Veränderungen myofibrillärer Proteine von Rindermuskeln während des postmortalen Reifungsprozesses

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Die Fragmentierung von Myofibrillen, d.h. die Spaltung von Myofibrillen in kürzere Bruchstücke an oder nahe der Z-Scheibe während der postmortalen Reifung von Rindermuskel ist eine wesentliche Ursache für die Zartheit des Fleisches. Diese Fragmentierung steht in enger Beziehung zu sensorischen und physikalischen Messungen der Zartheit. Untersuchungen sprechen dafür, daß der Calciumaktivierende Faktor (CAF) die Ursache der Fragmentierung ist. Die Beziehung zwischen Myofibrillen-Fragmentations-Index (MFI) und sensorischen Zartheitswerten macht über 50% der Zartheitsveränderungen von Rindersteaks aus (M. long. dorsi von konventionell gereiften Rinderschlachttierkörpern -A maturity-).In SDS-Polyacrylamid Gelen verschwindet während der Reifung gleichzeitig mit dem Abbau der Z-Scheiben und der Fragmentierung von Myofibrillen auch Troponin T und es erscheint eine 30 000 Dalton Komponente. Untersuchungen zeigen, daß CAF für diese Veränderungen verantwortlich ist. Höhere Temperaturen während der Reifung von Schlachttierkörpern und Ca^{2+} Zusatz zu zerkleinertem Muskel beschleunigen den Abbau von Troponin und das Auftreten der 30 000 Dalton Komponente. Weiterhin findet sich die 30 000 Dalton Komponente nur im zarten aber nicht im zähen M. long. dorsi von Schlachttierkörpern (A maturity). Es wird vorgeschlagen, die myofibrilläre Fragmentierung als ein geeignetes Parameter zu verwenden, um das Ausmaß der Zartheit von konventionell behandelten Rinderschlachttierkörpern zu bestimmen.

Changes in myofibrillar proteins during postmortem tenderization of bovine muscle

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Myofibril fragmentation, the separation of myofibrils into shorter segments at or near the Z-disk, is a significant event during postmortem tenderization of bovine muscle. It is strongly related to sensory and physical measures of tenderness and there is evidence to support the hypothesis that calcium activated factor (CAF) is the causative agent in myofibril fragmentation. The relationship between myofibril fragmentation index (MFI) and sensory tenderness scores account for over 50 percent of the variation in tenderness of beef steak from longissimus muscle of conventionally aged A-maturity bovine carcasses. Coupled with the degradation of Z-disks and fragmentation of myofibrils is the simultaneous disappearance of troponin T and appearance of a 30,000 dalton component on SDS polyacrylamide gels during postmortem aging. Evidence shows that CAF is responsible for these changes in myofibrillar proteins. High temperature postmortem aging of carcasses and Ca^{2+} added to muscle minces accelerate the degradation of troponin T and the presence of the 30,000 dalton component. Moreover, the 30,000 dalton component is found only in tender and not in tough bovine longissimus from A-maturity carcasses. Myofibril fragmentation tenderness is suggested as an appropriate term to describe a state of tenderness of conventionally handled beef carcasses.

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Changements des proteines myofibrillaires des muscles bovins pendant le processus de maturité post mortem

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La fragmentation des myofibrilles, c`est à dire, la séparation des myofibrilles en segments plus courts à ou pas loin d`une disque Z pendant la maturation post mortem du muscle bovin est une raison essentielle pour la tendresse de la viande. Cette fragmentation est en relation étroite avec des mesurages de la tendresse sensorique et physique. Des études constatent que le facteur activant le calcium (CAF) est la raison de la fragmentation. La relation entre l`index des fragmentations myofibrillaires (MFI) et des valeurs de tendresse sensoriques font plus de 50% des changements de tendresse des beefsteaks (M. long. dorsi des bovins maturés d`une façon conventionnelle - A maturity -). Pendant la maturation dans les gels polyacrylamide SDS disparaissent aussi avec la dégradation des disques Z et de la fragmentation des myofibrilles Troponin T et une composante Dalton 30 000 apparait. Des analyses ont montré que CAF est responsible pour ces changements. Des temperatures élèvées pendant la maturation des bovins et l`adjonction de $Ca^{2+}$ au muscle concassé accelère la dégradation de Troponin et l`apparition de la composante Dalton 30 000. De plus la composante Dalton 30 000 est dans le tendre mais pas dans le dur M. long. dorsi des bovins (A maturity). Il est supposé d`utiliser la fragmentation myofibrillaire comme un paramètre approprié pour fixer la tendresse des bovins traités d`une façon conventionnelle.

ИЗМЕНЕНИЯ В МИОФИБРИЛЛЯРНЫХ БЕЛКАХ В ТЕЧЕНИЕ ПОСМЕРТНОГО СМЯГЧЕНИЯ БЫЧИХ МЫШЦ

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Миофибриллярная фрагментация, разделение миофибрилл на более короткие сегменты на или близко от z -диска--значительное происшествие в течение посмертного смягчения бычей мышцы. Это сильно относится к сенсорным и физическим измерениям мягкости, и есть данные, которые поддерживают предположение, что активированный кальцием фактор (CAF) --причинный агент в миофибриллярной фрагментации. Соотношение между миофибриллярным фрагментационным индексом (MFI) и счётом сенсорной мягкости объясняет выше 50 процентов изменчивости мягкости бифштекса из мышцы longissimus из обычно подверженных старению А-зрелости бычих тушек. С деградацией z -дисков и фрагментацией миофибрилл есть одновременное исчезновение тропонина Т и появление компонента 30 000 дальтонов на полиакриламидных с додециловым сульфатом натрия гелях в течение посмертного старения. Данные указывают, что CAF причина этих изменений в миофибриллярных белках. Посмертное старение тушек при высокой температуре и прибавление Ca^{2+} к мышечным фаршам ускоряют деградацию тропонина Т и появление компонента 30 000 дальтонов. Кроме того, компонент 30 000 дальтонов находится только в мягкой, а не в жёсткой бычей мышце longissimus из А-зрелости тушек. Миофибриллярная фрагментационная мягкость предлагается подходящим термином, которым описывать состояние мягкости обычно обработанных бычих тушек.

Changes in myofibrillar proteins during postmortem tenderization of bovine muscle

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Recent evidence indicates that the changes in myofibrillar proteins during postmortem aging are of primary significance in determining the level of tenderness of bovine longissimus muscle. Moreover, these changes are mediated by a calcium-active factor (CAF), a protease endogenous to the muscle cell, and these proteolytic changes are limited and specific. The purpose of this paper is to present evidence showing that proteolysis of myofibrils during postmortem aging is very likely caused by CAF and that this proteolysis is related to beef steak tenderness.

The phenomenon of myofibrils breaking into shorter segments at or near the Z-disk during postmortem 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). Myofibrils isolated from 1-day postmortem-aged bovine longissimus 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 intact (Fig. 1). As postmortem aging increases, however, myofibrils become shorter, more fragmented, and Z-disks are degraded. Degradation of Z-disk of bovine, porcine and rabbit myofibrils at different times and temperatures of postmortem storage has been described by Henderson et al. (1970). The postmortem changes observed with phase-contrast microscopy in myofibrils from longissimus (L) are similar in myofibrils from semitendinosus (ST) muscle; however, little change can be detected in myofibrils from psoas major (PM) muscle (Olson et al., 1976).

The discovery of a protease endogenous to the muscle fiber that was capable of degrading Z-disk of myofibrils from rabbit skeletal muscle by Busch et al. (1972) turned out to be of significance to postmortem studies on myofibrillar proteins and beef steak tenderness. 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. A protease was isolated from the sarcoplasm, and its ability to remove Z-disks in the presence and absence of Ca^{2+} was investigated to determine if it was involved in Z-disk degradation. The combination of Ca^{2+} and protease resulted in the removal of Z-disk from myofibrils. Thus, Ca^{2+} was necessary for proteolytic degradation of Z-disk structure, and the factor responsible for Z-disk degradation was named calcium-activated sarcoplasmic factor (CASF). Subsequently, Dayton et al. (1975, 1976 a, b) isolated this protease from porcine skeletal muscle to carry out purification and characterization studies. This time, the protease was termed calcium-activated factor (CAF). (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 and that 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 proteins. It was found that CAF degraded troponin T and I, tropomyosin and C protein, but not troponin C, myosin, actin or α-actinin.

A limited and specific proteolysis of myofibrillar proteins during postmortem storage of bovine longissimus muscle was shown to occur by Olson et al. (1977). To accomplish this, myofibrils were isolated at certain postmortem 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 postmortem 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 (Fig. 2). In addition, high-temperature aging of muscle at 25°C accelerated the disappearance of troponin T and the appearance of the 30,000-dalton component (Fig. 3) and did not have an effect on other myofibrillar proteins. Hay et al. (1973) and Penny (1974) had earlier shown the occurrence of a 30,000-dalton component in myofibrils isolated from chicken leg and breast muscle and bovine muscle, respectively.

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 bovine muscle. It was demonstrated that CAF, when added to myofibrils, resulted in loss of Z-disk structure (Fig. 4) and that myofibrils treated with CAF and CAF and troponin (CAF-TN) had a 30,000-dalton component (Fig. 5 and 6). 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 (Fig. 7). These parallel effects of incubation of myofibrils with CAF and postmortem storage suggest that the limited and specific proteolysis of myofibrillar proteins leading to myofibril fragmentation and improved tenderness is caused by a Ca^{2+} activated factor endogenous to the muscle cell.

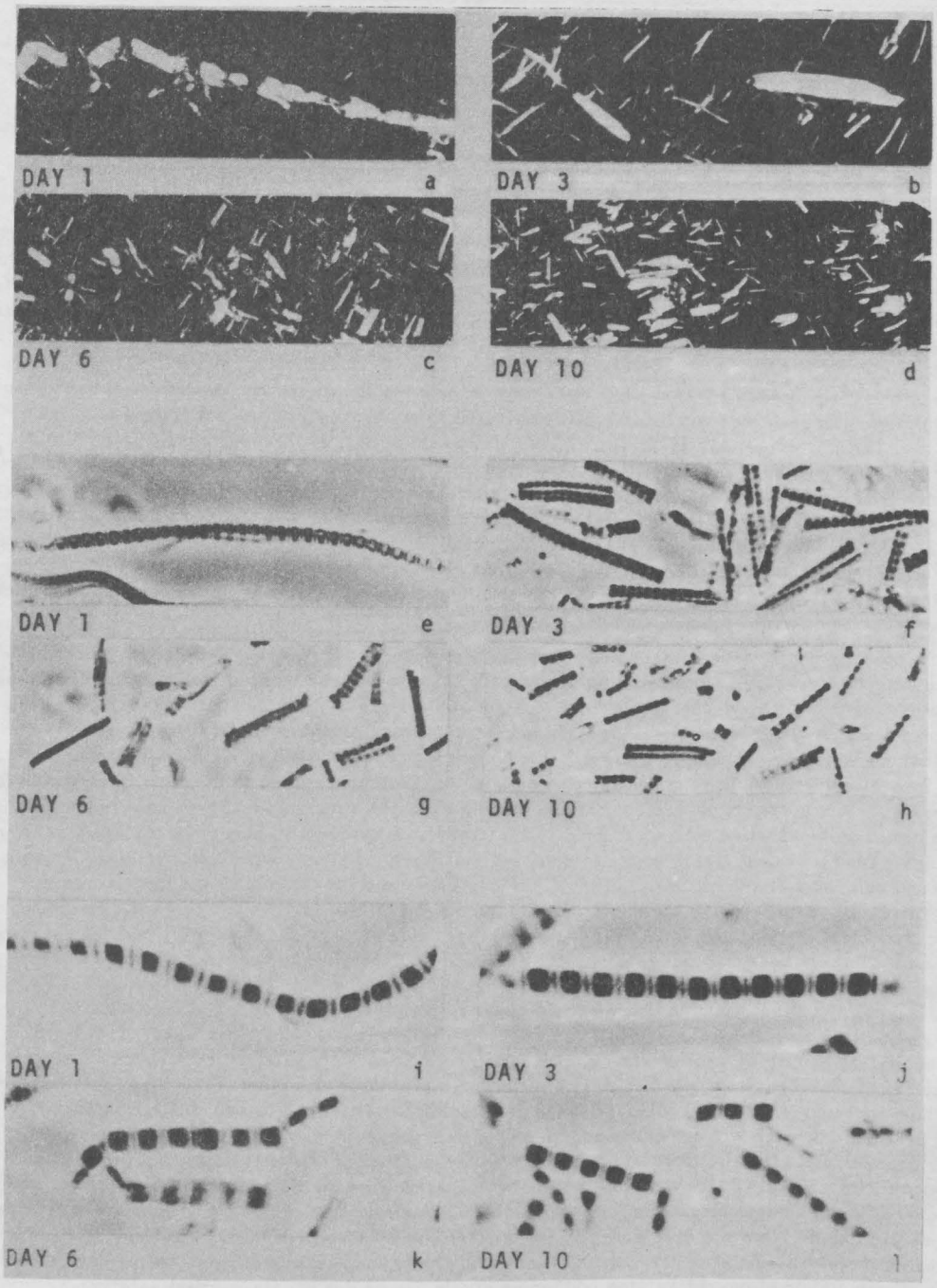

Figure 1. Polarized-light and phase micrographs of myofibrils prepared from bovine longissimus muscle at different times of postmortem storage at 2°C. (a) Polarized-light micrograph of myofibrils at 1 day postmortem. Both single myofibrils and fiber pieces are present (X100). (b) Polarized-light micrograph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

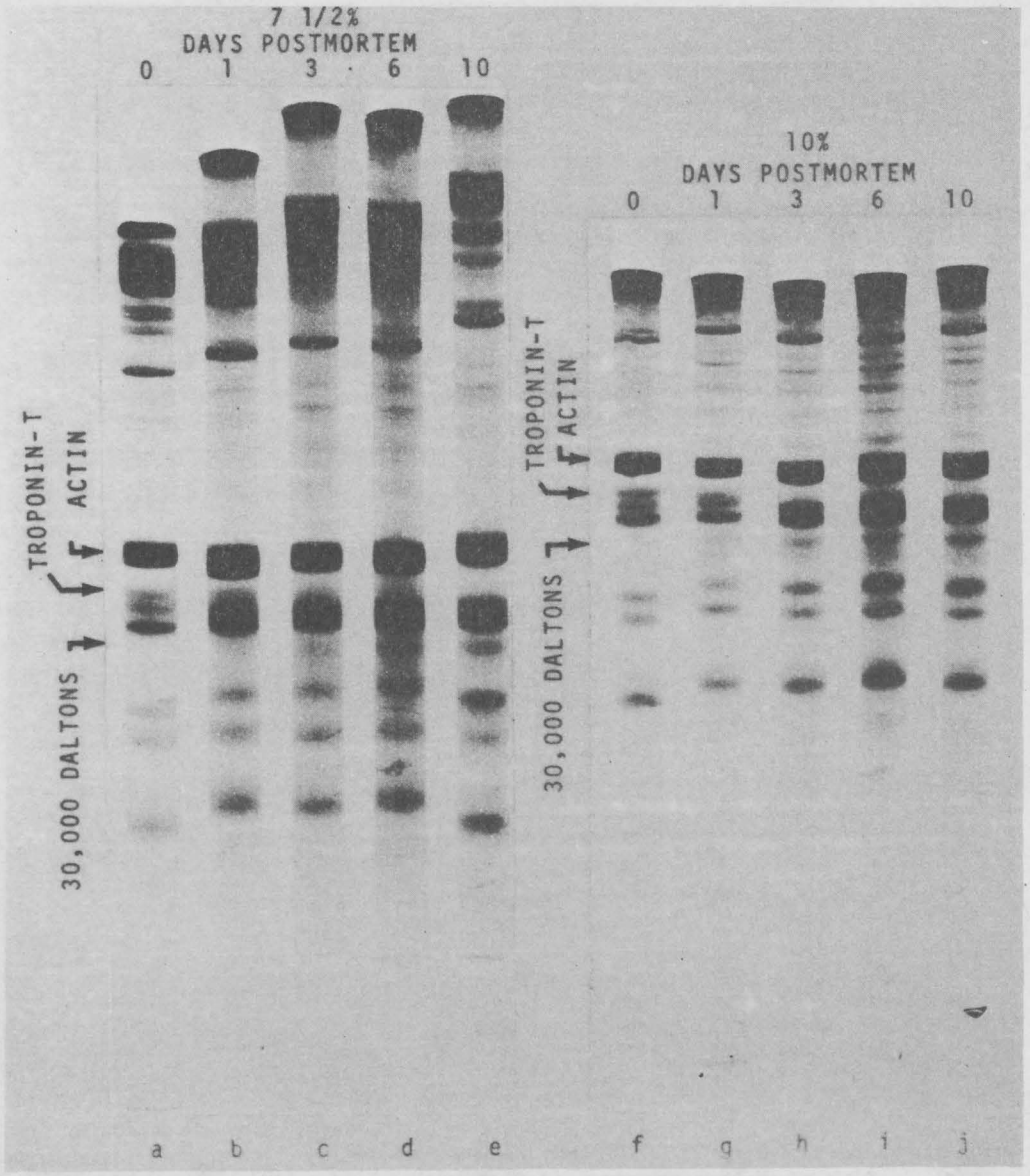

Figure 2. SDS 7½ and 10% polyacrylamide gels of myofibrils prepared from bovine longissimus muscle at death and at different times of postmortem storage at 2°C. (a-e): Note the gradual decrease of the troponin-T band and the gradual increase of the 30,000-dalton band from 0 to 10 days postmortem. No 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 to 10 days postmortem. From Olson et al. (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

We (Cheng and Parrish, 1978) have provided substantiating evidence that the change in myofibrillar proteins during postmortem aging was the disappearance of troponin T and the appearance of the 30,000-dalton component by extracting myofibrils with strong salt solution (Hasselbach-Sneider). With dilute salt solution (1 mM Tris, pH 8.5), our results indicated that α-actinin was a major component released, and C protein seemed to be more extractable from myofibrils during postmortem storage. Moreover, results (Cheng and Parrish, 1977) showing that myofibrils isolated from minced 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 in contrast to myofibrils isolated from control and oxalate samples (Fig. 8). In addition, α-actinin was much more readily extracted with 1 mM Tris, pH 8.5 solution from isolated myofibrils of control and Ca^{2+} samples than it was from oxalate-treated samples (Fig. 9). This evidence suggests that CAF and Ca^{2+} have a profound role in degradation of myofibrillar proteins and tenderization.

The fragmentation of myofibrils, reported as a myofibril fragmentation index (MFI), and the appearance of the 30,000-dalton component have been shown to have practical significance, too. A strong relationship between MFI and tenderness (Table 1) of longissimus muscle from bovine carcasses with varying maturities and quality has been shown (Olson and Parrish, 1977; Culler et al., 1978). Furthermore, the 30,000-dalton component seemed related to more tender than to less tender beef steak (Olson and Parrish, 1977). Indeed, MacBride and Parrish (1977) have recently shown that the 30,000-dalton component occurred only in tender and not in tough bovine longissimus postmortem-aged in the carcass for 1 day at 2°C (Fig. 10).

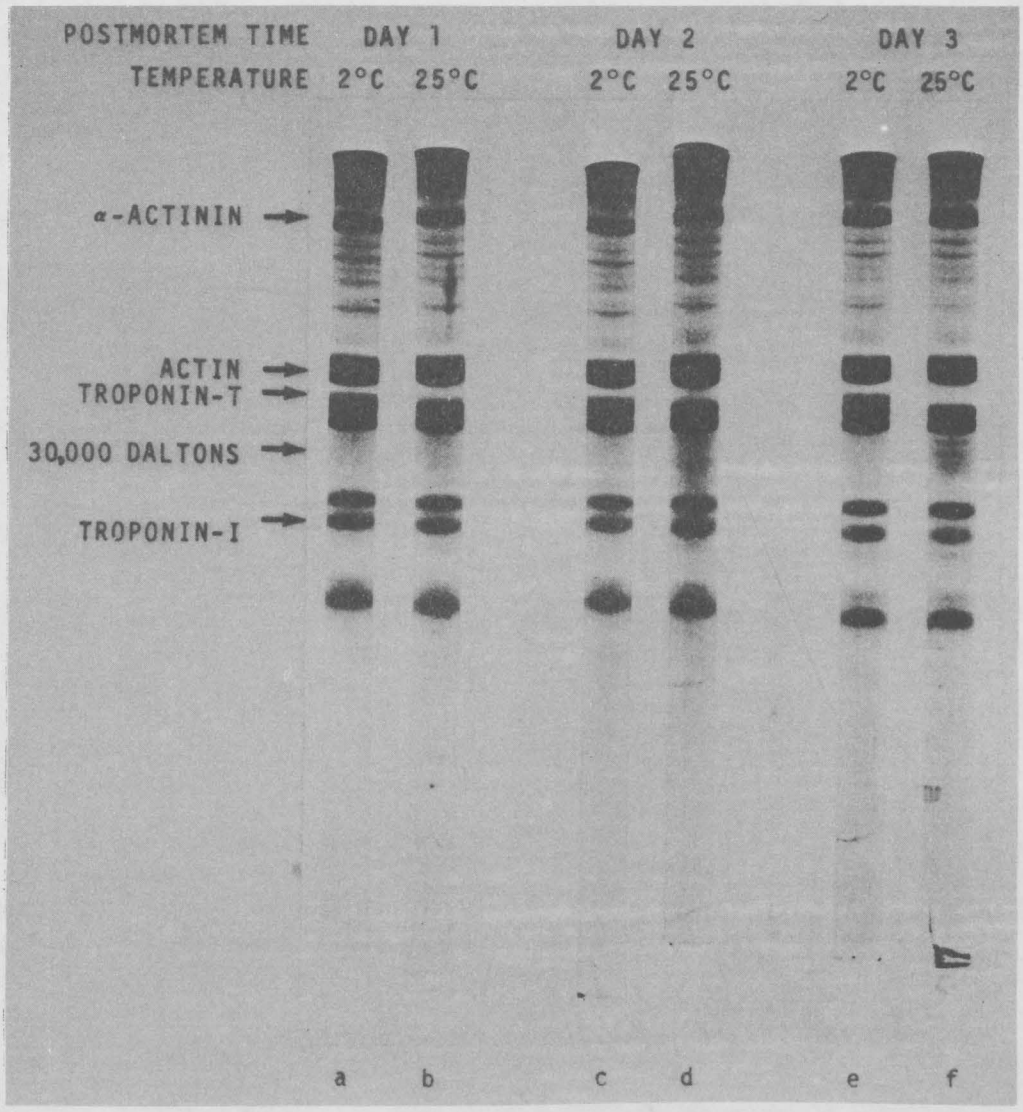

Figure 3. SDS 10% polyacrylamide gels of myofibrils prepared from bovine longissimus muscle at different times of postmortem storage at 2°C and 25°C. (b) Note the decreased intensity of the troponin-T band compared with (a). (d) Note the slight appearance of the 30,000-dalton band and the decreased intensity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

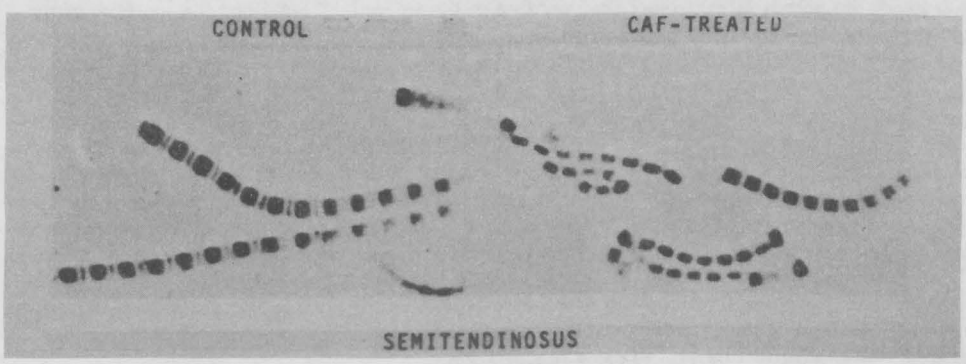

Figure 4. Phase micrographs of at-death (control) and CAF-treated, at-death myofibrils of bovine semitendinosus muscle (X2000). Note the presence of prominent Z-lines (control). Myofibrils are fragmented, and Z-lines are absent (CAF-treated). From Olson et al. (1977) and reproduced by permission of the Institute of Food Technologists and the author.

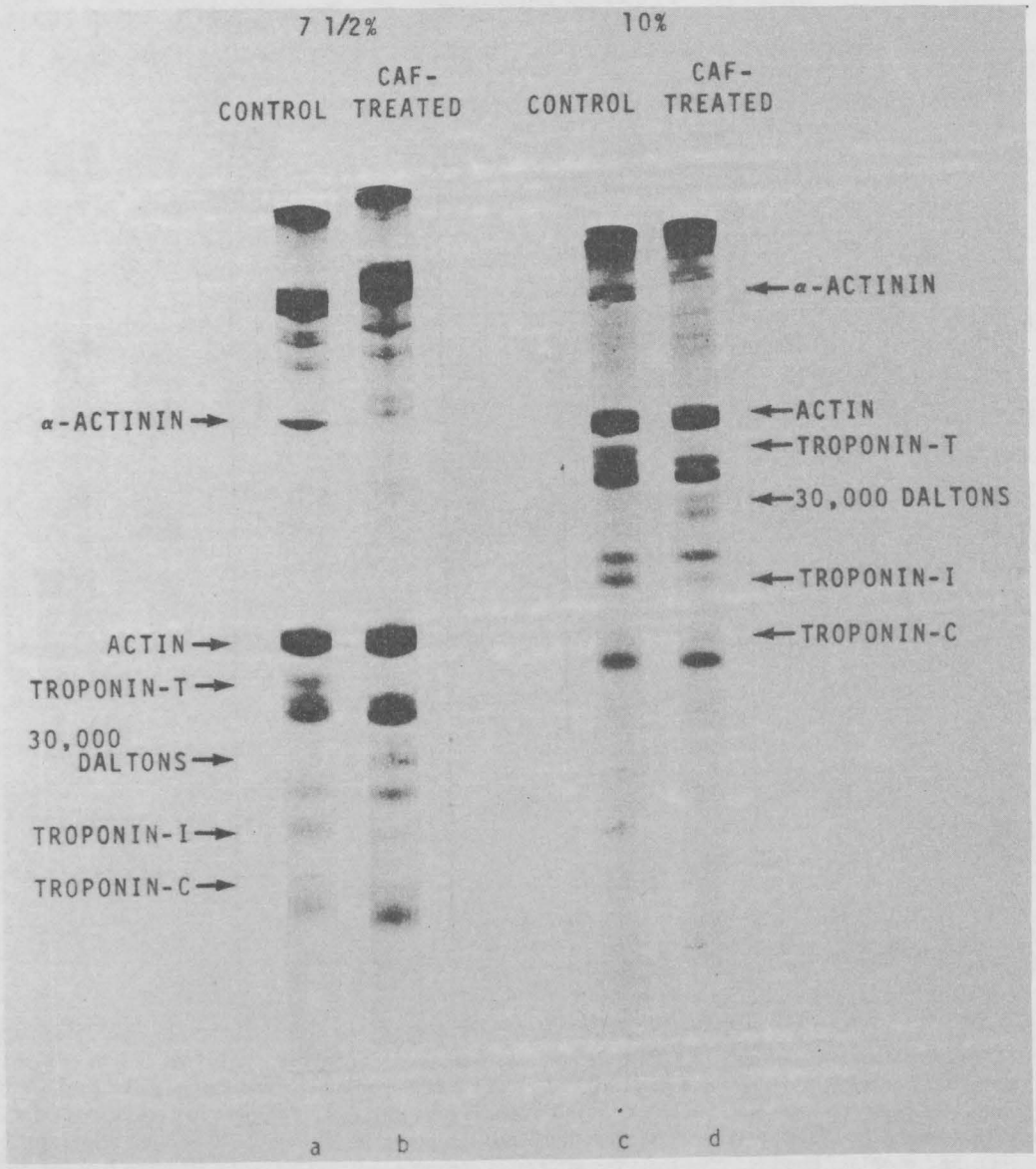

Figure 5. SDS-7½ and 10% polyacrylamide gels of at-death bovine longissimus myofibrils incubated in the absence and presence of CAF. (a) Note the presence of α-actinin, troponin-T and troponin-I bands. (b) Note the absence or decreased intensity of α-actinin, troponin-T and troponin-I bands and the presence 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.

Other 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 freeze-dried steaks from bovine semitendinosus were reconstituted in a solution of this proteinase. The CASF-treated steaks were more tender than controls, and this tenderness increase was attributed to a structural weakening of the myofibrils at or near the Z-line.

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

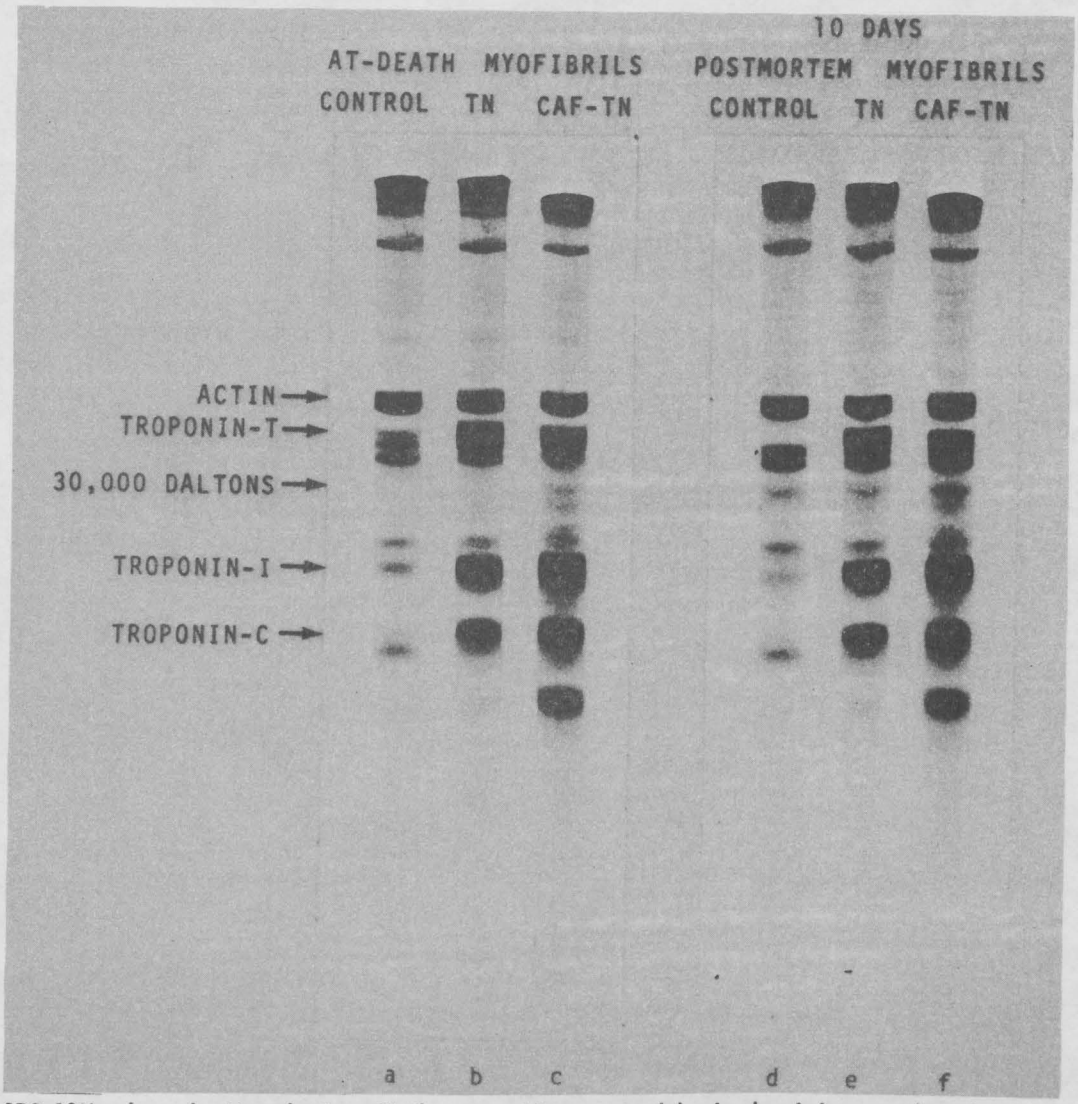

Figure 6. SDS-10% polyacrylamide gels of myofibrils prepared from at-death bovine longissimus muscle and at 10 days of postmortem storage in the absence (control) and presence of troponin (TN) and CAF-treated troponin (CAF-TN). (b) Note the increased intensities in the troponin subunit bands. (c) Note 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.

of the 30,000-dalton component in tender muscle during postmortem 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 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. 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 (Culler et al., 1978; MacBride and Parrish, 1977).

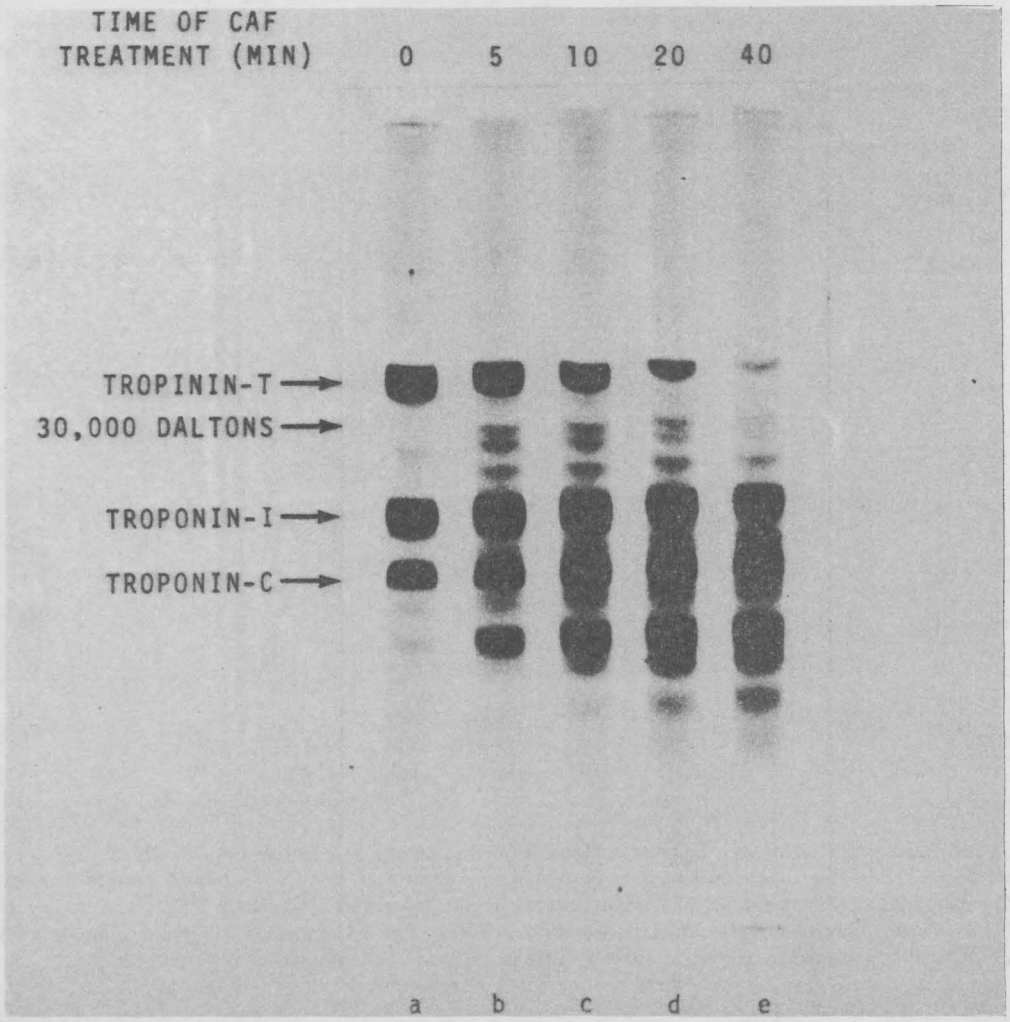

Figure 7. SDS-10% polyacrylamide gels of purified troponin prepared from the at-death bovine longissimus muscle and incubated with CAF for 0, 5, 10, 20 and 40 min. (a) Note troponin subunits T, I and C. (b) Note the proteolytic breakdown products in the 30,000-dalton band region and below the I and C 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.

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.

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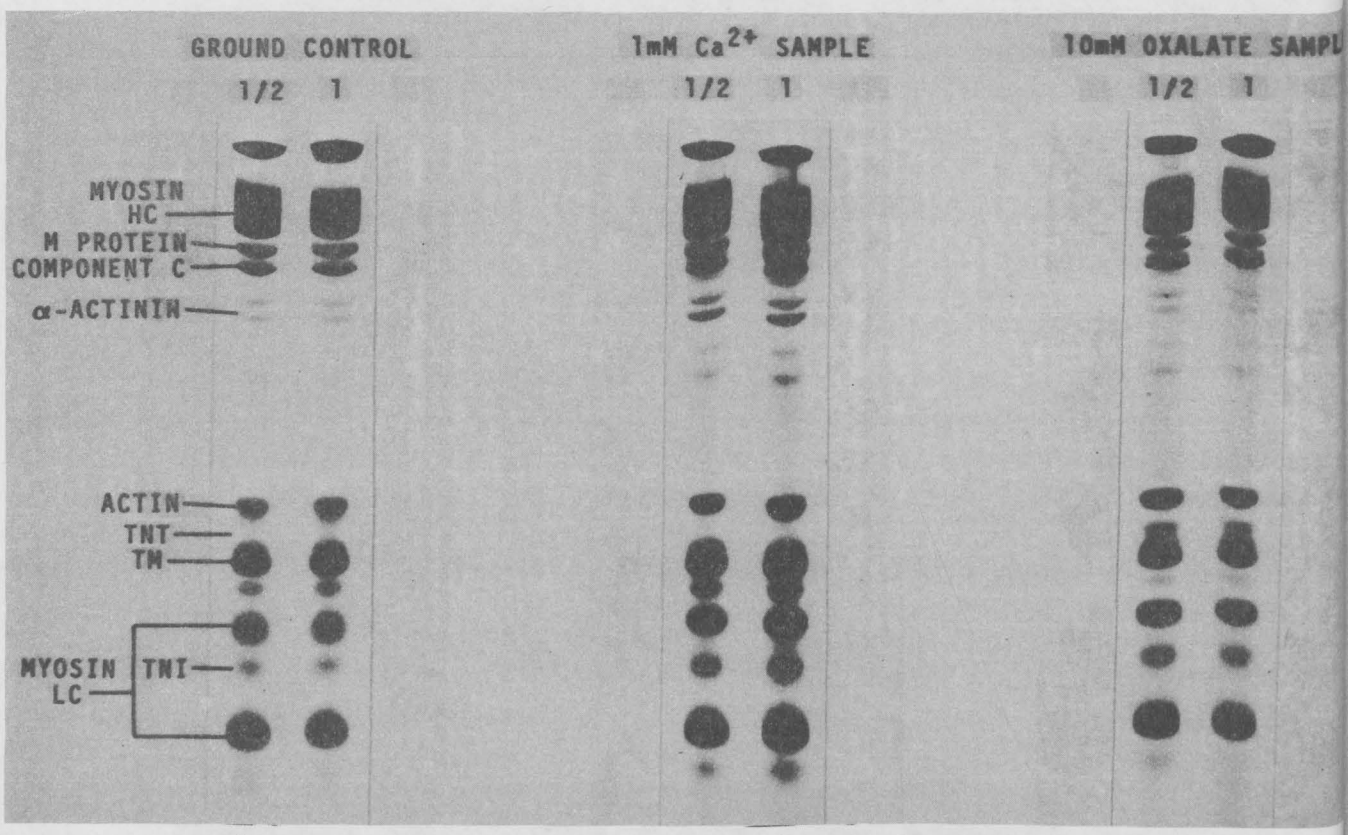

Figure 8. SDS-7½% polyacrylamide gels of protein extracts (H-S solution) from isolated myofibrils of 10-day postmortem stored at 2°C bovine longissimus muscle (ground muscle control, 1 mM Ca^{2+}, 10 mM oxalate sample). Numbers indicate hours extraction. Myosin heavy chain (myosin HC), Troponin T (TNT), Tropomyosin (TM), Myosin light chains (Myosin LC), Troponin I (TNI). Protein load: 30 μg. From Cheng and Parrish (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

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 different maturity groups.

Maturity	Days Postmortem	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, B, C and E maturity[d]	10-14	-0.72**	0.75**

[a]Six veal
[b]35 carcasses
[c]12 carcasses
[d]78 carcasses

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

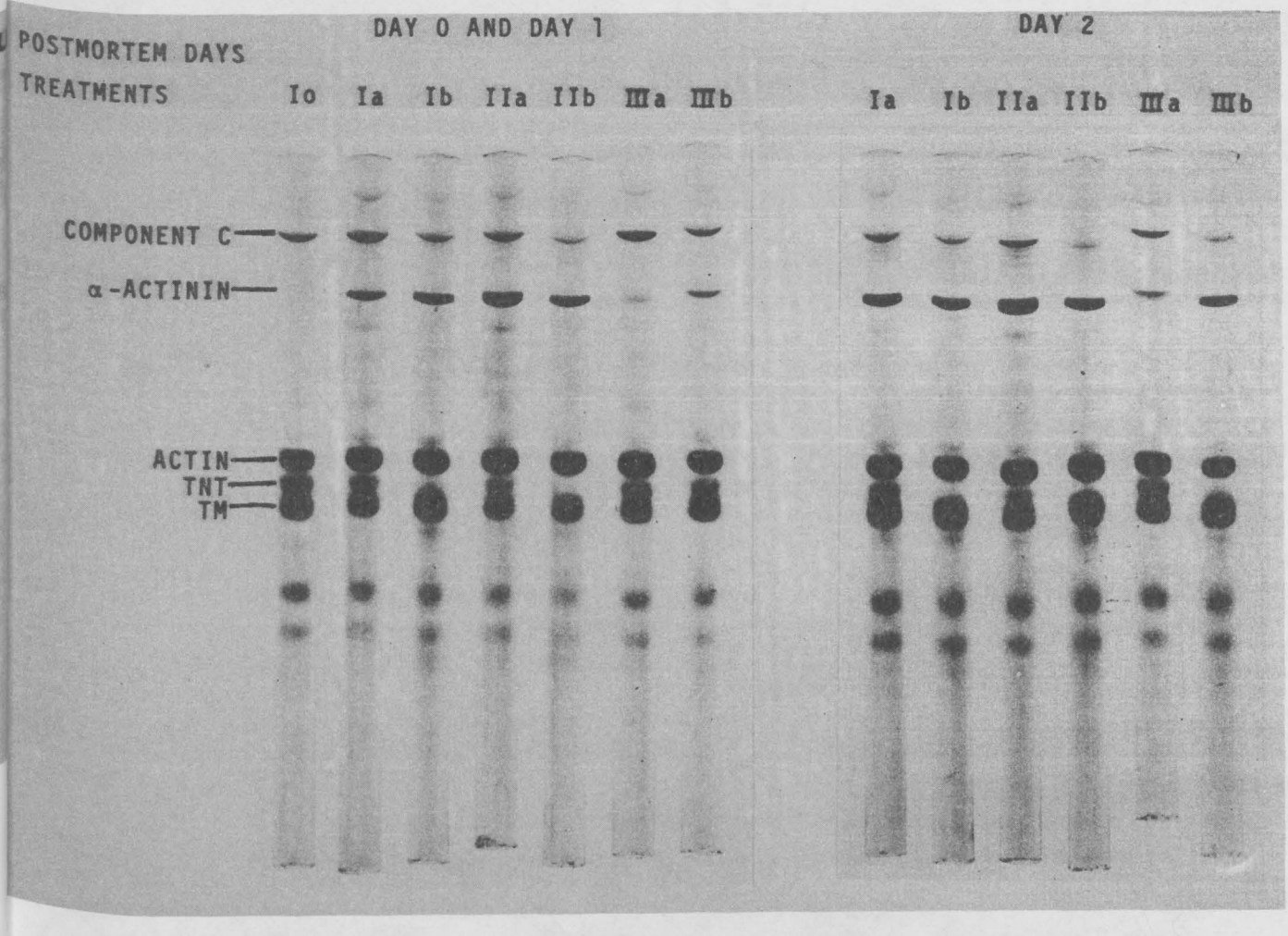

Figure 9. SDS-7½% polyacrylamide gels of protein extracts (1 mM Tris, pH 8.5 solution, 1-hr extraction) from isolated myofibrils of bovine longissimus muscle at-death (Io) and with different treatments (I, ground muscle control; II, 1 mm Ca^{2+} sample; III, 10 mM oxalate sample) postmortem-stored at 2°C (a) or at 23°C (b). Troponin T (TNT), Tropomyosin (TM). Protein load: 10 μg. From Cheng and Parrish (1977) and reproduced by permission of the Institute of Food Technologists and the authors.

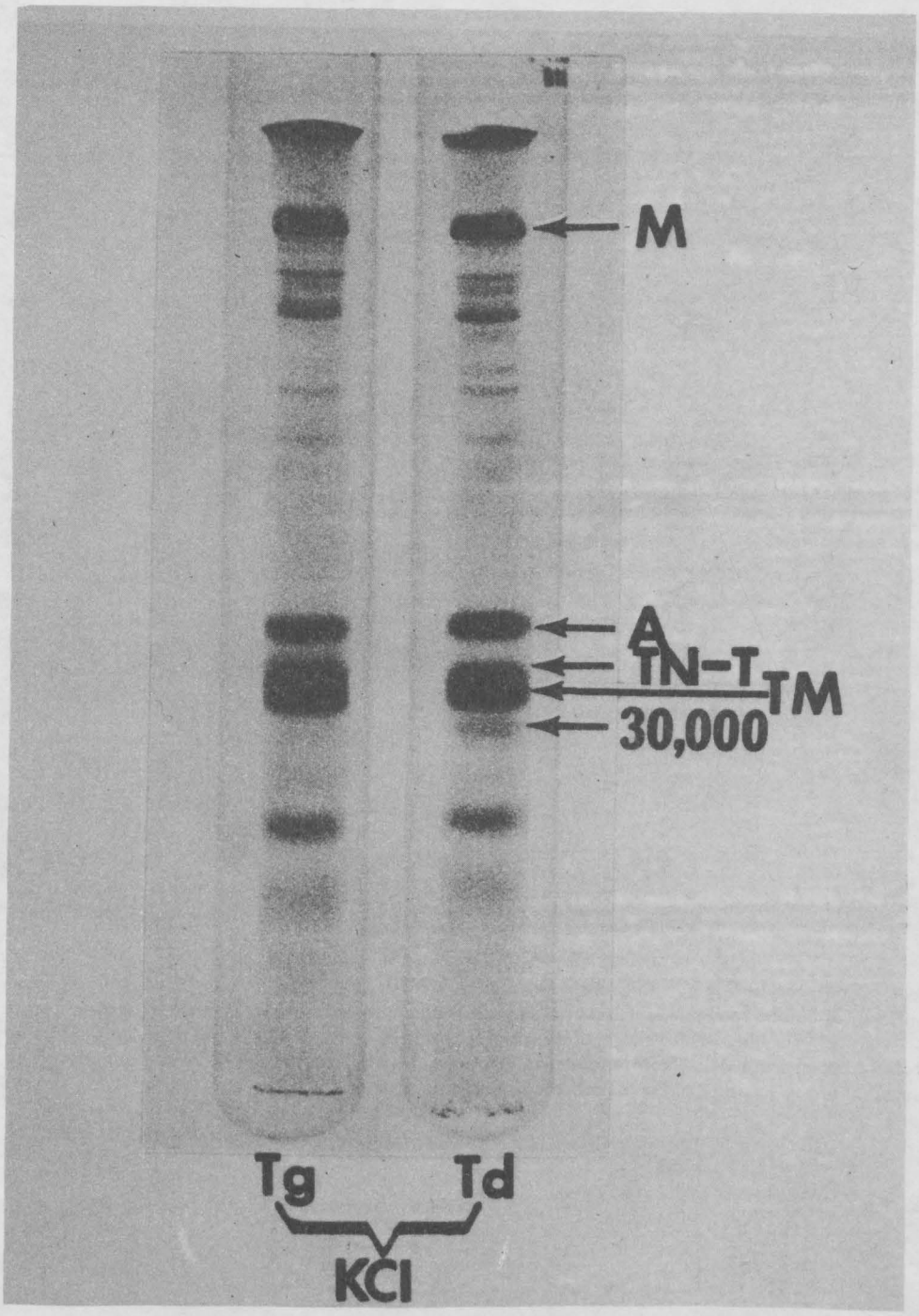

Figure 10. SDS-7½% polyacrylamide gels of myofibrillar proteins extracted with high ionic-strength salt solution (0.6 M KCl, 0.1 M K phosphate, pH 7.4) from 1 day postmortem (2°C storage) bovine tough (Tg) and tender (Td) longissimus muscle. Myosin (M), actin (A), troponin T (TN-T), tropomyosin (TM) and 30,000 (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.

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