

# Role of the Neutral Proteinases in Postmortem Muscle Protein Degradation and Meat Tenderness

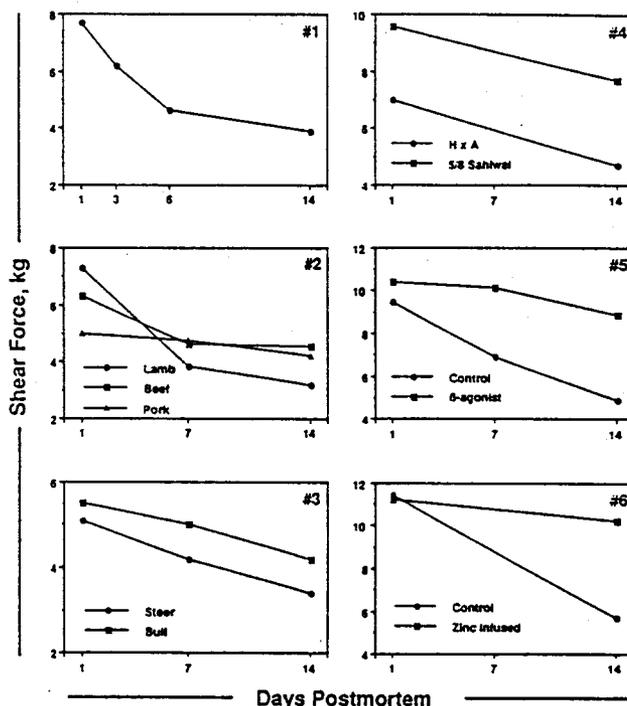
Mohammad Koohmaraie\*

## Introduction

Consumers consider tenderness to be the single most important component of meat quality. Recently, it has finally become clear that a lack of consistency in meat tenderness is one of the main problems facing the meat industry (Morgan et al., 1991b; Smith, 1992). A West coast supermarket chain (annual beef sales of \$130 million) requests that its customers return any meat purchased, if they are not satisfied. Over the last 3 years, customers have returned meat valued at \$364,000/year, of which \$286,000 (i.e., 78%) were related to inadequate or inconsistent beef tenderness, even after blade tenderization (Morgan, 1992). The magnitude of the tenderness problem is realized when one considers the following facts as stated by Wilks (1992), "Data reveal that: a) only one-tenth of one percent of tough, dry or bland steaks are returned for replacement or to get money back, b) for every one complaint that is vocalized, ten complaints are never heard, and c) most consumers who have such experiences don't complain—they just don't return." Additionally, consumers are also demanding meat with a minimal quantity of visible fat. To satisfy this consumer demand, retailers have begun to trim excess fat. Clearly, this practice contributes to inefficient production and, therefore, methods of producing lean carcasses is an intensive area of research. In many cases, lean carcasses are produced at the expense of tenderness (such as dietary administration of some  $\beta$ -adrenergic agonist) or inconsistent meat tenderness (intact male; Field, 1971; Seideman et al., 1982; Koohmaraie, 1988a). Therefore, it is important to identify factors regulating meat tenderness so that methodology can be developed to manipulate the process advantageously. Such methodology may also be used to supplement the current U.S. quality grading system which is based primarily on the quantity of intramuscular fat. The inability of marbling to explain a large portion of total variation in tenderness is well documented (for review, see Parrish, 1974).

Of all the observed variation in meat tenderness (obtained from animals of similar age), approximately 15% is explained by differences in marbling and connective tissue. I believe that differences in postmortem events that lead to improvement in meat tenderness probably explain the majority, if not all, of the 85% of the variation in tenderness not

Figure 1



The rate and extent of postmortem tenderization in beef (#1, from Koohmaraie et al., 1987); different species (#2, from Koohmaraie et al., 1991a); bulls and steers (#3, from Morgan et al., 1992); *Bos indicus* and *Bos taurus* cattle (#4, from Whipple et al., 1990); lambs fed  $\beta$ -adrenergic agonist, L644,969 (#5, from Koohmaraie et al., 1991b); and control and lamb carcasses infused with zinc chloride (#6, from Koohmaraie, 1990).

accounted for (Figure 1). It is for this reason that we have studied and continue to study the mechanism of meat tenderization during postmortem storage (for review, see Koohmaraie, 1988b, 1992a,b).

The objective of this manuscript is to discuss the role of neutral proteinases in postmortem protein degradation and meat tenderness.

## Postmortem Changes in Skeletal Muscle

Because postmortem changes in skeletal muscle were the subject of a previous speaker (Bandman, 1992), only key postmortem changes relevant to this manuscript will be discussed. During postmortem storage of carcasses, nu-

\*M. Koohmaraie, USDA-ARS, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68833

Reciprocal Meat Conference Proceedings, Volume 45, 1992.

merous changes occur in skeletal muscle (for information on original source, see Goll et al. 1983a; Koohmaraie, 1988b, 1992a), some of which result in the loss of tissue integrity, which is translated into the improvement in meat tenderness. These changes include: 1) Z-disk weakening and/or degradation which leads to fragmentation of myofibrils; 2) degradation of desmin which leads to fragmentation of myofibrils, probably through disruption of transverse crosslinking between myofibrils; 3) degradation of titin (speculatively, degradation of titin would improve meat tenderness due to loss of tensile strength of myofibrils); 4) degradation of nebulin (because of the location of nebulin in the myofibrils [I-band], it is not clear how nebulin degradation will affect meat tenderness); 5) disappearance of troponin-T and simultaneous appearance of polypeptides with molecular weight of 28 to 32 (because of the location of troponin-T in myofibrils [i.e., I-band], it is doubtful that degradation of troponin-T by itself will have a direct effect on meat tenderness); however, these changes [i.e., the disappearance of troponin-T and appearance of 28 to 32 kDa polypeptides] seem to be good indicators of the extent of postmortem proteolysis); 6) appearance of a polypeptide with a molecular weight of 95 (neither the origin nor its significance to meat tenderness is known); and 7) perhaps the most important observation is that the major contractile proteins (myosin and actin) are not affected. One of the important changes that occurs in the tissue is the ease of fragmentation of myofibrils under controlled homogenization, which does not occur in unaged tissue (Davey and Gilbert, 1969). The extent of myofibril fragmentation is now routinely measured by a number of laboratories and is called myofibril fragmentation index (MFI). Speculatively, the weakening and/or degradation of Z-disks and degradation of desmin (and probably degradation of titin) are responsible for the increased fragility of myofibrils during postmortem storage.

### Experimental Evidence Supporting Proteolysis Theory

Because all of the above changes are due to proteolytic action, proteinases indigenous to skeletal muscle cells must have a major role in the regulation of these changes and ultimate meat tenderness. There is substantial evidence indicating that the rate and extent of postmortem proteolysis are the principal causes of the observed variation in meat tenderness. Some of this evidence includes: 1) differences in the rate of muscle protein degradation are probably the reason for differences in the rate of postmortem tenderization in meat from pork, lamb and beef carcasses (Koohmaraie et al., 1991a); 2) infusion of carcasses with zinc chloride, which is a potent inhibitor of several classes of proteinases, prevents postmortem proteolysis and the tenderization process (Koohmaraie, 1990); 3) of all the parameters that are thought to affect meat tenderness, reduced rate and extent of postmortem proteolysis is the principal reason for the tenderness differences of meat from *Bos taurus* and *Bos indicus* breeds of cattle (Wheeler et al., 1990; Whipple et al., 1990; Shackelford et al., 1991); 4) neither detectable postmortem proteolysis nor meat tenderization occurs in muscle from carcasses of animals (lamb

and beef) fed some  $\beta$ -adrenergic agonists (Fiems et al., 1990; Kretchmar et al., 1990; Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991b; Pringle et al., 1992; Wheeler and Koohmaraie, 1992); 5) incubation of muscle slices with calcium chloride induces Z-disk degradation, myofibril fragmentation (Busch et al., 1972; Koohmaraie et al., 1988a) and degradation of myofibrillar proteins (Koohmaraie, 1988a); 6) incubation of muscle slices with calcium chelators (EDTA or EGTA) prevents Z-disk degradation, myofibril fragmentation (Busch et al., 1972; Koohmaraie et al., 1988a) and degradation of myofibrillar proteins (Koohmaraie et al., 1988a); 7) infusion of lamb carcasses with calcium chloride accelerates postmortem proteolysis and tenderization (Koohmaraie et al., 1988b; Koohmaraie et al., 1989; Koohmaraie and Shackelford, 1991; St. Angelo et al., 1991); and 8) differences in tenderness of meat obtained from intact and castrated males are probably due to differences in the rate and extent of postmortem proteolysis (Morgan et al., 1992).

### Proteinases Involved in Postmortem Proteolysis

Skeletal muscle contains numerous proteinases; however, because of their ability to degrade myofibrillar proteins, two proteolytic systems have received considerable attention. These include the lysosomal cathepsins and the calpain proteolytic system. Recently, this list has been expanded to include the multicatalytic proteinase complex (MCP). Because the lysosomal proteinases were discussed by a previous speaker (Zeece, 1992), the focus of this manuscript will be on the neutral proteinases which include both calpains and MCP. I shall first discuss the biochemical properties of each of these proteolytic systems and then, using current experimental data, examine their possible roles in postmortem muscle protein degradation.

### Biochemical Properties of the Calpain Proteolytic System

The first report documenting the existence of calpain is perhaps that of Guroff (1964) who reported the existence of a calcium- and sulfhydryl-dependent proteinase from rat brain. Meyer et al. (1964) reported the existence of a similar proteinase in skeletal muscle and it was later purified from porcine skeletal muscle by Dayton et al. (1976a,b). Since then, calpains have been shown to exist in a wide variety of cells, tissues and species. The following paragraphs will summarize some of the key characteristics of this proteolytic system. The sources for this information are reviews written on calpain (Goll et al., 1983a,b, 1985, 1986, 1989, 1992; Mellgren, 1987; Murachi, 1983, 1984, 1989; Murachi et al., 1981a,b; Pontremoli and Melloni, 1986; Suzuki, 1987; Suzuki et al., 1987; Croall and DeMartino, 1991). Original information sources will be given only when the subject has not been addressed in these review articles.

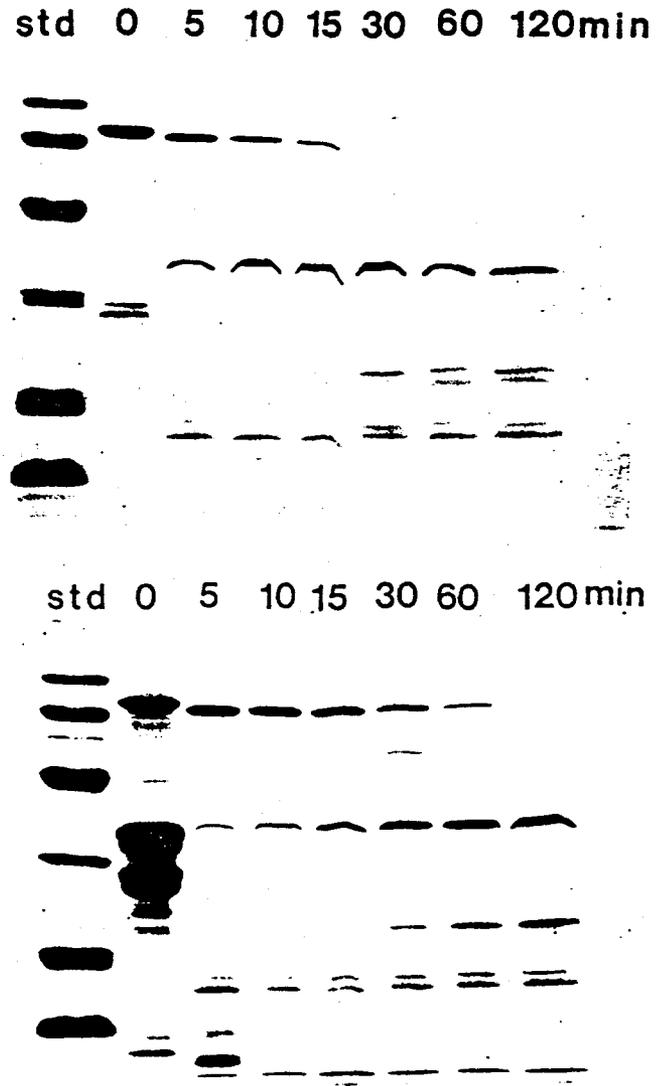
The calpain proteolytic system has been called by a variety of names including kinase activating factor (KAF), calcium-activated factor (CAF), calcium-activated neutral protease (CANP), calcium-dependent sulfhydryl protease

(CDSP) and calcium-dependent protease (CDP). It is now generally accepted to be called "calpain" by the International Union of Biochemistry (EC 3.4.22.17). The calpain proteolytic system consists of  $\mu$ -calpain (the form of the proteinase active at micromolar concentration of calcium, thus  $\mu$ -calpain), m-calpain (the form of the proteinase active at millimolar concentration, thus called m-calpain), calpastatin (a protein that specifically inhibits both forms of calpain at their respective calcium concentration required for proteolytic activity) and an activator (a protein that greatly enhances calpain activity).

Skeletal muscle calpains (both  $\mu$ - and m-calpain) have a molecular weight of about 110 kDa under non-denaturing gel electrophoresis (PAGE) which is dissociated to two subunits with molecular weight of 80 kDa and 30 kDa, by SDS-PAGE. The large subunit which is different, though homologous, between  $\mu$ - and m-calpain is the catalytic subunit. The large subunit consists of four domains. Domain II has been assigned as the catalytic subunit because of its amino acid sequence identity to other sulfhydryl proteinases such as papain. Within domain II, cysteine-108 and histidine-265 are considered to be reactive amino acids. Domain IV is the calcium binding subunit because of its amino acid sequence identity to other calcium binding proteins such as calmodulin and troponin C. The function of domain I (the N terminus) and III (the C terminus) is not known at the present. cDNA for the large subunit of both  $\mu$ - and m-calpain has been cloned and sequenced. These results indicate that although there is similarity in nucleotide sequence, the large subunit of  $\mu$ - and m-calpain are clearly different gene products. The small subunit, which is identical in both forms, is the regulatory subunit of the proteinase.

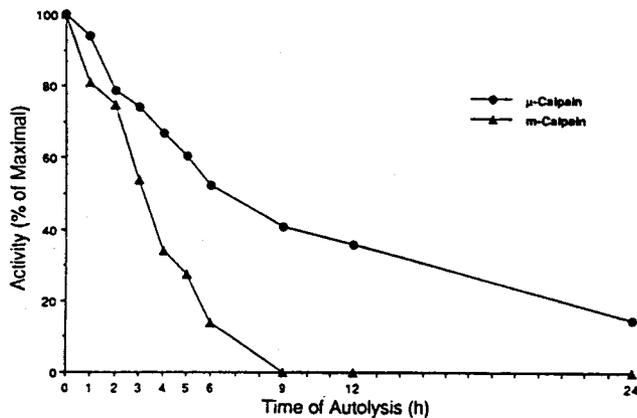
One of the important and well characterized properties of calpains is their susceptibility to calcium-induced autoprolysis. Prolonged exposure to sufficient calcium results in the loss of proteolytic activity and ultimate destruction of the enzyme. As a result of autoprolysis, both the 80 kDa and 30 kDa subunits are degraded to produce polypeptides with molecular mass ranging in size from 78 kDa to 18 kDa. The autolysis and subsequent loss of proteolytic activity of calpains is highly dependent on the presence of substrate, pH and temperature. The rate of autolysis and subsequent loss of proteolytic activity is decreased by the presence of substrate (Figure 2), decreasing temperature and increasing pH (Koochmariaie, 1992c). Autoprolysis is one of the major factors that regulates the activity of the  $\mu$  calpain under postmortem conditions (Koochmariaie, 1992c). Though autolysis of  $\mu$  and m-calpain is similar for the most part, it differs in one significant way. Autolysis of  $\mu$  calpain is an intermolecular process (i.e., each  $\mu$  calpain will degrade its neighboring  $\mu$ -calpain molecules but not itself) and, therefore, autolysis will not go to completion. As a result, even after extensive autolysis either in situ or in vitro, some  $\mu$ -calpain activity will remain. On the other hand, because autolysis of m-calpain is both an inter- and intramolecular process (i.e., each m-calpain molecule will degrade itself and neighboring m-calpain molecules), autolysis of m-calpain proceeds to completion. As a result, after extensive autolysis, no m-calpain activity can be detected (Figure 3).

Figure 2



Time course of bovine skeletal muscle  $\mu$ -calpain autolysis in the absence (top) and presence (bottom; 900  $\mu$ g of casein) of substrate at pH 7.0 and 25°C. Autolysis was done in 40 mM Tris-acetate, pH 7.0, 0.5 mM EDTA, 10 mM MCE with 1.58 mg/ml purified  $\mu$ -calpain. Tubes were preincubated at 25°C for 10 min and then the reaction was initiated with the addition of 3.8 mM  $\text{CaCl}_2$ . The zero h samples were removed prior to the addition of  $\text{CaCl}_2$ . At indicated times, after vortexing an aliquot of the reaction was removed and immediately mixed with equal volume of protein denaturing buffer (to stop the reaction) protein denaturing buffer and heated in a boiling water bath for 5 min, cooled to room temperature and then electrophoresed on a 12.5% polyacrylamide gel. Lane 1 is standard which consisted of Rabbit muscle phosphorylase b (97.4 kDa), Bovine serum albumin (66.2 kDa), Hen egg white ovalbumin (45.0 kDa), Bovine carbonic anhydrase (31 kDa), Soybean trypsin inhibitor (21.5 kDa) and Hen egg white lysozyme (14.4 kDa). (Koochmariaie, unpublished data)

Figure 3



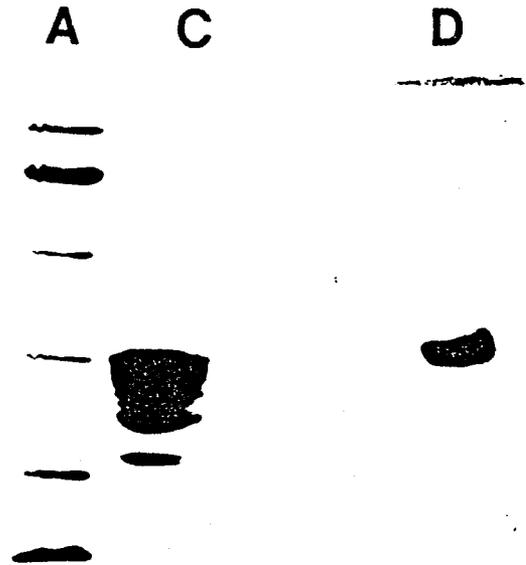
Effect of autolysis at pH 7.0 and 5°C on the proteolytic activity of bovine skeletal muscle  $\mu$ -calpain (top) and m-calpain (bottom). For details see Figure 1. From Koohmaraie (1992c).

All cells that contain calpain also contain calpastatin, although the ratio of calpains to calpastatin varies from cell to cell. In skeletal muscle, this ratio is species dependent. The ratio of calpastatin: $\mu$ -calpain + m-calpain is approximately 2.0 in bovine, 1.2 in ovine and 0.7 in porcine skeletal muscle (Koohmaraie et al., 1991a). Calpastatin inhibits the activity of  $\mu$ - and m-calpain at the respective calcium concentration required for catalytic activity and has no inhibitory effect on any other known proteinase. There has been considerable confusion regarding the molecular weight of calpastatin. The molecular weight of calpastatin is reported to be between 68 and 400 kDa. In meat animals (sheep, pigs, and cattle), we routinely isolate calpastatin with the molecular weight of 68 kDa (Kendall et al., 1992; Koohmaraie, 1992c). Calpastatin is an unusual protein, in that it is highly susceptible to proteolysis while in the tissue. It has been proposed that one of the major reasons for the large variation in molecular weight of calpastatin is its degradation during extraction from the tissue. However, once isolated, it is very stable. For example, heating even at boiling temperature or SDS does not affect its activity.

In addition to calpastatin, there is some evidence indicating that calpains are also regulated by an activator protein (DeMartino and Blumenthal, 1982; Takeyama et al., 1986; Pontremoli et al., 1988, 1990; Shiba et al., 1992). The first report of the existence of a calpain activator is that of DeMartino and Blumenthal (1982), who discovered it in experiments designed to examine the possible effects of calmodulin on the calpains. These so-called calpain activators isolated from various sources have different properties and characteristics and, therefore, the field of calpain activators (endogenous proteins) is not well understood.

Subcellular localization studies indicate that both  $\mu$ - and m-calpain and calpastatin are present throughout muscle cells, including nuclei and mitochondria, but they are localized at high concentrations at the Z-disk region (Kumamoto et al., 1992).

Figure 4



SDS-Polyacrylamide gel electrophoresis (PAGE) (C) and nondenaturing PAGE (D) analysis ovine skeletal muscle multicatalytic proteinase complex. Lane A is the molecular weight standards consisting of rabbit muscle phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), hen egg white ovalbumin (45.0 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa) and hen egg white lysozyme (14.4 kDa). From Koohmaraie (1992d).

### Biochemical Properties of the Multicatalytic Proteinase Complex

The multicatalytic proteinase complex (MCP) was first isolated from the bovine pituitary (Orlowski and Wilk, 1981) and, subsequently, purified from a number of mammalian tissues. All indications are that MCP is also a ubiquitous proteinase, found in every species and cell type examined from archaebacterium to man. The following paragraphs will summarize key characteristics of this proteolytic system. The sources for this information are recent reviews on MCP (Rivett, 1989; Orlowski, 1990). Original information sources will be given only when the subject has not been addressed in these review articles.

The proteinase complex has been referred to by a variety of names, some of which include high-molecular-weight protease, high-molecular-weight cysteine proteinase, latent alkaline multifunctional proteinase, macropain, multicatalytic proteinase, proteasome, 700-kDa multisubunit proteinase and 20S protease. However, there is now a general agreement to call it MCP. Though its function is not known, it is proposed to play a significant role in nonlysosomal protein turnover. It has also been hypothesized that it could be involved in the degradation of muscle proteins (Goll et al., 1989).

MCP is a nonlysosomal proteinase with a native molecular weight of about 650 to 700 kDa. It is composed of a series of low molecular weight, nonidentical subunits.

Electron microscopy of MCP from different sources has indicated similar cylinder-shape particles with dimensions of about 15 by 11 nm. Recently, a significant number of these subunits have been cloned and sequenced. These results indicate that none of the MCP subunits has any sequence identity to any known proteinase. Furthermore, while the MCP subunits are homologous to one another, they are clearly the product of different but homologous genes. Therefore, the components of MCP are distinct subunits of the large complex rather than products of autodigestion (Lee et al., 1990; DeMartino et al., 1991).

When isolated from tissues, MCP does not exhibit proteolytic activity, but can be activated by heat, polylysine, pre-treatment with low concentrations of SDS, and dialysis against water. The proteinase complex was first recognized as "multicatalytic" by Wilk and Orlowski (1980, 1981), because multiple synthetic substrates were degraded. These activities include: trypsin-like (cleavage on the carboxyl side of basic residues), chymotrysin-like (cleavage on the carboxyl side of hydrophobic residues), and peptidylglutamyl-peptide hydrolyzing (cleavage of the carboxyl side of the glutamyl residues) activity (Orlowski, 1990). Examination of the effect of SDS on proteolysis has indicated that the main component responsible for degradation of protein is the peptidylglutamyl-peptide hydrolyzing activity (Orlowski and Nichaud, 1989).

Recently, a protein inhibitor of MCP was isolated from bovine red blood cells (Ma et al., 1992b). This inhibitor appears to be specific for MCP because it had no effect on other proteinases tested. Also, a protein activator of MCP has been isolated from human red blood cells (Fagan and Waxman, 1992) and bovine red blood cells and bovine heart (Ma et al., 1992a). Though it is not clear how MCP activity is regulated, it seems plausible that its activity is regulated by these proteins (inhibitor and activator) and possibly by other factors yet to be identified.

Because MCP has been hypothesized to be involved in the regulation of muscle protein degradation and because of the lack of knowledge regarding its potential involvement in postmortem proteolysis, we have recently (Koochmaraie, 1992d) purified and characterized MCP from ovine skeletal muscle. All indications are that MCP from ovine skeletal muscle is similar, if not identical, to MCP from other sources. Some of the characteristics of ovine skeletal muscle MCP are: 1) molecular mass of about 600 kDa as determined by chromatography on Sephacyl S-300, which dissociates into a series of low molecular weight polypeptides ranging in molecular masses from 21 to 31 kDa; 2) it has no proteolytic activity as isolated from tissue, but it can be reversibly activated by heating at 60°C and with pre-treatment with a low concentration of SDS; 3) maximum proteolytic activity is observed at pH 7.5 to 8.0 and 45°C and it retains about 2% of its maximum activity at 5°C and pH 7.5 and about 22% of its maximum activity at pH 5.5 and 45°C; 4) calcium chloride has no effect on its proteolytic activity; and 5) using radiolabeled casein as a substrate, the specific activities of  $\mu$ -, m-calpain and MCP were 44.0, 59.7 and 2.0 (mg casein degraded/mg proteinase), respectively.

As yet, the subcellular location of MCP in skeletal muscle has not been determined. However, based on immunoreac-

tivity after subcellular fractionation, Tanaka et al. (1986) reported that in rat liver MCP was largely (83%) found in the cytosolic fraction.

### Experimental Evidence Indicating that Calpains are Probably Responsible for Postmortem Proteolysis that Results in Tenderization

As mentioned before, proteinases must have the following characteristics to be considered as possible candidates in causing postmortem muscle protein degradation (Koochmaraie, 1988b): 1) be located within the skeletal muscle cell; 2) have access to the substrate, i.e., myofibrils; and 3) have the ability to degrade similar proteins that are degraded in muscle during postmortem storage. Of the three proteolytic systems that have the potential to be involved in postmortem tenderization, the calpain proteolytic system is the only one that has all of the above characteristics.

Although MCP is localized within skeletal muscle cells, it cannot degrade myofibrillar proteins (Koochmaraie, 1992d). Of all of the myofibrillar proteins, troponin-C and myosin light chains-2 and -3 were the only proteins degraded by MCP and it had no detectable effect on the morphology of myofibrils (Koochmaraie, 1992d). Therefore, MCP does not appear to have a direct role in the tenderization process. However, MCP might have an indirect role by acting as a regulator of the proteolytic system involved in the tenderization.

In contrast to MCP, substantial experimental data exist that support a direct role for the calpain proteolytic system in bringing about postmortem changes resulting in meat tenderization. The calpains: 1) are located within skeletal muscle cells; 2) are localized on the myofibril structures that are affected during postmortem storage (dispersed throughout myofibrils and highest concentration is found on the Z-disks; Kumamoto et al., 1992); and 3) have the ability to precisely reproduce postmortem changes in myofibrils under in-vitro conditions. The experimental data in support of the role of calpains have been analyzed in detail elsewhere (Koochmaraie, 1988b, 1992a,b).

Results of several experiments had convinced us that calpains are the primary enzyme systems responsible for postmortem meat tenderization (particularly Koochmaraie et al., 1988a). To test the validity of our hypothesis, we infused lamb carcasses with calcium chloride to increase intracellular concentration of calcium to activate the calpains (Koochmaraie et al., 1988b). Results supported our hypothesis in that maximum postmortem tenderization occurred in the first 24 hours of postmortem storage. Since then, the effectiveness of this method (calcium chloride acceleration of postmortem tenderization) has been examined under a variety of conditions and the process has consistently produced tender meat regardless of animal source (Table 1). Moreover, we have recently demonstrated that calcium chloride injection at 24 hour postmortem tenderizes meat as effectively as injection at 0 hour (Wheeler et al., 1992).

There are some important characteristics of the calcium chloride infusion/injection that deserve notation: 1) maximum tenderness value is obtained in the first 24 hours of postmortem storage; 2) it consistently produces uniformly

Table 1. Effect of Calcium Chloride Infusion/Injection Warner-Bratzler Shear Value.

	Species	Muscle	Treatment	Control			Calcium Chloride		
				Days post-slaughter			Days post-slaughter		
				1	7	14	1	7	14
Koohmaraie et al. (1988b)	Lamb	LD	None	7.6	4.6	—	3.6	2.7	—
Koohmaraie et al. (1989)	Lamb	LD	None	10.3	8.6	—	3.4	3.0	—
Koohmaraie et al. (1989)	Lamb	LD	None	8.9	6.3	—	4.4	4.1	—
Koohmaraie et al. (1990)	Lamb	LD	None	8.9	5.4	—	4.1	3.5	—
Koohmaraie et al. (1990)	Lamb	LD	None	9.0	—	6.2	6.0	—	5.0
St. Angelo et al. (1991)	Lamb	LD	None	8.3	5.3	—	3.3	3.8	—
Koohmaraie and Shackelford (1991)	Lamb	LD	None	10.0	7.7	6.9	3.0	3.1	2.7
Koohmaraie and Shackelford (1991)	Lamb	LD	BAA	11.4	9.6	9.1	6.0	6.0	5.2
Wheeler et al. (1991)	Beef	SM	HT	8.9	8.5	7.3	3.7	3.2	3.2
Wheeler et al. (1991)	Beef	BF	HT	6.4	5.5	4.9	3.7	3.3	3.7
Wheeler et al. (1991)	Beef	BF	None	5.6	4.6	4.6	3.6	3.8	3.3
Wheeler et al. (1992)	Beef	LD	None	8.4	7.2	—	—	2.8	—
Morgan et al. (1991a)	Cow	LD	None	8.9	7.5	6.4	4.2	3.7	3.7
Morgan et al. (1991a)	Cow	GM	None	9.8	7.2	6.9	3.7	4.1	4.1
Morgan et al. (1991a)	Cow	SM	None	9.6	7.2	6.9	6.0	6.2	6.0

LD=Longissimus dorsi; BF=Biceps femoris; SM=Semimembranosus; GM=Gluteus medius; BAA= $\beta$ -adrenergic agonist; HT=Hot-boned.

tender meat; 3) because of the unique built-in control (auto-proteolysis of calpains in the presence of calcium; for detail see Koohmaraie, 1992b), meat is never over-tenderized as with other proteases (e.g., papain); 4) because calcium accelerates the rate of glycolysis (shortly after calcium chloride infusion or injection of prerigor meat, the rigor process as determined by pH is completed), the process can easily be applied to hot-boning (Wheeler et al., 1991) which has traditionally been considered as a means of decreasing energy and labor costs associated with chilling and fabricating carcasses; and 5) from a nutritional point of view, this process could be used as method of increasing calcium intake. The importance of calcium in the human diet is well recognized (Heaney, 1992). In fact, Heaney and Barger-Lux (1991) suggested that calcium fortification of processed meat, because meat is widely consumed, is one of the best methods of increasing calcium intake in the human population.

Although the process was developed (see Koohmaraie, 1988b, 1992a,b) to accelerate the postmortem tenderization process by activating calpains, we do not know the precise mechanism(s) through which calcium chloride infusion or injection accelerates postmortem events leading to meat tenderization. Undoubtedly, mechanisms other than the activation of calpains are involved. However, there are several lines of evidence suggesting that the primary mode of action of calcium, whether from exogenous (calcium injection or infusion) or endogenous (sarcoplasmic reticulum and mitochondria) sources, is through activation of calpains (Table 2).

Regardless of the mechanism of action, calcium chloride infusion or injection has the potential to have a significant impact on the meat industry. The usefulness of this process will depend on successful modification to allow practical application by the meat industry. We are now in the process of addressing some of these issues such as time of treatment (prerigor vs. postrigor), volume and concentration of calcium chloride, effect on lean color and meat microflora, and flavor profiling.

## Future Directions

The recently completed National Tenderness Survey and National Beef Quality Audit has clearly demonstrated that excess fat and inconsistent tenderness are the major problems facing the beef industry. The major cause of the tenderness problem is our inability to consistently produce tender meat compounded by our inability to accurately sort carcasses based on their tenderness values prior to consumption. This is perhaps the best demonstration of the need to improve our methodology so that carcasses producing tough meat can be identified. It is sobering to realize that the only time we actually know the tenderness value of the meat we produce is when it is eaten. For this reason, until recently, lack of tenderness or inconsistency of tenderness was not recognized as a problem. The problem is compounded by the fact that only 0.1% of the dissatisfied consumers actually return meat for replacement or refund (Wilks, 1992). We must, therefore, develop the ability to predict meat tenderness before it is purchased by the consumer. This new method should be an objective method that is directly related to meat tenderness. This approach necessitates a thorough understanding of the factors regulating meat tenderness. Over the last several decades, numerous factors have been proposed to be involved in the determination of meat tenderness which include: rate of pH and temperature decline, amount and quality (crosslinking) of collagen, sarcomere length, ionic strength and postmortem proteolysis. If we restrict ourselves to one muscle (e.g., longissimus muscle, because of its economical value) and a constant age, then I believe the rate and extent of postmortem proteolysis is most important of all. Moreover, I speculate that other factors such as ionic strength and the rate of pH and temperature decline exert their influence on postmortem proteolysis and meat tenderness through the calpain proteolytic system. In fact, some of our recent data suggest that pH and temperature have a profound effect on the rate of inactivation of  $\mu$ -calpain (Koohmaraie, 1992c).

**Table 2. Some of the Experimental Data Indicating that Calcium Acts through Activation of the Calpains.**

1. Incubation of muscle slices with calcium chloride induces proteolysis of myofibrillar proteins, fragmentation and activates calpains (Koochmaraie et al., 1988a). However, incubation of muscle slices with calcium chelators (EDTA and EGTA) prevents proteolysis of myofibrillar proteins, myofibril fragmentation and calpains activation (Koochmaraie et al., 1988a). Davey and Gilbert (1969) demonstrated that incubation of fiber pieces with EDTA prevented both weakening of lateral attachments and the disappearance of Z-disks. Busch et al. (1972) demonstrated that myofibrils fragmentation and disappearance of Z-disks were accelerated by incubating muscle slices with calcium chloride and both processes were inhibited by using EDTA instead of calcium chloride.
2. When carcasses were infused with different concentrations of calcium chloride (75, 150 and 300 mM), acceleration of meat tenderness occurred only at the concentration which activated calpains (Koochmaraie et al., 1989).
3. Alarcon-Rojo and Dransfield (1989) reported that the calcium chloride acceleration of postmortem tenderization was inhibited in the presence of inhibitors that are specific for calpains.
4. Infusion of carcasses with zinc chloride, which is a potent inhibitor of calpains, inhibited proteolysis of myofibrillar proteins, myofibril fragmentation and tenderization during 14-day postmortem storage (Koochmaraie, 1990).
5. Of the three proteolytic systems thought to be involved in postmortem proteolysis and tenderization, only calpains are activated by calcium. Calcium has no effect on the multicatalytic proteinase complex (Koochmaraie, 1992d) and has no stimulatory effect on the lysosomal proteinases. In fact at 10 mM, calcium chloride inhibits the activity of cathepsin B by 39% (Barrett, 1973).
6. Muscle from animals fed  $\beta$ -adrenergic agonist does not undergo (or undergoes minimal) postmortem proteolysis and produces tough meat. Lack of postmortem proteolysis and meat tenderization in BAA-fed animals has been attributed to a reduction in the activity of the calpain proteolytic system (Fiems et al., 1990; Wang and Beermann, 1988; Kretchmar et al., 1989, 1990, Koochmaraie and Shackelford, 1991; Koochmaraie et al., 1991b; Pringle et al., 1992; Wheeler and Koochmaraie, 1992). However, calcium chloride infusion of carcasses from BAA-fed lambs induces activation of calpains, degradation of myofibrillar proteins and meat tenderization (Koochmaraie and Shackelford, 1991).

Additional reasons for such speculations are discussed by Koochmaraie (1992b).

As detailed in the previous section, current experimental data suggest that the calpain proteolytic system is probably the single most important proteolytic system directly involved in postmortem proteolysis and improvement in meat tenderness. Further support for our hypothesis (Koochmaraie, 1988b, 1990, 1992a,b; Koochmaraie et al., 1986, 1987, 1988a,b,c) has recently been reported by Dransfield (1992). He reported that 68% of the variation in meat toughness was accounted for by variation in the activity of  $\mu$ -calpain. We have, therefore, decided that understanding the regulation of calpain under postmortem conditions (entirely different than in living muscle,

Koochmaraie, 1992a) is perhaps one of the key items of knowledge required for the development of a method to predict meat quality.

#### ACKNOWLEDGMENTS

I am grateful to Drs. J. R. Arbona, T. L. Wheeler, and G. Whipple for their constructive criticism and invaluable suggestions during preparation of this manuscript; to K. Theer for preparation of figures and slides; and to M. Bierman and C. Grummet for secretarial assistance. I am also grateful to Drs. E. Bandman, B. B. Marsh, R. A. Merkel, H. J. Mersmann, G. C. Smith, S. B. Smith, and J. D. Tatum for their review, constructive criticism, and invaluable suggestions prior to the submission of this manuscript.

#### References

- Alarcon-Rojo, A.; Dransfield, E. 1989. Effect of calcium ions on texture of beef during conditioning. *Proc. Int. Cong. Meat Sci. Technol.* 35:1141.
- Bandman, E. 1992. Changes in myofibrillar and cytoskeletal proteins in postmortem muscle. *Proc. Recip. Meat Conf.* (This issue).
- Barrett, A. J. 1973. Human cathepsin B1: Purification and some of the properties of the enzyme. *Biochem. J.* 131:809.
- Busch, W. A.; Stromer, M. H.; Goll, D. E.; Suzuki, A. 1972.  $Ca^{2+}$ -specific removal of Z lines from rabbit skeletal muscle. *J. Cell Biol.* 52:367.
- Croall, D. E.; DeMartino, G. N. 1991. Calcium-activated neutral protease (calpain) system: Structure, function, and regulation. *Physiol. Rev.* 71:813.
- Davey, C. L.; Gilbert, K. V. 1969. Studies in meat tenderness. 7. Changes in the fine structure of meat during aging. *J. Food Sci.* 34:69.
- Dayton, W. R.; Goll, D. E.; Zeece, M. G.; Robson, R. M.; Reville, W. J. 1976a. A  $Ca^{2+}$ -activated protease involved in myofibrillar protein turnover. Purification from porcine muscle. *Biochemistry* 15:2150.
- Dayton, W. R.; Reville, W. J.; Goll, D. E.; Stromer, M. H. 1976b. A  $Ca^{2+}$ -activated protease possibly involved in myofibrillar protein turnover. Partial characterization of the purified enzyme. *Biochemistry* 15:2159.
- DeMartino, G. N.; Blumenthal, D. K. 1982. Identification and partial purification of a factor that stimulates calcium-dependent proteases. *Biochemistry* 21:4297.
- DeMartino, G. N.; Orth, K.; McCullough, M. L.; Lee, L. W.; Munn, T. Z.; Moomaw, C. R.; Dawson, P. A.; Slaughter, C. A. 1991. The primary structures of four subunits of the human, high-molecular-weight proteinase, macropain (proteasome), are distinct but homologous. *Biochim. Biophys. Acta* 1079:29.

- Dransfield, E. 1992. Modelling post-mortem tenderisation—III: Role of calpain I in conditioning. *Meat Sci.* 31:85.
- Fagan, J. M.; Waxman, L. 1992. Identification of an activator of the multicatalytic proteinase from human red blood cells. *FASEB J.* 6:A1968.
- Field, R. A. 1971. Effect of castration on meat quality and quantity. *J. Anim. Sci.* 32:849.
- Fiems, L. O.; Buts, B.; Cottyn, B. G. 1990. Effect of a  $\beta$ -agonist on meat quality and myofibrillar protein fragmentation in bulls. *Meat Sci.* 27:29.
- Goll, D. E.; Edmunds, T.; Kleese, W. C.; Sathe, S. K.; Shannon, J. D. 1985. Some properties of the  $\text{Ca}^{2+}$ -dependent proteinase. In: E. A. Khairallah et al. (Ed.) *Intracellular Protein Catabolism*. p. 151. Alan R. Liss, New York.
- Goll, D. E.; Kleese, W. C.; Sloan, D. A.; Shannon, J. D.; Edmunds, T. 1986. Properties of the Ca-dependent proteinases and their inhibitor. *Cienc. Biol.* 11:75.
- Goll, D. E.; Kleese, W. C.; Szpacenko, A. 1989. Skeletal muscle proteases and protein turnover. In: D. R. Campion, G. J. Hausman and R. J. Martin (Ed.) *Animal Growth Regulation*. p. 141. Plenum Publishing, New York.
- Goll, D. E.; Otsuka, Y.; Nagainis, P. A.; Shannon, J. D.; Sathe, S. K.; Muguruma, M. 1983a. Role of muscle proteinases in maintenance of muscle integrity and mass. *J. Food Biochem.* 7:137.
- Goll, D. E.; Shannon, J. D.; Edmunds, T.; Sathe, S. K.; Kleese, W. C.; Nagainis, P. A. 1983b. Properties and regulation of the  $\text{Ca}^{2+}$ -dependent proteinase. In: B. de Bernard et al. (Ed.) *Calcium-Binding Proteins*. p. 19. Elsevier, Amsterdam.
- Goll, D. E.; Thompson, V. F.; Taylor, R. G.; Christensen, J. A. 1992. Role of the calpain system in muscle growth. *Biochimie* (In press).
- Guroff, G. 1964. A neutral, calcium-activated proteinase from the soluble fraction of rat brain. *J. Biol. Chem.* 239:149.
- Heaney, R. P. 1992. Calcium in the prevention and treatment of osteoporosis. *J. Intern. Med.* 231:169.
- Heaney, R. P.; Barger-Lux, M. J. 1991. Calcium in nutrition and prevention of disease. *Food Nutr. News* 63:7.
- Kendall, T. L.; Koohmaraie, M.; Arbona, J. R.; Williams, S. E.; Young, L. L. 1992. Effect of pH and ionic strength on bovine m-calpain and calpastatin activity. *J. Anim. Sci.* (Submitted)
- Koohmaraie, M. 1988a. Palatability of meat from bull and steer carcasses. *ISI Atlas of Science: Animal and Plant Sci.* 1:124.
- Koohmaraie, M. 1988b. The role of endogenous proteases in meat tenderness. *Proc. Recip. Meat Conf.* 41:89.
- Koohmaraie, M. 1990. Inhibition of postmortem tenderization in ovine carcasses through infusion of zinc. *J. Anim. Sci.* 68:1476.
- Koohmaraie, M. 1992a. The role of  $\text{Ca}^{2+}$ -dependent proteases (calpains) in postmortem proteolysis and meat tenderness. *Biochimie* 74:239.
- Koohmaraie, M. 1992b. Muscle proteases and meat aging. *Proc. of the 38th Int. Cong. Meat Sci. Technol.* Meat Sci. (Submitted)
- Koohmaraie, M. 1992c. Effect of pH and temperature and inhibitors on autolysis and catalytic activity of bovine skeletal muscle  $\mu$ -calpain. *J. Anim. Sci.* (Accepted).
- Koohmaraie, M. 1992d. Ovine skeletal muscle multicatalytic proteinase complex (proteasome): purification, characterization and comparison of its effects on myofibrils with  $\mu$ -calpain. *J. Anim. Sci.* (Submitted).
- Koohmaraie, M.; Babiker, A. S.; Merkel, R. A.; Dutson, T. R. 1988a. Role of  $\text{Ca}^{2+}$ -dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. *J. Food Sci.* 53:1253.
- Koohmaraie, M.; Babiker, A. S.; Merkel, R. A.; Dutson, T. R. 1988b. Acceleration of postmortem tenderization in ovine carcasses through activation of  $\text{Ca}^{2+}$ -dependent proteases. *J. Food Sci.* 53:1638.
- Koohmaraie, M.; Crouse, J. D.; Mersmann, H. J. 1989. Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and ionic strength. *J. Anim. Sci.* 67:934.
- Koohmaraie, M.; Schollmeyer, J. E.; Dutson, T. R. 1986. Effect of low-calcium-requiring calcium activated factor on myofibrils under varying pH and temperature conditions. *J. Food Sci.* 51:28.
- Koohmaraie, M.; Seideman, S. C.; Schollmeyer, J. E.; Dutson, T. R.; Crouse, J. D. 1987. Effect of post-mortem storage on  $\text{Ca}^{2+}$ -dependent proteases, their inhibitor and myofibril fragmentation. *Meat Sci.* 19:187.
- Koohmaraie, M.; Seideman, S. C.; Schollmeyer, J. E.; Dutson, T. R.; Babiker, A. S. 1988c. Factors associated with the tenderness of three bovine muscles. *J. Food Sci.* 53:407.
- Koohmaraie, M.; Shackelford, S. D. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a beta-adrenergic agonist. *J. Anim. Sci.* 69:2463.
- Koohmaraie, M.; Shackelford, S. D.; Muggli-Cockett, N. E.; Stone, R. T. 1991b. Effect of the  $\beta$ -adrenergic agonist  $\text{L}_{644,969}$  on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs. *J. Anim. Sci.* 69:4823.
- Koohmaraie, M.; Whipple, G.; Crouse, J. D. 1990. Acceleration of postmortem tenderization in lamb and Brahman-cross beef carcasses through infusion of calcium chloride. *J. Anim. Sci.* 68:1278.
- Koohmaraie, M.; Whipple, G.; Kretchmar, D. H.; Crouse, J. D.; Mersmann, H. J. 1991a. Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. *J. Anim. Sci.* 69:617.
- Kretchmar, D. H.; Hathaway, M. R.; Dayton, W. R. 1989. In vivo effect of a  $\beta$ -adrenergic agonist on activity of calcium-dependent proteinases, their specific inhibitor, and cathepsins B and H in skeletal muscle. *Arch. Biochem. Biophys.* 275:228.
- Kretchmar, D. H.; Hathaway, M. R.; Dayton, W. R. 1990. Alterations in postmortem degradation of myofibrillar proteins in muscle of lambs fed a  $\beta$ -adrenergic agonist. *J. Anim. Sci.* 68:1760.
- Kumamoto, T.; Kleese, W. C.; Cong, J. Y.; Goll, D. E. 1992. Localization in electron micrographs of the Ca-dependent proteinases and their inhibitor in normal, starved, and denervated rat skeletal muscle. *Anat. Rec.* 232:60.
- Lee, L. W.; Moomaw, C. R.; Orth, K.; McGuire, M. J.; DeMartino, G. N.; Slaughter, C. A. 1990. Relationships among the subunits of the high molecular weight proteinase, macropain (proteasome). *Biochim. Biophys. Acta* 1037:178.
- Ma, C-P; Slaughter, C. A.; DeMartino, G. N. 1992a. Identification, purification and characterization of a protein activator of the 20 S proteasome (macropain). *J. Biol. Chem.* (In press).
- Ma, C-P; Slaughter, C. A.; DeMartino, G. N. 1992b. Purification and characterization of a protein inhibitor of the 20S proteasome (macropain). *Biochim. Biophys. Acta* 1119:303.
- Mellgren, R. L. 1987. Calcium-dependent proteases: An enzyme active at cellular membranes? *FASEB J.* 1:110.
- Meyer, W. L.; Fischer, E. H.; Krebs, E. G. 1964. Activation of skeletal muscle phosphorylase b kinase by  $\text{Ca}^{2+}$ . *Biochemistry* 3:1033.
- Morgan, J. B. 1992. Tenderness problems and potential solutions. *National Quality Audit, Final Report*. p. 180.
- Morgan, J. B.; Miller, R. K.; Mendez, F. M.; Hale, D. S.; Savell, J. W. 1991a. Using calcium chloride injection to improve tenderness of beef from mature cows. *J. Anim. Sci.* 69:4469.
- Morgan, J. B.; Savell, J. W.; Hale, D. S.; Miller, R. K.; Griffin, D. B.; Cross, H. R.; Shackelford, S. D. 1991b. National beef tenderness survey. *J. Anim. Sci.* 69:3274.
- Morgan, J. B.; Wheeler, T. L.; Koohmaraie, M.; Savell, J. W.; Crouse, J. D. 1992. Meat tenderness and the calpain proteolytic system in longissimus muscle of young bulls and steers. *J. Anim. Sci.* (Submitted)
- Murachi, T. 1983. Calpain and Calpastatin. *TIBS* 8:167.
- Murachi, T. 1984. Calcium-dependent proteinases and specific inhibitors: Calpain and calpastatin. *Biochem. Soc. Symp.* 49:167.
- Murachi, T. 1989. Intracellular regulatory system involving calpain and calpastatin. *Biochem. Int.* 18:263.
- Murachi, T.; Hatanaka, M.; Yasumoto, Y.; Nakayama, N.; Tanaka, K. A. 1981a. A quantitative distribution study on calpain and calpastatin in rat tissues and cells. *Biochem. Int.* 2:651.
- Murachi, T.; Tanaka, K.; Hatanaka, M.; Murakami, T. 1981b. Intracellular  $\text{Ca}^{2+}$ -dependent protease (calpain) and its high-molecular weight endogenous inhibitor (calpastatin). *Adv. Enzyme Regul.* 19:407.
- Orlowski, M. 1990. The multicatalytic proteinase complex, a major extralysosomal proteolytic system. *Biochemistry* 29:10289.

- Orlowski, M.; Michaud, C. 1989. Pituitary multicatalytic proteinase complex. Specificity of components and aspects of proteolytic activity. *Biochemistry* 28:9270.
- Orlowski, M.; Wilk, S. 1981. A multicatalytic protease complex from pituitary that forms enkephalin and enkephalin containing peptides. *Biochem. Biophys. Res. Commun.* 101:814.
- Parrish, F. C. Jr. 1974. Relationship of marbling to meat tenderness. *Proc. Meat Indus. Res. Conf.* p. 117.
- Pontremoli, S.; Melloni, E. 1986. Extralysosomal protein degradation. *Annu. Rev. Biochem.* 55:455.
- Pontremoli, S.; Melloni, E.; Michetti, M.; Salamino, F.; Sparatore, B.; Horecker, B. L. 1988. An endogenous activator of the Ca-dependent proteinase of human neutrophils that increases its affinity for Ca. *Proc. Natl. Acad. Sci. USA* 85:1740.
- Pontremoli, S.; Viotti, P. L.; Melloni, E. 1990. Identification of an endogenous activator of calpain in rat skeletal muscle. *Biochem. Biophys. Res. Commun.* 171:569.
- Pringle, T. D.; Calkins, C. R.; Koohmaraie, M.; Jones, S. J. 1992. Response time of animal performance, muscle growth, endogenous muscle proteinase activities, muscle metabolism and meat tenderness to  $\beta$ -adrenergic agonist feeding in wether lambs. *J. Anim. Sci.* (Submitted).
- Rivett, A. J. 1989. The multicatalytic proteinase of mammalian cells. *Arch. Biochem. Biophys.* 268:1.
- Seideman, S. C.; Cross, H. R.; Oltjen, R. R.; Schanbacher, B. D. 1982. Utilization of the intact male for red meat production: A review. *J. Anim. Sci.* 55:826.
- Shackelford, S. D.; Koohmaraie, M.; Miller, M. F.; Crouse, J. D.; Reagan, J. O. 1991. An evaluation of tenderness of the longissimus muscle of Angus by Hereford versus Brahman crossbred heifers. *J. Anim. Sci.* 69:171.
- Shiba, E.; Ariyoshi, H.; Yano, Y.; Kawasaki, T.; Sakon, M.; Kambayashi, J.; Mori, T. 1992. Purification and characterization of a calpain activator from human platelets. *Biochem. Biophys. Res. Commun.* 182:461.
- Smith, G. C. 1992. Results of the face-to-face interviews (phase I) from the national beef quality audit. National Quality Audit, Final Report. p. 100.
- St. Angelo, A. J.; Koohmaraie, M.; Crippen, K. L.; Crouse, J. 1991. Acceleration of tenderization/inhibition of warmed-over flavor by calcium chloride-antioxidant infusion into lamb carcasses. *J. Food Sci.* 56:359.
- Suzuki, A. 1987. Calcium activated neutral protease: Domain structure and activity regulation. *TIBS* 12:103.
- Suzuki, K.; Imajoh, S.; Emori, Y.; Ohno, S. 1987. Calcium-activated neutral protease and its endogenous inhibitor. *FEBS Lett.* 220:271.
- Takeyama, Y.; Nakanishi, H.; Kishimoto, A.; Nishizuka, Y. 1986. A calcium-protease activator associated with brain microsomal-insoluble elements. *FEBS Lett.* 194:110.
- Tanaka, K.; Ii, K.; Ichihara, A.; Waxman, L.; Goldberg, A. L. 1986. A high molecular weight protease in the cytosol of rat liver. I. Purification, enzymological properties, and tissue distribution. *J. Biol. Chem.* 261:15197.
- Wang, S. Y.; Beermann, D. H. 1988. Reduced calcium-dependent proteinase activity in cimaterol-induced muscle hypertrophy in lambs. *J. Anim. Sci.* 66:2545.
- Wheeler, T. L.; Koohmaraie, M. 1992. Effects of  $\beta$ -adrenergic agonist L644,969 on muscle protein turnover, endogenous proteinase activities and meat tenderness in steers. *J. Anim. Sci.* (Accepted).
- Wheeler, T. L.; Crouse, J. D.; Koohmaraie, M. 1992. The effect of postmortem injection time and freezing on the effectiveness of calcium chloride at improving meat tenderness. *J. Anim. Sci.* (Submitted).
- Wheeler, T. L.; Koohmaraie, M.; Crouse, J. D. 1991. Effects of calcium chloride injection and hot-boning on the tenderness of round muscles. *J. Anim. Sci.* 69:4871.
- Wheeler, T. L.; Savell, J. W.; Cross, H. R.; Lunt, D. K.; Smith, S. B. 1990. Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. *J. Anim. Sci.* 68:4206.
- Whipple, G.; Koohmaraie, M.; Dikeman, M. E.; Crouse, J. D.; Hunt, M. C.; Klemm, R. D. 1990. Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. *J. Anim. Sci.* 68:2716.
- Wilk, S.; Orlowski, M. 1980. Cation-sensitive neutral endopeptidase isolation and specificity of the bovine pituitary enzyme. *Neurochemistry* 35:1172.
- Wilks, D. 1992. Introductory remarks: issuance of the challenge to the workshop participants. National Quality Audit, Final Report. p. 92.
- Zeece, M. G. 1992. Role of the lysosomal (acidic) proteases and inhibitors in postmortem muscle. *Proc. Recip. Meat Conf.* (This issue).