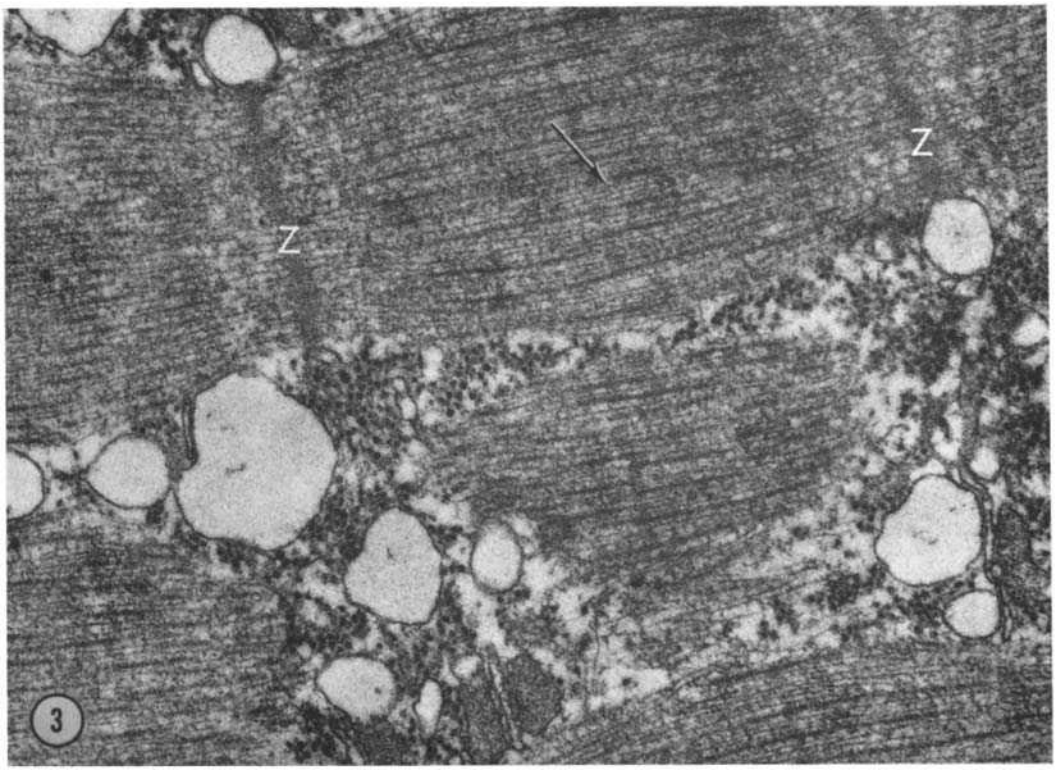

FIGURE 3 Although not supercontracted, this section of muscle sampled after 24 hr storage at 2°C shows quite clearly that sliding of filaments has occurred. In the center of the A band (arrow) four thin filaments are seen between adjacent, thick filaments. Section stained with uranyl acetate and lead citrate. Z, Z line. × 48,000.

ments. To eliminate any variability in sample preparation, this same technique was used to remove all postmortem samples discussed in this paper although identical results could be obtained with rigor muscle by simple excision of the muscle strip.

The strip of muscle was then immersed in cold 2.5% glutaraldehyde in 0.2 M Sorensen's phosphate buffer at pH 7.15. After 1 hr, the glutaraldehyde was drawn off and replaced with fresh fixative solution. At the end of the second hour, the glutaraldehyde was removed, and the fixed fibers were washed with Sorensen's phosphate buffer for 1–3 min. After removal of this rinsing solution, the central part of the strip was freed from the glass rod, placed on dental wax, flooded with buffer, and cut into 1 mm cubes. These cubes were then transferred to cold 1% osmium-tetroxide solution that was similarly buffered for 2 hr with a change to fresh solution after the first hour. All fixative and rinse solutions were adjusted by buffer dilution to an osmotic strength of 480 milliosmols calculated by the method of Powell et al. (1964). Dehydration in graded acetone was followed by infiltration and embedding in an Epon-Araldite mixture as reported by Anderson and Ellis (1965).

After polymerization at 60°C for 24 hr and a 2–3 day curing period, thin sections were cut with a diamond knife on a Reichert ultramicrotome, model Om U2. All biopsy samples of intact muscle were oriented so the long fiber axis was parallel to the knife edge. However, isolated myofibrils pelleted prior to embedding had random orientation and therefore could not be oriented with respect to the knife edge. Sections of approximately 75 mμ or less, as judged by interference colors, were used with a strong preference for those of 60 mμ or less. Uncoated, 300-mesh grids were used to mount the sections, which were then stained for 50–60 min by using 2% uranyl acetate in methanol and were rinsed in two changes each of methanol, 50% methanol, and water. After thorough drying, undiluted lead-citrate stain was applied for 12 min by using the method of Reynolds (1963). A second staining method used an aqueous uranyl acetate–phosphotungstate solu-

tion. Separate aqueous stock solutions, containing either 2% uranyl acetate or phosphotungstate were made up in the usual manner. Immediately prior to staining, equal parts of the 2% uranyl acetate pH 4.32 and of the 2% phosphotungstate pH 1.95 solutions were mixed together. The pH of the resultant mixture was 3.32. After a 15–20 min staining period, grids were washed with water and allowed to air dry. All electron micrographs were taken with a RCA EMU 3-F instrument operated at 50 kv.

Myofibrils for trypsin experiments were prepared by grinding a portion of the muscle through an Oster Model 516 grinder and homogenizing this ground muscle in 5 volumes of a solution of 0.25 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.05 M Tris, pH 7.6 by using three 15-sec homogenizations. After 1 hr, myofibrils were collected by centrifugation, resuspended in the solvent, and extracted for another hour. Centrifugation of this suspension yielded a precipitate which was then suspended in 0.05 M Tris, 1 mM EDTA, pH 7.6, and passed through a strainer to remove connective tissue. The myofibrils were again sedimented and resuspended in the following solutions in the order given: (*a*) 0.15 M KCl; (*b*) 1 mM EDTA, pH 7.6; (*c*) deionized glass distilled water; (*d*) 0.15 M KCl. After the final centrifugation, myofibrils were suspended in 0.15 M KCl and stored at 2° for biuret determinations. Biuret analyses were done by suspending the myofibrils in 0.1 N NaOH for 30 min followed by addition of biuret reagent. This treatment resulted in solubilization of the myofibrillar protein. This method of myofibril isolation yielded preparations in which the Z line structure and regularity of filament orientation were better preserved, when compared with sections from intact portions of the same muscle, than were Z lines or filament orientation in myofibrils prepared by KCl or glycerol solutions. Myofibrils were prepared from muscle immediately after death and from muscle kept 24 hr postmortem at 2°.

For trypsin treatment, all suspensions were diluted to a protein concentration of 4–5 mg/ml. Hydrolysis of myofibrils was done by using trypsin to myofibril ratios of 1:100 (w/w) at 24°C and pH 7.6. Purified,

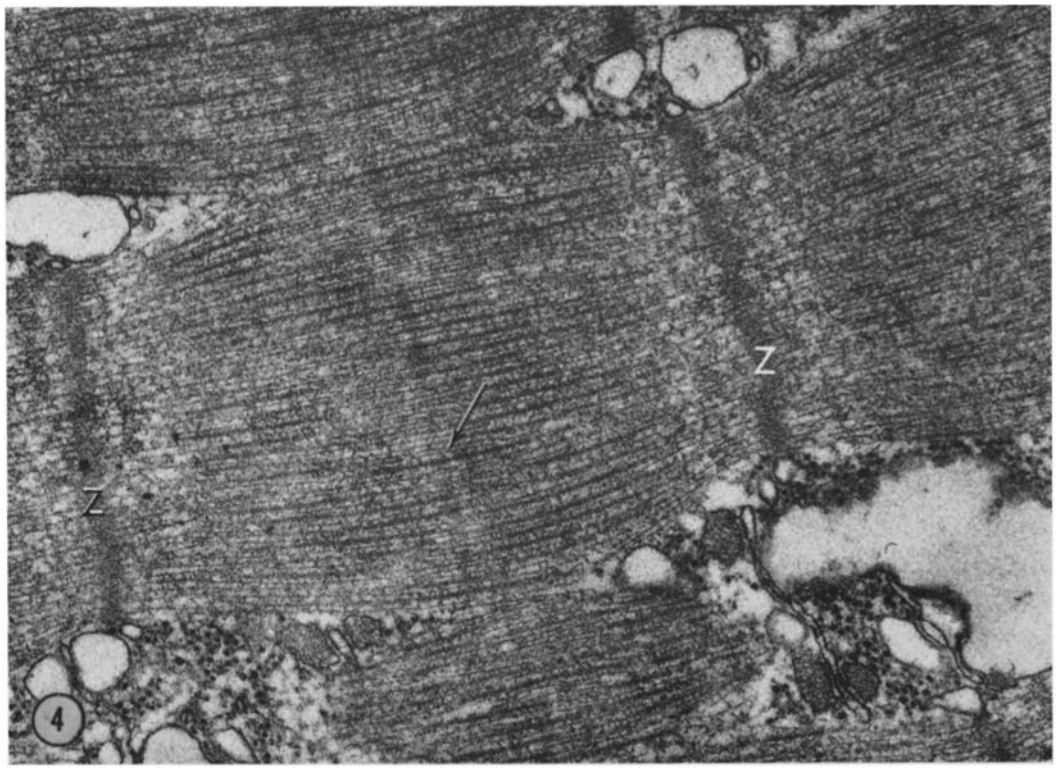

FIGURE 4 Thin section of muscle sampled 24 hr postmortem after storage at 2°C. The state of contraction is similar to that seen in Fig. 3. Plane of sectioning shows two thin filaments between adjacent thick filaments in the center of the A band (arrow). This, too, indicates that sliding of filaments has occurred. *Z*, Z line. Section stained with uranyl acetate and lead citrate. × 47,500.

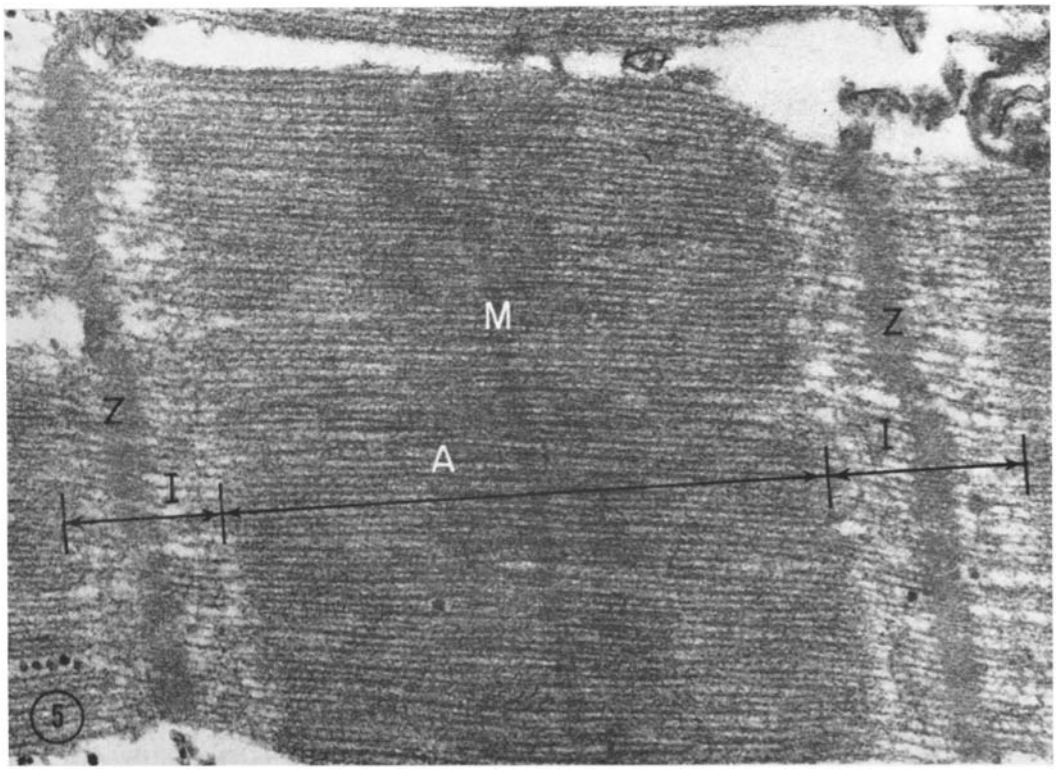

FIGURE 5 Muscle sampled after 24 hr storage at 16°C. Muscle with this treatment is slightly contracted as indicated by an absence of H zones and a small degree of sarcomere shortening. Section stained with uranyl acetate and lead citrate. × 61,500.

crystalline trypsin, essentially salt free, was purchased from Sigma Chemical Co. (St. Louis, Mo.) and dissolved in 0.001 N HCl just prior to use. Hydrolysis was terminated by the addition of a fourfold excess of either ovomucoid or soybean trypsin inhibitor. An aliquot of this reaction mixture was placed in glass centrifuge tubes and sedimented. The supernatant was discarded, and 2–3 ml of 2.5% glutaraldehyde were carefully layered onto the pellet which was removed from the tube, flooded with fixative, and cut into 1 mm cubes. The remainder of the specimen preparation for electron microscopy was performed as described in the preceding paragraphs. It should be noted that the term muscle refers to intact muscle sampled with the biopsy technique and that the term myofibrils refers to isolated homogenates taken from the same muscles as the biopsy samples.

OBSERVATIONS

The morphology of muscle sampled immediately after death will first be described and then will be compared with the appearance of rigor-shortened muscle. In all cases, the biopsy technique was used so that valid comparisons might be made.

Muscle sampled immediately after death and fixed as described had the appearance of resting muscle. More complete descriptions may be found in the literature (Huxley, 1960; Bloom and Fawcett, 1962), but, for purposes of orientation, structural features pertinent to this study are reviewed briefly. Wide I bands and a prominent but narrow H zone are evident in Fig. 1; lateral edges of the A band are straight as is the M line. When Huxley's (1966) technique for obliquely viewing micrographs is used on Fig. 1, the M line shows a substructure consisting of three lines, one in the center and one on each edge. Glycogen granules, Z lines, and elements of the sarcoplasmic reticulum also are evident.

The supercontracted condition similar to that seen in barnacle muscle by Hoyle et al. (1965) is particularly evident in muscle stored at 2°C and

sampled 24 hr postmortem (Fig. 2). Sarcomeres are decidedly shortened, and filamentous material has accumulated around the Z line. In some locations, it appears that thick filaments have passed through the Z line although the thickness of the sections does not permit a definite conclusion in this regard. Segments of the M line appear to be displaced either to the right or left of the normal position. Of the seven animals in this group, the only animal from which 2°C, 24 hr muscle was not supercontracted is shown in Figs. 3 and 4. These relatively thin sections are included to show quite clearly that the shortening occurring here is due to a sliding of filaments since the plane of sectioning shows two thin filaments between adjacent thick filaments at the lateral edges of the A band, and four thin filaments are observed in the center of the A band between the thick filaments (Fig. 3). A section of similar thickness (Fig. 4) shows one thin filament between pairs of thick filaments at the edges of the A band but two thin filaments in the center of the A band. Sarcomere lengths in the 2°C, 24 hr muscle were always considerably shorter than those observed in muscle sampled immediately postmortem. Although some variability in sarcomere lengths is commonly observed, even within a single fiber, measurements of sarcomeres showed a mean sarcomere length of 1.2 ± 0.02 μ after 24 hr at 2°C compared with 2.7 ± 0.05 μ immediately after death. A band length in muscle sampled immediately postmortem was 1.5 ± 0.02 μ, so shortening in the 2°C, 24 hr muscle must involve some overlap or crumpling of the thick filaments in the region of the Z band. This shortening is most easily observed by noting the characteristic banding changes which were always evident in the postmortem muscle. These banding pattern changes will be the principal topics of discussion in the subsequent paragraphs.

After storage at 16°C, muscle sampled 24 hr

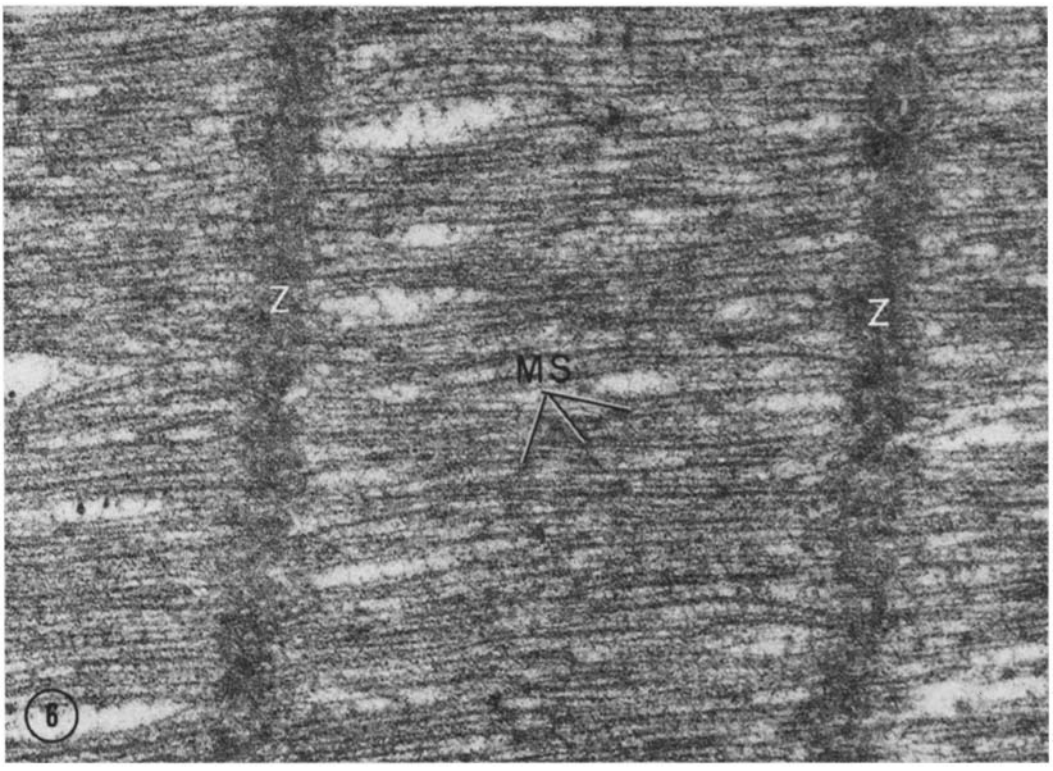

FIGURE 6 Structural disruption of this sample removed from muscle after 24 hr storage at 37°C is apparent. M line segments (*MS*) are displaced both to the left and to the right of the normal M line location. Absence of I bands and thickening of the area around the Z line indicates that supercontraction has occurred. Section stained with uranyl acetate and lead citrate. × 59,500.

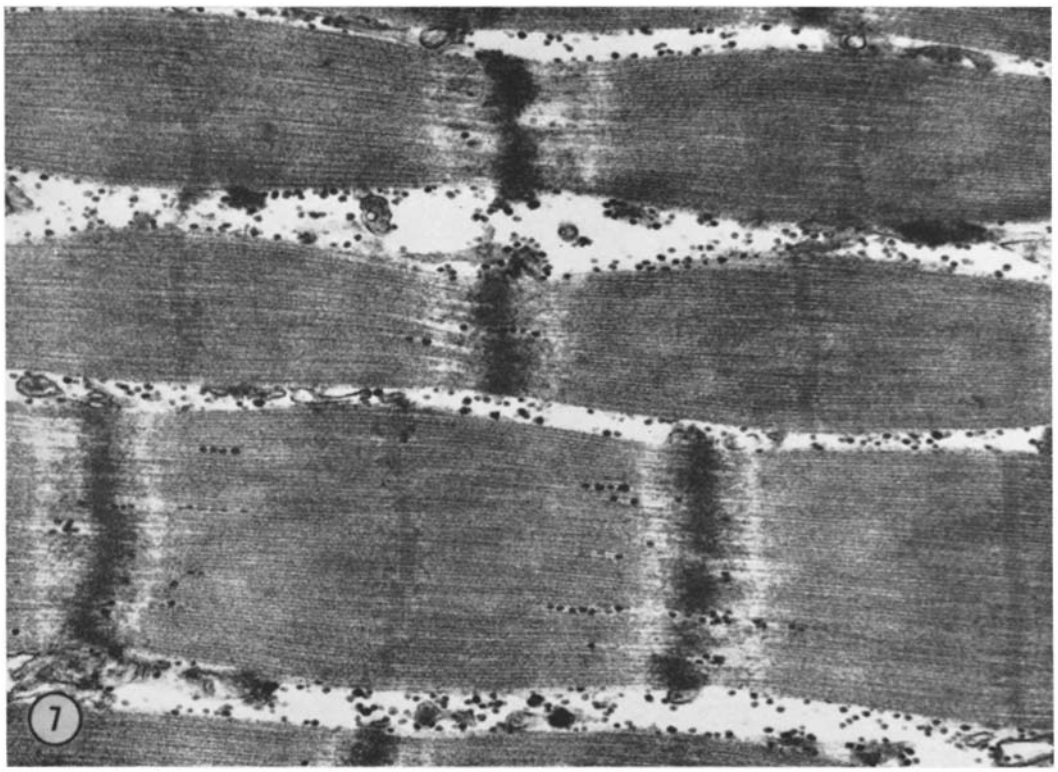

FIGURE 7 Muscle stored at 2°C and sampled 312 hr postmortem. The moderately contracted state seen here has replaced the supercontracted pattern seen after 24 hr storage at 2°C. Section stained with uranyl acetate and lead citrate. × 42,000.

postmortem shows slight shortening (Fig. 5) to a mean sarcomere length of 2.0 ± 0.06 μ. H zones are always absent, and I bands are usually 25% narrower than prerigor muscle. The sarcoplasmic reticulum has degenerated; the concentration of glycogen granules has decreased, but mitochondria retain their structural integrity.

Muscle stored at 37°C and sampled 24 hr postmortem also shows drastically shortened sarcomeres (Fig. 6). The mean sarcomere length of muscle subjected to this treatment was 1.5 ± 0.04 μ. The filaments of the contractile apparatus appear straight, and thick filaments can be followed into the area of increased density around the Z line. Segments of the M line are, at best, difficult to find; therefore, information about whether bundles of thick filaments are displaced, as seems to be the case at 2°C, remains obscure.

A striking departure from the supercontracted state is observed in all cases in muscle held at 2°C for 312 hr (Fig. 7). The 312-hr samples in this study were all taken from muscle which had been previously sampled at death and at 24 hr so direct comparison to Figs. 1 and 2 is possible. After 312 hr at 2°C, the mean sarcomere length was increased to 1.8 ± 0.04 μ. Although narrow I bands are again present, the area next to the Z line still shows some increase in density compared with the normal I band. The regularity of the thick filaments and the realignment of the previously displaced elements of the M line clearly indicate that a rearrangement occurred. Storage at 16°C for 312 hr resulted in muscle (Fig. 8) in a state of contraction similar to that observed after 24 hr at 16°. However, after 312 hr, mean sarcomere length was slightly decreased to 1.7 ± 0.04 μ. Storage for 312 hr at either 2° or 16°C resulted in a similar degree of contraction (Figs. 7, 8). It is interesting to note the large amount of glycogen or glycogen-like granules in the 312 hr muscle, particularly in the 16°C samples (Fig. 8).

Morphology of myofibrils isolated with sucrose

solution is shown in Figs. 9 and 10. Myofibrils isolated immediately after death appear relaxed, as evidenced by wide I bands (Fig. 9). The effects of the isolation procedure have caused disorganization in the A band and obscured the H zone. Although the Z line exhibits some discontinuities, ultrastructure of the Z line (inset) has been preserved. Myofibrils isolated from muscle stored at 2°C for 24 hr no longer show I bands, but thick filaments are more highly ordered than in myofibrils from at-death muscle (Fig. 10). This indicates that (*a*) the muscle is shortened when the sample is removed and (*b*) rigor-shortened muscle is better able to withstand the stress of the isolation procedure.

The effects of trypsin treatment on myofibril fine structure is shown in Figs. 11–13. The myofibrils in these micrographs were treated with trypsin for varying lengths of time but originally were part of the untreated homogenate shown in Figs. 9 and 10. The first effect of trypsin treatment is the removal of Z lines after 1–1½ min of hydrolysis. Myofibrils isolated from muscle sampled immediately after death and treated with trypsin for 1–2 min frequently show widened I bands due to a crumpling or withdrawal of thick filaments to a narrow band on both sides of the H zone (Fig. 11). However, thick filaments remain straight across the H zone region and, after 5 min of trypsin treatment, again become straight throughout most of their length (Fig. 13). Concomitantly, supercontracted rigor-shortened muscle extends, and a rather sudden departure from the contracted state occurs. After the sarcomere becomes extended, the degree of order appears much higher in rigor muscle than in prerigor muscle similarly treated with trypsin. This higher degree of order is evident within the sarcomere from muscle stored at 2°, sampled 24 hr after death, and treated with trypsin for 5 min (Fig. 12). An example of typical morphology of myofibrils isolated from muscle sampled at death and treated with trypsin for 5 min shows

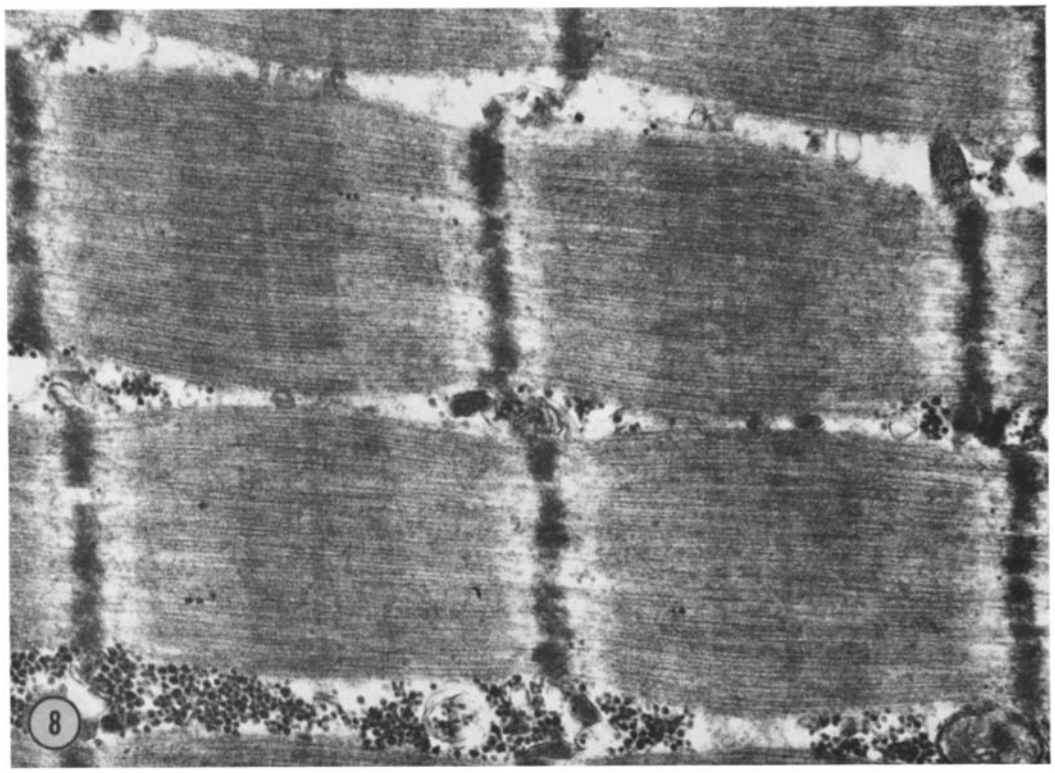

FIGURE 8 Muscle stored at 16°C and sampled 3½ hr postmortem. The degree of contraction evident here is quite similar to that seen in Fig. 7. Note the accumulation of particles which resemble glycogen. Section stained with uranyl acetate and lead citrate. × 38,000.

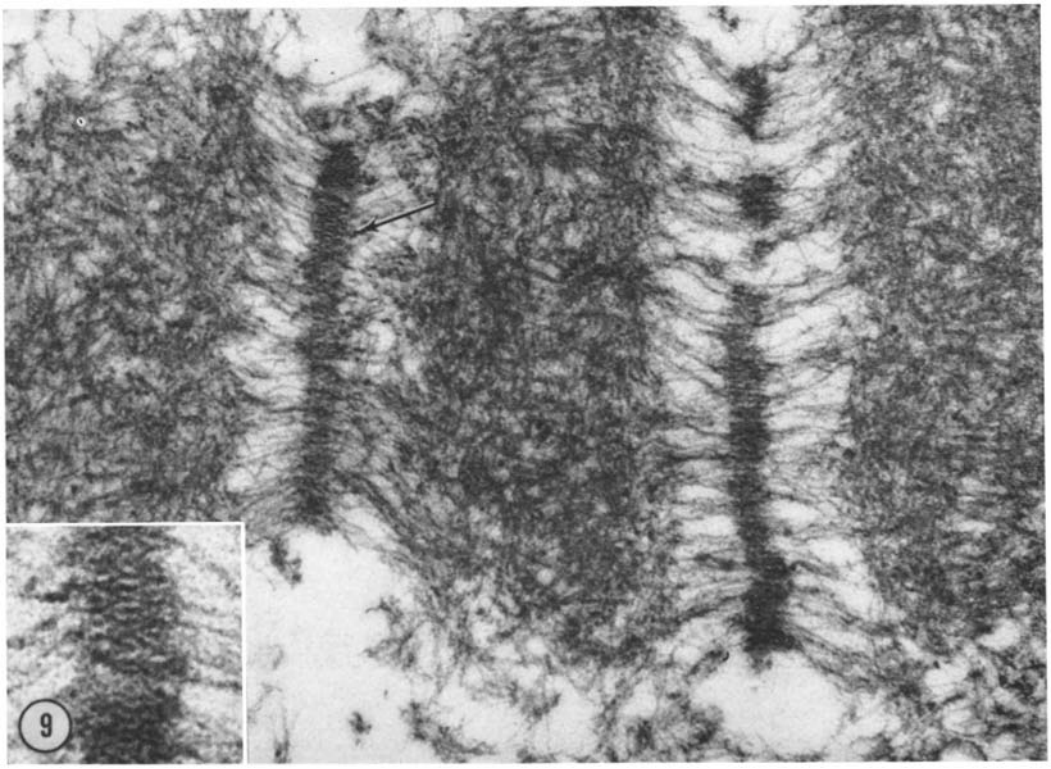

FIGURE 9 Myofibrils isolated at death by using sucrose solution. Arrow indicates location where ultrastructure of Z line may be seen. Section stained with uranyl acetate-phosphotungstate solution. × 48,000. Inset is a higher magnification of the area pointed out by the arrow. × 120,000.

A and I bands and H zones but relatively few thin filaments protrude from the disorganized A band (Fig. 13). That the relationship between interdigitating thick and thin filaments has been undisturbed is evident; after extended periods of trypsin treatment, more thin filaments extend from the A band in rigor than in prerigor muscle.

DISCUSSION

Muscle in Rigor

The results of this study show that postmortem shortening accompanying the onset of rigor mortis involves changes in banding patterns which are in agreement with Huxley and Hanson's (1954) sliding filament model and also demonstrate in mammalian muscle the occurrence of supercontraction which resembles that reported by Hoyle et al. (1965) in a barnacle muscle. That sliding of filaments also occurs during postmortem shortening at 2°C has been demonstrated by the presence of a double overlap of actin filaments in the center of the sarcomere. This finding substantiates Locker's (1959) conclusion and suggests that postmortem shortening may be a useful system to gain information about actin-myosin interactions leading to relative movements among thick and thin filaments. In addition, it appears that some perforation of Z lines may occur during postmortem shortening of bovine muscle, although the penetration is not as extensive as that demonstrated by Hoyle et al. (1965). However, it is not yet possible to determine whether the sliding of filaments accounts for all the morphological changes observed or whether supercontraction of bovine muscle will occur in vivo. The effects of temperature on the contractile apparatus suggest that other changes or mechanisms may be involved, e.g. a high degree of shortening occurs if muscle is stored at 2°C for 24 hr, but considerable elongation from the supercontracted pattern is seen after 312 hr at this temperature.

That bovine muscle exhibits a minimal degree of postmortem shortening at 16°C but that rabbit muscle undergoes minimal shortening at 0° (Bendall, 1960; Stromer and Goll, unpublished observations) indicates that care must be taken in extrapolation to other species and that differences in reactive groups or structures probably exist between species. The departure from a supercontracted state after 312 hr storage at 2°C is difficult to explain if rigor shortening is totally irreversible as thought by some researchers. It is doubtful for several reasons, that the relaxation in the 312 hr muscle was caused by an autolytic removal of the cross-bridges. Firstly, it would be surprising to find autolytic changes after storage at 2°C and not see any effects after storage at 16°C. Secondly, the nucleosidetriphosphatase activities of myofibrils prepared from muscle after 312 hr postmortem at 2°C was very similar to those prepared from muscle immediately after death (Goll and Robson, 1967). Removal of the cross-linkages should have altered the typical modification of myosin ATPase caused by actin. Thirdly, these cross-linkages are probably composed principally of heavy meromyosin (Huxley, 1963) which is water-soluble. Thus, had autolytic removal occurred, it should have been possible to extract heavy meromyosin directly from the 312 hr muscle. Attempts to perform this extraction with 0.15 M KCl, 0.03 M Tris, pH 7.6, were unsuccessful.

The extreme shortening in the 2°C, 24 hr muscle is accompanied by displacement of bundles of thick filaments connected by segments of the M line either to the right or left of the center of the A band. For this to occur, either certain actin-myosin bonds would need to be broken, or some actin filaments would need to be stretched. Neither of these events would have to occur if each thick filament interacted with actin on only one side at the H zone. Hoyle et al. (1965) have previously indicated that the apparent shrinkage of A bands during supercontraction or stretching could be explained in terms of sliding filaments if displacement of thick filaments occurred, either to the left

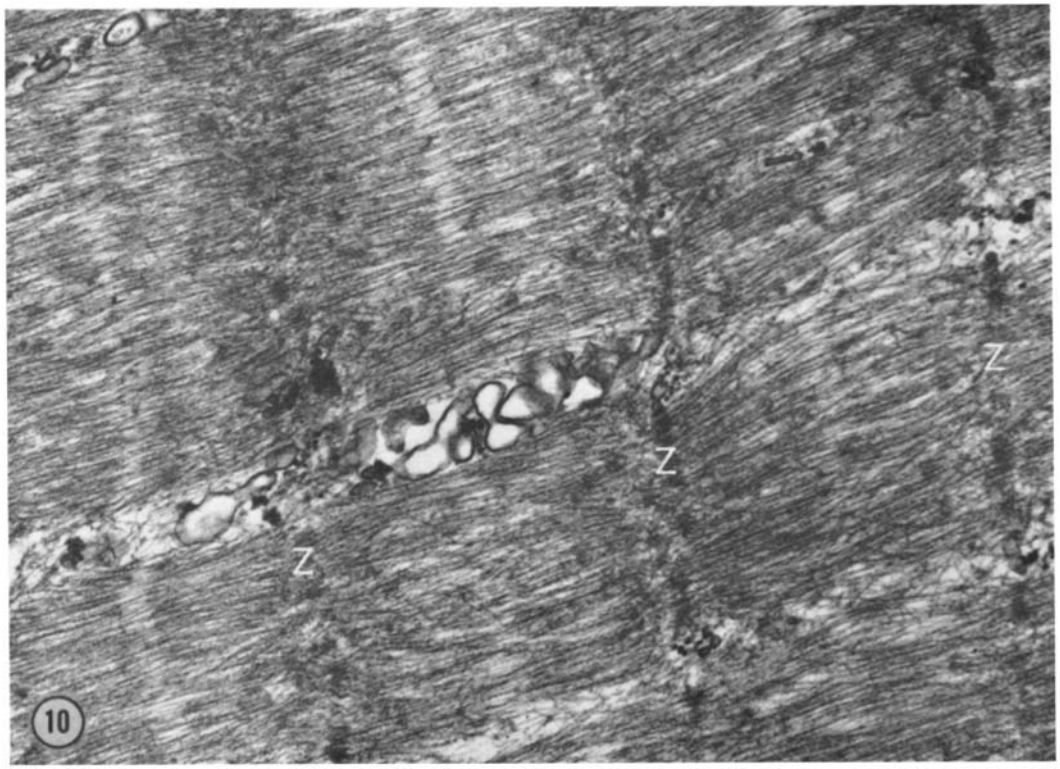

FIGURE 10 Myofibrils isolated with sucrose solution after 24 hr storage at 2°C. Note the accumulation of filaments around the Z lines. Section stained with uranyl acetate–phosphotungstate solution. × 38,000.

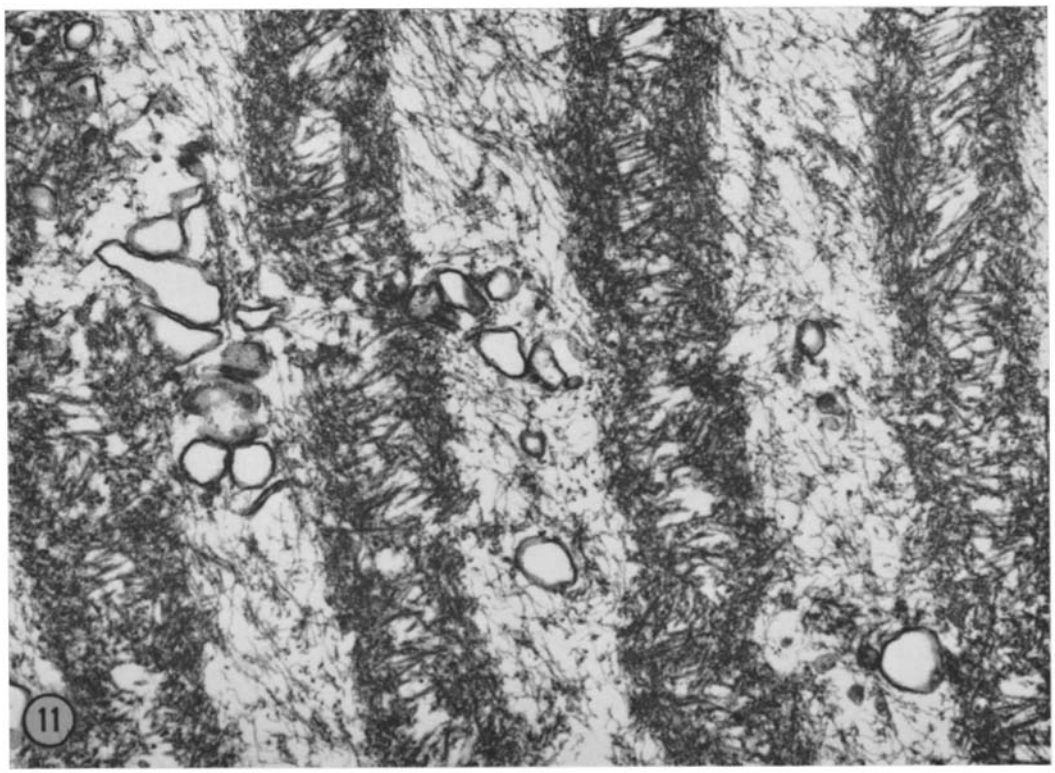

FIGURE 11 Myofibrils isolated at death and treated with trypsin for 2 min. Note the retraction of thick filaments to either side of the H zone and also the absence of Z lines. Section stained with uranyl acetate and lead citrate. × 39,500.

or right of their equilibrium positions. Hoyle et al. do not, however, offer any suggestions for the mechanism of these displacements. It is possible that the myosin-myosin bonds discussed by Hoyle et al. are in fact the elements of the M line. Although we have frequently observed what appears to be penetration of the Z line by thick filaments in intact muscle, just as did Hoyle et al., in neurally-evoked contraction, this phenomenon was not observed in rigor-shortened myofibrils. The intact muscle samples and the samples for myofibril preparation were removed from the same muscle; therefore, it seems likely that the differences between the myofibril and the intact muscle samples may have been caused by our myofibril isolation procedure.

Other studies (Busch, Parrish, and Goll, unpublished results) have shown that shortening at 2°C begins within 3 hr after death, while ATP levels are still above 1 mM. Shortening at 37°C, however, begins later, about 5–8 hr after death, when ATP levels are below 0.2 mM. These same studies have shown that if shortening is measured by isometric tension development, the ability to maintain tension is gradually lost after 24–48 hr at 2°C, but the ability to maintain tension at 37°C remains constant until bacterial decomposition destroys the strip. For these reasons, it seems possible that postmortem shortening at 2°C represents a true contraction initiated by either (*a*) a lowering of ATP to a level that permits actin-myosin interaction in the presence of 5–10 mM Mg^{++}, 100–150 mM KCl, and low ($<10^{-6}$–10^{-7} M) levels of Ca^{++} (Levy and Ryan, 1965), or (*b*) a gradual efflux of Ca^{++} from the membranes of the sarcoplasmic reticulum caused by a lowering of ATP to a level insufficient to fully maintain the activity of the Ca^{++} pump (Hasselbach, 1964). Shortening at 37°C is a different process, possibly initiated by the low (<5.5) pH values which occur very quickly postmortem in muscle stored at 37°. The sliding filament theory suggests that muscle

contraction occurs through a repetitive making and breaking of bonds between the actin and myosin filaments. In view of the commonly accepted theories regarding the role of ATP in dissociation of actin and myosin, it is quite surprising that any shortening can occur at the low ATP levels existing 5–8 hr postmortem in the 37°C muscle. Marsh (1953) has shown that loss of extensibility and shortening occur simultaneously in postmortem bovine muscle at 37°C. Since loss of extensibility in rigor muscle is usually interpreted as a manifestation of a combination of thick and thin filaments due to low ATP levels, Marsh's results also indicate that shortening at 37°C may not be a typical contraction involving breaking of actin-myosin bonds by ATP.

Trypsin Treatment

Because, after extended periods of trypsin treatment, more thin filaments extend from the A band in rigor than in prerigor muscle, it appears that rigor shortened myofibrils are more resistant to the hydrolytic action of trypsin than are prerigor myofibrils. Superficially, this could be explained on the basis of some steric hindrance caused by the shortening. However, even after Z lines have been removed and the supercontracted myofibrils have relaxed, these myofibrils continue to have increased resistance to trypsin. This information suggests that either rigor-shortening itself or some other phenomenon accompanying rigor mortis is responsible for subtle changes in thick and thin filaments.

The departure from the supercontracted state after trypsin treatment is accompanied by a substantial lateral movement of the thin filaments relative to the thick filaments. In addition a realignment of the thick filaments occurs, even though an M line is not visible after rearrangement. If the Z line were composed principally of tropomyosin, as has been suggested by Corsi and Perry (1958) and Huxley (1963), it might be ex-

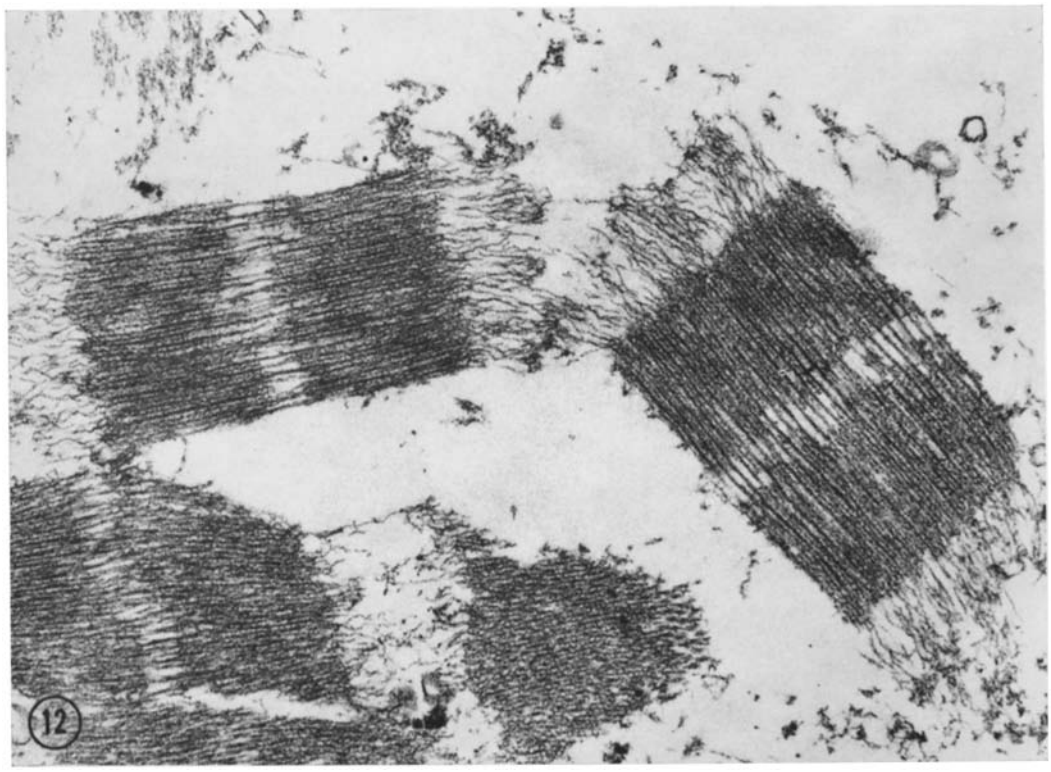

FIGURE 12 Myofibrils from muscle stored at 2°C, sampled 24 hr postmortem and treated with trypsin for 5 min. The two sarcomeres show thin filaments extending beyond the ends of the thick filaments. Section stained with uranyl acetate and lead citrate. × 39,500.

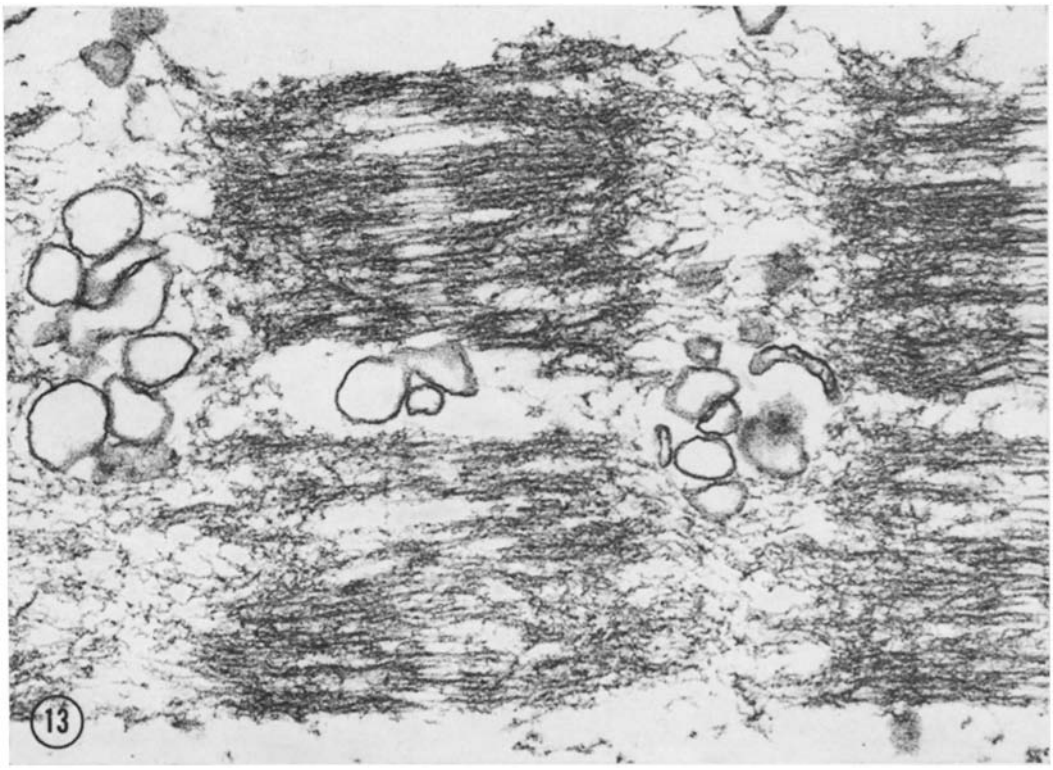

FIGURE 13 Myofibrils isolated at death and treated with trypsin for 5 min. Structural integrity of this sample is poorer than that seen in Fig. 12. However, thin filaments are still present in the I band and between thick filaments. Section stained with uranyl acetate and lead citrate. × 54,500.

pected to be quite sensitive to proteolytic degradation in view of the reported sensitivity of tropomyosin to trypsin (Laki, 1957).

The authors are grateful to Dr. R. A. Jenkins for stimulating and helpful discussions and to Mrs. Nancy Roth for her review of the manuscript.

This study was supported in part by Public Health Service Research Grant GM 12488. Journal Paper No. J-5540 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1549.

Received for publication 7 November 1966.

REFERENCES

ANDERSON, W. A., and R. A. ELLIS. 1965. Ultrastructure of Trypanosoma lewisi: flagellum, microtubules and the kinetoplast. *J. Protozool.* **12**:483.

BATE-SMITH, E. C. 1939. Changes in elasticity of mammalian muscle undergoing rigor mortis. *J. Physiol.* **96**:176.

BATE-SMITH, E. C., and J. R. BENDALL. 1947. Rigor mortis and adenosinetriphosphate. *J. Physiol.* **106**:177.

BATE-SMITH, E. C., and J. R. BENDALL. 1949. Factors determining the time course of rigor mortis. *J. Physiol.* **110**:47.

BENDALL, J. R. 1951. The shortening of rabbit muscle during rigor mortis: its relation to the breakdown of adenosinetriphosphate and creatine phosphate and to muscular contractions. *J. Physiol.* **114**:71.

BENDALL, J. R. 1960. Post-mortem changes in muscle. *In* Structure and Function of Muscle. G. H. Bourne, editor. Academic Press Inc., New York, **3**:227.

BLOOM, W., and D. W. FAWCETT. 1962. Muscular tissue. *In* A Textbook of Histology. W. B. Saunders Co., Philadelphia. 187.

CORSI, A., and S. V. PERRY. 1958. Some observations on the localization of myosin, actin and tropomyosin in the rabbit myofibril. *Biochem. J.* **568**:12.

ELLIOTT, G. F., J. LOWY, and B. M. MILLMAN. 1965. X-ray diffraction from living, striated muscle during contraction. *Nature.* **206**:1357.

ELLIOTT, G. F., J. LOWY, and C. R. WORTHINGTON. 1963. An x-ray and light diffraction study of the filament lattice of striated muscle in the living state and in rigor. *J. Mol. Biol.* **6**:295.

GOLL, D. E., and R. M. ROBSON. 1967. Molecular properties of post-mortem muscle. I. Myofibrillar nucleosidetriphosphatase activity of bovine muscle. *J. Food Sci.* In press.

HANSON, J., and H. E. HUXLEY. 1955. The structural basis of contraction in striated muscle. *Symp. Soc. Exptl. Biol.* **9**:228.

HASSELBACH, W. 1964. Relaxing factor and the relaxation of muscle. *Progr. Biophys. Mol. Biol.* **14**: 167.

HOYLE, G., J. H. MCALEAR, and A. SELVESTER. 1965. Mechanism of supercontraction in a striated muscle. *J. Cell Biol.* **26**:621.

HUXLEY, A. F., and R. NIEDERGERKE. 1954. Structural changes in muscle during contraction. Interference microscopy of living muscle fibers. *Nature.* **173**:971.

HUXLEY, H. E. 1957. The double array of filaments in cross-striated muscle. *J. Biophys. Biochem. Cytol.* **3**:631.

HUXLEY, H. E. 1960. Muscle cells. *In* The Cell. J. Brachet and A. F. Mirsky, editors. Academic Press Inc., New York. **4**:366.

HUXLEY, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* **7**:281.

Huxley, H. E. 1966. The fine structure of striated muscle and its functional significance. *In* The Harvey Lectures. Academic Press Inc., New York. **60**:85.

HUXLEY, H. E., W. BROWN, and K. C. HOLMES. 1965. Constancy of axial spacings in frog sartorius muscle during contraction. *Nature.* **206**:1358.

HUXLEY, H. E., and J. HANSON. 1954. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature.* **173**:973.

LAKI, K. 1957. The composition of contractile muscle proteins. *J. Cell. Comp. Physiol.* **49** (Suppl. 1): 249.

LEVY, H. M., and E. M. RYAN. 1965. Evidence that calcium activates the contraction of actomyosin by overcoming substrate inhibition. *Nature.* **205**: 703.

LOCKER, R. H. 1959. Striation patterns of ox muscle in rigor mortis. *J. Biophys. Biochem. Cytol.* **6**:419.

MARSH, B. B. 1953. Shortening and extensibility in rigor mortis. *Biochim. Biophys. Acta.* **12**:478.

POWELL, T. E., C. W. PHILPOTT, and M. D. MASER. 1964. On the hydrogen ion concentration and osmolality of fixative components. *J. Cell Biol.* **23**: 110A.

PRICE, H. M., E. L. HOWES, D. M. SHELDON, O. D. HUTSON, R. T. FITZGERALD, J. M. BLUMBERG, and C. M. PEARSON. 1965. An improved biopsy technique for light and electron microscopic studies of human skeletal muscle. *Lab. Invest.* **14**: 194.

REEDY, M. K., K. C. HOLMES, and R. T. TREGEAR. 1965. Induced changes in orientation of the cross-bridges of glycerinated insect flight muscle. *Nature.* **207**:1276.

REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.