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ACCELERATION OF POSTMORTEM TENDERIZATION IN OVINE CARCASSES THROUGH INFUSION OF CALCIUM CHLORIDE: EFFECT OF CONCENTRATION AND IONIC STRENGTH

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ABSTRACT

Ovine carcasses were arterially infused with a volume equal to 10% of the live weight after electrical stimulation. The infusion solutions contained .075 *M*, .15 *M* or .3 *M* calcium chloride. Results indicated that .3 *M* calcium chloride treatment was the most effective concentration of $CaCl_2$ to reduce the shear force value measured at 24 h postmortem. To examine the contribution of ionic strength to tenderization that occurs by infusion of carcasses with .3 *M* $CaCl_2$, ovine carcasses were infused with $CaCl_2$ and NaCl solutions of identical ionic strength. Results indicated that the tenderization that occurred by infusion of carcasses with $CaCl_2$ was not due to ionic strength of the $CaCl_2$ solution. Results also indicated that, compared to control animals, NaCl-infused carcasses were more tender after 6 d of postmortem storage (but not after 1 d, as observed with $CaCl_2$-infused carcasses). Evidence is presented that indicates that activation of calcium-dependent proteases could be responsible for the observed tenderization (reduction in shear force) due to infusion of ovine carcasses with $CaCl_2$.
(Key Words: Tenderness, Proteases, Postmortem Changes, Aging, Proteolysis.)

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Introduction

Proteolysis of myofibrillar proteins is reported to be a key event in meat tenderization during postmortem storage of carcasses at refrigerated temperatures (for review, see Penny, 1980; Goll et al., 1983a). Infusion of lamb carcasses with .3 *M* calcium chloride immediately after death accelerated tenderization and proteolysis of myofibrillar proteins so that they were completed by 24 h postmortem (Koohmaraie et al., 1988b). We have suggested that activation of Ca^{2+}-dependent proteases is responsible for acceleration of these events. Wu and Smith (1987) presented evidence and concluded that the elevation of ionic strength during postmortem storage (Dubuisson, 1950) is one of the mechanisms responsible for postmortem tenderization of meat. The objectives of the present study were 1) to determine the effect of variation in concentration of $CaCl_2$ in the infusion solution, 2) to examine whether an increase in ionic strength due to infusion of carcasses with $CaCl_2$ is responsible for the observed effects and 3) to further examine the accuracy of our hypothesis that activation of a Ca^{2+}-dependent protease is the mechanism through which infusion of ovine carcasses with $CaCl_2$ accelerates postmortem changes.

Materials and Methods

Animals. These experiments were conducted in two stages. In the first experiment, the effect of different concentrations of $CaCl_2$ was examined. For this experiment, a total of 20 lambs (8 to 12 mo old, 34 to 50 kg live

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weight) was slaughtered. Lambs were slaughtered in groups of five; one for each of the five treatments: 1) control (animals were slaughtered according to normal procedures); 2) electrically stimulated immediately after death (2 HZ; 100 volts; total of 360 pulses; 10 s on, 10 s off); 3) electrically stimulated and then infused with a volume equal to 10% of the live weight of .075 *M* $CaCl_2$; 4) same as Treatment 3 but with .15 *M* $CaCl_2$; and 5) same as Treatment 3 but with .3 *M* $CaCl_2$. The second experiment was conducted to examine the contribution of ionic strength to the increased tenderness that results from $CaCl_2$ infusion of ovine carcasses. For this experiment a total of 18 lambs (8 to 12 mo old, 36 to 50 kg live weight) was slaughtered. Lambs were slaughtered in groups of three, one of each of three treatments: 1) electrically stimulated, as indicated above; 2) electrically stimulated and then infused with NaCl and 3) electrically stimulated and then infused with $CaCl_2$. Because the objective of this study was to examine the contribution of ionic strength, NaCl and $CaCl_2$ solutions had the same ionic strength. A .3 *M* solution of $CaCl_2$ was employed because in the first experiment .3 *M* was found to be the most effective concentration of $CaCl_2$ to reduce the shear force measured at 24 h postmortem. Its conductivity was measured by a conductivity meter. A concentration of NaCl (.6 *M*) that gave the same conductivity reading was prepared and used during this experiment.

Infusion and Sampling. After completion of electrical stimulation, the lamb carcasses were transferred to a lamb cradle and the carotid artery was exteriorized. Solutions were pumped into the artery with a pumping device[4] (during the infusion process, one jugular vein remained intact and the other was opened and the carotid artery not used for infusion was clamped). After completion of the infusion, the carcasses were dressed and transferred to a holding cooler (1 to 2°C). Twenty-four hours after slaughter, the entire loin was removed and divided into two sections and assigned to d 1 or d 7 postmortem for the following determinations: 1: shear force, activities of Ca^{2+}-dependent protease-I, -II and their inhibitor, and mineral analysis (i.e., Ca and Na content); and d 7: shear force.

Shear Force Determination. Shear force of the cooked chops (2·time postmortem^{-1}·animal^{-1}) were determined according to the procedure described by Koohmaraie et al. (1988c).

Calcium and Sodium Determination. Water-extractable Ca and Na content of longissimus muscle was determined by atomic absorption according to the procedure described by Nakamura (1973a,b).

pH Determination. pH was determined at the completion of infusion process (0 h) and 24 h postmortem using a pH meter[5] equipped with a combination electrode.

Preparation of Ca^{2+}-Dependent Proteases and Their Inhibitor. Low and high calcium-requiring forms of Ca^{2+}-dependent protease (**CDP-I** and **CDP-II**, respectively) and their inhibitor (**CDP-inhibitor**) were prepared from 100 g of longissimus muscle at 24 h postmortem. Muscles were trimmed of fat and connective tissue, cut into 2-cm^2 pieces, frozen in liquid N and stored at –70°C. At the time of extraction (no more than 2 wk storage) frozen samples were allowed to stand at 2 to 4°C for 90 min and homogenized in 2.5 volume (vol/wt) of extraction solution, which consisted of 150 m*M* Tris base, containing 50 m*M* ethyleneglycol-bis (β, aminoethyl ether) N,N,N′, N′-tetraacetic acid (**EGTA**) and 10 m*M* 2-mercaptoethanol (**MCE**); pH was adjusted to 8.3 with 6 *N* HCl. Tissue was homogenized with a blender[6], twice at low speed and twice at high speed, each for 30 s with a 30-s cooling period interspersed between bursts. The homogenate was centrifuged at 16,000 × g_{max} for 60 min. The supernatant fluid was filtered through cheesecloth and dialyzed against 20 m*M* Tris base, containing 5 m*M* EGTA and 10 m*M* MCE with pH adjusted to 7.5 with 6 *N* HCl (18 to 24 h with two changes). After dialysis, the supernatant fluid was centrifuged (16,000 × g_{max} for 3 h), filtered through cheesecloth and applied to a DEAE-Sephacel column that had been equilibrated with 20 m*M* Tris base, containing .1 m*M* EGTA and 10 m*M* MCE with pH adjusted to 7.4 with 6 *N* HCl. The column was washed with the same buffer to remove unbound proteins, until absorbency of the outflow was less than .4 at 278 nm. The bound proteins were eluted with a continuous gradient of NaCl from 0 to 500

[4]Presto Precision Products, Inc., Farmingdale, NY.
[5]Digi-sense, Model No. 599 4-10, Cole-Parmer Inst. Co., Chicago, IL.
[6]Waring Prod. Div., Dynamics Corp. of America, New Hartford, CN.

TABLE 1. EFFECT OF TREATMENTS ON pH, CALCIUM CONTENT, SHEAR FORCES AND ENZYME ACTIVITIES IN OVINE LONGISSIMUS MUSCLE

	Treatments				
Traits	Control	ES[a]	ES + .075 *M* $CaCl_2$	ES + .15*M* $CaCl_2$	ES + .3 *M* $CaCl_2$
pH (0 h)[b]	6.96 ± .02[h]	6.18 ± .10[i]	6.19 ± .08[i]	6.13 ± .10[i]	6.14 ± .08[i]
pH (24 h)[b]	5.86 ± .05[h]	5.75 ± .01[i]	5.69 ± .04[ij]	5.64 ± .03[j]	5.62 ± .01[j]
pH (24 h)[c]	5.78 ± .08[h]	5.70 ± .05[hi]	5.58 ± .03[i]	5.58 ± .03[i]	5.60 ± .05[i]
Calcium, μg/g tissue	3.45 ± .41[h]	4.00 ± .60[h]	132.29 ± 32.90[i]	214.76 ± 48.40[j]	553.23 ± 116.31[j]
Shear force, d 1[d]	10.32 ± .80[h]	8.62 ± .98[hi]	8.47 ± .16[i]	6.20 ± .62[j]	3.45 ± .55[k]
Shear force, d 6[d]	8.64 ± .92[h]	5.45 ± .80[i]	6.09 ± .24[i]	4.67 ± .26[ij]	2.97 ± .40[j]
CDP-I[e]	37.78 ± 6.76[h]	37.02 ± 4.01[h]	29.45 ± 2.06[h]	7.85 ± 1.38[i]	NM[l]
CDP-II[f]	70.18 ± 10.62[h]	77.92 ± 4.13[h]	70.47 ± 2.76[h]	65.1 ± 3.77[h]	18.22 ± 1.05[i]
CDP-inhibitor[g]	47.05 ± 7.15[h]	48.00 ± 2.38[h]	55.46 ± 11.22[h]	50.75 ± 15.98[h]	NM[l]

[a]Electrically stimulated.

[b]Semimembranous muscle.

[c]Longissimus muscle.

[d]kg/1.27 cm.

[e]Low Ca^{2+}-requiring Ca^{2+}-dependent protease total activity/100 g muscle (caseinolytic activity).

[f]High Ca^{2+}-requiring Ca^{2+}-dependent protease total activity/100 g muscle (caseinolytic activity).

[g]Inhibitor of CDP-I and CDP-II, A_{278}/100 g muscle (inhibition of casein hydrolysis by CDP-II).

[h,i,j,k]Means within the same row with different superscripts differ ($P < .05$).

[l]Not measurable; below level of detection.

TABLE 2. EFFECT OF TREATMENTS ON SODIUM, CALCIUM CONTENTS, SHEAR FORCES AND ENZYME ACTIVITIES IN OVINE LONGISSIMUS MUSCLE

Traits	Treatments		
	ES[a]	ES + NaCl	ES + $CaCl_2$
Na, μg/g	527.7 ± 17.3[b]	2491.2 ± 202.6[c]	588.6 ± 28.0[b]
Calcium, μg/g	7.6 ± .7[b]	6.5 ± .9[c]	505.2 ± 83.7[c]
Shear force, d 1, kg[d]	8.87 ± .80[b]	8.07 ± .93[b]	4.41 ± .62[c]
Shear force, d 7, kg[d]	6.29 ± .95[b]	3.82 ± .48[c]	4.06 ± .39[c]
CDP-I[d]	59.10 ± 3.51[b]	54.96 ± 2.23[b]	NM[c]
CDP-II[d]	78.10 ± 3.42[b]	77.16 ± 3.48[b]	41.20 ± 2.89[c]
CDP-inhibitor[d]	78.10 ± 5.79[b]	70.60 ± 9.14[b]	22.78 ± 2.73[c]

[a]Electrically stimulated.

[b,c]Means within the same row with different superscripts differ ($P < .05$).

[d]See Table 1 for abbreviations.

m*M* in equilibrating buffer (column size, 2.6 × 40 cm; flow rate 30 ml/h; fraction volume 6.5 ml). Fractions were assayed for CDP-I, CDP-II and CDP-inhibitor activities. To determine the activities of CDP-I and CDP-II, .5 ml of each fraction was added to 1.5 ml of reaction mixture that consisted of 100 m*M* Tris base, 10 m*M* MCE, 5 mg/ml casein and 5 m*M* $CaCl_2$ and pH adjusted to 7.5 with 1 *N* acetic acid. To determine Ca^{2+}-independent activities (background), .5 ml of each fraction was added to 1.5 ml of a reaction mixture in which 5 m*M* $CaCl_2$ was replaced by 10 m*M* EDTA. Incubation times were 60 min at 25°C. The reaction was stopped with 2 ml of 5% trichloroacetic acid, centrifuged at 2,000 × g_{max} for 30 min and the absorbance at 278 nm of the supernatant fluid determined. CDP-I was eluted starting at fractions 55 to 57 and ending at 65 to 68, and CDP-II was eluted starting at 82 to 84 and ending at 96 to 98. Total CDP-I and CDP-II activities were determined by pooling these respective fractions and assaying as before.

The activity of CDP-inhibitor was determined according to Pontremoli et al. (1987). Briefly, a 1.0-ml aliquot of each fraction between 25 and 65 was heated at 90°C for 3 min and centrifuged at 2,000 × g_{max} to remove heat-denatured proteins. Supernatant fluid from each heated fraction was assayed for inhibitor activity. Three tubes were necessary to assay the inhibitor: 1) enzyme alone (calcium-dependent protease-II); 2) enzyme + inhibitor fraction assayed in Ca^{2+}-containing reaction mixture; and 3) inhibitor fraction assayed alone in EDTA-containing reaction mixture (to determine Ca^{2+}-independent activity).

Inhibitor activity was calculated according to the following formula: inhibitor activity = a − (b − c) × dilution factor.

Chronologically, the experiment to determine the effect of different Ca concentrations was conducted first. In this experiment, the inhibitor activity (Table 1) was determined according to Koohmaraie et al. (1987). However, preliminary work indicated that the heating of the inhibitor fractions prior to assay for inhibitor activity (Pontremoli et al., 1987) increased the recovery of inhibitor, so that for the second experiment this procedure was adopted (Table 2).

Autolysis of Ca^{2+}-Dependent Protease-II. Fractions of CDP-II obtained from control animals were pooled and dialyzed (two changes, 18 to 24 h) against 20 m*M* Tris-HCl buffer, pH 7.5, containing .1 m*M* EGTA and 10 m*M* MCE (dialysis buffer). After dialysis, the CDP-II preparation was autolyzed in dialysis buffer containing 6 m*M* $CaCl_2$ for different times and temperatures. To ascertain that the observed effects were due to the presence of Ca^{2+} and not a temperature effect, parallel controls that contained 10 m*M* EDTA instead of $CaCl_2$ accompanied the autolysis experiments. At specified times, aliquots were withdrawn and assayed for CDP-II activity.

Chromatography of Autolyzed CDP-II. Pooled CDP-II from control animals was dialyzed and autolyzed for 30 min at 0°C. Because the rate of autolysis at 25°C was very fast, autolysis was conducted at 0°C. The autolysis was stopped by addition of neutralized EDTA. After dialysis to remove Ca^{2+} and

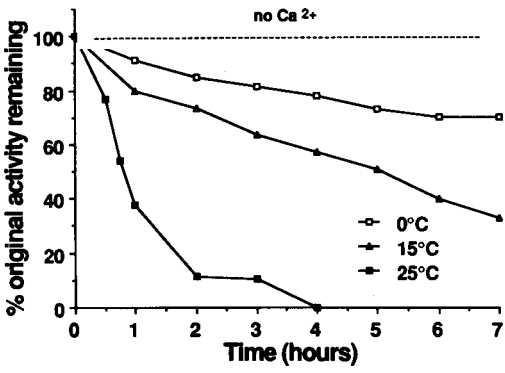

Figure 1. Effect of temperature on the autolysis of Ca^{2+}-dependent protease-II. CDP-II (in 20 m*M* Tris base, containing .1 m*M* EGTA, 10 m*M* 2-Mercaptoethanol with pH adjusted to 7.4 with 6 *N* HCl) was incubated at different temperatures in the presence of either no $CaCl_2$ or 6 m*M* $CaCl_2$. At various times, sample were assayed for caseinolytic activity.

EDTA complexes, the autolyzed CDP-II was loaded onto a DEAE-Sephacel column that was equilibrated with dialysis buffer and eluted with a linear gradient of NaCl from 0 to 500 m*M*, as indicated previously. For comparative purposes, non-autolyzed CDP-II was brought to the same EDTA concentration, dialyzed and chromatographed.

Statistical Analysis. Animals were assigned randomly to treatments. Data were analyzed by one way analysis of variance. When significant differences among treatments were detected, treatment means were compared using the least significant difference method. The comparison error rate was .05 (SAS, 1982).

Results and Discussions

General. It has been documented clearly that postmortem storage of carcasses at refrigerated temperature improves meat tenderness. Considerable effort has been directed toward understanding the mechanism(s) responsible for this improvement in meat tenderness during postmortem storage. The majority, if not all, of this improvement is the result of proteolytic degradation by proteases endogenous to the skeletal muscle cell (Koohmaraie, 1989). Examination of changes in the collagen structure during postmortem storage has not revealed transformations comparable to those of myofibrillar proteins (Tarrant, 1987). Therefore, the principal mechanism of meat tenderization during postmortem storage may be limited to proteolysis of myofibrillar proteins by endogenous muscle proteases (Goll et al., 1983a). Either Ca^{2+}-dependent proteases or lysosomal enzymes or the synergistic action of both classes of these proteases is responsible for postmortem tenderization (Goll et al., 1974; Moeller et al., 1976; Olson et al., 1977; Dutson, 1983; Goll et al., 1983a; Koohmaraie et al., 1986; Etherington et al., 1987; Koohmaraie et al., 1987, 1988a,b,c; Ouali et al., 1987). The Ca^{2+}-dependent proteases are located in the cytosol and at sites where most of the changes occur in the muscle during postmortem storage, namely Z-disks (Dayton and Schollmeyer, 1981). In contrast, lysosomal enzymes normally are localized inside membranes and presumably have to be released from their compartments to act on sarcoplasmic or myofibrillar proteins (Greaser, 1986).

Calcium Concentration. The principle objective for electrical stimulation in this process was to deplete ATP so that Ca^{2+}-induced contraction with subsequent toughening would not occur after $CaCl_2$-infusion. Because high-frequency electrical stimulation (60 Hz) results in meat tenderization, low-frequency (2 Hz) electrical stimulation was used (Takahashi et al., 1984, 1987). To ensure the effectiveness of electrical stimulation, the pH of the semimembranous muscle was determined immediately after treatment. This method of electrical stimulation significantly lowered the pH value compared to the unstimulated control group (Table 1).

As previously reported (Koohmaraie et al., 1988b), infusion of lamb carcasses with .3 *M* $CaCl_2$ reduced the shear force value at d 1 dramatically compared with control carcasses or carcasses that were electrically stimulated but not infused (Table 1). Infusion of carcasses with .075 *M* $CaCl_2$ had no effect on shear force value, whereas infusion of .15 *M* $CaCl_2$ decreased shear force by 2.5 kg compared to the carcasses that were electrically stimulated but not infused.

Activities of CDP-I, CDP-II and their inhibitor at 24 h postmortem were similar for control, electrically stimulated and carcasses infused with .075 *M* $CaCl_2$ (Table 1). Carcasses infused with .15 *M* $CaCl_2$ had lower CDP-I activities (lower protease activity indicates activation of the protease by autolysis) than controls; there was no activity remaining in the carcasses infused with .3 *M* $CaCl_2$. The

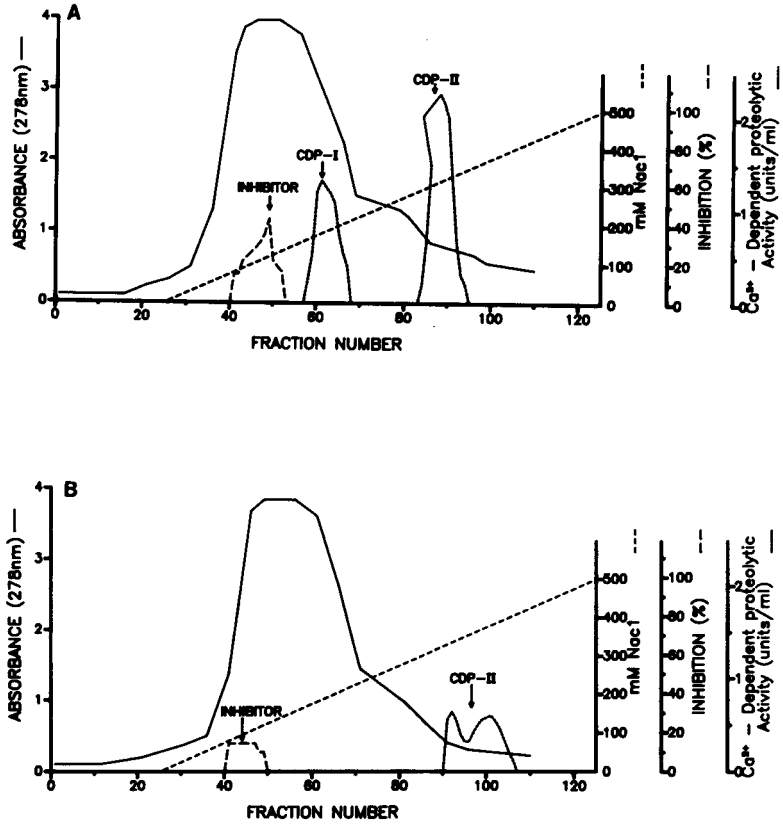

Figure 2. Elution profile of muscle extracts from control (a) or carcasses infused with .3 *M* $CaCl_2$ (b). The muscle extracts were loaded to a DEAE-Sephacel column (2.6 × 40 cm; flow rate of 30 ml/h), washed with elution buffer to remove unbound proteins and the bound proteins were eluted with a continuous gradient of NaCl from zero to 500 m*M* (400 ml of each) in elution buffer. Fractions of 6.5 ml were collected.

CDP-II activity and inhibitor activity were lowered only in carcasses infused with .3 *M* $CaCl_2$.

When carcasses were infused with .075 *M* $CaCl_2$ the amount of water-extractable Ca^{2+} (assumed to be free Ca^{2+}) in longissimus muscle was about 132 μg/g of tissue (Table 1). This amount of free Ca^{2+} corresponds to 3.3 m*M* Ca^{2+} distributed homogeneously in the muscle. This concentration should be sufficient to activate both CDP-I and CDP-II (Goll et al., 1985; Murachi, 1985); yet no tenderization effect was observed, nor was there any effect on CDP-I, -II or their inhibitor. Assuming that activation of Ca^{2+}-dependent proteases is the mechanism through which this tenderization is achieved (Koohmaraie et al., 1988b), speculatively, the majority of Ca^{2+} in the muscle after infusion of .075 *M* $CaCl_2$ must be compartmentalized differently from CDP-I or CDP-II, so that neither of these enzymes was activated to produce tenderization.

The effect of these treatments on the inhibitor of Ca^{2+}-dependent proteases is interesting. It has been demonstrated that both CDP-I (Goll et al., 1983b) and CDP-II (Goll et al., 1983b, Shannon and Goll, 1985; Mellgren et al., 1986) are capable of hydrolyzing the specific inhibitor of this proteolytic system in vitro. The infusion of carcasses with .15 *M* $CaCl_2$ caused a dramatic decrease in CDP-I activity but not in CDP-II or the inhibitor activities. However, when CDP-II activity was enhanced (as evident by loss of enzyme activity due to autolysis) by infusion of carcasses with .3 *M* $CaCl_2$, no inhibitor activity could be detected. Thus, the inhibitor of this proteolytic system was affected only when CDP-II was activated. These results suggest that CDP-II and inhibitor might be located in a

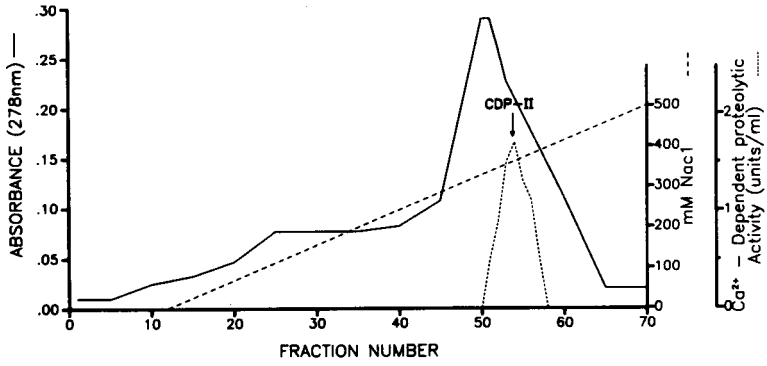

Figure 3. Rechromatography of pooled Ca^{2+}-dependent protease-II from control animals. Pooled CDP-II (in 20 m*M* Tris base, containing .1 m*M* EGTA and 10 m*M* MCE with pH adjusted to 7.4) was incubated at 0°C for 30 min. After incubation neutralized EDTA was added to final concentration of 10 m*M*, dialyzed against the same buffer and loaded to a DEAE-Sephacel column (1.6 × 30 cm; flow rate 30 ml/h) and eluted with a continuous gradient of NaCl from zero to 500 m*M* (200 ml of each) in elution buffer. Fractions of 4.5 ml were collected.

different subcellular compartment than CDP-I in the skeletal muscle cell.

Ionic Strength. On the basis of in vitro experiments, Wu and Smith (1987) suggested that elevation of ionic strength in muscle during postmortem storage is one of the mechanisms for the observed tenderization of meat during this period. Because of high ionic strength of the infusion solution (i.e., .3 *M* $CaCl_2$), a treatment that included infusion of carcasses with NaCl at the same ionic strength as the $CaCl_2$ solution was included. Theoretical ionic strength of a .3 *M* $CaCl_2$ solution is .9. However, the actual ionic strength probably is lower because of the dissociation constant of $CaCl_2$ and the ionic activity coefficient of Ca^{2+} (Pecsok et al., 1976). The conductivity of a .3 *M* $CaCl_2$ solution was determined, and the NaCl solution for infusion was prepared to have the same conductivity (i.e., 38 mS, as determined by a conductivity meter[7]). Results indicated that infusion of ovine carcasses with .6 *M* NaCl (conductivity = 38 mS) did not decrease in shear force at d 1 as was observed in carcasses infused with $CaCl_2$ (Table 2). However, the aging response (decrease in shear force between d 1 and d 6 postmortem time) was much higher in NaCl-infused carcasses than in control carcasses. Therefore, it appears that elevation of ionic strength during postmortem storage (Dubuisson, 1950) could contribute to the tenderization process. However, our results seem to indicate that acceleration of postmortem tenderization (by d 1) due to infusion of $CaCl_2$ is not the result of an increase in ionic strength, because the tenderization process in $CaCl_2$-infused animals already had occurred by d 1 postmortem. We have suggested that the observed tenderization is due to activation of Ca^{2+}-dependent proteases. The rationale for this suggestion is derived from the observations that both CDP-I and CDP-II undergo autolysis in in vitro assays where sufficient Ca^{2+} is available for CDP activation (Guroff, 1964; Suzuki et al., 1981a,b; Hathaway et al., 1982; Mellgren et al., 1982; Goll et al., 1983a,b; Parkes et al., 1985; De Martino et al., 1986; Inomata et al., 1986; Imajoh et al., 1986; Crawford et al., 1987). Some also have suggested that initial autolysis due to Ca^{2+} is required for the protease to become active (Inomata et al., 1986). Because continued incubation in the presence of Ca^{2+}; eventually results in loss of protease activity and infusion of lamb carcasses with $CaCl_2$ results in loss of CDP-I activity with a significant decrease in the activity of CDP-II (Table 1), activation of CDP appears to be the major causative factor associated with accelerated tenderization (at d 1) in $CaCl_2$-infused ovine carcasses.

Autolysis of Ca^{2+}-Dependent Proteases. Because Ca^{2+}-dependent autolysis of CDP is highly temperature-dependent (Suzuki et al., 1981a), an experiment was conducted to

[7]Radiometer America, Inc., Westlake, OH.

examine the effect of temperature on the rate of inactivation of ovine skeletal muscle CDP-II (Figure 1). Our results support those of Suzuki et al. (1981a). Results indicate that after 60 min at 25°C, CDP-II had less than 40% of its original activity, and after 4 h it had lost all of its caseinolytic activity. Because infusion of carcasses with $CaCl_2$ was conducted immediately after death while the muscle temperature was about 37°C, the question becomes whether CDP could remain catalytically active for a long enough time to cause tenderization or whether autolysis is so extensive and rapid that these proteases could not cause the accelerated tenderization after $CaCl_2$ infusion. DeMartino et al. (1986) demonstrated that the presence of substrate greatly reduced the rate of autolysis in the presence of Ca^{2+}. Therefore, the presence of substrate (i.e., myofibrils) under in vivo conditions even at high temperature (i.e., conditions that existed during and after infusion of carcasses with $CaCl_2$) should reduce the rate of loss of enzymatic activity and the proteases may remain active much longer than expected from incubations in vitro (Figure 1).

Representative elution patterns of muscle extracts from longissimus dorsi of electrically stimulated lamb carcasses and carcasses electrically stimulated and infused with .3 *M* $CaCl_2$ are indicated in Figure 2 (extracts from electrically stimulated carcasses infused with NaCl had a similar elution profile to electrically stimulated, but not infused, carcasses). In samples obtained from $CaCl_2$-infused animals, no CDP-I activity was detected and CDP-II activity was decreased, as indicated previously (Table 2). The present results (Figure 2) indicate that some CDP-inhibitor activity was detected, whereas previously we reported that no CDP-inhibitor activity should be detected. Detection of inhibitor activity (Figure 2) resulted not from a discrepancy between current and previous results but from a different assay procedure for inhibitor activity. This was verified by reassay of inhibitor fractions from the $CaCl_2$-infused carcasses (Figure 2) using previous procedures (Koohmaraie et al., 1987); we were unable to detect CDP-inhibitor activity (data not shown).

We have suggested the loss of catalytic activity in $CaCl_2$-infused carcasses was due to autolysis of CDP in the presence of Ca^{2+}. To verify this suggestion further, a representative sample of CDP-II was incubated with 6 m*M* $CaCl_2$ for 30 min at 0°C and then it was rechromatographed on DEAE-Sephacel. Results indicated that when non-autolyzed CDP-II (treated the same as autolyzed CDP-II, but incubated with EDTA instead of $CaCl_2$) was rechromatographed, a peak of activity could be detected at the same location as for CDP-II in muscle extract (Figure 3). However, although autolyzed CDP-II (for detail see Material and Methods) had 90% of its original activity when assayed prior to addition of EDTA to stop autolysis and had an identical protein profile upon chromatography, no activity could be detected in the fractions eluted from the column (data not shown). These results support those of Suzuki et al. (1981a,b). These results, therefore, suggest that the reason for inability to detect CDP-I activity and a significant ($P < .05$) decrease in CDP-II activity in samples obtained from carcasses infused with $CaCl_2$ could be due to their autolysis caused by an increase in intracellular concentration of Ca^{2+} achieved during infusion of carcasses with $CaCl_2$. However, our findings do not exclude other Ca^{2+}-mediated processes that could result in proteolysis of CDP-I and CDP-II by proteases other than CDP itself.

The tenderization process that occurs in the control animals in this experiment and during the normal aging process may involve mechanisms in addition to the proteolysis of myofibrillar proteins by CDP. These may include effects of ionic strength.

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