A Review of the Muscle Cell Cytoskeleton and Its Possible Relation to Meat Texture and Sarcolemma Emptying

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SARCOLEMMA EMPTYING

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Abstract

A review of the muscle cytoskeleton
is presented. Current evidence leads to
the concept of a muscle cell cytoskeleton
consisting of at least two elements - gap
filaments which are located parallel to
the fiber axis and provide intracellular
elasticity and tensile strength and
intermediate filaments found in the Z-
disc area that function to connect
adjacent Z-discs and promote lateral
registration. The former constituent
consists of the high molecular weight
protein connectin (titin) while the
latter is composed of the smaller protein
desmin (skeletin). Both proteins exist
in filamentous form, are susceptible to
proteolysis and are insoluble in
physiological solutions. It is proposed
that these two elements may interact in
the region of the Z-disc to form a three-
dimensional network that functions to
hold myofibrils in place and provide an
ordering of the contractile mechanism.
Degradation of the cytoskeleton during
post-mortem conditioning of muscle may be
partially responsible for the tenderizing
phenomenon observed in aged meat.
Original work is described in which beef
skeletal muscle cell segments are induced
to empty, leaving behind the sarcolemmal
sheath. Conditions necessary for this
reaction to occur included the presence
of Ca^{++} ions and six days of post-mortem
conditioning at 0-5°C. On the basis of
these data, it is further proposed that
muscle cell emptying may be a consequence
of the action of an endogenous
proteolytic enzyme that breaks down the
cytoskeleton.

Introduction

Meat texture is an important, if not
the most important, quality factor
dictating its acceptance as a food.
Scientists have used genetic,
nutritional, chemical, microstructural,
instrumental and sensory methodology in
order to understand the factors
responsible for tenderness - toughness in
meat. At present, the most widely
accepted theory of meat texture holds
myofibrillar contraction and
intramuscular connective tissue to be the
two major factors determining physical
properties of muscle tissue (see Harris,
1976 for review). More recently, Currie
and Wolfe (1980) have introduced the
concept of intrafiber water as a third
factor to be considered in meat texture.
These authors found a high correlation
among the tensile and adhesive properties
of muscle strips undergoing rigor and
changes in extracellular space, which, in
turn, is inversely proportional to
intrafiber water content. High levels of
intrafiber water are thought to
facilitate slippage of the myofibrils
under tensile and adhesive forces.

The purpose of this review is to
consider another structural factor which
may play a significant role in meat
texture. As will be seen, the muscle
cell cytoskeleton, although postulated
for many years, has only recently come
under close scrutiny and its true
relation to meat texture is unknown.
However, both theoretical and
experimental reasons exist for carefully
considering the cytoskeleton as a
potential contributor to the physical
properties of meat.

The Cytoskeletal Concept

It is not greatly surprising that
the initial examination of cells using
relatively primitive light microscopes
led to the view of a membrane surrounding

a structureless cytoplasm embedded in which were various organelles and inclusions. It became apparent, however, that the multitude of enzymatic processes taking place within a cell could not occur with the required frequency if substrate and enzyme were not ordered in some way. This, and the advent of better ways of looking at cells, led to a dynamic concept in which organized arrays of fibrous elements interact to form a highly integrated structural network. These interconnected members are collectively called the cytoskeleton. This term appears to have been introduced in the literature by Peters (1956) although fibrous networks of filaments were reported prior to this by early electron microscopists. It is of interest to note that Peters hypothesized the presence of a cytoskeleton without the benefit of direct microscopic evidence. Concerning the role of the cytoskeleton, he wrote "I felt forced to postulate the presence of a fluid anatomy in the geography of the cell, being some tenuous network by the action of which the cell's enzymic activities were coordinated."

This living framework called the cytoskeleton is now known to exist in all eukaryotic cells. It is composed of various fibrous elements which have been grouped into three major structural categories including microtubules, microfilaments and intermediate filaments. Various functions have been attributed to the cytoskeleton related to cell motilities, e.g. cytoplasmic streaming, organelle movement, cytokinesis, phagocytosis secretion and cell surface modulation (Brinkley, 1982). It is of importance to realize that the cytoskeleton is an active dynamic system involved with cell movement and changes in shape and not simply a passive network as its name might imply.

Cytoskeleton of the Muscle Cell

It will be appreciated that because of the unique architecture of the muscle cell, the function of a cytoskeleton would of necessity be different from that of nonmotile cells. As connective tissue serves as an extracellular source of support for the fiber so would the cytoskeleton be presumed to hold myofibrils in place and provide an ordering of the contractile mechanism.

The structural evidence for a muscle cell cytoskeleton would seem to have had its origin in early theories attempting to explain muscle elasticity. Hanson and Huxley (1955) proposed the presence of very thin but elastic "S-filaments" linking actin filaments and a similar

proposal was advanced by Hoyle (1967) that featured "ultrathin filaments", independent of actin (Figure 1). A number of workers (Sjöstrand, 1962; Carlsen et al., 1965; McNeill and Hoyle, 1967) used electron microscopy to demonstrate the actual occurrence of such filaments. Evidence was gathered using muscle tissue from which the contractile proteins had been extracted (Walcott and Ridgeway, 1967; dos Remedios and Gilmour, 1978) and from highly stretched muscle fibers (Locker and Leet, 1975, 1976). In all cases, very fine filaments persisted in the preparations (Figure 2). The names given to these structures and their exact spatial location have varied from worker to worker; the latter workers observed filaments occurring in the A-I gap in highly stretched fibers and used the name 'gap filaments', first conferred on them by Sjöstrand (1962). This name will be used in this review. Regarding location, it would seem reasonable to entertain the following possibilities for thin myofibrillar filaments running parallel to the fiber axis: 1) they could connect an A band to a Z-disc; 2) they could connect two A bands through a Z-disc; 3) they could connect two adjacent Z-discs; 4) or they could connect numerous Z-discs, thus linking to transverse elements, and via these to the sarcolemma. The definitive answer to the exact location of gap filaments awaits further research.

Chemical characterization of the proposed elastic component of muscle was provided by Maruyama and coworkers (Maruyama et al., 1976, 1977) who obtained a rubbery, insoluble protein from extracted myofibrils, termed it "connectin" and concluded that it functions as an elastic component of muscle. The electron microscope showed the isolated protein to consist of thin filaments and antibody studies located the protein along the sarcomere except at the Z-discs (Maruyama et al., 1981). Connectin, alternatively called titin (see Wang and Ramirez-Mitchell, 1983), has also been identified in the sarcolemma where it might join the membrane and myofibrils.
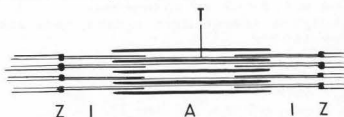

Figure 1. Diagram showing proposed model for muscle including a very thin elastic filament(T) extending between Z-discs (Z) and parallel to the A band (A) and I band (I). Source: Hoyle (1967); used with permission.
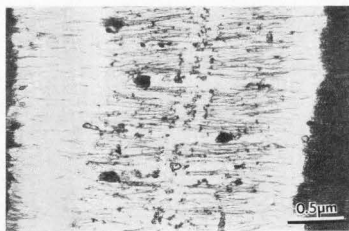
Figure 2. Transmission electron micrograph of ox *sterno-mandibularis* muscle glycerinated at twice the excised length and incubated in calcium-activated factor and calcium ions. Most of the Z-disc material has been removed and numerous small filaments can be seen spanning the A-I gap. Source: Locker *et al*. (1977); used with permission.
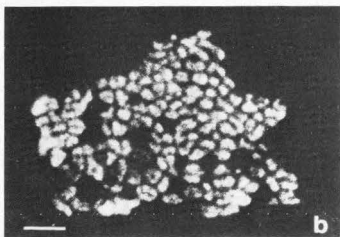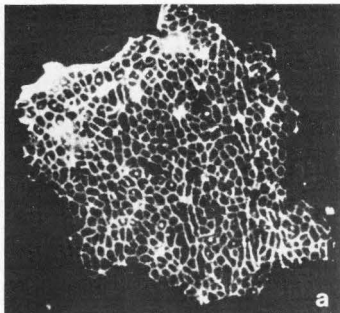

Figure 4. a. Indirect immunofluorescence showing distribution of desmin at the periphery of Z-discs in isolated Z-disc sheet from chicken skeletal muscle. b. Similar technique showing distribution of α-actinin in the interior of Z-discs. Bar = 2.5 μm. Source: Lazarides (1982); used with permission.
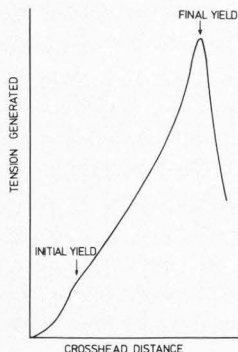

Figure 3. Typical force-deformation curve obtained from applying tensile force to uncooked beef muscle. A more pronounced effect may be seen in Locker and Wild (1982b). Source: Currie and Wolfe (1980); used with permission.

Another line of evidence for the presence of thin filaments located parallel to the fiber is based on studies of the tensile properties of muscle. In these experiments force is applied to muscle strips that acts to pull sarcomeres apart longitudinally and the resulting force-elongation curve recorded. Locker and his group (Locker and Carse, 1976; Locker *et al*., 1977; Locker and Wild, 1982a) have reported that gap filaments may make a significant contribution to the tensile strength of muscle. Upon the application of tensile force in an Instron testing machine, the muscle strips exhibit an initial yield (e.g. Figure 3). This appears to be the same as the "yield point" obtained in a more pronounced way with simpler methodology by Locker and Wild (1982b). This point occurs at a low extension where the connective tissue network is still slack, thus suggesting that the failure takes place in the myofilaments. The location of this yield point remains quantitatively constant even up to the point where the myofibril is becoming "cooked". Locker and Wild (1982a) concluded that the yield point is due to I-filaments snapping at the Z-line or pulling. On the basis of these ultrastructural and chemical observations, it would seem reasonable to conclude that gap filaments, composed of connectin, function as the axial element of the muscle cytoskeleton and are related to elasticity and strength.

Another possible cytoskeletal element was suggested by the observation

that sarcomeres exist in axial register and exhibit lateral organization leading to the characteristic striated appearance of muscle tissue. Lazarides (1980) has reviewed this subject and cites work demonstrating that the fibrous elements that have been observed to link myofibrils laterally and to link them to the sarcolemma are so-called "intermediate filaments". This term refers to an ubiquitous class of filaments distinguished morphologically by an average diameter of 10 nm and found in many types of cells. Intermediate filaments are particularly abundant in adult smooth muscle. Exhaustive extraction of smooth muscle cells to remove contractile proteins results in a residual cytoskeleton and subsequent electrophoretic separation led to the isolation of a 50-55,000 dalton protein termed "desmin" by Lazarides and Hubbard (1976). This protein has now been prepared from adult mammalian skeletal muscle (O'Shea et al., 1981). These authors also reported the reconstitution of 10 nm filaments from purified desmin. In skeletal muscle desmin, alternatively called skeletin, has been identified in the periphery of the Z-disc in filamentous form (Robson et al., 1981). The unique honeycomb structure of desmin in interconnected Z-discs (Figure 4) leads to the conclusion that this protein, along with actin and α-actinin, comprise this structure. The latter protein gives a fluorescence pattern that is complementary to that of desmin, indicating their intimate association. Possible roles for desmin in muscle were proposed by Lazarides (1980, 1982) including the linking of Z-discs leading to an integration and alignment of contractile elements as well as functions during biogenesis. Figure 5 shows how desmin might function as a part of the muscle cell cytoskeleton. The evidence gathered to date on desmin strongly supports its inclusion as a major component of the muscle cell cytoskeleton.
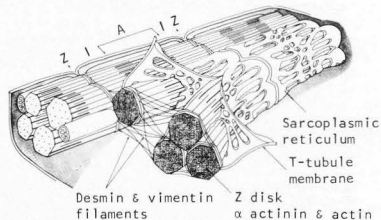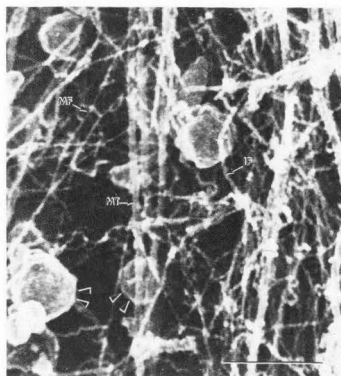

Figure 6. High resolution scanning electron micrograph of cytoskeleton of 1 wk old chick skeletal muscle culture. MT - microtubules; IF - intermediate filament; MF - actin microfilament; arrowheads - presumed elements of the sarcoplasmic reticulum. Bar = 0.5 μm. Source: Ip and Fischman, 1979; used with permission.

Thus far two components of the muscle cell cytoskeleton, a gap filament composed of the protein connectin and an intermediate filament composed of the protein desmin, have been identified. Table 1 summarizes their characteristics. Certainly other elements may exist and be demonstrated in the future; new myofibrillar proteins continue to be reported (Greaser et al., 1981). With what is already known, however, an integrated muscle cell cytoskeleton can be envisaged that consists of a three-dimensional framework made up of lateral components (intermediate filaments) linked to axial components (gap filaments) at the level of the Z-disc. Whether the two elements interact directly or through a third constituent such as α-actinin is, at present, unknown but see Wang and Ramirez-Mitchell (1983).

It will be noted that most of the direct evidence gathered thus far to support the existence of a cytoskeleton in muscle cells has been gathered by transmission electron microscopy and protein isolation techniques followed by immunofluorescence. It is always more convincing if parallel proof can be gained by a different approach and, considering the three-dimensional information that can be obtained using the scanning electron microscope (SEM), this would appear to be a useful tool in the study of cytoskeletal elements. The

Figure 5. Diagram of desmin location in skeletal muscle. Source: Lazarides (1980); used with permission.

Table 1: Characteristics of gap filaments and intermediate filaments of skeletal muscle cytoskeleton.

Characteristic	Gap filaments	Intermediate filaments
Physical form	$\sim$ 2 nm dia. filaments	$\sim$ 10 nm dia. filaments
Location	Connect A band to Z-disc (?) - extend to sarcolemma	Periphery of Z-disc - extend to sarcolemma
Function	Intracellular elasticity and tensile strength	Connect adjacent Z-discs, provide lateral registration
Constituent protein	Connectin (titin)	Desmin (skeletin)
Molecular Weight	760,000-1,000,000	55,000
Yield (% of myofibrillar protein)	5.5%	0.35%
Post-mortem degradation	Susceptible to proteolysis	Susceptible to proteolysis
Influence of temperature	Survive cooking	?
Solubility	Insoluble in physiological solutions	Insoluble in physiological solutions
Enzymatic function	None	None

problem, of course, is one of inadequate resolution. Ip and Fischman (1979) have advanced this goal through the use of high resolution SEM to study isolated and *in situ* cytoskeletal elements from several sources (Figure 6). These authors conclude that with suitable specimen preparation it is possible to resolve and identify various elements of the cytoskeleton and to obtain direct three-dimensional information on their organization *in situ* by using the SEM.

Possible Relation of the Cytoskeleton to Meat Texture

In the following discussion it will be assumed that gap filaments and intermediate filaments do exist in muscle cells and that they approximate the structural functions outlined in the previous section. To postulate a role for these elements in meat texture, one must investigate how they are stabilized *in situ*, the influence of the post-mortem environment and the effect of cooking.

Evidence for cytoskeletal stabilizers is scarce. McCollester and Semente (1966) reported the nucleotide pyrophosphate flavin adinine dinucleotide (FAD) effective in this role but these findings have been questioned (see next section). While this seems the only direct observation recorded, one can speculate that since the former workers found calcium ions and incubation necessary for cytoskeletal breakdown an endogenous enzyme calcium activated neutral protease (CANP seems a likely candidate) is involved in degradation. Whether this enzyme would hydrolyze filaments or their attachments to other structures is not known.

Post-mortem muscle tissue is degraded by proteolytic enzymes (Penny, 1980; Dayton *et al.*, 1981) and it has been found that both desmin (Robson *et al.*, 1981) and connectin (Takahashi and Saito, 1979) are susceptible to proteolysis. The latter authors reported connectin levels fell to zero within one day in chicken breast muscle and within 7 days in rabbit muscle; Robson *et al.* (1982) stated 80% of the desmin present in adult mammalian muscle was degraded in seven days at cold room temperature. Thus, whatever contribution these structures may have made to the physical properties of fresh muscle would be diminished as a result of conditioning and variations in tenderness may be related to the degree of cytoskeletal breakdown. Less is known about the effect of heat on the cytoskeleton. Gap filaments from unaged tissue are reported to survive cooking (Locker *et al.*, 1977) but little is known of its influence on intermediate filaments.

It would seem, therefore, that both the known cytoskeletal components have the capacity to be qualitatively related to meat tenderness and the physical properties of muscle tissue. Evidence, or even speculation, for their direct involvement in these processes is limited, however. The existing literature may be summarized as follows:

Gap filaments

A) The disappearance of connectin correlates with the loss of about 30% of muscle elasticity (Takahashi and Saito, 1979).

B) Based on tensile properties and electron micrographs Locker and Wild (1982c) concluded that only gap filaments and collagen define the tensile strength of cooked muscle.

Intermediate filaments

A) Structural changes occur in the Z-disc, the location of desmin, as a result of conditioning which are reflected in increased tenderness (see review by Penny, 1980).

B) There may be some relation between post-mortem alterations in desmin and water holding capacity (Robson et al., 1981).

It is possible to postulate events in the post-mortem muscle cell that would make the breakdown of the cytoskeleton a major factor in tenderness. If the result of endogenous proteolysis was disconnection of the previously integrated units and not their total breakdown, the cytoskeleton would lose its functional role without any great change in its structure. Regarding this point, Locker et al. (1977) observed that the changes produced in gap filaments by muscle proteases were not evident in uncooked tissue but that heating caused their disappearance. Thus, if the function of the cytoskeleton is lost, the myofibrils may disengage and present less resistance to masticatory forces.

Relation of the Muscle Cell Cytoskeleton to Sarcolemmae Isolation

General evidence for the presence of a cytoskeleton in muscle cells resulted from efforts to isolate their sarcolemmae. Reports by McCollester (McCollester, 1963; McCollester and Semente, 1964) described a procedure to obtain skeletal muscle cell membranes based upon the extraction of intracellular contents by water. Dissolution of the cell contents could only be accomplished following homogenization in the presence of Ca^{++} ions and an incubation step at $37^{\circ}C$. This led the authors to invoke the concept of a cytoskeleton which must be disrupted before the cell contents could be solubilized. The yield of membranes was used to monitor the extent to which the cytoskeleton had been broken down and this reaction was attributed to endogenous enzymes.

Further experimentation by this group (McCollester and Semente, 1966) resulted in the conclusion that nucleotide pyrophosphates, especially FAD. stabilized the cytoskeleton since it

inhibited the emptying reaction. This finding was later challenged (Stanley et al., 1968). With our present knowledge of muscle proteolytic enzymes, it seems quite possible that the enzyme responsible for cytoskeletal breakdown is CANP which is located in the Z-disc and in or near the sarcolemma (Dayton et al., 1981). While this enzyme has been reported to be responsible for Z-disc breakdown post-mortem, and thus be of importance in tenderness, it is of interest to note that the yield and properties of α-actinin are unaltered by conditioning (see Penny, 1980). On the other hand, desmin, another major component of the Z-disc, is susceptible to proteolysis. Thus, it may be proposed that an action of the proteolytic enzymes active during conditioning of meat is to attack the cytoskeleton. If this is the case, it should be possible to measure this process indirectly through the yield of sarcolemmae since this reflects cytoskeletal breakdown. The subsequent section describes the results of experiments aimed at testing this supposition.

Preparation of Sarcolemmae from Beef Muscle and its Relation to Post Mortem Conditioning

Procedure

Two procedures were employed to obtain sarcolemmae from beef muscle. The first was adapted from McCollester (1962) and involved homogenization of 20 g of tissue (sternomandibularis muscle from steer carcasses obtained at the time of slaughter and held for 1-6 days at $0-5^{\circ}C$; this muscle contains predominantly red fibers) with 200 mL of cold ($5^{\circ}C$) 50 mM $CaCl_2$ in an Osterizer-type blender fitted with a Polytron Model BEW cutter (Will Scientific, Inc., Rochester, N.Y.) for four bursts of 15 sec. The homogenate was strained through cheesecloth to remove fibrous connective tissue and the muscle cell segments recovered by centrifugation at room temperature for 30 sec in a clinical centrifuge set at full speed. The cells, still retained in four 50 mL centrifuge tubes, were then washed three times in a solution of 25 mM NaCl, 2.5 mM histidine buffered to pH 7.4 with Tris by gently resuspending the lightly packed cells in approximately 45 mL of solution and centrifuging as before. Following the washing steps, the cells were resuspended in the same solution and incubated at $37^{\circ}C$ for 30 min. The cells were then centrifuged, washed once in the solution and the cloudy supernatant discarded. The residue was taken up in distilled, deionized water adjusted to pH 7.4 with Tris, centrifuged, and the sediment resuspended in Tris-buffered

Figure 7. Interference light microscopy of bovine **sternomandibularis** muscle cell segments. a. Following homogenization in 50 mM CaCl$_2$. Note presence of crimp in large cell; b,c. Unemptied cells following extraction procedure; d,e. Partially emptied cell. Note presence of transverse striations in unemptied portions; f. Empty cell showing adhering collagen fibril.
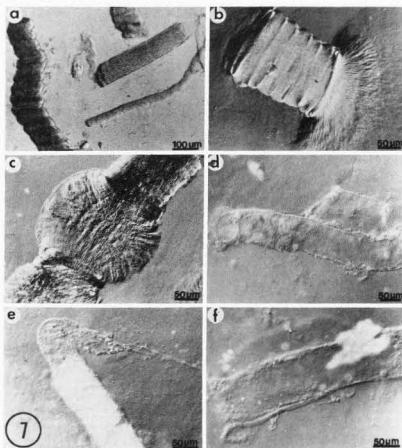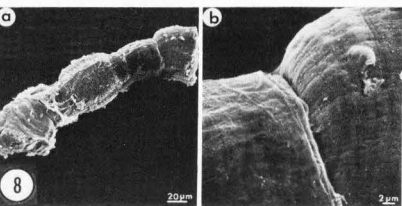
Figure 8. Scanning electron microscopy of unemptied cell. Note presence of transverse striations in b.

water. The tubes were then shaken vigorously and the contents added to approximately 800 mL of Tris-buffered water stirred with a magnetic bar. The preparation was held overnight at 0-5°C and the degree of emptying quantitated by examination with a light microscope. Three slides with coverslips were prepared using drops of the stirred preparation and counts were made of full or partially emptied cells versus totally empty cells by scanning the entire area of each coverslip at 400x. Toluidine Blue O dye may be used to help differentiate full and empty cells, although with some experience this is not difficult. Data are reported as the percentage of empty cells.

The second method used to prepare empty cell segments was similar to that of Westort and Hultin (1966) and differing only in incubation temperature. The preparation was held at room temperature (ca. 20°C) instead of 37°C. In both cases, controls were added in which the tissue was homogenized in water but otherwise carried through the rest of the standard procedure.

Results

The purpose of these series of experiments was to investigate the role of post-mortem conditioning, Ca^{++} ions and high temperature incubation in the production of beef skeletal muscle sarcolemmae since all of these parameters have been implicated in cytoskeletal breakdown. Thus, the methods described above were applied to fresh beef muscle and tissue that had been conditioned for up to 6 days at 0-5°C. The data from this work (Table 2) show the absolute requirement for Ca^{++} ions. They also show that emptying did not occur for

Table 2. Yield of sarcolemmae from beef skeletal muscle. Effect of post-mortem conditioning and procedural variation on percentage of empty cells. (Data are average of two animals).

Post-mortem conditioning (days)	Homogenizing media			
	water		50 mM CaCl$_2$	
	Temp. of incubation			
	Room Temp.	37°C	Room Temp.	37°C
1	0	0	0	0
3	0	0	0	0
6	0	0	81	69

rigor muscle or tissue kept only 3 days
at 0-5°C, but occurred readily after 6
days of conditioning. Incubation
temperature did not produce a significant
effect under these conditions.

Micrographs were taken of the cells
at various stages of the emptying
process. Figure 7a shows a typical field
of the homogenized tissue under low light
microscope magnification. Note that
several of the cell segments indicate the
presence of crimp. As mentioned, the
muscle tissue used in these experiments
was removed from the carcass immediately
post-mortem and thus went into rigor
unrestrained which may explain their
contracted appearance. It was observed
that these bands were no longer apparent
in unemptied cells at the end of the
procedure. In Figures 7b and c may be
seen two cell segments that have
undergone the complete procedure but
which did not empty. It will be observed
that in both cases the cell contents have
ballooned out, in 7b from the cut end and
in 7c in the middle of the cell segment.
Scanning electron micrographs of a
similar cell gathered on a 45 µm
Millipore filter and subsequently
critical point dried and coated (Figure
8) show what appears to be a constricting
sarcolemmal envelope holding in the cell
contents; where these have escaped from
the envelope characteristic transverse
bandings of myofibrils can be observed
which indicates that in unemptied cells
Z-discs remain intact. The ballooning
effect was more prevalent as conditioning
time increased, suggesting that it
results from a failure of transverse
structures. Presumably it is this
failure of transverse elements impinging
upon the sarcolemma that makes emptying
possible.

Partially emptied cells are shown in
Figures 7d and e. The unemptied portions
may show transverse banding and the
emptied tube is characterized by what may
be interpreted as collagen fibers.
Figure 7f, an emptied cell segment,
displays in the lower left corner, a
typical fiber thought to be an adhering
strand of collagen. An isolated cell
membrane was photographed at higher
magnification using three different
planes of focus (Figure 9). Presumed
collagen fibers may be observed in
apparently random orientation.

Conclusions

It is not difficult to reconcile the
results of these experiments with what
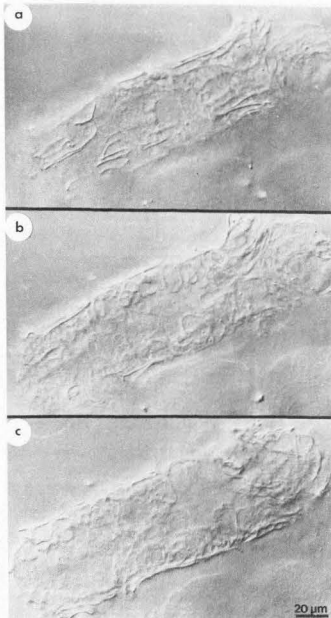is known of the muscle cell cytoskeleton.

Figure 9. Interference light microscopy
of empty cell segments taken at three
planes of focus. Note presence of
collagen fibrils and their random
orientation.

The requirement for Ca^{++} ions and six
days of post-mortem conditioning are
consistent with a hypothesis that
ascribes muscle cell emptying to a
breakdown of the cytoskeleton by a
calcium activated endogenous proteolytic
enzyme. Of particular interest is that,
in this system, emptying only occurs
following a conditioning period which
normally produces significant tenderizing
in beef muscle (Stanley, 1983). While
much more research is required in this
area, these preliminary results indicate
that muscle cell emptying may be a
simple, albeit indirect, measure of
breakdown of at least the transverse
elements of the cytoskeleton which, in
turn, may reflect the tenderization
phenomenon occurring in conditioned meat.

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Discussion with Reviewers

S.H. Cohen: Would the author comment on the conclusion made by Ullrick et al. (Ullrick W C, Tosell P A, Chase D and Dasse K. (1977). Are there extensions of thick filaments in the Z-line in vertebrate and invertebrate striated muscle? J. Ultrastruct. Res. 60: 263-271) that "extension of the myosin-containing filaments to the Z-line are present in invertebrate flight muscle, but that we find no evidence to suggest that they are present in vertebtate muscle"?
Author: Although the existence of 'gap filaments' has been challenged by the above authors, there is a compelling body of both experimental and theoretical evidence dating from the mid-50's (see text) that lead to the conclusion that, in fact, this proposal must be taken seriously.

S.H. Cohen: Does the author have any experimental evidence on the integrity of the sarcolemmae as indicated by the retention of enzymatic activity?
Author: No, our experiments thus far have centered on determining the percentage of empty tubes produced by various experimental conditions through microscopic rather than chemical means. It should be noted that it has been shown that isolated sarcolemmae exhibit ATPase activity but several glycolytic enzymes were not found (McCollester D L and Randle P J (1961). Isolation and some enzymatic activities of muscle cell membranes. Biochem. J. 78: 27 p).

P.B. Bell: Can the author relate the results obtained by the "sarcolemmal emptying" assay to more direct ways of examining the cytoskeleton of muscle fibers?

Author: Work is presently underway in this laboratory to attempt to determine the relationship between the emptying reaction and both chemical and structural analyses.

S.H. Cohen: The author considers only CANP as contributing to the breakdown of the cytoskeleton. Could any other enzymes be involved?

Author: After reviewing the available evidence, it was concluded (Penny, 1980) that "CANP has clearly been shown to be the enzyme most likely to be involved (in conditioning) because its activation by Ca^{++} ions explains the accelerating effects of Ca^{++} ions on conditioning and it can mimic the changes observed during conditioning." It remains to be established if other proteolytic enzymes found in muscle cells such as cathepsins B and D have a role in breakdown of the cytoskeleton.

R.H. Locker: Since the paper begins on the theme of meat texture and tenderness, it should have paid more attention to cooked meat. Tenderness is assessed on cooked meat, where the relative significance of filaments is totally changed. I have attempted to discuss this in detail for myofibrillar filaments in papers presented at the Reciprocal and European Meat Conferences in 1982. The G-filaments have proved to be the survivors, even on extreme cooking.

The fate of the transverse "honeycomb" of desmin on cooking has had little attention. However in 1976 Davey, Niederer and Graafhuis (J. Sci. Fd. Agric. 27, 251) showed that links between the Z-lines of adjacent myofibrils survived cooking (40 min., $80°C$). This evidence of course says nothing about strength.

The questions of whether the critical junctions between axial and transverse elements deform under tension to provide axial strength also remain unanswered.

Author: I agree with you that these are important considerations for future work in this area.

J.M. Squire: Are micrographs, such as those shown, thought to represent hard evidence about the state of a particular cell? Would it not be possible to compare pelleted and soluble mass under the various conditions used, to get good quantitative data on the emptying of cells?

Author: The answer to the first part is yes. Work is ongoing regarding the second part and we hope to report on it in near future.