ELECTRICAL STIMULATION OF MATURE COW CARCASSES AND ITS EFFECT ON TENDERNESS, MYOFIBRIL PROTEIN DEGRADATION AND FRAGMENTATION.

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INTRODUCTION

Electrical stimulation has been demonstrated to improve tenderness in beef, lamb and goats (Savell et al. 1977) as well as in cows (Mekeith et al., 1980) but the mechanism of its tenderizing effect has not been ascertained. Cross (1979) reviewed the three theories proposed to explain this mechanism. The three theories are: reduction in cold shortening capacity (Davey et al., 1976; Chrystall and Hagyard, 1976), disrupture of the myofibrils due to the massive contraction provoked by electrical stimulus (Savell et al., 1978) and increased activity of acid proteases due to the low pH produced by electrical stimulation before rigor sets in (Savell et al., 1977).

Since electrically stimulated carcasses that are not frozen or rapidly chilled still show increased tenderness over non-stimulated carcasses under the same conditions, it would appear that reduction in cold shortening cannot explain the effect of electrical stimulation. The formation of contracture bands and disintegration of the myofibril have been attributed to rapid postmortem glycolysis (Cassens et al., 1963) and electrical stimulation (Will et al., 1980). Increased activity of cathepsins and B-glucuronidase has been reported (Sorinmade et al., 1978) and accelerated autolysis has been observed (Will et al., 1980) in electrically stimulated muscles.

Increases in tenderness have been related to increased myofibril fragmentation (Olson et. al., 1976) and to the appearance of the 30,000 dalton protein on SDS-polyacrylamide gel (MacBride and Parrish, 1977).

EXPERIMENTAL

The effect of electrical stimulation (ES) on pH, temperature, myofibril fragmentation index (MFI) and on the time of appearance of the 30,000 dalton protein in SDS-polyacrylamide gels was studied. Five mature cows were slaughtered and dressed. Each carcass was split into sides; one side was electrically stimulated while the other side was not stimulated and served as control.

Electrical stimulation was performed, within 30min. of bleeding, with an Elther hog stunner set to deliver 300v., 60 Hz a.c. for 1.5sec. duration after each 0.5 sec. interval. Total stimulation time was 120sec. Bacon hangers were used as electrodes and were inserted in the biceps femoris muscle and the sternocephalicus muscle or the intercostal muscle.

Temperature and pH were measured and samples were taken for MFI determination and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) from the semimembranosus, longissimus dorsi and triceps brachii muscles at 0, 3, 6, 24, 72 and 168 hours post-stimulation (PS). Warner - Bratzler shear force measurement was made on steaks removed from the three muscles from both electrically stimulated and control sides at 24, 72 and 168 hours PS only.

Temperature and pH measurement

Temperature was measured with a portable Koch "Electrotherm" digital thermometer equipped with a stainless steel probe while pH was monitored with an Orion gel-filled combination electrode connected to a Corning Model 12 research pH meter or to a Digi-Sense LED portable pH meter. The thermometer and pH electrode were inserted in fresh locations for each measurement. Crossed knife incisions were made to admit the blunt electrode.

Myofibril fragmentation index

MFI was determined by following the procedure of Olson et al., (1976) with the following modifications: 2g muscle samples, obtained with a coring device, were used instead of 4g samples. Homogenization was performed with a Polytron instead of the Waring blendor and sedimentation was conducted by centrifugation at 2,500 x G for 10 min. instead of 1000 x G for 15 min., otherwise the procedure followed was identical to that of Olson et al., (1976). The changes in speed and time of centrifugation were made to avoid freezing the samples as preliminary studies showed that freezing increased MFI especially when ES is applied. The chosen speed and time were found to give similar results to those of Olson et al., (1976) i.e. samples stored for 72 hours gave higher MFI than samples stored for 24 hours.

SDS - polyacrylamide gel electrophoresis

Myofibril isolation and purification were performed according to the procedure of Goll and Robson (1967). Gel electrophoresis was performed by the method of Weber and Osborn (1969) for muscle proteins. To 50 ul of 10% SDS solution was added 0.5ml purified myofibril suspension (0.5 mg/ml protein content). The mixture was boiled for 5 min., cooled and then 25 ul of 1% B-mercaptoethanol

were added. Thirty microliter samples (15 ug protein content) were electrophoresed at a constant current in 7.5% acrylamide gel columns in 12.5 cm x 0.5 cm glass tubes at 5 ma/tube. The gels were placed in staining solution (0.125% Coomassie blue in 45.4% methanol, 4.6% glacial acetic acid and 50% water) for 2 hr. at room temperature and destained in a solution of 7.5% acetic acid, 5% methanol and 87.5% water for 24-48 hours. Band positions were measured and relative mobility calculated. The molecular weights of the bands were determined using BIO-RAD low molecular weight standards (Lysozyme: 14,300; Soybean trypsin inhibitor: 21,000; Carbonic anhydrase 30,000; Ovalbumin; 43,000; Bovine serum albumin (BSA); 68,000 and Phosphorylase B; 94,000) and high molecular weight standards (Ovalbumin; BSA; Phosphorylase B; B-galactosidase: 116,500 and Myosin: 200,000). Due to the large number of gels in this study, a computer program using FORTRAN was developed to calculate relative mobilities using the Weber - Osborn equation, and molecular weight using the method of least squares as outlined by Pollard (1977).

Warner - Bratzler shear force determination.

Steaks, 3.5cm thick, were cooked in a preheated Blodgett electric forced air convection oven (165C) to an internal temperature of 65.5C (as determined by both thermocouple and thermometer). All steaks were allowed to cool to room temperature before six (for the SM and TB) and three (for the LD) 1.27cm cores were removed parallel to the muscle fibers. Each core was sheared twice and the average shear force for each steak was recorded as kg of force per cm^2.

Statistical analysis.

Data were analysed using the "DOS FORTRAN: ANVCRH" Computer program of Henderson (1974) which is an analysis of variance subroutine for balanced designs. Data transformation, linear, multiple and polynomial regression analyses, pooled and two-sample t-test as well as data plotting were executed by MINITAB II (Ryan 1976).

RESULTS & DISCUSSION

Myofibril fragmentation

MFI increased ($P \leq 0.01$) with time in both stimulated and control muscles (Fig 1). Neither treatment nor muscle had a significant ($P \geq 0.05$) effect on MFI although stimulation consistently produced numerically higher MFI at all times. In general the triceps brachii muscle had higher MFI than the semimembranosus and longissimus dorsi muscle (Table 1). The MFI values observed in this study were uniformly higher than those reported by Olson et al., (1976) for the same post-mortem period. This may reflect the differences in procedure for myofibril isolation between this study and Olson et al's. Myofibrils have been isolated at different speeds of centrifugation (Goll and Robson, 1967; Moller et al. 1973; Olson et al., 1976) but it is not unlikely that the isolation procedure could affect MFI. Further studies are needed to ascertain the effect of different speeds of centrifugation on MFI. The 24 hr. PS. MFI in this study were abnormally high (Fig 1) and lie outside the expected asymptomic curve for MFI (Olson et al., 1976). This anomaly cannot be easily explained and may be due to experimental error. However, the marked agreement in the shape of the MFI plots of this study and that of Olson et al., (1976) seem to indicate that the relationship between MFI and time is truly described by an asymptotic regression and is decidedly non-linear.

SDS - PAGE

The time of appearance of the 30,000 dalton protein was noted. In some replicates, traces of this protein appeared as early as 3hr. PS. (Table 2) but was present in greatest intensity in all muscles by 72 hr. PS., and by 168 hr. PS. was already fading in intensity or had disappeared. In general, there did not seem to be a marked treatment effect on the time or extent of appearance of the 30,000 dalton protein.

An increase in MFI has been associated with degradation of troponin T and the Z - disk by the calcium activated factor (CAF) and the appearance of the 30,000 dalton protein (MacBride and Parrish, 1977). In this study the level of resolution of protein bands on SDS gels was insufficient to show specific degradation of troponin T, and the appearance of the 30,000 dalton protein was coincident with decreased MFI. These results indicate that electrical stimulation did not produce the prescribed effects of CAF activity. Penny and Ferguson-Pryce (1979) reported that the major contribution to proteolysis of troponin T came from the enzyme system containing cathepsins. Schwartz and Bird (1977) observed that cathepsin D degraded isolated F-Actin at about 10% the rate of attack on myosin. The proteolysis that is promoted by the condition of low pH at high temperature produced by electrical stimulation appear to be due principally to cathepsins (Savell et al., 1977; Sarinwade et al., 1978).

Temperature and pH

Electrical stimulation lowered pH values significantly ($P \leq 0.01$) in all muscles as compared with the control (Table 1). Differences in pH between the three muscles - SM, LD, TB - were not significant ($P \geq 0.05$) suggesting that electrical stimulation was equally effective throughout the carcass. Electrical stimulation raised carcass temperature but this increase was negated by 3 hr. PS. (Table 1). However, differences in temperature between treatments were not significant ($P \geq 0.05$) while differences due to muscles were highly significant ($P \leq 0.01$).

Warner - Bratzler shear force.

Shear force values were significantly ($P \leq 0.01$) affected by electrical stimulation. At all sampling times, shear force was lower in stimulated muscles than in control muscles (Fig 2). Among the three muscles, TB had the highest shear force followed by SM and finally the LD with the lowest shear force (Table 3). Increased meat tenderness has been explained by increased MFI (Olson et al., 1976). In this study the increases in MFI of electrically stimulated muscle explained about 21% of the decreases in shear force.

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Table 1. MFI, pH and temperature of muscles from control and electrically stimulated cow carcasses

	Muscle	Treatment	Time post-stimulation (hr) 0	3	6	24	72	168
MFI[a,c]	Semimembranosus		59.2±0.8	85.1±3.3	85.7±2.3	113.6±1.6	97.8±2.1	104.2±2.1
	Longissimus dorsi		59.2±1.1	73.3±2.0	92.5±1.1	108.6± 2.0	92.9±0.3	97.4±1.3
	Triceps brachii		64.5±1.3	84.0±2.9	93.7±2.9	118.0±2.1	108.6±2.2	102.7±1.6
pH[b,c,d]		Control	6.64±0.03	6.31±0.06	6.09±0.07	5.61±0.04	5.32±0.07	5.18±0.06
		Electrical stimulation	6.35±0.03	5.85±0.03	5.69±0.06	5.55±0.07	5.46±0.05	5.26±0.06
Temperature[b,c,d]		Control	37.6±0.9	30.2±5.7	23.2±7.9	5.9±1.2	2.0±0.1	1.5±0.2
		Electrical Stimulation	38.3±1.3	28.4±8.1	22.0±8.3	5.5±3.3	2.0±0.1	1.6±0.2

a. Means ± standard errors of ten samples
b. Means ± standard errors of fifteen samples
c. Means not underscored by the same line are significantly different ($P<0.05$)
d. Muscles sampled were semimembranosus, longissimus dorsi and triceps brachii

Table 2. Time of appearance of the 30,000 dalton protein on SDS gels of muscle samples from stimulated and unstimulated sides of cow carcasses. [abc]

Muscle	Treatment	Time post-stimulation (Hr.)					
		0	3	6	24	72	168
Semimembranosus	Control		1	1		4	1
	Electrical Stimulation					3	2
Longissimus dorsi	Control					3	2
	Electrical Stimulation			1	1	2	1
Triceps brachii	Control		1			1	1
	Electrical Stimulation		1	1		2	1

a Results of five replicates. b Numbers depict the number of replicates that showed the protein at the particular time. c Figures between 29,500 to 30,500 MW were rounded to 30,000 MW

Table 3. Warner - Bratzler shear force (kg/cm^2) values for electrically stimulated and unstimulated muscles of cow carcasses [a]

Muscle	Time post-stimulation (Hr.)	Treatment	
		Control	Electrical Stimulation
Semimembranosus	24	6.8 ± 1.3^{kj}	5.3 ± 0.2^{kj}
	72	5.5 ± 0.5^{ij}	5.0 ± 0.2^{j}
	168	4.6 ± 0.5^{i}	4.2 ± 0.3^{i}
Longissimus dorsi	24	6.1 ± 0.3^{kj}	5.2 ± 0.9^{kj}
	72	5.6 ± 0.1^{j}	4.3 ± 0.5^{ij}
	168	4.6 ± 0.4^{i}	3.9 ± 0.8^{i}
Triceps brachii	24	7.4 ± 1.0^{k}	6.2 ± 0.3^{k}
	72	6.4 ± 1.1^{kj}	5.7 ± 0.4^{k}
	168	5.1 ± 0.2^{i}	4.7 ± 0.5^{ij}

a Means ± standard errors of thirty shear measurements.

ijk Means in the same row bearing the same superscript are not significantly different ($P > 0.05$)

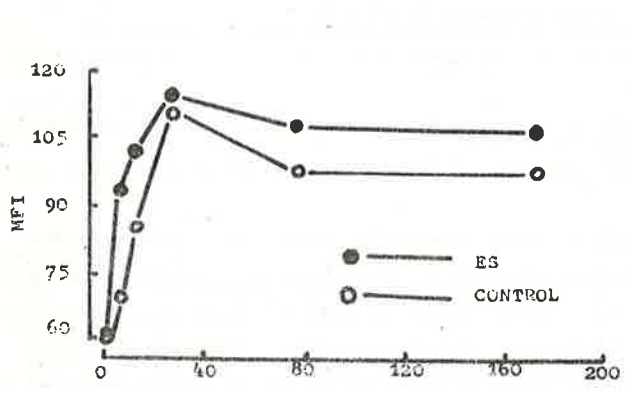

Figure 1.

Myofibril fragmentation index of electrically stimulated and control muscles of cow carcasses.

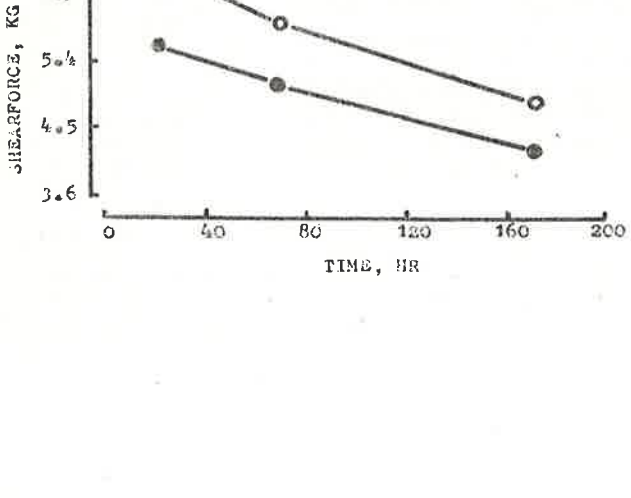

Figure 2.

Warner - Bratzler shear force values for steaks from electrically stimulated and control sides of mature cows.