# What Causes Postmortem Tenderization?

DARREL E. GOLL*, MERIN L. BOEHM[1], GEERT H. GEESINK[2], and VALERY F. THOMPSON

## Introduction

Inconsistency and variability in tenderness have been identified as a major problem facing the meat industry (Morgan et al., 1991). Tenderness increases with increasing time of postmortem storage (Calkins and Seideman, 1988; Goll et al., 1964; Wheeler and Koohmaraie, 1994), and it has generally been assumed that part of this increase is caused by postmortem proteolysis mediated primarily by the calpain system (Goll et al., 1974; 1983; 1992; Koohmaraie, 1988; 1992a; 1992b). Consequently, considerable effort has been directed at determining what myofibrillar proteins are degraded during postmortem storage and whether this degradation is related to tenderness and to calpain activity (Goll et al., 1992; Koohmaraie, 1988; 1992a; 1992b). These studies have shown that the large cytoskeletal proteins, nebulin and titin, along with troponin T and vinculin are degraded during postmortem storage (Anderson and Parrish, 1989; Ho et al., 1994; 1996; 1997; Huff-Lonergan et al., 1995. Paxhia and Parrish, 1988; Taylor et al., 1995). Degradation of nebulin, titin, and vinculin would be expected to contribute to increased tenderness. These same studies, however, have also shown that very little proteolytic degradation of these cytoskeletal proteins occurs during the first 24 hr postmortem (Anderson and Parrish, 1989; Ho et al., 1994; 1996; 1997; Taylor et al., 1995) and that proteolytic degradation of muscle proteins is minimal even up to 3 days postmortem (Huff-Lonergan et al., 1995; Taylor et al., 1995). Yet, the largest changes in tenderness occur during the first 3 days postmortem (Calkins and Seideman, 1988; Dransfield, 1992; Goll et al., 1964; Wheeler and Koohmaraie, 1994). Is the limited amount of proteolysis that occurs during the first 3 to 4 d of postmortem storage sufficient to cause the large changes in tenderness that are observed during this period or are other factors involved? If other factors are involved in postmortem tenderization, what are these factors and how important is their role? This article will summarize the evidence suggesting that postmortem changes in tenderness involve at least two different phenomena: 1) proteolytic degradation of cytoskeletal proteins, particularly nebulin, titin, and vinculin, during postmortem storage; this proteolytic degradation is caused primarily by the calpain system; and 2) changes in the nature of the actin/myosin interaction: these changes result from alterations in the manner in which myosin cross-bridges bind to the actin thin filament. We suggested several years ago that both changes in the actin/myosin interaction and calpain-induced proteolysis are involved in postmortem tenderization (Goll et al., 1995), and this article will elaborate on some of the points made in this previous paper.

*Abbreviations used are: AM, actomyosin; M, myosin; AM·ATP, AM-ADP.P, etc., ATP bound to actomyosin, ADP and inorganic phosphate bound to actomyosin, etc.; m-calpain, the micromolar $Ca^{2+}$-requiring $Ca^{2+}$-dependent proteinase; m-calpain, the millimolar $Ca^{2+}$-requiring $Ca^{2+}$-dependent proteinase*

*Darrel E. Goll, Muscle Biology Group, Universityof Arizona, Tucson, Arizona 85721.

[1]Merin L. Boehm, 5344 Roberts Road, Hamburg, New York 14075.

[2]Geert H. Geesink, USDA, ARS Roman L. Hruska Meat Animal Research Center, Clay Center, Nebraska 68933.

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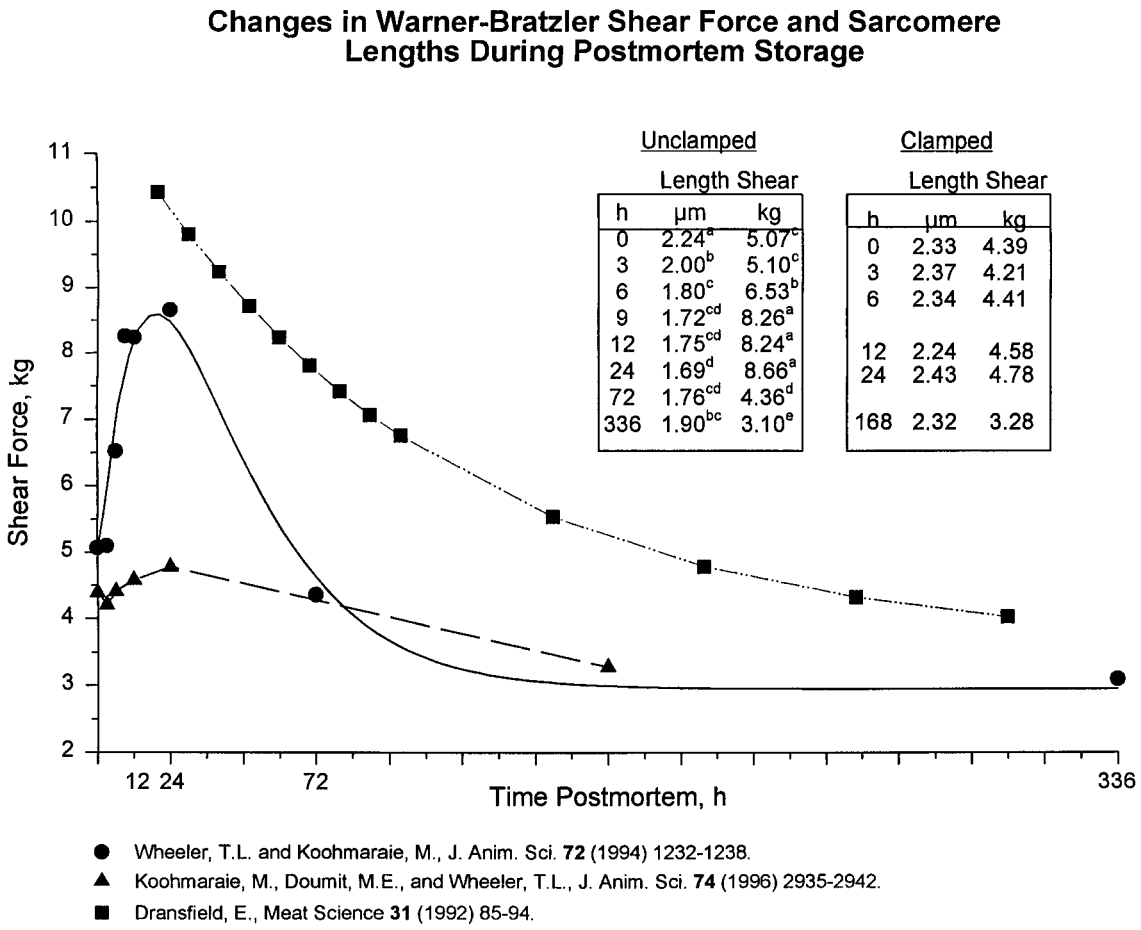
## Postmortem Changes in Tenderness

Before discussing the potential causes of postmortem tenderization, it is necessary to know as precisely as possible how tenderness changes during postmortem storage. Relatively few studies have examined postmortem changes in tenderness beginning immediately after death, probably because it is very difficult to measure tenderness during the first 24 hr postmortem. Muscle ATP concentration does not decrease to levels too low to support contraction until 6 to 12 hr postmortem, depending on state of the animal at death and conditions of postmortem handling, and any attempt to shear or cook postmortem muscle that contains ATP results in severe contraction. This contraction markedly increases toughness and results in anomalously high shear (low tenderness) values for such muscle. Notwithstanding these difficulties, several attempts have been made during the last 5 to 6 years to obtain accurate measurements of tenderness during the first 24 hr postmortem (Figure 1). These attempts

**FIGURE 1.**




*Results of three different procedures for measuring changes in tenderness during postmortem storage. The Dransfield procedure used a modeling approach and is based on beef pectoralis profucdus muscle. The "unclamped" and "clamped" approaches are measurements made on lamb longissimus muscle. The data in this figure were from Dransfield (1992), Wheeler and Koohmaraie (1994; "unclamped") and Koohmaraie et al (1996, "clamped").*

have either involved clamping muscle to prevent shortening or have used a modeling approach based on estimates of changes in μ-calpain activity and the rate of postmortem tenderization at the pH and temperature of postmortem muscle (assumed ultimately to reach 5.5 and 5°C, respectively). Although the patterns of changes in postmortem tenderization obtained when using these three approaches differ substantially, they all share two common features: 1) except for the clamped model, the largest changes in tenderness occur during the first 72 hr postmortem (Table 3); a number of other studies in which tenderness measurements started at 1 day postmortem have also shown that the rate of change in tenderness is greatest during the first 72 hr postmortem (Calkins and Seideman, 1988; Dransfield et al., 1992; Goll et al., 1964; Olson, et al., 1977; Ouali, 1990); and 2) after 72 hr postmortem, the toughness in all three models follows a slow exponential decrease. Attempts to understand what causes postmortem tenderization need to begin with an analysis of these three different patterns of postmortem tenderization (Figure 1).

**The Dransfield Model**–This model of postmortem tenderization is based on fitting measurements of bovine skeletal muscle tenderness and μ-calpain activity obtained at different times postmortem to a general first-order, rate equation of the form, $T_t = T_o e^{-kt}$, where $T_t$ is tenderness at some time postmortem, $T_o$ is at-death tenderness, k is a rate constant, and t is time. A sophisticated analysis involving contributions of temperature and rates of inactivation of μ-calpain in postmortem muscle produces the curve shown in Figure 1. First-yield-force measurements of bovine skeletal muscle toughness were highly related to toughness predicted from this μ-calpain-based model with an $R^2 = 0.68$ ($r = 0.82$). The model, however, is based on two assumptions that are questionable: 1) that μ-calpain retains 24 to 28% of its maximal activity (as measured at 25°C and pH 7.5) at 5°C and pH 5.5 (Koohmaraie et al., 1986); and 2) that the ability of calpastatin

to inhibit the calpains is much reduced below pH 6.5 and is lost completely below pH 5.7 (Cottin et al., 1981). Subsequent measurements have shown that μ-calpain retains only 14 to 18% of its maximal activity (as measured at 25°C and pH 7.7) at 25°C and pH 5.5 (Edmunds et al., 1991). Lowering the temperature to 4 to 5°C would reduce μ-calpain activity even further, and it seems unlikely that μ-calpain retains more than 5 to 8% of its maximal activity at the pH and temperature of postmortem muscle. The study comparing ability of calpastatin to inhibit calpain activity at different pH values used a very impure preparation that contained polypeptides of 70- and 13-kDa, rather than the 125-kDa form of intact skeletal muscle calpastatin. Moreover, a 10-fold excess of this impure calpastatin preparation (w/w) was required to inhibit the calpain being studied (Cottin et al., 1981). Purified skeletal muscle calpastatin, on the other hand, completely inhibits the calpains at a 1:4 ratio, w/w, of calpastatin to calpain. Studies using purified calpastatin and calpain (Otsuka and Goll, 1987) have shown that pH has no effect on the ability of calpastatin to inhibit the calpains down to a pH of 6.18. The calpains precipitate from aqueous solutions below pH 6.2, so measurements of calpastatin's ability to inhibit the calpains are difficult to interpret below this pH. It is unclear how much the adjustments needed to correct for these two questionable assumptions would affect the Dransfield model, especially during the critical first 72 hr postmortem.

**The Unclamped Model**–The "unclamped" or *in situ* aging data were the first attempt to experimentally measure tenderness of skeletal muscle during the first 24 hr postmortem without the confounding effects of ATP. After clamping to prevent its shortening during excision from the carcass, lamb *longissimus* muscle was removed at each of the specified times postmortem (Figure 1). The clamped muscle was rapidly frozen at -30°C for 1.5 hr and was then stored at -5°C for 10 days. Storage at -5°C permitted slow degradation of ATP, so the ATP concentration was zero or nearly zero after 10 days at -5°C. Hence, these samples could be cooked, and tenderness measurements made without the confounding effects of ATP-induced shortening. These measurements indicated that toughness of lamb *longissimus* muscle increases markedly during the first 24 hr postmortem, then decreases rapidly between 24 to 72 hr postmortem, and finally decreases more slowly out to 14 days postmortem (Figure 1). Clearly, the large increase in toughness during the first 24 hr cannot be associated with postmortem proteolysis, and if any significant amount of proteolysis occurred during the first 24 hr postmortem, the tenderizing effects of this proteolysis were far overshadowed by some other phenomenon that caused a large increase in toughness. The sarcomere lengths of the clamped muscle showed that this muscle shortened significantly while it was attached to the carcass skeleton during the first 24 hr postmortem. It is known that cold-shortening can significantly increase muscle toughness (Marsh and Leet, 1966), and this would be a simple explanation for the large increase in toughness between 0 and 24 hr postmortem. However, sarcomere lengths increase only slightly between 24 and 72 hr postmortem even though toughness decreases markedly. What causes this large decrease in toughness? Is it due to postmortem proteolysis or is it due to some other phenomenon? As will be argued later and as the results on clamped muscle suggest, the large decrease in toughness between 24 and 72 hr postmortem in the unclamped muscle is probably not caused by postmortem proteolysis but rather by a change in the actin/myosin interaction.

**The Clamped Model**–To circumvent the confounding effect caused by shortening in the unclamped muscle, Koohmaraie and coworkers did a second study in which shortening was prevented by removing sections of clamped lamb *longissimus* muscle immediately after death and allowing the clamped muscle to "age" at -1.1°C for various periods of time before rapidly freezing it at -30°C for 1.5 hr as was done in the unclamped study. After freezing, the clamped samples were stored at -5°C for 8 days to allow hydrolysis of all ATP. As shown in Figure 1, clamping effectively prevented sarcomere shortening during aging at -1.1°C. The clamped and unshortened muscles also did not undergo the marked increase in toughness between 0 and 24 hr postmortem that the unclamped muscles did. Three important conclusions may be drawn from these observations.

1. The large increase in toughness that occurred in the "unclamped" muscles attached to the carcass during the first 24 hr postmortem is associated with the shortening of these muscles during this period. Although the simple interpretation would be that this large increase in toughness is caused by the cold-shortening effect, this is unlikely to be the correct interpretation or the true cause of this toughness, as will be discussed subsequently.
2. The small amount of proteolysis that occurs during the first 72 hr postmortem (Ho et al., 1994; 1996; 1997; Huff-Lonergan et al., 1995; Taylor et al., 1995) does not result in any appreciable increase in tenderness. Clamping muscles at-death removes any confounding effects that shortening has on tenderness, and tenderness of the clamped muscles remains almost constant during the first 72 hr postmortem. If postmortem proteolysis contributed significantly to tenderness during this period, toughness would have been expected to decrease during the first 72 hr postmortem.
3. Because postmortem proteolysis does not contribute substantially to increased tenderness during the first 72 hr postmortem, the large decrease in toughness that occurs between 24 and 72 hr postmortem in the unclamped muscle (Figure 1) is not due to proteolysis. Neither is it due entirely to lengthening of the shortened sarcomeres. Although some sarcomere lengthening from 1.69 to 1.76 μM occurs (this lengthening was not statistically significant in Wheeler and Koohmaraie's study) between 24 and 72 hr postmortem, this increase is not nearly as great as the increase

from 1.76 to 1.90 μM that occurs between 72 and 336 hr postmortem and that is associated with a much smaller change in tenderness (from 4.36 kg to 3.10 kg or a 1.06 kg decrease from 72 to 336 hr compared with 8.66 kg to 4.36 kg or a 4.30 kg decrease for the 24 to 72 hr postmortem period).

Thus, a careful analysis of the most recent models for postmortem tenderization raises several questions relating to the causes of this tenderization: 1) what is the role/importance of proteolysis to postmortem tenderization? 2) What is the significance of sarcomere length/cold shortening to tenderness? 3) Are factors other than proteolysis and shortening important to postmortem tenderization and if so, what are these factors?

## Role/Importance of Proteolysis in Postmortem Tenderization

The evidence indicating that proteolysis probably does not have a significant role in changes in tenderness during the first 72 hr postmortem has already been discussed in the preceding sections. Briefly this evidence can be summarized as follows: 1) relatively little proteolytic degradation of cytoskeletal proteins can be detected during the first 72 hr postmortem; 2) there are relatively few changes in muscle ultrastructure during the first 72 hr postmortem; the changes observed during this period are limited to loss of costameres and sarcolemmal structure (Taylor et al., 1995) and loss of Z-disk integrity typified by loss of the fibrillar Z-disk structure and appearance of discontinuities in Z-disks (Ho et al., 1995; 1996); and 3) perhaps most importantly, despite the small amount of cytoskeletal protein degradation and some changes in the Z-disk, Z-disk/I-band integrity, and sarcomere/costamere integrity (presumably caused by proteolytic degradation), there is no change in tenderness of unshortened muscle during the first 72 hr postmortem. It should be noted that the preceding statements apply to muscle that has not been electrically stimulated, that has not been injected with $CaCl_2$ or other agents designed to enhance the rate of postmortem tenderization, and that has been placed in a cooler at 2°C or less immediately postmortem (i.e., no high temperature aging). Electrical stimulation increases the extent of ultrastructural disruption of myofibrils early postmortem (Ho et al., 1995; 1996), probably because of mechanical rupture due to the severe contractures induced by electrical stimulation, and $CaCl_2$ injection increases the rate of tenderization during the first 72 hr postmortem partly because of increases in the extent of postmortem proteolysis by the calpains (Koohmaraie et al., 1989; 1990).

Consequently, the available evidence suggests that, in untreated carcasses placed at 2 to 4°C immediately postmortem, proteolysis has a relatively small role in postmortem tenderization until after 72 hr and that the large changes in tenderness observed during the first 72 hr postmortem (Calkins and Seideman, 1988; Goll et al., 1964; Wheeler and Koohmaraie, 1994) are due largely to events other than postmortem proteolysis. Under these "normal" conditions, postmortem proteolysis probably accounts for 30 to 40% of total postmortem tenderization observed during the first 19 to 21 days postmortem, and the contributions due to proteolysis occur primarily after 72 hr postmortem.

**TABLE 1. Summary of Some of the Differences Between Living Skeletal Muscle Cells and Postmortem Skeletal Muscle That May Affect the AM Interaction.**

| |
|---|
| A. There is little or no ATP in postmortem muscle; the ATP concentration is 5-10 mM in living muscle. |
| B. Postmortem muscle has a higher free $Ca^{2+}$ concentration than living muscle [0.1 to 0.2 mM in living muscle (Harkins et al., 1993) to greater than 100 mM in postmortem muscle (Jeacocke et al., 1993; Parrish et al., 1981)], so the actomyosin interaction is constantly in the "ON" state. |
| C. The pH of postmortem muscle (5.4 to 5.8) is lower than in living muscle (6.8 to 7.3). |
| D. Is there limited proteolysis of myosin LC1? Of myosin S-1?<br>1. Myosin light chain has been reported to interact with actin in the "strong" binding state; cleavage of this light chain, therefore, could contribute to weakening of the actin/myosin interaction in postmortem muscle. |

## Significance of Sarcomere Length/Cold Shortening To Postmortem Tenderization

Following the initial observation that postmortem shortening at temperatures below 15 to 16°C has profound effects on toughness of muscle (Marsh and Leet, 1966), a number of studies found that shortened sarcomere lengths were related to increased toughness (Davis et al., 1979; Herring et al., 1965). The initial observation indicated that muscles having sarcomere lengths of 2.5 to 2.0 μM were tender, those having sarcomere lengths of 1.7 to 2.0 μM were moderately tough, and those having sarcomere lengths of 1.5 to 1.7 μM were extremely tough (Marsh and Leet, 1966). A decrease in sarcomere lengths to less than 1.5 μM resulted in gradually increasing tenderness. These results were interpreted to indicate that, as the degree of overlap between the thick and thin filaments gradually increased during sarcomere shortening, the "denser" myofilament lattice resulting from this shortening increased muscle toughness. At sarcomere lengths of 1.5 to 1.7, there is complete overlap of thick and thin filaments and a "double overlap" of thin filaments in the center of the sarcomere. This degree of shortening is the state of maximum toughness in cold-shortened muscle. As sarcomeres shorten to lengths less than 1.5 μM, the thick filaments, which are 1.5 μM in length and which abut up against the Z-disk in the 1.5 to 1.7 μM sarcomeres, now penetrate the Z-disk, disrupting its structure and the structure of the sarcomere, and resulting in an increase in tenderness.

This interpretation of how sarcomere length is related to tenderness is dialectically appealing and has received widespread acceptance. Several studies, however, have found

**TABLE 2. Summary of Some of the Evidence That the Actin/ Myosin Interaction Changes During Postmortem Storage.**

A. The $Mg^{2+}$-modified ATPase activity of myofibrils (Goll and Robson, 1967; Ouali and Valin, 1980; Ouali, 1990) or of actomyosin (Fujimaki et al., 1965; Robson et al., 1967) prepared from postmortem muscle increases by 20 to 50% during the first 24 hr postmortem and then decreases back to at-death levels after 13 days postmortem.

B. Rate of superprecipation of actomyosin extracted from postmortem muscle increases up to 7 days of postmortem storage and then decreases slightly after 14 days postmortem (Arakawa et al., 1970; Goll et al., 1970).

C. Rigor-shortened sarcomeres lengthen slightly between 3 and 14 days postmortem. (Gothard et al., 1966; Stromer et al., 1967; Takahashi et al., 1967; Wheeler and Koohmaraie, 1994).

D. Rigor-shortened mouse *biceps brachii* strips can be stretched from an average sarcomere length of 1.7 mm to 2.0 mm *in situ* after 4 hr postmortem (Hegarty, 1972; Herlihy et al., 1972); this stretching is accompanied by a widening of the h-zone.

   1. Rigor-shortened muscle strips from turkey or porcine muscle can also be stretched without rupturing (Hegarty et al., 1973).

E. Muscle strips clamped at constant length develop tension during the first 6 to 30 hr postmortem at 2°C and then gradually lose the ability to maintain that tension after 24 to 30 hr (Busch et al., 1972).

F. "At-death" actomyosin requires 0.6 mM ATP to dissociate the actin/ myosin complex in 0.5 M KCl at pH 7.0; under the same conditions, 0.2 mM ATP or 0.1 mM ATP will dissociate actomyosin prepared from muscle after 2 or 7 days postmortem at 4°C (Fujimaki et al., 1965).

G. Muscle becomes inextensible (less than 1 to 2% extensibility) as ATP concentrations decrease to zero and then gradually regain some of their extensibility (to 5 to 6%) with increasing time of postmortem storage; the time at which extensibility increases coincides with loss of ability to maintain isometric tension (Davey and Gilbert, 1977; Etherington et al., 1987).

   1. Extensibility of cooked *longissimus* samples have been shown to be very highly related to panel tenderness scores ( r = 0.88 to 0.92; Hostetler and Cover, 1961).

**TABLE 3. Changes in Tenderness During Postmortem Storage.**

| Model[a] | Change during[b] the 1st 72 hr (kg) | Change after[c] 72 hr. (kg) | Change during 1st[d] 72 hr Total change |
|---|---|---|---|
| Dransfield | 5.43 | 2.95 | 65.8% |
| Unclamped | 4.30 | 1.36 | 76.0% |
| Clamped | 0.48 | 1.30 | 27.0% |

[a] *See Figure 1 for references relating to these models.*
[b] *Difference between the maximum toughness (at 24-hr for unclamped and clamped models; a value of 12.5 kg was used for the 0-hr toughness in the Dransfield model) and the 72-hr toughness.*
[c] *Difference between toughness at 72 hr and the ultimate toughness (3.00 kg for the unclamped and clamped models, 4.12 kg for the Dransfield model).*
[d] *Total change in tenderness was calculated as the difference between maximum toughness (at-death in the Dransfield model and at 24 hr postmortem in the unclamped and clamped models) and the ultimate toughness.*

little or no relationship between sarcomere length and toughness (Aberle et al., 1981; Culler et al., 1978; DeVol et al., 1988; Parrish et al., 1979). Parrish et al. (1979) reported a correlation of 0.06 between sensory tenderness and sarcomere length measured on 123 animals of widely differing tenderness, and DeVol et al. (1988) found correlations of 0.07 and 0.01 between sarcomere length and Warner-Bratzler shear and sensory tenderness measurements, respectively, in a study involving 120 animals. It has been suggested (Lockner et al., 1980) that cold shortening is not a primary determinant of tenderness except in very rapidly chilled, lean carcasses. In a reexamination of the relationship between tenderness and postmortem shortening, Smulders et al. (1990) found that tenderness was completely independent of sarcomere length ( r = 0.16) in rapidly glycolyzing postmortem muscles that reached a pH of 6.3 or less within 3 hr postmortem. Indeed, some muscles in this class that had sarcomere lengths of 1.6 to 1.7 μM were among the most tender of the muscles examined in 67 animals.

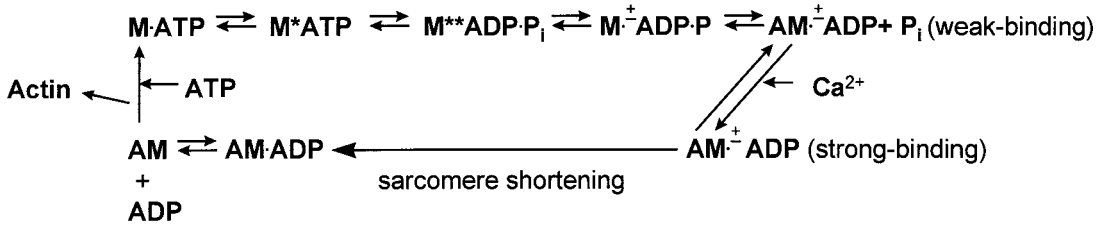
That it is possible for muscles having sarcomere lengths of 1.6 to 1.7 μM to be very tender indicates that there is no direct, causal link between short sarcomere lengths/high myofibrillar density and decreased tenderness as has been widely assumed. Rather, that different studies obtain widely different results for the relationship between sarcomere length and tenderness indicates that sarcomere length is variably associated with some factor that in turn is directly and causally related to tenderness. The effects of cold-shortening on tenderness occur during the first 72 hr postmortem, so it seems likely that this factor is directly related to the large increase in toughness that occurs during the first 24 hr postmortem in "unclamped" muscle (Figure 1).

## Are Factors Other Than Proteolysis Important to Postmortem Tenderization?

The preceding sections have summarized the reasons for believing that something other than proteolysis or sarcomere length has a critical role in tenderization during the first 72 hr postmortem. The available information suggests that changes in the actin/myosin cross-bridge interaction from a resting (weak) state to a contractile (strong) state followed by a gradual weakening of this strong state has an important effect on tenderness. The reasons for believing that state of the actin/myosin cross-bridge interaction is involved in postmortem tenderization and some details on how this interaction may change during postmortem storage have been discussed earlier (Goll et al., 1995), and only a summary of this evidence will be presented here.

As muscle ATP concentration decreases during postmortem storage, myofibrils attempt to contract, initiating the transition from a weak to strong actin/myosin cross-bridge state. This transition probably causes the large increase in toughness observed by Wheeler and Koohmaraie (1994) in their "unclamped" muscles (Figure 1). The energy needed for contraction is lost as muscle ATP concentrations fall to zero or nearly zero, and myofibrils/muscle become temporarily

**FIGURE 2.**




*Results of the transient state kinetic and fluorescence spectroscopy studies are summarized in the above scheme.*

"locked" in the strong actin/myosin cross-bridge state. Washing muscle strips *in vitro* to remove their ATP creates this same "locked" state, and muscle biologists call this the "rigor" state. In *in vitro* experiments at neutral pH and in the absence of ATP and $Ca^{2+}$, myofibrils can remain in this "rigor" or inextensible state almost indefinitely (assuming microbial action is inhibited). The environment in postmortem muscle, however, differs entirely from that in living muscle or from that in "rigor" myofibrils stored at neutral pH and in the absence of $Ca^{2+}$ (Table 1). Either these differences or some other factor contribute to a gradual weakening of the strong actin/myosin cross-bridge interaction with increasing time of postmortem storage. Whatever the cause, there is ample evidence to show that the actin/myosin interaction changes during postmortem storage (Table 2). Observations such as a loss of ability to maintain isometric tension (Busch et al., 1972), an increase in extensibility (Etherington et al., 1987; Herlihy et al., 1972), and a lengthening of rigor-shortened sarcomeres (Stromer et al., 1967; Wheeler and Koohmaraie, 1994) all indicate that the actin/myosin interaction becomes progressively weaker during postmortem storage (Table 2). It seems likely, therefore, that this weakening of the actin/myosin interaction is directly responsible for most of the large decrease in toughness between 24 and 72 hr postmortem of the unclamped muscle (Figure 1). Interestingly, the time-course of increasing toughness for 24 hr followed by a decrease in toughness after 24 hr roughly parallels the time-course of increasing isometric tension followed by a decrease in isometric tension of bovine *longissimus* muscle held at 2°C (Busch et al., 1972). Also, the increase in $Mg^{2+}$-modified myofibrillar ATPase during the first 24 to 36 hr postmortem is temporally related to increased toughness, and the gradual decrease in $Mg^{2+}$ -modified myofibrillar ATPase activity is roughly related temporally to the decrease in muscle toughness (Goll and Robson, 1967; Figure 1)..

The x-ray crystallographic structures of actin (Holmes et al., 1990; Kabsch et al., 1990) and of the S1 head of myosin (Rayment et al., 1993b) have now been determined, and the nature of the actin/myosin interaction has been ascertained at the molecular level by combining these crystallographic structures with "low resolution" (30Å) electron density maps of the actin/myosin complex (Rayment et al., 1993a). These studies have shown that, in the rigor (no ATP) state, at least four sites on the myosin head contact actin and that two adjacent actin monomers in the same strand of the double-stranded actin filament are involved in this rigor interaction (Rayment et al., 1993a). In addition, several lines of evidence indicate that the N-terminus of the myosin light chain 1 (the large essential light chain) may interact with actin after the initial actin/myosin contacts are made. This x-ray crystallographic information has been combined with information obtained from transient state kinetic and electron paramagnetic resonance studies to show that the actin/myosin cross-bridge interaction passes through at least two different states during each contractile cycle. In resting muscle, the actin/myosin cross-bridge is in a "weak-binding" state. After ATP hydrolysis and dissociation of the inorganic phosphate resulting from this hydrolysis, the actin/myosin cross bridge interaction undergoes a transition to a "strong binding" state; this is the state that produces the contractile force (Figure 2).

The $M^{*}$, $M^{**}$, and $M^{\pm}$ represent different conformational states of the myosin cross-bridge as indicated by fluorescence spectroscopy. Following shortening, the myosin cross-bridge is oriented approximately at a 45° angle relative to the actin filament. This is called the "rigor" state by muscle biologists and is the only state that has been possible to study by x-ray crystallographic and image reconstruction techniques. Hence, the structure of the cross-bridge in the "weak-binding" state is not known in detail and has been inferred from a number of fluorescence spectroscopy and electron spin resonance studies.

This recent information on the actin/myosin cross-bridge interaction has profound significance to postmortem tenderization. Changes in the actin/myosin cross-bridge interaction during postmortem storage of muscle have not been studied, but because postmortem muscle has little or no ATP, the actin/myosin cross-bridge interaction in this muscle is probably in one of four possible states:

$AM^{\pm}{\cdot}ADP{\cdot}P_i$, $AM^{\pm}{\cdot}ADP$, AM·ADP, or AM. The first of these is a weak-binding state. Because of the absence of ATP and the presence of adenylate kinase in postmortem muscle, other states such as $AM^{\pm}{\cdot}AMP$, $AM^{\pm}$ (weak-binding) may also exist. Moreover, the relative proportions of these different states will depend on the conditions at which muscle enters rigor mortis. For example, because contraction (strong-binding

state) is impeded by low pH values, the proportion of myosin cross-bridges in the strong binding state will be less if ATP levels decrease slowly postmortem so muscle pH is less than 6.3 before intracellular $Ca^{2+}$ concentrations increase to a level high enough to initiate contraction. Such a situation would decrease the proportion of myosin cross-bridges in the strong-binding state and account for the lack of relationship between sarcomere length and tenderness in muscles that reached a pH of 6.3 or less within 3 hr postmortem (Smulders et al., 1990). In these muscles, sarcomeres shorten, but the low pH decreases the proportion of cross-bridges in the strong-binding state. Because of the lack of information on changes in the actin/myosin cross-bridge interaction in postmortem muscle and because the relative strengths of the different possible actin/myosin cross-bridge states in postmortem muscle are unknown (*e.g.*, is AM a stronger binding state than AM±ADP, etc.?), it presently is possible to devise an explanation for almost any pattern of postmortem tenderization based on changes in the actin/myosin cross-bridge interaction. For example, clamping muscles to prevent their shortening during the onset of rigor mortis (Figure 1) would prevent the majority of myosin cross-bridges from "swiveling" relative to the actin filament and from forming the additional contacts with actin needed for the transition to the strong-binding state. Such muscle would not experience the large increase in toughness that unclamped muscle does and would remain tender regardless of the nature of the nucleotide bound to the myosin cross-bridge (Figure 1).

## Conclusions

As indicated in the earlier discussion of possible changes in the actin/myosin cross-bridge interaction in postmortem muscle (Goll et al., 1995), postmortem tenderization is a complex process that almost certainly is affected by a large number of factors including rate of postmortem pH decline, osmolarity of postmortem muscle cells, temperature, and genetic factors inherent to the animal, among others. However, it seems likely that these factors all exert their effects on rate of postmortem tenderization by affecting a few basic processes: 1) the nature of the actin/myosin cross-bridge interaction, especially the relative proportion of myosin cross-bridges in the strong-binding state, and the subsequent weakening of this interaction; and 2) calpain-induced cleavages of titin and nebulin that cause a weakening of the Z-disk/sarcomere connection, degradation of certain cytoskeletal proteins such as vinculin and dystrophin that constitute the costameres, and degradation of desmin and other intermediate filament proteins that form the intermyofibrillar linkages. Although postmortem proteolysis has been studied extensively, a careful analysis of the information available on postmortem tenderization suggests that proteolysis has a relatively small role in the large changes in tenderness that occur during the first 72 hr postmortem. On the other hand, postmortem proteolysis likely is responsible for most of the tenderization that occurs later during postmortem aging. Changes in the actin/myosin cross-bridge interaction during postmortem storage have received little attention from animal scientists. In view of the potentially major role that these changes may have during the first 72 hr of postmortem tenderization, future studies on the actin/myosin cross-bridge interaction in postmortem muscle would seem warranted.

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