SKELETAL MUSCLE TISSUE DISRUPTION

by

F. C. Parrish, Jr.*

Disruption of skeletal muscle tissue components and the events bringing about tissue disruption are important aspects of tenderization. Both natural (endogenous) and exogenous processes are responsible for tissue disruption and resultant tenderness. These processes involved in tissue disruption can vary from those occurring at the molecular to those occurring at the macro level of anatomical organization of skeletal muscle.

The emphasis in the first part of this paper will be on the post-mortem molecular changes of myofibrillar proteins and the relationship of these changes to tenderization in bovine muscle. For background information on muscle structure, myofibrillar proteins and the role of myofibrillar proteins in meat tenderness, the cogent review by Goll *et al.* (1974) is recommended. The remaining part of the paper will be devoted to exogenous forms of tissue disruption and their relationship to tenderization.

Natural (Endogenous) Tenderization

The phenomenon of myofibrils breaking into shorter segments at or near the Z-disk during post-mortem storage of muscle, termed myofibril fragmentation, has been observed by several investigators using microscopic methods (Davey and Gilbert, 1967, 1969; Parrish *et al.*, 1973; Olson *et al.*, 1976; Sayre, 1970; Takahashi *et al.*, 1967). Figure 1 clearly illustrates what happens to myofibril microstructure during post-mortem storage of *longissimus* muscle. Myofibrils isolated from 1 day post-mortem aged muscle at 2°C and observed under polarized-light or phase-contrast microscopy are relatively well-ordered, are long (they may contain over 20 sarcomeres per myofibril) and their Z-disks appear to be intact. As post-mortem aging increases, however, myofibrils become shorter, more fragmented and Z-disks are degraded. Also to be noted is that myofibril fragmentation occurs in the structural region of the Z-disk. Degradation of Z-disk of bovine, porcine and rabbit myofibrils at different times and temperatures of post-mortem storage has been described by Henderson *et al.* (1970). The post-mortem changes observed in myofibrils with phase-contrast microscopy from longissimus (L) are similar in myofibrils from semitendinosus (ST); however, little change can be detected in myofibrils from psoas major (PM) muscle (Olson *et al.*, 1976).

The microscopic changes in myofibrils observed during post-mortem storage of muscle seemed to coincide with improved tenderness (Parrish *et al.*, 1973). Quantitation of myofibril fragmentation by microscopy, however, has not been successfully attained, and therefore, the exact relationship between fragmentation and tenderness was unknown. Consequently, we were interested in finding a quantitative method of determining myofibril fragmentation, and then relating these values to tenderness values. Davey and Gilbert (1969) had earlier reported the measurement of a suspension of myofibrils by a light absorbance technique, but they did not use this method to determine the relationship between myofibril fragmentation and tenderness. However, Moller *et al.*, (1973) successfully used this method and obtained a correlation of .78 between myofibril fragmentation value and sensory tenderness of bovine longissimus muscle. Subsequently, we successfully adapted this method to our studies (Olson *et al.*, 1976). This method involved homogenation of a certain amount of finely minced muscle, followed by a series of centrifugation and decantation steps. Our recent efforts have reduced the number of washes (two) necessary to isolate myofibrils and make satisfactory measurements, and, therefore, the time to carry out the procedure has been shortened (Culler *et al.*, 1978). After adjusting the protein concentration of the myofibril isolate, the absorbance of a 10 ml aliquot of the diluted myofibril suspension is measured at 540 nm. This absorbance method is based on the premise that the amount of light absorbed by a myofibril suspension will be proportional to the relative size of myofibrils and fiber pieces in the suspension. For example, in two myofibril suspensions having the same protein concentration, the suspension having more fragmented myofibrils and smaller fiber pieces will be more turbid and have a higher absorbance value than the other suspension with longer myofibrils and larger fiber pieces. After the absorbance reading per 0.5 mg/ml myofibril protein was obtained, it was multiplied by 200. We (Olson *et al.*, 1976) termed this value myofibril fragmentation index (MFI).

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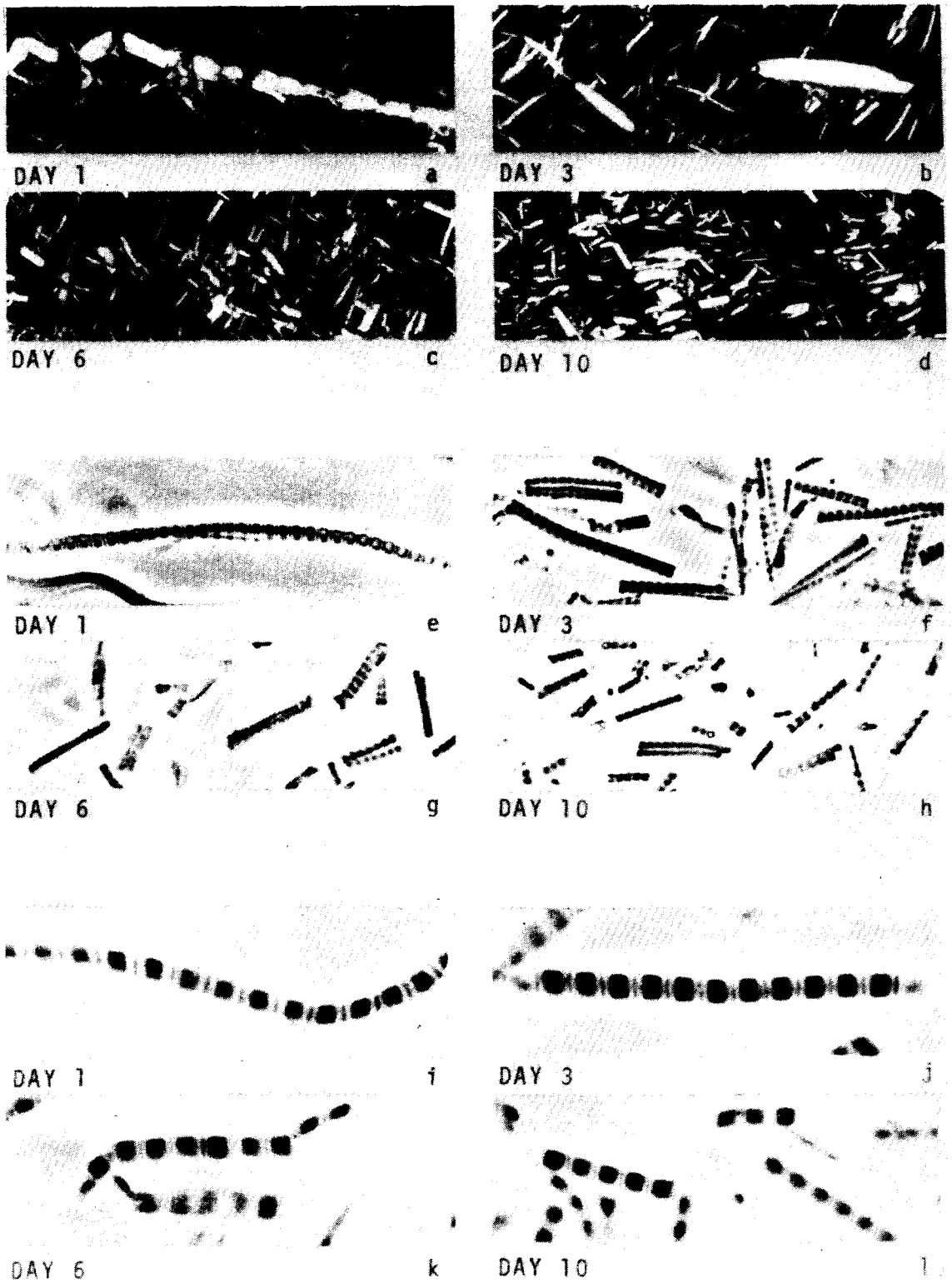

FIGURE 1

Polarized-light and phase micrographs of myofibrils prepared from bovine longissimus muscle at different times of post-mortem storage at 2°C. (a) Polarized-light micrograph of myofibrils at 1 day post-mortem. Both single myofibrils and fiber pieces are present (X100). (b) Polarized-light micrograph of myofibrils at 3 days post-mortem. Fiber pieces and myofibrils are more fragmented than those in Figure 1a (X200). (c) Polarized-light micrograph of myofibrils at 6 days post-mortem. Greater myofibril fragmentation is shown than in Figure 1a and 1b (X200). (d) Polarized-light micrograph of myofibrils at 10 days post-mortem. Myofibrils are highly fragmented (X200) (e) Phase micrograph of myofibrils at 1 day post-mortem (X800). (f) Phase micrograph of myofibrils at 3 days post-mortem. Myofibrils are fragmented more than myofibrils in Figure 1e (X800). (g) Phase micrograph of myofibrils at 6 days post-mortem. Myofibrils are more fragmented than those in Figure 1e and 1f (X800). (h) Phase micrograph of highly fragmented myofibrils at 10 days post-mortem (X500). (i) Phase micrograph of myofibril at 1 day post-mortem. Sarcomeres are relaxed and Z-lines are prominent (X200). (j) Phase micrograph of myofibrils at 3 days post-mortem. Some fragmentation is observed and Z-lines are prominent (X2000). (k) Phase micrograph of myofibrils at 6 days post-mortem. Myofibrils are more fragmented and Z-lines are less prominent (X2000). (l) Phase micrograph of myofibrils at 10 days post-mortem. Myofibrils are highly fragmented and Z-lines are very faint (X2000). From Olson et al. (1976) and reproduced by permission of the Institute of Food Technologists and the authors.

Figures 2 and 3 illustrate the relationship between MFI and post-mortem storage and Warner-Bratzler (W-B) shear values and post-mortem storage for ST and PM muscles, respectively. In addition, Figures 4 and 5 demonstrate the effect of post-mortem storage temperature on MFI and W-B shear values for L, ST and PM muscles, respectively. The conclusions that can be derived from these figures are: (1) the increase in myofibril fragmentation index coincides with the decrease in W-B shear values during post-mortem storage; (2) Z-disk degradation, MFI and W-B shear values were relatively similar for L and ST muscles, but were distinctly dissimilar for PM muscle; and (3) higher post-mortem storage temperature (25°C) decreased W-B shear values and increased MFI in L and ST muscles, whereas storage temperature had little effect on these properties of PM muscle.

Subsequently, we (Olson and Parrish, 1977) were able to find a strong relationship between MFI and tenderness of longissimus from veal, A and C-maturity carcasses (Table 1). These values represent some of the strongest relationships reported between a tissue component and tenderness. Furthermore, that 50% of the variation in tenderness of longissimus from A-maturity carcasses can be accounted for by myofibril fragmentation demonstrates the importance of myofibrillar proteins and their structural and chemical

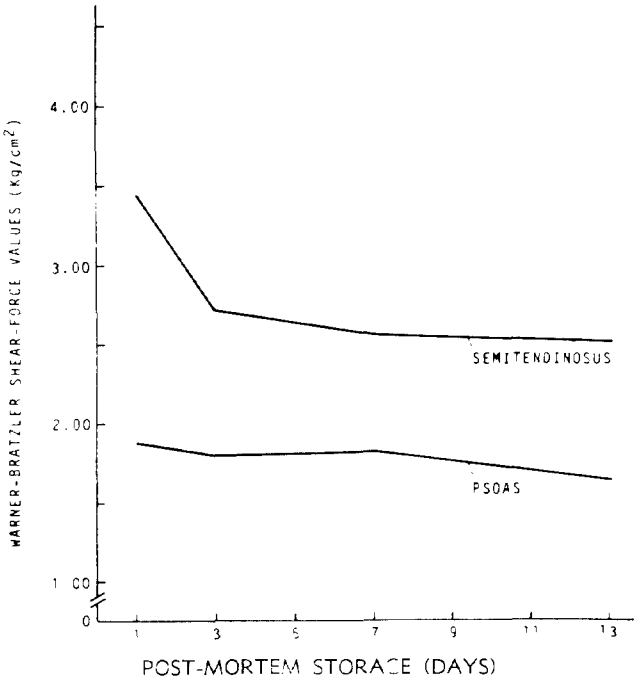

FIGURE 3

Effect of post-mortem storage time on Warner-Bratzler (W-B) shear-force values of bovine semitendinosus and psoas major muscles at different times of post-mortem storage at 2°C. From Olson et al. (1976) and reproduced by permission of the Institute of Food Technologists and the authors.

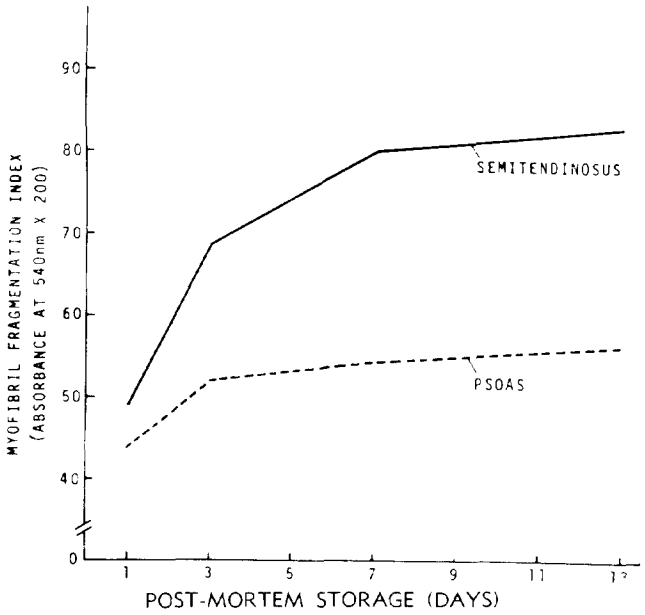

FIGURE 2

Effect of post-mortem storage time on the fragmentation index of myofibrils from bovine semitendinosus and psoas major muscles at different times of post-mortem storage at 2°C. From Olson et al. (1976) and reproduced by permission of the Institute of Food Technologists and the authors.

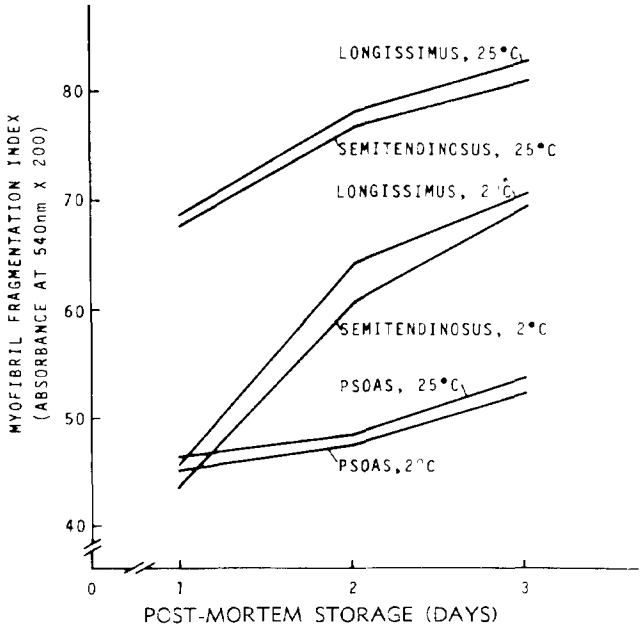

FIGURE 4

Effect of post-mortem storage time and temperature treatments on the myofibril fragmentation index of myofibrils prepared from bovine longissimus, semitendinosus and psoas major muscles at different times of post-mortem storage at 2°C and 25°C. From Olson et al. (1976) and reproduced by permission of the Institute of Food Technologists and the authors.

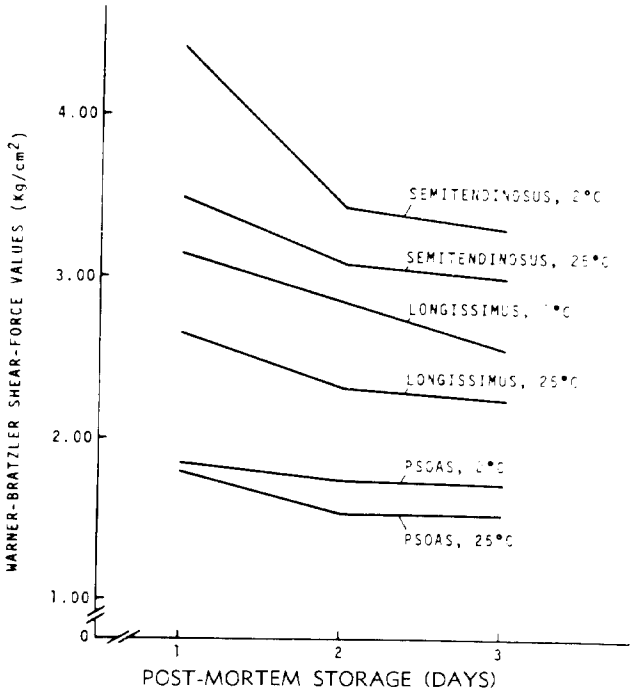

FIGURE 5

Effect of post-mortem storage time and temperature on Warner-Bratzler (W-B) shear-force values of bovine longissimus, semitendinosus and psoas major muscles at different times of post-mortem storage at 2°C and 25°C. From Olson et al. (1976) and reproduced by permission of the Institute of Food Technologists and the authors.

changes to beef steak tenderness. In addition, these results signify the potential importance of using MFI as a means of segregating carcasses into different tenderness categories. That is, a beef carcass grading system based on MFI offers more accurate assessment of tenderness and palatability attributes than the presently employed grading criteria. We have been unable, however, to find any carcass characteristics closely associated with MFI (Culler *et al.*, 1978). This is unfortunate because some simple means of identifying MFI would provide an extremely beneficial avenue of detecting carcasses with highly palatable meat. Our results clearly show that changes in myofibrils isolated from post-mortem muscle, and determined as a myofibril fragmentation index, are a good indicator of cooked meat (steaks from the longissimus broiled to an internal temperature of 65°C) tenderness.

The principal research problems requiring further investigation before MFI can be of practical benefit to a system of categorizing tenderness of carcasses and cuts are to (1) develop a rapid method of determining myofibril fragmentation, and (2) determine the ante- and post-mortem variables regulating myofibril fragmentation.

Although MFI offers a significant advance in relating a change of a skeletal muscle tissue component (myofibril fragmentation) to tenderness, an equally intriguing aspect of our studies was determining the causative factor of myofibril fragmentation (Olson *et al.*, 1977). We were fortunate that Busch *et al.* (1972) had previously discovered a protease endogenous to the muscle fiber that was capable of degrading Z-disk of myofibrils from rabbit skeletal muscle. They noticed that muscle strips incubated in Ca^{2+} lost Z-disk structure and the ability to maintain isometric tension, but strips incubated in EDTA and EGTA maintained their microscopic structure and isometric tension. To determine what factors were involved in Z-disk degradation, they isolated a protease from the sarcoplasm and tested its ability to remove Z-disks in the presence and absence of Ca^{2+}. The combination of Ca^{2+} and protease resulted in the removal of Z-disk from myofibrils. Thus, Ca^{2+} was necessary for protease activity in the loss of Z-disk structure, and the name given to the factor responsible for Z-disk degradation was calcium activated sarcoplasmic factor (CASF). Subsequently, Dayton *et al.* (1975, 1976a & b) carried out purification and characterization studies of the calcium activated protease isolated from porcine skeletal muscle. They called this protease calcium activated factor (CAF). Parenthetically, CASF and CAF are abbreviations for the same protease. It was determined that all Z-disk removing activity eluted in a single peak off of each column chromatography. Additionally the five-column chromatographic procedures produced a 17,800 fold increase in specific activity. Subsequently, purified CAF was incubated with purified myofibrillar proteins, and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was used to detect the possible degradative changes that CAF had on these

TABLE 1

CORRELATION COEFFICIENTS BETWEEN MYOFIBRIL FRAGMENTATION INDEX (MFI) AND WARNER-BRATZLER (W-B) SHEAR VALUES AND BETWEEN MFI AND SENSORY TENDERNESS SCORES (STS) OF LONGISSIMUS MUSCLE FROM THREE MATURITY GROUPS.

Maturity	Days Post-mortem	MFI vs WB	MFI vs STS
Veal[a]	1	−0.95**	0.88
	7	−0.97**	0.95**
A-maturity[b]	1	−0.65**	0.67**
	7	−0.75**	0.73**
C-maturity[c]	1	−0.68*	0.68*
	7	−0.72*	0.65*

a Six veal
b 35 A-mat
c 12 C-mat

*Significant at the 5% level
**Significant at the 1% level

proteins. It was found that CAF degraded troponin T and I, tropomyosin and C protein, but not troponin C, myosin, actin or α-actinin. These very interesting and original results by Busch *et al.* (1972) and Dayton *et al.* (1975, 1976a & b) provided an excellent base for our studies on beef tenderness.

First, a series of experiments was carried out to determine the changes in myofibrillar proteins that were related to tenderness and that could be attributed to CAF. Olson *et al.* (1977) were able to show that a limited and specific proteolysis occurred in myofibrillar proteins during post-mortem storage of bovine muscle. To accomplish this, myofibrils were isolated at specific post-mortem times and then analyzed on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. As it turned out, the sensitivity of SDS polyacrylamide gel electrophoresis was essential because only a very subtle change was observed to occur in myofibrils from post-mortem aged muscle. That is, there was no change in the major myofibrillar proteins, but there was a change in a subunit of troponin, troponin T. It was observed that there was a simultaneous disappearance of troponin T and appearance of a 30,000-dalton component (Figure 6). In addition, myofibrils from muscle aged at 25°C illustrated that high temperature aging only accelerated the disappearance of troponin T and the appearance of the 30,000-dalton component (Figure 7) and did not have an effect on other myofibrillar proteins.

These intriguing results of finding a 30,000-dalton component in post-mortem muscle then led to studies involving the determination of CAF activity of post-mortem aged L, ST and PM muscles (Olson *et al.*, 1977). Total CAF activity, based on the proteolysis of denatured casein by crude CAF, was found to be muscle and post-mortem time dependent (Figure 8). Results of total CAF activity suggest that the lack of myofibril fragmentation of PM was due to the small amount and rapid loss of CAF activity, and conversely that the high amount of myofibril fragmentation of L and ST was related to the relatively high level of CAF activity.

Further evidence showing that CAF was the agent responsible for myofibril fragmentation, Z-disk degradation and the disappearance of troponin T and the concurrent appearance of a 30,000-dalton component was the incubation of purified CAF with myofibrils isolated from at-death muscle. It was demonstrated that CAF when added to myofibrils resulted in loss of Z-disk structure (Figures 9) and myofibrils treated with CAF and CAF and troponin (CAF-TN) had a 30,000-dalton component (Figures 10 and 11). In addition, incubation of purified CAF with purified troponin gave direct evidence that the degradation of troponin-T to a 30,000-dalton component was caused by CAF (Figure 12).

Earlier Hay *et al.* (1973) and Samejima and Wolfe (1976) had shown that a 30,000-dalton component occurred during post-mortem storage in myofibrils isolated from chicken leg and breast muscle, but they were unable to determine the source of this component. Penny (1974) had also reported the appearance of a 30,000-dalton component in bovine muscle during post-mortem aging, but he was unable to show that CASF was the protease causing the appearance of a 30,000-dalton component.

From our studies it seems that CAF activity weakens the Z-disk and at about the same time degrades troponin T to a 30,000-dalton component. These parallel effects (incubation of myofibrils with CAF and post-mortem storage) add further evidence that the limited and specific proteolysis of myofibrillar proteins is very likely caused by a Ca^{2+} activated factor endogenous to the muscle cell. Although the action of lysosomal enzymes is still being proposed and investigated as causing tenderization (Moeller *et al.*, 1976), the evidence at the moment is overwhelmingly in favor of calcium activated factor as the major causative factor of tenderization.

We (Cheng and Parrish, 1977) have further substantiated by extracting myofibrils with strong salt solutions that the change in myofibrillar proteins during post-mortem aging is the disappearance of troponin T and the appearance of the 30,000-dalton component. With dilute salt solution (1 mM Tris, pH 8.5) our results indicate that more α-actinin and C protein seem to be extracted during post-mortem storage. Moreover, muscle samples treated with Ca^{2+} had more rapid degradation of troponin T and the concurrent appearance of a 30,000-dalton component, and there was more 30,000-dalton component and α-actinin observed on SDS compared with control and oxalate samples. This information suggests that CAF and Ca^{2+} have a profound role in degradation of myofibrillar proteins and tenderization.

The appearance of the 30,000-dalton component has been shown to have practical significance, too. Olson and Parrish (1977) have shown that the 30,000-dalton component seemed to be related to more tender than to less tender beef steak. More recently, MacBride and Parrish (1977) have shown that the 30,000-dalton component occurs only in tender and not in tough bovine longissimus post-mortem aged in the carcass for 1 day at 2°C (Figure 13). The appearance of the 30,000-dalton component offers the

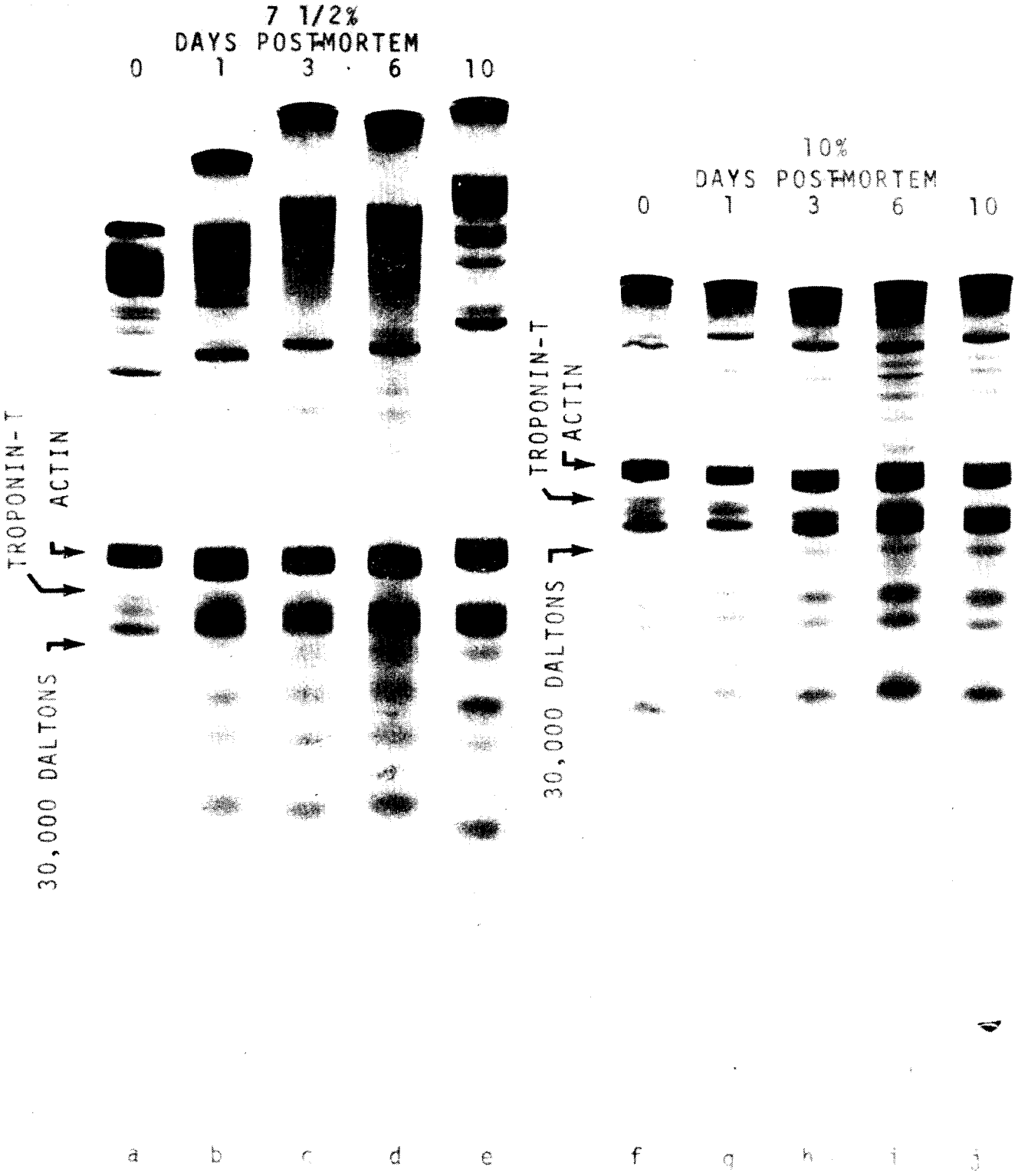

FIGURE 6

SDS 7½ and 10% polyacrylamide gels of myofibrils prepared from bovine longissimus muscle at death and at different times (1, 3, 6 and 10 days) of post-mortem storage at 2°C. (a-e): Note the gradual decrease of the troponin-T band and the gradual increase of the 30,000-dalton band from 0-10 days post-mortem. No other major changes of other bands are noted. (f-j): Note the decrease of the troponin-T band and the increase of the 30,000-dalton band from 0-10 days post-mortem. From Olson et al. (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

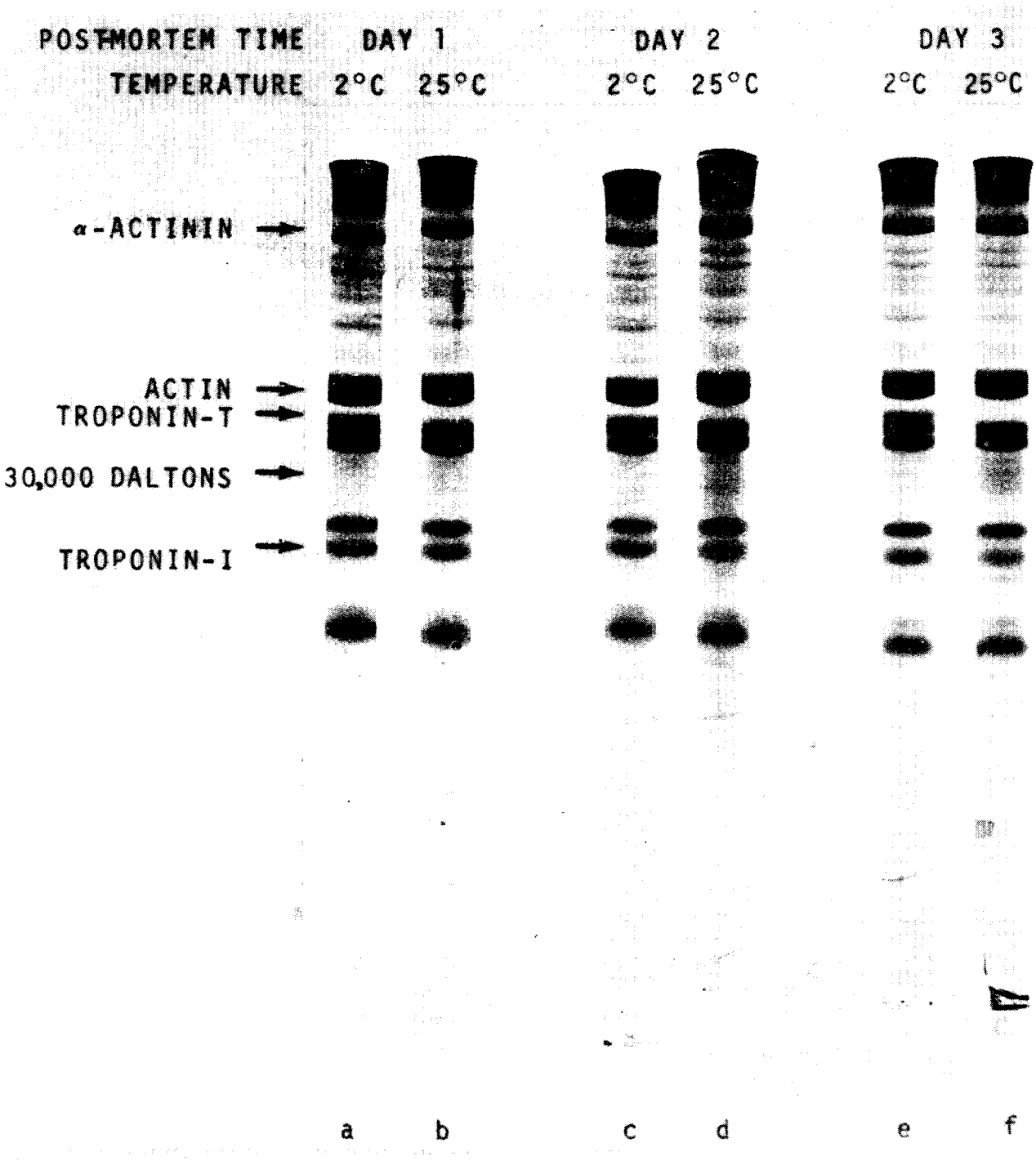

FIGURE 7

SDS 10% polyacrylamide gels of myofibrils prepared from bovine longissimus muscle at different times (after 1, 2 and 3 days) of post-mortem storage at 2°C and 25°C. (b) Note the decreased intensity of the troponin-T band compared with (a). (d) Note the slight appearance of the 30,000-dalton band and the decreased intensity of the troponin-T band. (f) Note the absence of the troponin-T band and the presnce of the 30,000-dalton band. From Olson et al. (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

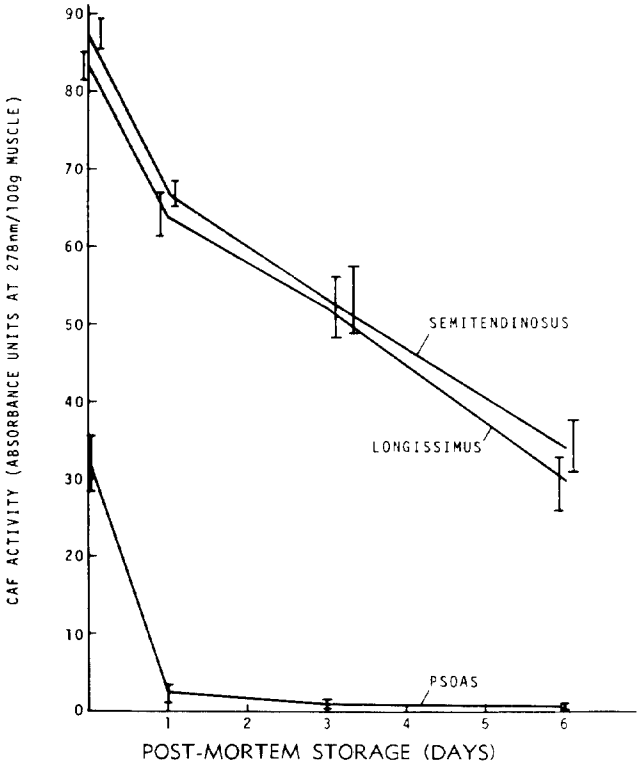

FIGURE 8

Effect of post-mortem storage on total CAF activity in bovine longissimus, psoas major and semitendinosus muscle at different times of post-mortem storage at 2°C. (Means ± SE of five carcasses). From Olson et al. (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

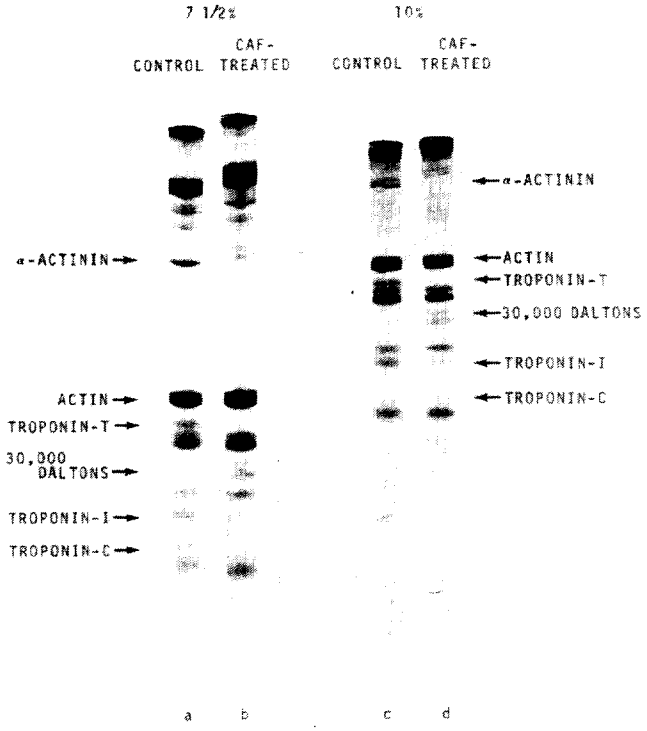

FIGURE 10

SDS-7½ and 10% polyacrylamide gels of at-death bovine longissimus myofibrils incubated in the absence and presence of CAF. (a) Note the presence of α-actinin, troponin-T and troponin-I bands. (b) Note the absence or decreased intensity of α-actinin, troponin-T and troponin-I bands and the presence of bands in the 30,000-dalton region. (c) Note the presence of α-actinin, troponin-T and troponin-I bands. (d) Note the absence or decreased intensity of α-actinin, troponin-T and troponin-I bands and the presence of bands in the 30,000-dalton region. From Olson et al. (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

FIGURE 9

Phase micrographs of at-death (control) and CAF-treated at-death myofibrils of bovine semitendinosus muscle (X2000). (c) Note the presence of prominent Z-lines. (d) Myofibrils are fragmented and Z-lines are absent. From Olson et al. (1977) and reproduced by the Institute of Food Technologists and the author.

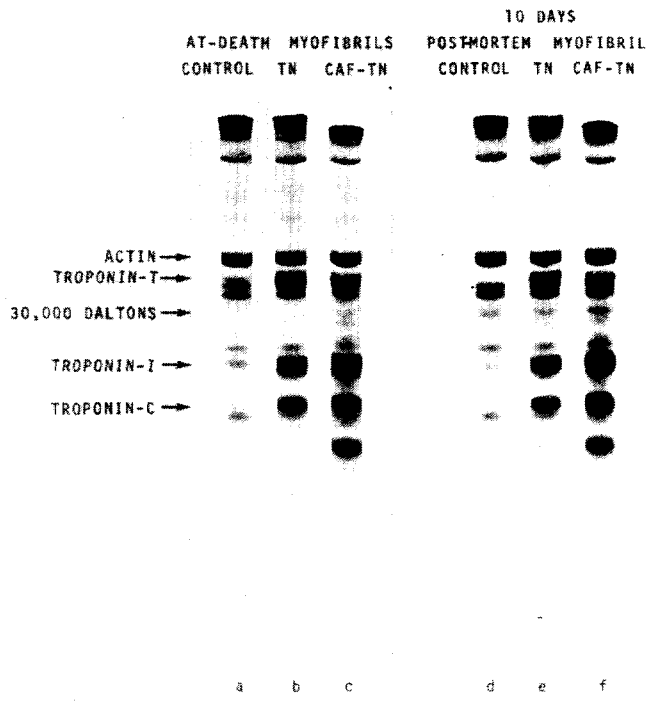

FIGURE 11

SDS-10% polyacrylamide gels of myofibrils prepared from at-death bovine longissimus muscle and at 10 days of post-mortem storage in the absence (control) and presence of troponin (TN) and CAF-treated troponin (CAF-TN). (b) Note the increased intensities in the troponin subunit bands. (c) Note the presence of the 30,000-dalton band. (d) Note the absence of troponin-T and the presence of the 30,000-dalton band. (e) Note that the troponin-T band is in the same location as in at-death myofibrils (11a). (f) Note that the 30,000-dalton band and the bands below troponin-C are of greater intensity than when CAF-treated troponin was not combined with myofibrils (11d and 11e). From Olson et al. (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

potential of objectively detecting tenderness early in the post-mortem aging cycle, and the presence, or absence, of it could be used as a criterion for tender or tough longissimus muscle.

Previously, the terms "background toughness" due to connective tissue and "actomyosin toughness" due to configurational changes of actin and myosin in cold shortened bovine muscle (Locker and Hagyard, 1963; Marsh and Leet, 1966) have been used to describe different states of tenderness. We (MacBride and Parrish, 1977) have introduced the term "myofibril fragmentation tenderness," which is related to the fragmenting of the myofibril at or near the Z-disk and the appearance of the 30,000-dalton component in tender muscle during post-mortem storage of conventionally aged bovine longissimus muscle. This would seem to be an appropriate term to describe a state of tenderness in conventionally aged carcass beef because:

(1) Myofibril fragmentation accounts for about 50 percent of the variation in beef steak tenderness (Culler *et al.*, 1978; MacBride and Parrish, 1977; Moller *et al.*, 1973; Olson and Parrish, 1977).

(2) The presence of the 30,000-dalton component occurs in tender muscle (MacBride and Parrish, 1977; Olson and Parrish, 1977).

(3) A strong interrelationship exists among Z-disk degradation, myofibril fragmentation, the 30,000-dalton component and beef muscle tenderization (Olson *et al.*, 1976; Olson *et al.*, 1977; Olson and Parrish, 1977).

(4) The relationship is low between sarcomere length and tenderness of conventionally aged bovine

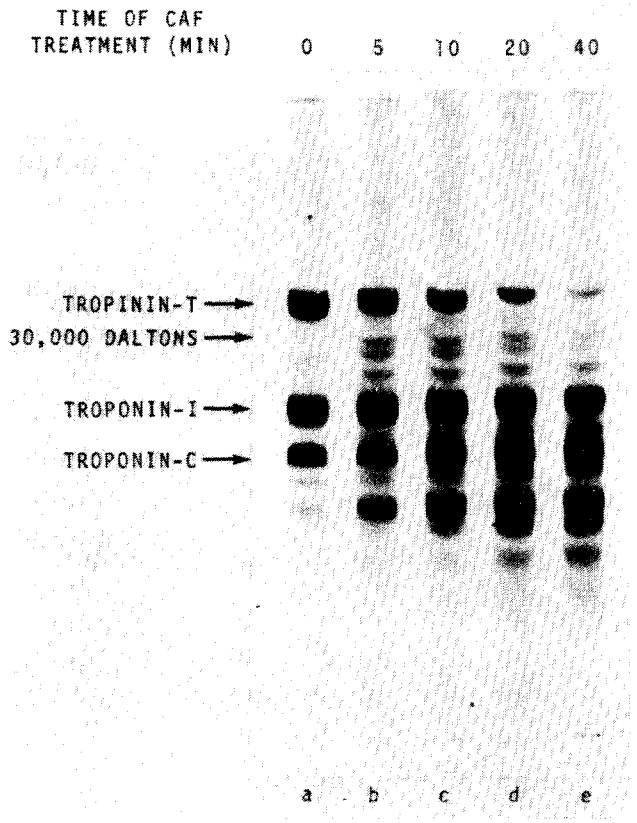

FIGURE 12

SDS-10% polyacrylamide gels of purified troponin prepared from the at-death bovine longissimus muscle and incubated with CAF for 0, 5, 10, 20 and 40 min. (a) Note troponin subunits T, I and C. (b) Note the proteolytic breakdown products in the 30,000-dalton band region and below the I and C subunits. (c) Note the increased intensity of the proteolytic breakdown products and the decreased intensity of the troponin-T band. (d) Note the increase in intensity of the bands below the I and C subunits and the decrease in the troponin-T band and the bands in the 30,000-dalton region. (e) Note the faint bands in troponin-T and in the 30,000-dalton region and the increased intensity in the bands below the I and C subunits. The troponin-I band has also decreased compared with the gel in (b). From Olson et al. (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

longissimus muscle (Parrish *et al.*, 1973; MacBride and Parrish, 1977; Culler *et al.*, 1978). Admittedly there are situations where sarcomere length has a very important effect on tenderness. Marsh and Leet (1966) and Herring *et al.* (1967) have shown that there is a strong relationship between sarcomere length and tenderness when muscle is excised in a prerigor condition and subjected to cold temperatures.

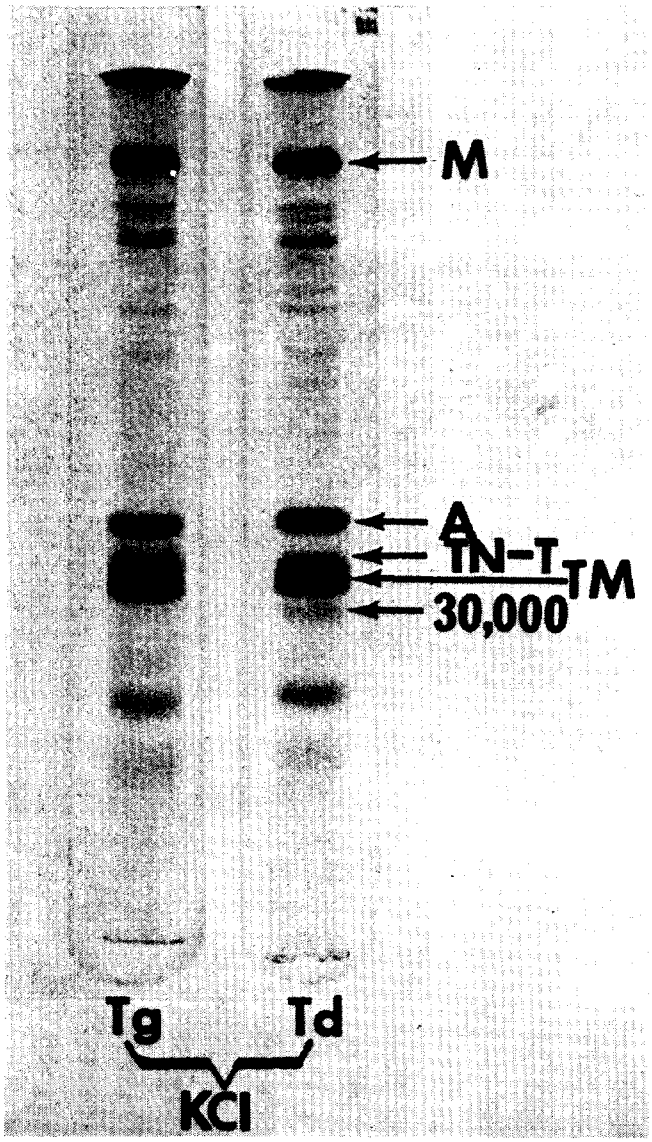

FIGURE 13

SDS-7½ percent polyacrylamide gels of myofibrillar proteins extracted with high ionic strength salt solution (0.6 M KCl, 0.1M K phosphate, pH 7.4) from 1 day post-mortem (2°C storage) bovine tough (Tg) and tender (Td) longissimus muscle. Myosin (M), actin (A), troponin T (TN-T), tropomyosin (TM) and (30,000-dalton component). Note the presence of the 30,000-dalton component in the tender sample and the absence of the 30,000-dalton component in the tough sample. Protein load: 25 μg. From MacBride and Parrish (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

In addition, evidence indicates that composition of the carcass is a factor in tenderization (Smith *et al.*, 1976). That is, carcasses having more subcutaneous and intramuscular fat are more tender because of a slower chilling rate and less shortening of sarcomeres.

(5) There is no significant difference in sarcomere length of myofibrils from tough and tender muscle (MacBride and Parrish, 1977).

Myofibril fragmentation tenderness gives meaning and explanation to the tenderization process; however, several questions require further investigation. These questions are related to the activity of CAF in tender muscle, or lack of activity in tough muscle, regulation and inhibition of CAF activity, concentration of Ca^{2+} in the muscle cell available to activate CAF, and how the 30,000-dalton component and myofibril fragmentation can be determined practically for the purpose of identifying tender and tough carcasses or cuts. Determination and relationship of factors to the 30,000-dalton component would seem to contribute significantly to our knowledge of the process of tenderization, and early and accurate identification of tender and tough cuts would seem to be beneficial to a quality beef operation.

Exogenous Enzyme Tenderization

It is well known that adding plant proteolytic enzymes to meat results in improved tenderness. Wang *et al.* (1958) microscopically characterized the effect of a variety of proteolytic enzymes on the muscle components of freeze-dried steaks. The amount of proteolysis on muscle components varied depending upon enzyme specificity; however, a general sequence of events observed was as follows: 1) the sarcolemma and the muscle fiber envelope were attacked and disappeared, 2) nuclei and cells of the endomysium disappeared, 3) cross striations of actomyosin disappeared, and 4) actomyosin finally dissolved. Each enzyme varied somewhat in its action on muscle constituents, and they all had different temperatures of activation and inactivation. Actually, in practice, the tenderizing action of the enzyme occurs only during the cooking process, which can be both beneficial and detrimental.

Poor penetration and distribution of the enzyme have been problems associated with surface application of proteolytic enzymes derived from plant sources. This results in variable and unsatisfactory levels of tenderness throughout the steak. The Swift Pro Ten process of injecting an enzyme (papain) solution into the vascular system of the animal shortly before slaughter has reduced these problems (Goeser, 1961). In this way the vascular system acts to uni-

formly distribute the enzyme throughout the musculature. The exact action of the Pro Ten process to bring about tissue disruption and tenderness is not fully known, but papain may, in addition to its general action, specifically degrade Z-disks structure to cause increased myofibril fragmentation.

Further evidence supporting a calcium activated protease as the agent causing tenderization is the work reported by Penny *et al.* (1974). They isolated crude CASF from rabbit muscle and then reconstituted freeze-dried steaks from bovine semitendinosus in a solution of this Ca^{2+} activated muscle proteinase. The CASF treated steaks were more tender than untreated controls, and this tenderness increase was attributed to a structural weakening of the myofibrils at or near the Z-line.

Mechanical Tenderization

The use of mechanical tenderizers to disrupt muscle tissue, its relationship to tenderness, and its potential usefulness in the meat trade has been a recently active area of research. The subject of mechanical tenderizing of beef, pork, lamb and goat has been comprehensively reviewed by Huffman (1975). Recently, Davis *et al.* (1977) blade tenderized both beef rounds and loins and found that the tenderized steaks were more tender than non-tenderized ones. In addition, Savell *et al.* (1977a) have shown that mechanically tenderized beef from Light Good, Heavy Good and Heavy Choice had improved in tenderness without any detrimental effect on other sensory attributes or weight losses. Glover *et al.* (1977) found highly significant improvements in tenderness of round roasts and round and loin steaks in mechanically tenderized U.S. Good grade beef. Hence, mechanical tenderization seems to be a beneficial means of improving tenderness; however, Davis *et al.* (1975) reported that mechanical tenderization provided little improvement of those steaks having inherent acceptable quality.

Although the mechanism of tissue disruption and improved tenderness is not specifically known, it is probably an effect upon severing both muscle fibers and connective tissue components of skeletal muscle.

Pressure-heat Induced Tenderization

Bouton *et al.* (1977) have shown that pressure-heat treatment can improve the tenderness of meat through some form of tissue disruption. In this treatment meat is subjected to pressures of about 100 meganewtons/m^2 (MNm^{-2}), then heated while under pressure to a temperature of approximately 60°C. It is not known exactly how the structure of muscle is disrupted by pressure-heat treatment to bring about improved tenderness, but it is thought that myofibrillar structure is broken down or weakened rather than there being some effect of pressure-heat on the connective tissue component of muscle (Bouton *et al.*, 1977; Ratcliff *et al.*, 1977).

Electrical-stimulated Tenderness

This is another method that has received a great deal of recent attention. Smith *et al.* (1977) have reviewed the promising practical use of electrical stimulation for improving meat tenderness. Savell *et al.* (1977b) have shown that meat tenderness can be improved and tenderness variability reduced by electrically stimulating sides of beef, lamb and goats. How electrical-stimulation improves tenderness, however, is not known. Some think that tenderness is improved because electrical-stimulation prevents cold-shortening (Chrystall and Hagyard, 1976). However, Savell *et al.* (1977b) could find no difference in sarcomere length of myofibrils from treated and control sides. A possible explanation is that electrical-stimulation causes a more rapid lowering of pH at high carcass temperature, which disrupts lysosomal membranes and releases hydrolases to break down tissue components (Savell *et al.*, 1977b). Another possibility is that electrical-stimulation releases Ca^{2+} from the sarcoplasmic reticulum, or other Ca^{2+} binding membrane structures, which in turn activates the protease, calcium activated factor. C F then proceeds to degrade Z-disks, degrades troponin-T to the 30,000-dalton component, resulting in increased myofibril fragmentation and improved tenderness.

Summary

Current evidence strongly supports myofibril fragmentation and the 30,000-dalton component relationship to bovine tenderization as the natural (endogenous) tenderization process. This type of tissue disruption is caused by calcium activated factor, a protease endogenous to the muscle cell.

"Myofibril fragmentation tenderness" is an appropriate term to describe a state of tenderness because of the evidence associated with myofibril fragmentation and tenderization of conventionally aged beef.

Proteolytic enzymes and mechanical, pressure-heat induced and electrical-stimulated tenderness are extrinsic agents used to bring about tissue disruption and improved meat tenderness.

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