

MUSCLE SAMPLES FOR SCANNING ELECTRON MICROSCOPY: PREPARATIVE TECHNIQUES AND GENERAL MORPHOLOGY

ABSTRACT

Bovine semitendinosus and longissimus dorsi, both freshly slaughtered and aged, were prepared by various techniques prior to examination by SEM. The important problems of sample preparation were freeze damage, production of surface artifacts, and tissue shrinkage. Ethanol freeze fracture of fixed material followed by critical point drying or air drying from solvent circumvented most of the difficulties and provided the most useful specimens. Cleavage of muscle tissue by freeze fracture exposed muscle cell structures for observation. Useful magnifications up to 20,000 \times were obtained and identification was made of A, I and H bands, nuclei, and structures suggestive of mitochondria and muscle cell triads. The structural consequences of cold shortening and rigor were observed.

INTRODUCTION

THE STRUCTURE of muscle tissue and the changes associated with rigor and postmortem aging in various muscles have been extensively studied using conventional transmission electron microscopy (TEM) (Ramsbottom and Strandine, 1949; Stromer et al., 1967; Weidemann et al., 1967; Henderson et al., 1970; McCallister and Hadek, 1970; Dutson et al., 1974). Scanning electron microscopy (SEM) has been used to describe structures in mammalian skeletal muscle (Schaller and Powrie, 1971, 1972; Stanley and Geissinger, 1972; Eino and Stanley, 1973) and cardiac muscle (Sybers and Sheldon, 1975).

However, the SEM of muscle tissue is relatively new; there is by no means agreement among researchers concerning interpretation of several structures which appear in SEM micrographs of muscle (q.v., Sybers and Sheldon, 1975) or concerning preparative techniques for muscle samples.

Preparation of muscle tissue for viewing by SEM presents severe problems. Muscle cells seem especially sensitive to water removal and tissue shrinkage is difficult to avoid. Sample preparations involving cutting of any kind produce surface artifacts which make interpretation of micrographs difficult. Indeed, because SEM images surfaces, any abrasions of the fragile muscle specimen during conventional sample preparation limits the information from that sample. In some published micrographs, the morphology shown has clearly been affected by sample preparation.

In the course of our investigation of the relationship of muscle morphology to meat tenderness, we found it absolutely essential to find a method of sample preparation which gave reasonable, consistent preservation and gave samples for examination by SEM which were comparable in prior treatment in order to permit comparisons of structure. In the present paper, we report on the general morphology of bovine skeletal muscle as viewed by SEM after various preparative techniques. Hopefully, information presented here will provide a guide to sample preparations that produce SEM micrographs of consistently high quality which will aid in identifying and elucidating structures whose interpretation is controversial.

MATERIALS & METHODS

Muscle samples

Muscle tissue was either bovine longissimus dorsi or semitendinosus. Longissimus was obtained from a steer graded "good," 45 min postmortem (and fixed in the cold immediately), or from the same muscle stored 24 hr at 6°C. Semitendinosus was obtained from a supermarket [commercial beef is normally aged 2–4 wk at temperatures between 32° and 40°F (Ramsbottom and Strandine, 1949)]. The specimens for SEM were segments of muscle fiber bundles (1 cm² \times 4 cm), excised intact, and small blocks (1 cm³) of muscle, blade cut or freeze fractured to expose interior surfaces.

Fixation

Fixatives used were 2.5% glutaraldehyde in 0.1M phosphate buffer or a modified Karnovsky's-type fixative (Heald, 1971) containing 2% paraformaldehyde, 2% glutaraldehyde, and a trace of Ca⁺² (2.5 \times 10⁻³% CaCl₂) in 0.05M phosphate. The post-fix, when used, was 1% OsO₄ in 0.05M phosphate. Fixative was used at pH 7 for freshly slaughtered muscle and pH 6 for aged muscle.

Muscle bundles were fixed overnight. Other samples, less than 1 cm on a side, were immersed in fixative for 1 hr to firm the tissue, then trimmed to a smaller size and returned to fresh fixative for another hour. These steps were repeated until the tissue pieces were the desired sizes. Continuous attention was given to the eventual surface to be viewed in the microscope. Trimming was confined to wet samples as much as possible because of extreme fragility of specimens after drying. Total fixation time for tissue blocks was about 6 hr at room temperature. All fixed samples, bundles and pieces, were rinsed in three successive changes of 0.05M phosphate buffer for 10 min each and a final water rinse. Some samples were post-fixed for 1 hr at room temperature. Water-ethanol exchange was carried out by immersing the tissue in aqueous solutions of 70, 95, 95, 100, 100% ethanol, successively, for 10 min each. Acetone, as well as other concentrations of ethanol, were used in the dehydration series for comparison. The number of steps in the series and the immersion time at each step were also varied.

Freeze fracturing

This general procedure for exposing internal surfaces of tissues was done both by fracturing from water and from ethanol. In preliminary experiments, fixed and unfixed tissue specimens (1 cm \times 6 mm \times 5 mm) were frozen in Freon 22 cooled to near liquid nitrogen temperature. Many samples fractured spontaneously; others were snapped with a scalpel while the sample was submerged in liquid nitrogen. If specimens were unfixed at this stage, they were dropped immediately into fixative. Some of the samples were post-fixed. The preferred method of freeze-fracture was that suggested by Humphreys et al. (1974). Ethanol-impregnated fixed tissue (1.5 \times 1.5 \times 3 mm) was sealed in a liquid ethanol environment within a Parafilm cylinder which had been formed around a wooden applicator stick. The packets were frozen in liquid nitrogen and, while supported on a liquid nitrogen-cooled brass block, were fractured by pressure from a sharp, cold scalpel blade. Frozen halves were returned to fluid ethanol to thaw before drying by the critical point method or at room temperature in a stream of nitrogen gas.

Drying procedures

Methods of drying tissues were freeze drying, critical point drying, and air drying at room temperature in a stream of nitrogen gas. Freeze drying was carried out by conventional lyophilization (freezing in Freon 22 at liquid nitrogen temperature and allowing to warm up fairly

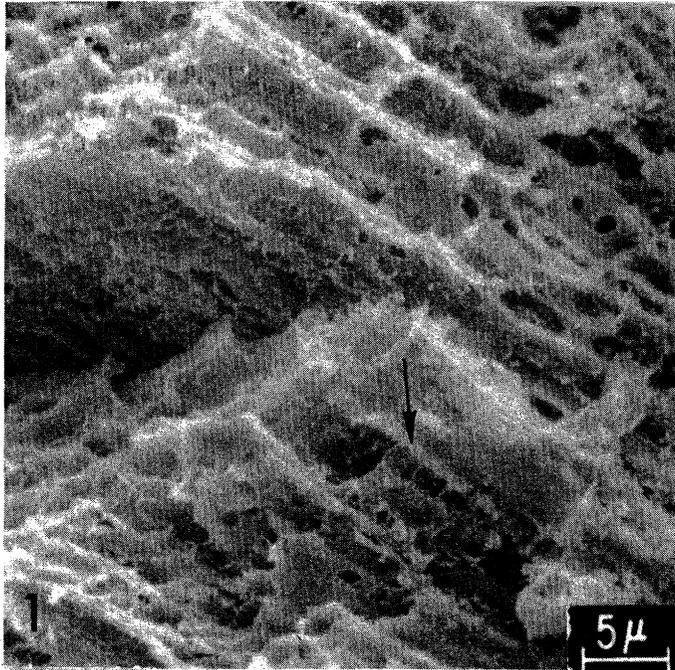


Fig. 1—Fracture surface through unfixed semitendinosus muscle fibers showing severe ice damage. Some remains of myofibrillar structure (arrow) and many ice crystal voids are seen.

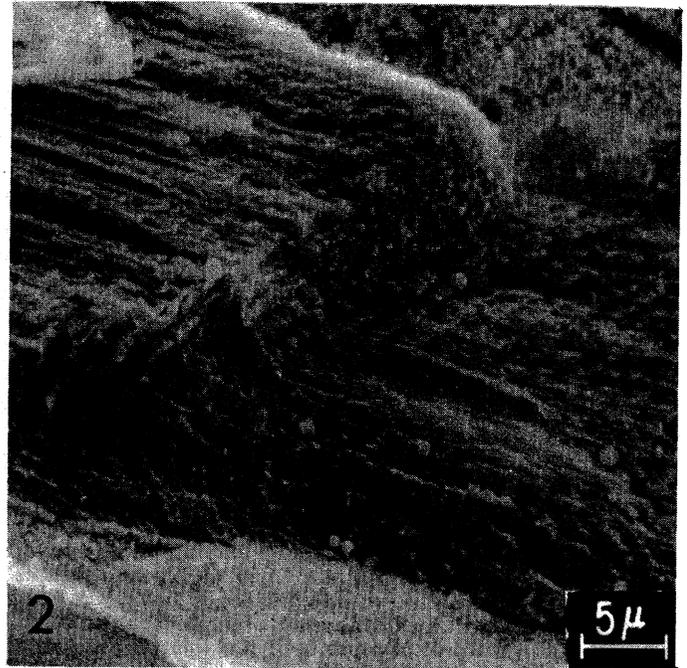


Fig. 2—Fracture surface through fixed semitendinosus muscle fibers. Myofibril structure maintained. Cleavage occurred parallel to fiber, with occasional vertical steps (arrow).

rapidly at a pressure of about 10^{-4} torr near a cold trap containing acetone-dry ice mixture; time, 2 hr) and by the conventional tissue-drying technique (freezing as above, laying samples in holes in a brass block chilled in liquid nitrogen, and placing block in a vacuum evaporator at 10^{-5} torr; time, 18 hr). The second method keeps the sample at a lower temperature (-60° to -70°C) while it dries. There is less risk of tissue damage by ice crystals reforming in the temperature range -40°C to 0°C (Boyde and Echlin, 1973).

For critical point drying, liquid CO_2 was used as transition fluid in a Denton Critical Point Drying apparatus. Samples were treated by dehydration in ethanol or acetone, followed by amyl acetate (Nemanic, 1972) or simply by dehydration followed by CO_2 exchange in the bomb (Humphreys et al., 1974). Exchange time in the bomb was determined by the size of the sample: 20 min for small samples (1 mm^3) or 45 min for larger pieces (5 mm^3). Air drying was used only with small (1 mm^3) samples which were impregnated with alcohol. Samples were placed in a shallow container which was flushed with a stream of nitrogen gas for about 2 hr.

All samples were stored in a desiccator when dry.

Mounting and metal coating for SEM

Dried tissue was attached to specimen stubs with conductive silver paint, with the surface of interest carefully oriented uppermost. Fractured surfaces when dry were usually easy to identify because they were flat and shiny when viewed through a dissecting microscope. Samples were painted on all sides except the top with conductive silver paint to minimize charging in the microscope, and were coated with approximately 150 nm of gold-palladium (60/40) on a rotary tilting stage in a vacuum evaporator. Coated samples were stored in a desiccator. Specimens were observed in a JSM-50A with an accelerating voltage of 15 kV and a specimen current of 2×10^{-12} amps.

RESULTS & DISCUSSION

Effects of preparative treatment on structure preservation

Certain biological tissues (for example, some plant tissues) can be prepared for observation by SEM without fixation. However, the results obtained with muscle tissue are critically dependent not only on the fixation but on all parts of the

preparative procedure as well. The accompanying micrographs will illustrate the effects of a variety of different sample preparations on the preservation of structure as viewed by SEM.

Figure 1 shows the results of freeze-fracturing before fixing the tissue. This sample of semitendinosus was freeze fractured in water and then fixed in modified Karnovsky's fixative and freeze dried from water in the vacuum evaporator. Some ragged remains of myofibril cross structure (arrow) are the only suggestion of the well-known fiber and myofibril arrangement of muscle. Large ice crystal voids are seen throughout the tissue. Figure 2 is similar tissue fixed before fracturing and freeze drying. The fracture occurred in the direction of the fiber, but caused some cleavage in other planes as well (arrow). Myofibrillar organization has been retained with the typical transverse banding of the fiber in evidence. At greater magnification, however, the fine structure of fixed, freeze-dried tissue (not fractured) discloses the poor preservation in the myofibril structure (Mf) (Fig. 3). In some places, voids occur between myofibrils near Z-lines where the T-tubule system probably interrupted the muscle fiber. A robust-looking fiber, disrupted from its origin outside this particular muscle cell, can be seen lying across the myofibrils. It is possibly connective tissue.

Figure 4 is another sample of semitendinosus, fixed before fracturing, then freeze dried by lyophilization. This tissue was prepared essentially in the same way as the previous specimen, but is noticeably better preserved. Well-defined myofibril structure is recognizable. The sarcomeres in this micrograph measure approximately 1.6μ and zones on sarcomeres are identifiable from their topography. Sarcomeres are delimited by prominent bands (unmarked arrow) which either overlie or are a part of the Z-discs. The bands are bumpy and tubular in appearance and are continuous across the fiber. Immediately next to each Z-area are smaller raised bands in the I-band regions (arrowhead). These correspond in location to the triads (T-tubules plus sarcoplasmic reticulum cisternae) of the mam-

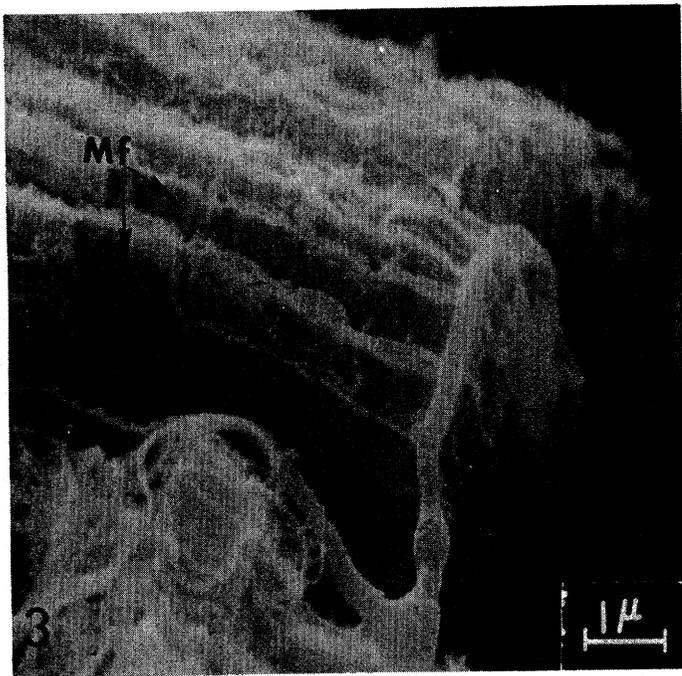


Fig. 3—Semitendinosus fixed, freeze dried. Structure recognizable but damage to myofibrils (Mf) apparent. Voids occur where T-tubules pulled away.

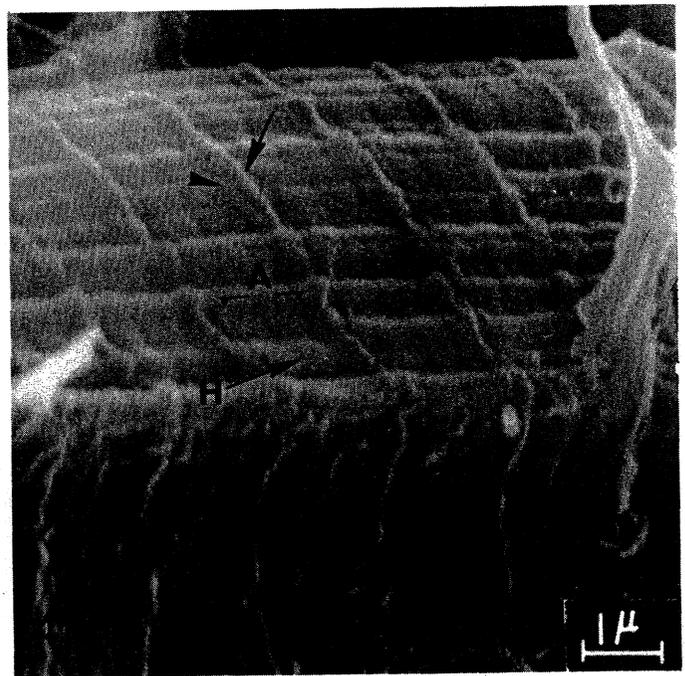


Fig. 4—Semitendinosus, fixed, freeze dried. Tissue well preserved, showing raised structures at the Z-line (unmarked arrow), A bands (A), H bands (H), and possible terminal cisternae (arrowhead).

malian muscle cell (Peachey, 1970) identifiable by transmission electron microscopy (TEM). The myosin bands (A-bands) are demarcated at their midpoints by a slight channel with a ridge running down the center (H). This depression corresponds to the H-band as defined by TEM. It is probable that this much structure is visible only because of a certain amount of shrinkage in the tissue, leaving some areas high, others low.

Post-fixation with osmium imparted a quality to samples which enhanced image contrast in the SEM. The specimen in Figure 5 was prepared by fixation, post-fixation, and freeze drying. The micrograph shows the surface of adjacent fibers of a semitendinosus bundle. The fiber surfaces are covered by endomysial collagen and, beneath that, the surface of the sarcolemma, all of which obscure underlying cell structure. It would be necessary to clear away the covering material completely, or to fracture, if underlying tissues were to be imaged. Scattered over the surface are patches of material (arrow), possibly lipid, deposited during the preparative procedure. Post-fixation was judged to be unnecessary for general sample preparation for SEM, but was useful in producing good quality micrographs.

In general, results from freeze-drying methods were quite variable. Fixation was a primary requirement, but it alone was not sufficient to ensure adequate specimen preservation when using freeze drying. One variable which was judged controllable, in this and all drying methods, was sample size. Smaller specimens were more likely to be well preserved.

Results obtained with the critical point drying technique are illustrated in the next two micrographs (Fig. 6 and 7). Figure 6 shows semitendinosus, fixed, fractured in water, and dried by the critical point method after ethanol and amyl acetate dehydration. The fracture followed fiber surfaces and occasionally stripped some contractile elements near the surface, as shown in the micrograph. The general appearance is that of a rather granular and abraded surface (unmarked ar-

row). Well-preserved myofibrils without much surface definition are imaged; possible mitochondria (M) can be seen in spaces between adjacent myofibrils. Figure 7 shows similar tissue prepared by ethanol fracturing and critical point drying directly from ethanol. Sarcomeres measure approximately 1.6μ . The general surface appearance is somewhat grainy or powdery with moderate surface definition. Raised structures again occur at the Z-lines and are referred to in this figure as T. I-bands (I) are faintly demarcated and the H-band areas (H) of the myosin are raised over a considerable width. Myofibrils are slightly separated from each other, except in the H-bands. In fact, adjacent myofibrils in most samples were in intimate contact at the H-band and at the Z-line. The sarcomeres are observed to narrow slightly as they approach the Z-lines, also noted by TEM by Stromer and Goll, 1967.

Comparing this micrograph to Figure 4 will point up some of the differences between well-preserved freeze dried (or air dried from solvent) and well preserved critical point dried meat tissue. Material which was freeze dried or air dried from solvent usually had better surface definition, gave "crisper," clearer micrographs, but had somewhat more shrinkage. Tissue which was critical point dried had less well defined topography, a mealy or abraded surface, but less overall shrinkage. Selection of technique depended upon the object of the particular experiment.

SEM of fresh muscle and the effects of cold shortening

The most useful preparative technique was to freeze fracture very small specimens in ethanol and either air dry or critical point dry from ethanol. This technique was employed for samples of longissimus dorsi excised at slaughter. Tissue fixed 45 min after slaughter was kept in fixative unrestrained for 24 hr at 6°C . The material was then freeze fractured in

ethanol and pieces about 1 mm³ were air dried in a stream of nitrogen gas. The results are shown in Figure 8.

The fibers (F) in this figure lie nearly horizontal to the plane of the micrograph. The fracture has exposed several par-

allel fibers, 20–40μ in diameter, which have sharply defined contraction waves. The contractions occur in tandem, so that the deep clefts (unmarked arrow) appear to form nearly straight lines across the fibers. The fracture follows fiber sur-

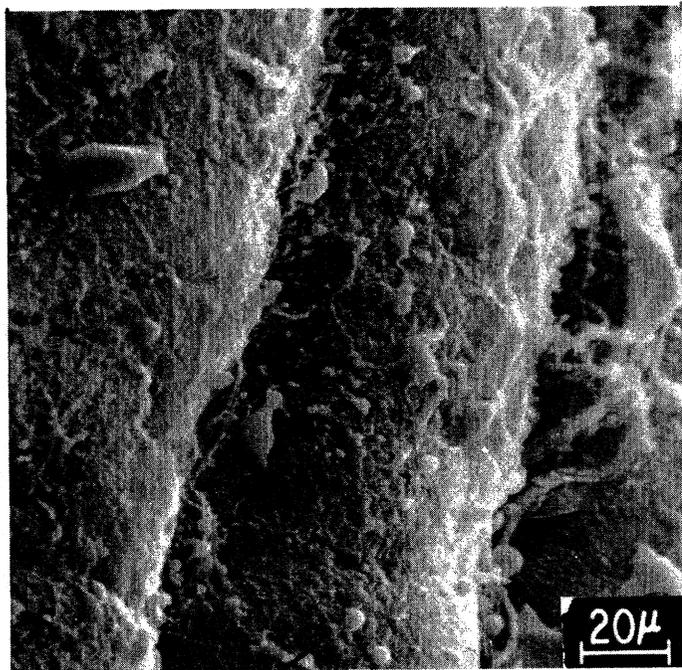


Fig. 5—Fiber surfaces of semitendinosus bundle. Collagen and other reticular material obscure underlying structure. Droplets of extraneous material (arrow), possibly lipids, seen on surface.

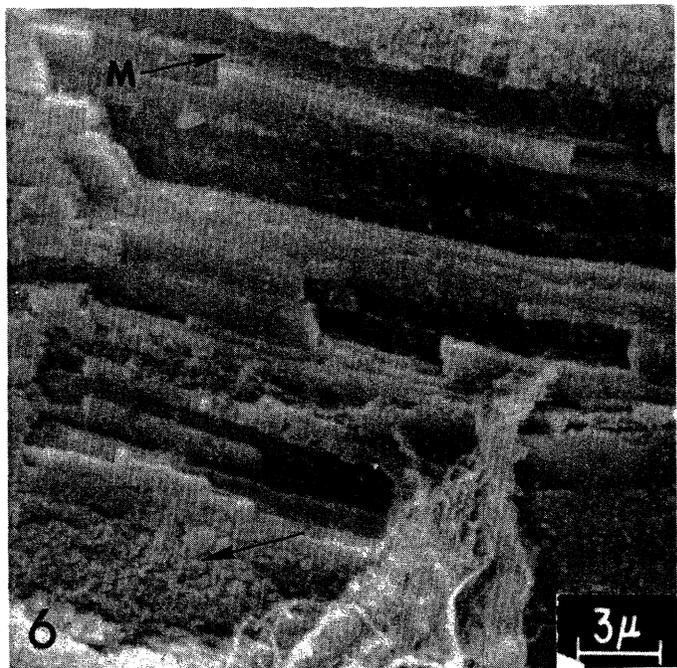


Fig. 6—Semitendinosus, fixed, fractured, and critical point dried. Fibrils well preserved without much surface definition. Mitochondri (M) visible. Some graininess in sample surface (arrow).

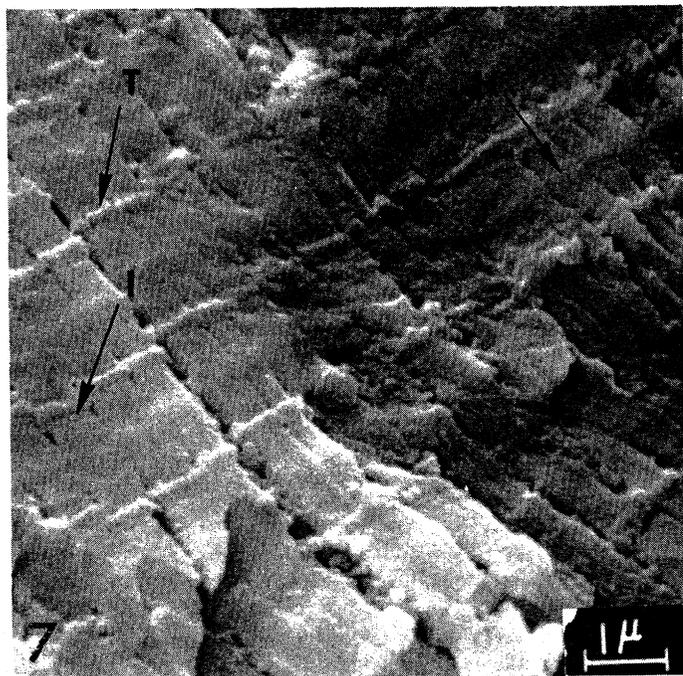


Fig. 7—Semitendinosus, critical point dried. Sarcomeres 1.6μ, with raised structures at the Z-lines (T), I-band (I) and H-band (H) raised to form ridge.

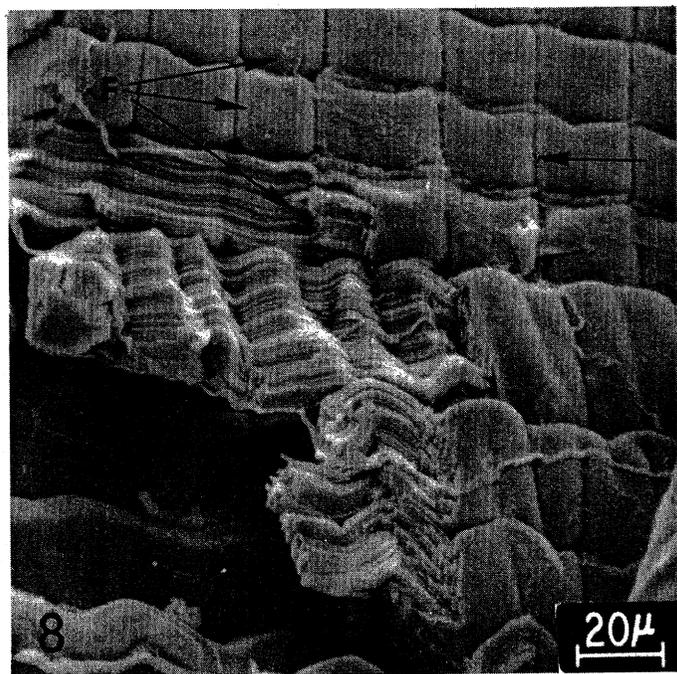


Fig. 8—Fracture through longissimus dorsi, fixed immediately after slaughter. Fiber (F) surfaces exposed, extreme contraction produced deep clefts (unmarked arrow), with some breakage of myofibrils.

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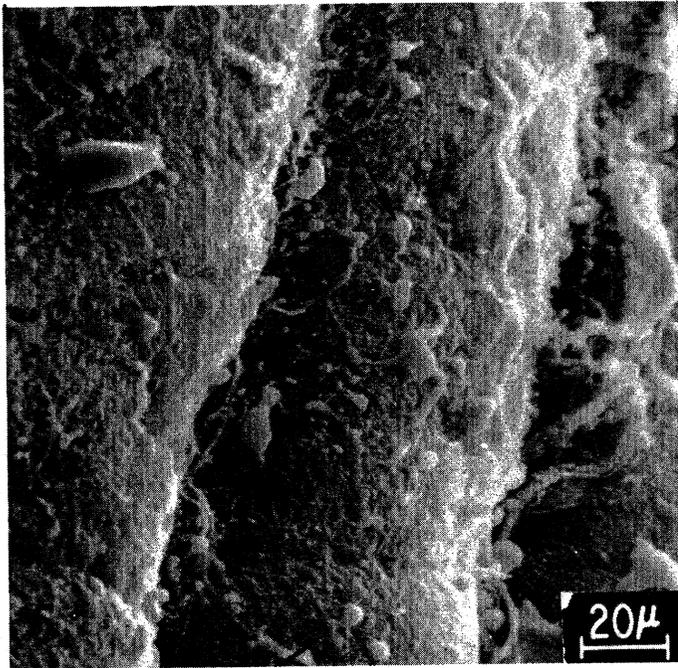


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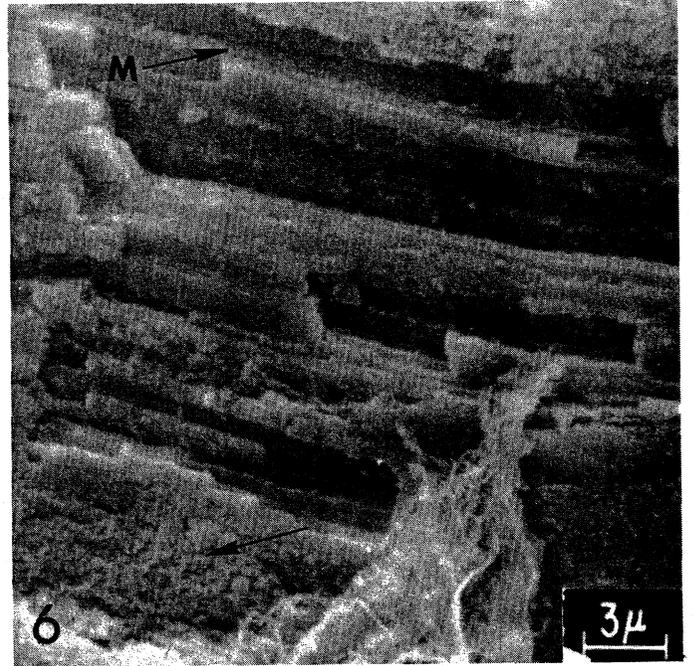


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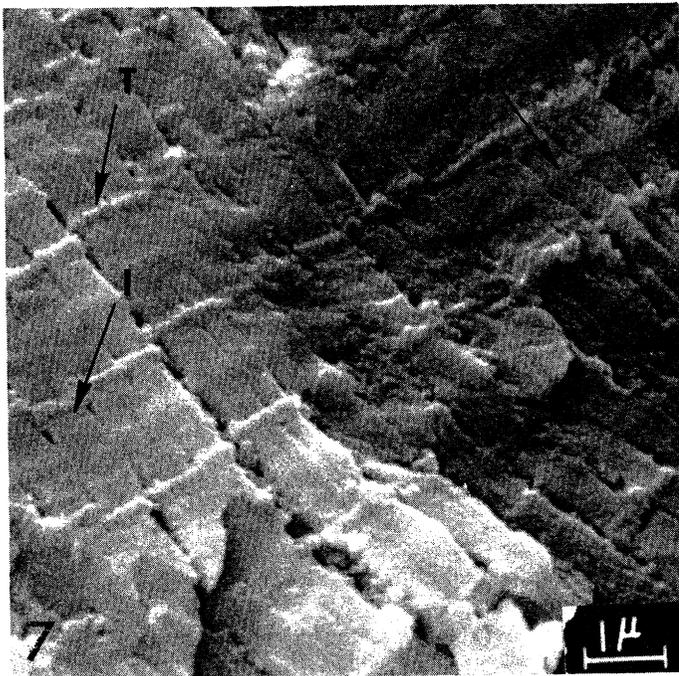


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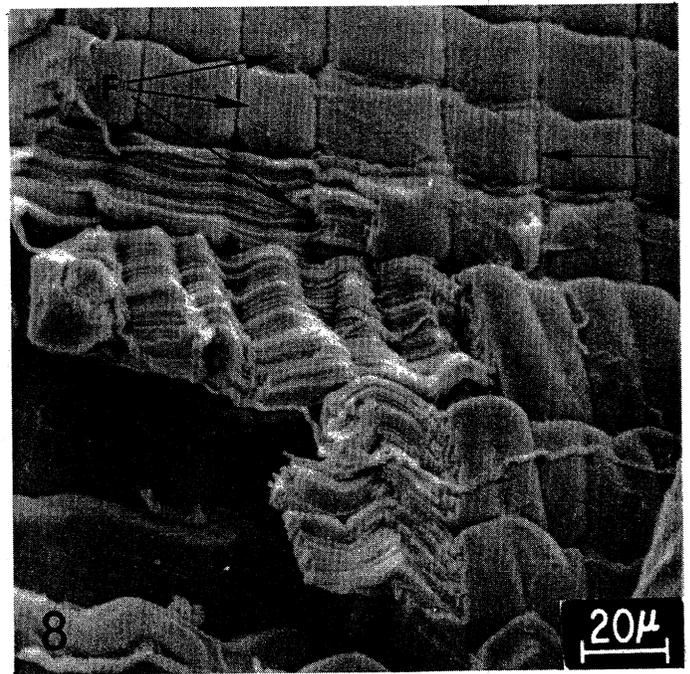


Fig. 8—Fracture through longissimus dorsi, fixed immediately after slaughter. Fiber (F) surfaces exposed, extreme contraction produced deep clefts (unmarked arrow), with some breakage of myofibrils.

faces except in the lower portion of the micrograph where fibers have been sheared longitudinally. Orderly rows of myofibrils in that area repeat the wave pattern of the surface. Some rupture of myofibrils is observed in the cleft area of the

wave pattern. The periodicity of the wave pattern is 20–30 μ in this case.

The presence of well defined contraction waves in muscle tissue is associated with cold-shortening. This condition occurs

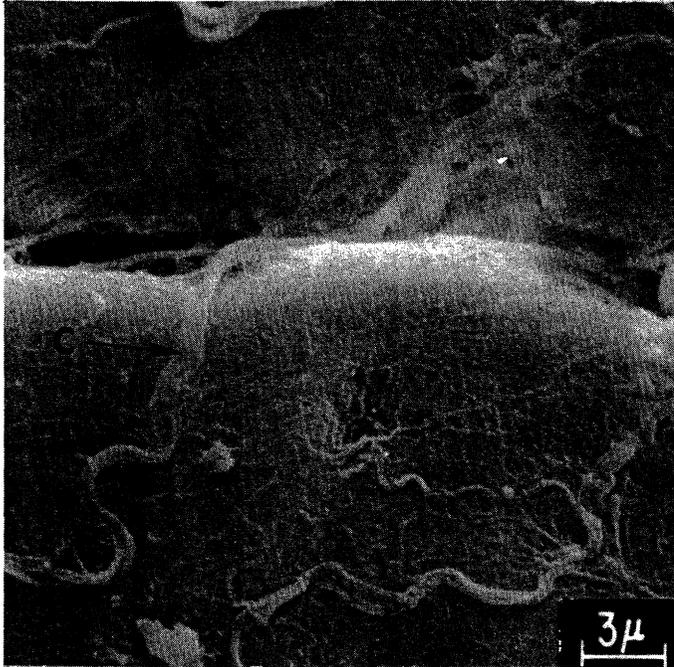


Fig. 9—Exposed surface of cold-shortened longissimus dorsi fibers, fixed immediately after slaughter. Collagen fibers (C) and reticular fibers cover contractile elements.

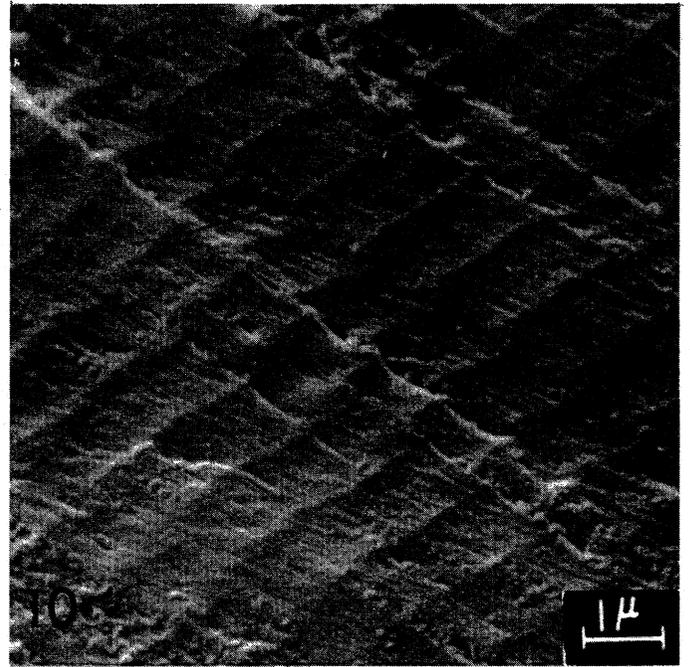


Fig. 10—Longissimus dorsi, cold-shortened, fixed immediately after slaughter, and air dried from ethanol. Surface definition moderate. Sarcomeres are 1.0–1.1 μ . Raised structures (arrow) are continuous with sarcomere surface.

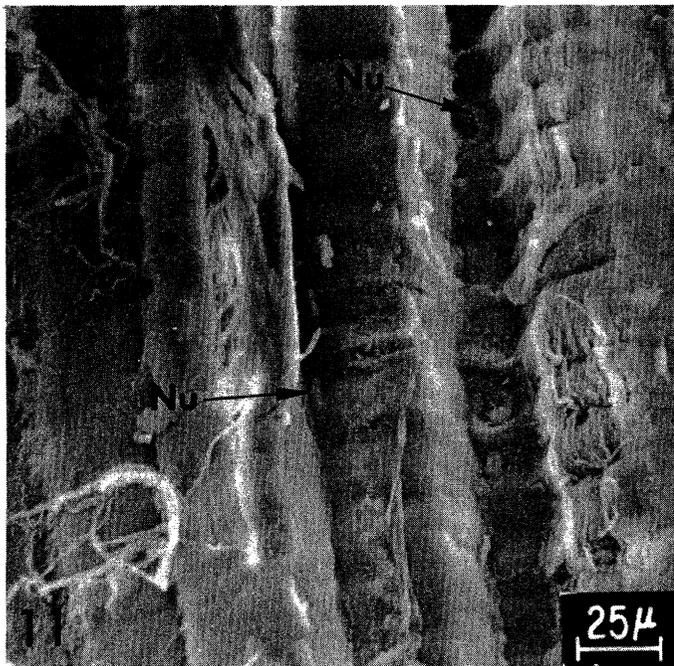


Fig. 11—Fracture longissimus dorsi fixed immediately after slaughter. Fractures occur around fiber surfaces. Numerous nuclei (Nu) present.

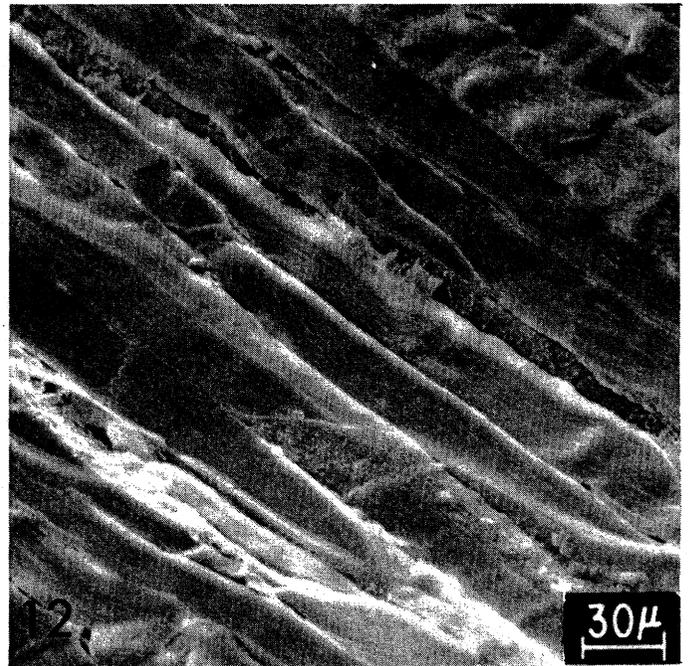


Fig. 12—Fracture through longissimus dorsi, fixed 24 hr after slaughter. Fracture plane is through fibers.

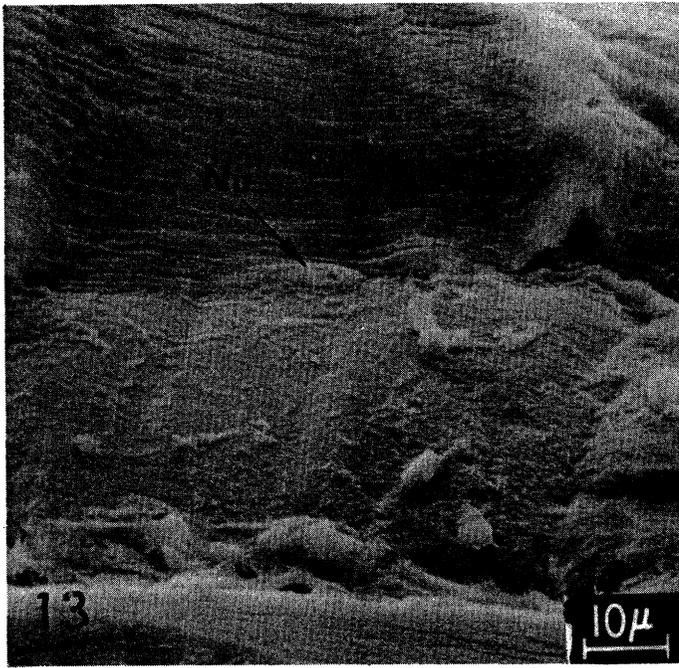


Fig. 13—*Longissimus dorsi*, fixed 24 hr after slaughter. Top and bottom fibers fractured through; middle fiber sarcolemma exposed. Nucleus (Nu) between fibers.

before or during rigor when muscle is chilled before it has passed fully into rigor (Locker and Hagyard, 1963).

A higher magnification of the surfaces of two cold-shortened fibers is shown in Figure 9. Occasional heavy endomysial collagen fibers (C) lie on top of an extensive network of randomly oriented fibrils, presumably the outer layers of the sarcolemma which encloses the individual muscle fiber. A transverse banding pattern is recognizable beneath the random fibrils.

The myofibril structure of the cold-shortened longissimus is shown in Figure 10. Sarcomere lengths of approximately $1.0\text{--}1.1\mu$ give evidence of the high degree of contraction in the tissue. Boundaries between myofibrils are indistinguishable in many places, and sarcomere banding is absent except for raised Z-band-I-band regions (arrow). At this degree of contraction, myosin filaments may buckle against the Z-band, causing bulging. Marsh et al. (1974) suggest that thick filaments actually penetrate the Z-line in highly contracted sarcomeres. This causes overlap of myosin from one sarcomere with actin from the next, a circumstance which could cause distention of the sarcomere in the I-Z area.

The age at which the sample is fixed appears to influence how the samples will fracture. Longissimus dorsi fixed immediately after slaughter exhibited a pronounced tendency to fracture around fiber surfaces (Fig. 11). On the other hand, samples of the same muscle held at 6°C and fixed 24 hr postmortem show fracture planes which shear across the fibers as seen in another low magnification micrograph (Fig. 12). A higher magnification view of the latter (Fig. 13) shows three adjacent fibers; the outer two are fractured through while the sarcolemma is exposed on the center fiber.

The presence of numerous nuclei (Nu) is evident in the freshly fixed samples (both fixed at slaughter and at 24 hr postmortem). These are readily apparent in Figures 11 and 13 as oblong bodies approximately 10μ in length lying along the boundaries between muscle fibers. Nuclei were not found in aged semitendinosus samples.

Sarcomere lengths: effects of fixation and cold shortening

An investigation was carried out to determine if and when shrinkage was occurring in sample preparation. Estimates of sarcomere lengths of unfixed and fixed tissue were made with a compound light microscope on thick muscle sections of commercially-aged semitendinosus suspended in 0.1M phosphate buffer, pH 6.0. Sarcomeres were counted and measured before fixation, after glutaraldehyde fixation, and after osmium postfixation. Unfixed tissue measured 2.5μ /sarcomere. Each fixation step contributed a 4% reduction in sarcomere length. Solvent dehydration resulted in further shrinkage, easily detected by eye when 2 mm^3 blocks of tissue, glutaraldehyde and osmium-fixed, were taken through the solvent series. Shrinkage was not appreciable until the specimen was immersed in 85% ethanol. When dehydration was continued through the series to higher ethanol concentrations, additional shrinkage did not appear to occur. Even with more steps in the dehydration series (8–10 concentrations; 10 min each) shrinkage still occurred at 85% solvent, without any apparent difference between ethanol and acetone as the solvent. The series finally adopted for general use was 70, 95, 95, 100, 100% ethanol for 5–10 min each depending upon sample size. Drying techniques introduced additional shrinkage; this was kept to a minimum by using small samples. Final sarcomere lengths of the semitendinosus as determined by SEM were approximately 1.9μ .

Cold shortening produced the most drastic shrinkage observed. In the freshly prepared longissimus dorsi, sarcomere lengths were as short at 1.0μ .

CONCLUSION

THE MOST IMPORTANT consideration in obtaining adequate micrographs from muscle tissue was careful preparation of specimens. Fixation was essential. Freeze fracture from ethanol proved to be the most dependable method and produced surfaces untouched by knife or blade. Each drying technique had its good and bad aspects when applied to muscle tissue. Freeze drying and air drying from alcohol produced some shrinkage, but the morphology was crisp and produced good micrographs. Critical point drying produced less shrinkage, but the surface when observed by SEM usually appeared abraded and grainy. In all cases, drying of smaller samples (2 mm or less on a side) produced the best results. It was also necessary to ground the specimens very carefully with silver paint and to coat with metal in the evaporator before observation with the microscope because dried muscle is very porous and charges badly when inadequately grounded. Small samples were advantageous here also.

Most tissue samples which were fractured cleaved through fibers, with the exception of longissimus fixed immediately after slaughter, which tended to cleave around fibers. Commercially-aged semitendinosus had well defined myofibril surface definition. Identification of Z, A, I and H bands was made. Structures suggestive of muscle cell triads were found. Freshly slaughtered longissimus myofibrils were observed in the cold-shortened condition; they were highly contracted and showed well defined contraction waves. Freshly prepared longissimus displayed many nuclei arranged longitudinally along the boundaries of the fibers.

REFERENCES

- Boyd, A. and Echlin, P. 1973. Freezing and freeze drying—A preparative technique for scanning electron microscopy. In "Scanning Electron Microscopy," Proceedings of the 6th Annual Scanning Electron Microscope Symposium, Ed. Johari, O. and Corvin, I., p. 759. IIT Research Institute, Chicago, Ill
- Dutson, T.R., Pearson, A.M. and Merkel, R.A. 1974. Ultrastructural postmortem changes in normal and low quality porcine muscle fibers. *J. Food Sci.* 39: 32.

- Eino, M.F. and Stanley, D.W. 1973. Catheptic activity, textural properties, and surface ultrastructure of postmortem beef muscle. *J. Food Sci.* 38: 45.
- Heald, C.W. 1971. Private communication. Virginia Polytechnic Institute, Blacksburg, Va.
- Henderson, D.W., Goll, D.E. and Stromer, M.H. 1970. A comparison of shortening and Z-line degradation in postmortem bovine, porcine, rabbit muscle. *Am. J. Anat.* 128: 117.
- Humphreys, W.J., Spurlock, B.O. and Johnson, J.S. 1974. Critical point drying of ethanol-infiltrated, cryofractured biological specimens for scanning electron microscopy. In "Scanning Electron Microscopy," Proceedings of the 7th Annual Scanning Electron Microscope Symposium, Ed. Johari, O. and Corvin, I., p. 275. IIT Research Institute, Chicago, Ill.
- Locker, R.H. and Hagyard, C.J. 1963. A cold shortening effect in beef muscles. *J. Sci. Food Agric.* 14: 787.
- Marsh, B.B., Leet, N.G. and Dickson, M.R. 1974. The ultrastructure and tenderness of highly cold-shortened muscle. *J. Food Technol.* 9: 141.
- McCallister, L.P. and Hadek, R. 1970. Transmission electron microscopy and stereo ultrastructure of the T system in frog skeletal muscle. *J. Ultrastruct. Res.* 33: 360.
- Nemanic, M.K. 1972. Critical point drying, cryofracture and serial sectioning. In "Scanning Electron Microscopy," Proceedings of the 5th Annual Scanning Electron Microscopy Symposium, Ed. Johari, O. and Corvin, I., p. 297. IIT Research Institute, Chicago, Ill.
- Peachey, L.D. 1970. Form of the sarcoplasmic reticulum and T-system of striated muscle. In "The Physiology and Biochemistry of Muscle as a Food," 2, Ed. Briskey, E.J., Cassens, R.G. and Marsh, B.B., p. 273. The University of Wisconsin Press, Madison, Wisc.
- Ramsbottom, J.M. and Strandine, E.J. 1949. Initial physical and chemical changes in beef as related to tenderness. *J. Anim. Sci.* 8: 398.
- Schaller, D.R. and Powrie, W.D. 1971. Scanning electron microscopy of skeletal muscle from rainbow trout, turkey, and beef. *J. Food Sci.* 36: 552.
- Schaller, D.R. and Powrie, W.D. 1972. Scanning electron microscopy of heated beef chicken and rainbow trout muscles. *Can. Inst. Food Sci. Technol. J.* 5: 184.
- Stanley, D.W. and Geissinger, H.D. 1972. Structure of contracted porcine psoas muscle as related to texture. *Can. Inst. Food Sci. Technol. J.* 5: 214.
- Stromer, M.H., Goll, D.E. and Roth, L.E. 1967. Morphology of rigor-shortened bovine muscle and the effect of trypsin on pre- and post-rigor myofibrils. *J. Cell Biol.* 34: 431.
- Sybers, H.D. and Sheldon, C.A. 1975. SEM techniques for cardiac cells in fetal adult and pathological heart. In "Scanning Electron Microscopy," Proceedings of the 8th Annual Scanning Electron Microscope Symposium, Ed. Johari, O. and Corvin, I., p. 275. IIT Research Institute, Chicago, Ill.
- Weidemann, J.F., Kaess, G. and Carruthers, L.D. 1967. The histology of pre-rigor and post-rigor ox muscle before and after cooking and its relation to tenderness. *J. Food Sci.* 32: 7.

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