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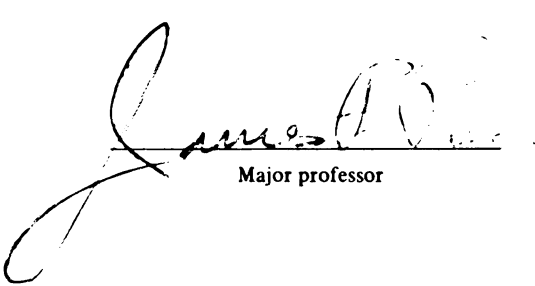
EFFECT OF SLAUGHTER METHOD, ELECTRICAL STIMULATION

AND HOLDING TEMPERATURE ON THE TENDERNESS
OF BEEF STEAKS
presented by

Emmanuel Uche Odume

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of the requirements for

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EFFECT OF SLAUGHTER METHOD, ELECTRICAL
STIMULATION AND HOLDING TEMPERATURE ON
THE TENDERNESS OF BEEF STEAKS

By

Emmanuel Uche Odume

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ABSTRACT

EFFECT OF SLAUGHTER METHOD, ELECTRICAL
STIMULATION AND HOLDING TEMPERATURE ON
THE TENDERNESS OF BEEF STEAKS

By

Emmanuel Uche Odume

The effect of slaughter method of steers, electrical stimulation (100 volts for approx. 100 secs.) of the carcass halves and holding temperature of the carcasses on the tenderness of beef steaks were evaluated. The influences of these treatments on changes in pH, ATP, internal temperature and microbial load were also determined. 12 grass-fed steers were involved in this study. 6 were slaughtered conventionally and the other 6 were confined, haltered and throat slashed with a sharp knife. Each carcass was split into sides and one side was electrically stimulated. 12 carcass sides were held in a 1°C chilling cooler and the other 12 in a 20°C storage atmosphere. Panel evaluation, Warner-Bratzler and Allo-Kramer shear values indicated that longissimus muscle samples from electrically stimulated sides of all steers were significantly ($P < 0.01$) more tender than

samples from untreated sides. Differences in tenderness due to slaughter method were not significant. Panel ratings favored the 20°C held over the 1°C held carcasses. ATP and pH decline were faster in electrically stimulated and 20°C-held carcasses. Higher microbial load was recorded for 20°C-held carcasses.

To You, Obi

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TABLE OF CONTENTS

Chapter	Page
LIST OF TABLES.	vi
LIST OF FIGURES	viii
INTRODUCTION.	1
LITERATURE REVIEW	4
Pre-slaughter Handling.	4
Slaughter Methods	6
Post-Mortem Changes in Meat	7
Chemical Changes.	8
Physical and Biophysical Changes	11
pH Changes.	14
Muscle Shortening	16
Factors Affecting Muscle Tenderness	19
Genetic Factors	19
Sex Influence	19
Age Influence	20
Connective Tissue Effects	21
Shortening Effect	22
Aging Effect...	23
Marbling Effect	25
Meat Tenderization.	27
MATERIALS AND METHOD.	33
Experimental Animals.	33
Slaughter Procedure	34
Electrical Stimulation.	35

Chapter	Page
Measurement of pH and Temperature	37
Microbial Count	37
Holding and Sampling.	38
Cooking Method.	39
Measurement of Tenderness	39
Panel Evaluation.	39
Warner-Bratzler Shear Force	40
Allo-Kramer (Texture Recorder) Shear Force	40
Adenosine Triphosphate (ATP) Determination	40
Statistical Analysis.	41
RESULTS AND DISCUSSION.	44
Postmortem Changes of pH.	44
ATP Changes	50
Postmortem Temperature Changes.	55
Tenderness Evaluation	60
Panel Scores.	60
Shear Values.	62
Microbial Count	66
SUMMARY	72
LITERATURE CITED.	75
APPENDIX.	91

LIST OF TABLES

Table	Page
1 Group distribution of experimental animals.	34
2 Muscle pH changes as affected by electrical stimulation, slaughter method and holding temperatures.	45
3 Simple correlation coefficients between various physical measurements, chemical and microbial analysis of beef steaks	51
4 <u>Longissimus</u> muscle ATP (μ Moles/gm tissue) changes as affected by electrical stimulation, slaughter method and holding temperature	54
5 <u>Longissimus</u> muscle temperature ($^{\circ}$ C) changes as affected by electrical stimulation, slaughter method and holding temperature.	56
6 Comparison of means and standard deviations of panel tenderness ratings for beef <u>longissimus</u> muscle	61
7 Comparison of means and standard deviations of Warner-Brazler shear	

	force values (Kg/cm^2) for beef	
	<u>longissimus</u> muscles.	63
8	Comparison of means and standard deviations of Allo-Kramer shear force values (Kg/gm) for beef	
	<u>longissimus</u> muscles.	64
9	Microbiological count ($\log_{10}$) of loin surface as affected by electrical stimulation, slaughter and holding temperature.	67

LIST OF FIGURES

Figure		Page
1	Diagram of the electrical set-up used for stimulating the carcasses	36
2	The effect of electrical stimulation on the rate of <u>longissimus</u> muscle pF and ATP decline.	46
3	The effect of slaughter method on the rate of <u>longissimus</u> muscle pH and ATP decline.	47
4	The effect of holding temperature on the rate of <u>longissimus</u> muscle pH and ATP decline.	48
5	Rate of temperature decline in <u>longissimus</u> muscles of stimulated and control carcasses.	55
6	Rate of temperature decline in <u>longissimus</u> muscles of stunned and unstunned steers	56
7	Rate of temperature decline in <u>longissimus</u> muscles of carcasses held at 1°C and 20°C.	57
8	Microbial growth rate on loin sur- faces of stimulated and control carcasses.	68

Figure		Page
9	Microbial growth rate on loin surfaces of stunned and unstunned steer carcasses.	69
10	Microbial growth rate on loin surfaces of carcasses held at 1°C and 20°C	70

LIST OF APPENDIX TABLES

Appendix		Page
I	Raw data of panel scores between stimulated and unstimulated beef <u>longissimus</u> muscles.	90
II	Raw data of Warner-Bratzler shear force (Kg/cm ²) between stimulated and unstimulated beef <u>longissimus</u> muscles.	91
III	Raw data of Allo-Kramer shear force (Kg/gm) between stimulated and unstimulated beef <u>longissimus</u> muscles.	92
IV	Raw data of pH decline in beef <u>longissimus</u> muscles of stimulated and unstimulated carcasses	93
V	Raw data of Adenosine triphosphate (ATP- Moles/gm tissue) in beef <u>longissimus</u> muscles of stimulated and unstimulated carcasses	94
VI	Raw data of temperature (°C) decline in beef <u>longissimus</u> muscles of stimulated and unstimulated carcasses.	95
VII	Raw data of microbial count (log ₁₀) of loin surfaces of stimulated and unstimulated carcasses	96

INTRODUCTION

In Nigeria, unpublished information indicates that steaks from the local Zebu cattle (Bos indicus) which had been shackled and slaughtered by direct throat slashing are generally tougher to bite when compared with steaks obtained from steers which had been immobilized pre-slaughter in packing houses.

Work in the U.S. (Carlo et al., 1970) shows that steers of Brahman (Bos indicus) breeding produce roasts and steaks which are less tender than most of the cattle of standard British breeds. The toughness difference has been attributed to "genetic" factors and shown to be moderately heritable (Brackelsberg et al., 1971). It is also known that the fat deposition pattern in Bos indicus breeds (Zebu or Brahman) is different from that in Bos taurus (humpless) breeds.

Cold induced muscle shortening is now known to result in a decrease in the tenderness of steaks (Marsh and Leet, 1966). Since the elucidation of this fact, little research has been directed toward discovery of how much of the breed related tenderness difference can be explained by differences in chill rate or fat deposition pattern. Lee (1976) have shown that steaks from slowly chilled beef carcasses were more tender than those from fast chilled carcasses, and that steaks from fat carcasses tended to be more tender than those from thin carcasses.

The relevance of chill induced muscle toughness in the cattle as they are produced and slaughtered in most slaughter houses in Nigeria, is open to question. While most of those cattle are not very fat, since they are principally grass fed and have to be trucked for very long distances in search of lush grass (Oyenuga, 1968) and may be old (chronologically) for their weight, the carcasses or parts may never be subjected to refrigeration prior to cooking.

Some other stresses imposed upon the animals (Sorinmade et al., 1978) or the muscles during slaughter and handling could induce muscle shortening or rigor type of toughness problems. Recent studies involving electrical stimulation of carcasses or muscles immediately post-mortem rely upon the concepts that pre-rigor acceleration of glycolysis (ATP disappearance) will avoid or negate the effects of cold induced shortening (Carse, 1973; Chrystall and Hagyard, 1975; 1976) or increased activity of acid proteases (Savell et al., 1978). It is thus conceivable that electrical stimulation might also affect the ultimate tenderness of beef muscle which is never chilled.

The technique of immobilization or slaughter of the animal is also believed to influence, to a large extent, the tenderness of the steaks and roasts that result. Various techniques of immobilization and slaughter as

defined by the Humane Slaughter Act of 1958, include electrical, chemical and mechanical methods, and their effects on meat quality are properly documented (Sybesma and Groen, 1970; Leest et al., 1970; Overstreet, 1975).

This study is therefore designed to investigate the effects which a variation in slaughter technique, post-mortem holding temperature conditions and electrical stimulation of beef carcass halves, have on tenderness of beef steaks from "grass-fed" cattle. A comparison of the Zebu and British type cattle would be desirable but impracticable due to the lack of availability of cattle with Bos indicus breeding of the nature similar to those native to Nigeria.

LITERATURE REVIEW

Pre-slaughter Handling

Several stress factors are brought to bear on beef steers during procedures required to convert the tissues into edible food. These are essentially environmental in origin (Forrest et al., 1975). Some studies on beef (Houston et al., 1962; and Henrickson et al., 1965) suggest that high energy content diets yield meat with increased flavor, tenderness and juiciness, although Paul et al. (1964) found no such trend in lamb. Different dietary schedules may, however, produce widely differing growth rates (Garrigus et al., 1969; and Sherry et al., 1978).

Starvation is known to reduce the size of muscle fibers (Yeates, 1964) with consequent increase in percentage of connective tissue, and meat toughness. But a period of refeeding after starvation permitted recovery of meat quality (Yeates, 1964; Hill, 1967). Kirton et al., (1972) recorded a loss of edible meat and excess fat from the carcasses of starved cattle as well as weight loss from non-carcass components of live weight. Carr et al. (1971) observed that fasting reduced slaughter weight of bovine carcasses in the first 24 hours, but no further effect on carcass yield up to 2 days. They also noted no detrimental effects on marbling score, maturity score

or grade but fasting improved the color of lean. Lambs fed on white clover species were reported to have stronger flavor of fat and lean and intense odor than those fed on perennial rye grass (Shorland et al., 1970).

Animal body muscles tend to be tough if subjected to very heavy exercise. The effects of various stress conditions on the palatability characteristics of the resulting meat have been investigated. The basic cause for many of the effects observed appears to be treatment influence on glycogen stores, with consequent alteration in the rate and extent of postmortem glycolysis and in the ultimate pH attained by the muscle. Exercise immediately ante-mortem reduced the glycogen level and influenced postmortem glycolysis (Lawrie, 1966; Sorinmade et al., 1978). Briskey et al (1959) showed that severe exercise of hogs immediately before slaughter depleted the muscle glycogen and produced high pH meat that was dark in color and dry in appearance. On the other hand, short-term excitement and exercise of swine immediately prior to slaughter produced rapid postmortem glycolysis and yielded muscle with inferior water binding capacity and low color and texture scores (Sayre et al., 1963).

Electrical stimulation (Lewis et al., 1963; Chrystall and Devine, 1978), abrupt change to a cold environment (Sayre et al., 1961) or elevated environmental temperature (Sayre et al., 1963) and the fatness of carcasses

(Lee, 1976) are known to influence the depletion of muscle glycogen reserves, alter the ultimate pH of the muscle, and influence water binding, color and firmness of the resulting meat.

Slaughter Methods

In the U.S., the Humane Slaughter Act of 1958 resulted in the adoption of humane methods of pre-slaughter immobilization in most packinghouses requiring that livestock must be insensible to pain at the time of exsanguination. As a result, three categories of immobilization techniques were approved: (i) Electrical, (ii) Chemical, and (iii) Mechanical. Marple (1977) has investigated which of these methods was most desirable for use since none of these methods of stunning meets the requirements of an ideal method. Althen et al. (1975) and Ono et al. (1976) suggested that the mechanical methods of stunning stimulate the release of epinephrine and norepinephrine and induce a corresponding increase in muscle cyclic-AMP levels eight times those of electrically stunned pigs and 28 times those of non-stunned pigs. They concluded that electrical stunning is less stressful to pigs than captive-bolt stunning.

Sybesma and Groen (1970) have studied the comparative responses of animals to CO₂ immobilization using a commercial tunnel facility, 70 volt electrical stunning

and 70 volt stunning after animals had passed through the tunnel with CO₂ present. They observed that the stress of getting the animals into the CO₂ tunnel accelerated the rate of postmortem glycolysis. CO₂ use for immobilization has also been linked to reduced bleed-out of carcasses (Leest et al., 1970). They also observed that animals immobilized with CO₂ and shackled prior to exsanguination had significantly lower blood and muscle pH at death, and lower muscle pH at 35 minutes postmortem. Muscle rigor value was also higher at 35 minutes and muscle ATP levels were lower at 35 minutes postmortem.

Overstreet et al. (1975) noted that pigs slaughtered without restraint or stunning had the slowest rate of postmortem glycolysis over those subjected to CO₂ immobilization. McLoughlin (1971) found that muscle ATP concentrations declined to their minimum value by two hours postmortem in pigs slaughtered normally, although little depletion of ATP occurred until three hours postmortem in muscle from pigs anesthetized prior to exsanguination.

Postmortem Changes in Meat

Marked alterations in palatability characteristics are known to be caused by a number of factors. These result from the changes that occur in muscle postmortem as well as certain inherent differences.

Chemical Changes. The chemistry of postmortem changes in muscle is essentially that of high energy phosphate compounds and the mechanisms involved in their synthesis and degradation (Pearson, 1970). This is anaerobic glycolysis and it involves the conversion of glycogen to lactic acid and is easily monitored by following pH decline (Bendall, 1960 and Cassens, 1966). Postmortem glycolysis in mammalian muscle frequently results in the pH decline to an ultimate level of 5.4 - 5.5, if glycogen content is adequate. However, values higher than this have been recorded (Lawrie et al., 1959; Bendall and Rhodes, 1976) or even less than 5.1 in pig muscle (Lawrie et al., 1958). Complete inhibition of glycolysis has been known to result at pH levels of 5.3 (Bate-Smith and Bendall, 1949). This was attributed to the possibility that one or more enzymes involved in glycolysis was increasingly inhibited as the pH declined. In some muscles where glycolysis ceases at high pH rates, Briskey and Lawrie (1961) suggested that either the phosphorylase was inactivated more readily or the glycogen was less accessible to attack.

Postmortem decline in pH of certain skeletal muscles varies considerably (McLoughlin, 1963; Elliot, 1965). Muscle temperature significantly influences the rate of pH and ATP change (Moeller et al., 1977; Dutson, 1977). The greatest decline in pH and loss of ATP occurs during

the first 2 to 3 hours postmortem (Kastner et al., 1973). Cassens and Newbold (1967) found that in the range of 5°C to 37°C the pH of the ox sternomandibularis (neck) muscle fell more slowly at lower temperatures and there was a period of several hours during which the pH of muscle stored at 1°C fell faster than that of muscle at 5°C. The ultimate pH attained at 1°C or 5°C was significantly higher than that attained at 15°, 25° or 37°C. Moeller et al. (1977) reported similar results, and Bendall (1960) stated that the lower the temperature in the range of 0°C to 37°C, the more slowly the pH fall in the psaos muscle of the rabbit.

The levels of ATP (Bendall and Rhodes, 1976) and/or glycogen present in the muscle at time of slaughter may also influence the extent of pH decline. The depletion of glycogen can be affected through starvation, exhaustive exercises or even by struggling at the time of slaughter (Lawrie, 1966). Preslaughter injection of insulin (Howard and Lawrie, 1957) or adrenalin (Klose et al., 1970; Khan and Nakamura, 1970; Bouton et al., 1971) can also reduce the level of muscle glycogen. Commercial handling practices after slaughter can influence the subsequent quality of meat but only within limits set by the physiological and biochemical characteristics of an animal before and at the time of slaughter. After death, the minimal rate of breakdown of ATP is probably that

required by the Ca^{++} pump (Bendall, 1969), for it is known that myofibrillar adenosine triphosphatase activity is strongly inhibited by falling pH in the range found during rigor onset (Wisner-Pedersen, 1966).

Bate-Smith and Bendall (1956) suggest that to ensure a slow rate of decline in the pH of muscle after death, it is necessary to have high levels of ATP and creatine phosphate (CP) present in the muscle initially. To meet these criteria, therefore, it is essential, both before and after death, that the rate of breakdown of ATP in muscle must be adequate for current needs and to maintain an appreciable balance of CP. Needham (1960) concludes that any condition which increases the rate of breakdown of ATP, (e.g., the increase of myofibrillar adenosine triphosphatase activity when muscle is stimulated) or which reduces the rate of resynthesis, must bring about a reduction in pH. Struggling at death will accelerate the rate of postmortem glycolysis because under such conditions phosphocreatine is depleted and the resynthesis of ATP is primarily dependent on the anaerobic breakdown of glycogen (Bendall, 1960). Goll (1968), Lawrie (1968) and, Buege and Marsh (1975) have similarly reviewed postmortem chemical changes in muscles and have identified some major chemical events.

In stunned animals, ATP hydrolysis is caused by muscular contractions that occur as a result of neural

stimuli during and immediately after stunning (Van der Wal, 1978). Eikelenboon and Sybesma (1969) had earlier observed that this decrease in ATP concentrations simultaneously led to a drop in pH. This hydrolysis of ATP may itself contribute up to 10% of this pH decrease through the protons which are thus set free (Honikel and Hamm, 1974). Bendall and Rhodes (1976) reported that at pH 6, 50% of the resting content of ATP had disappeared, and at pH 5.7 more than 90% and that rapid cooling of muscles to 2°C could be started without danger of cold shortening. But since rigor mortis manifests itself as soon as ATP concentration drops below a certain value - 2 to 4 $\mu\text{Moles/gm}$ - (Bendall, 1960) it means that the onset of rigor mortis is related to the initial ATP content of muscular tissue. Paul (1972) explains that the degradation of ATP leads initially to accumulation of ADP, followed by AMP, then IMP, and eventually inosine. When ATP decreases to 50-80% of its initial concentration, the muscle loses extensibility and becomes hard.

Physical and Biophysical Changes. The thermodynamic equilibrium of living muscle is profoundly altered when the animal is slaughtered. At time of slaughter, muscle is plastic and highly extensible.

Paul (1972) stated that as the oxygen supply is exhausted, the oxidation-reduction potential drops, and

aerobic energy production ceases. These changes are accompanied by loss of ability to maintain, (i) body temperature, (ii) in vivo osmotic equilibrium, (iii) membrane permeability and polarization, and (iv) normal ion concentrations in various parts of the tissues. The in vivo controls on muscle contraction are disrupted by the diminishing supply of ATP, so actin and myosin unite to form actomyosin, the irritability and extensibility of the muscle decrease, and the muscle becomes hard. This is rigor mortis, a condition of rigidity or contraction which develops in a matter of hours after death (Wierbicki et al., 1954). The shortening that results from the sliding of the thick and thin filaments is the primary cause of carcass stiffening during the development of rigor (Okubanjo and Stouffer, 1975). A study by Buck and Black (1967) on bovine longissimus muscle strips in which two degrees of stretch-tension were applied during rigor indicated that the average muscle fiber diameter was significantly smaller in the stretched muscle strips. Rigor onset involves a period of increasing isometric tension development (Jungk et al., 1967; Goll et al., 1970; 1971). Huxley (1969) found that the active tension generated by a living muscle decreased to zero when the muscle was stretched enough to eliminate any overlap of actin and myosin filaments.

Postmortem contraction depends on the muscle being

stimulated to contract before the ATP supply is exhausted, since ATP furnishes the immediate energy source to drive the contraction process (Newbold and Harris, 1972). Goll et al. (1970) attribute this contraction to the decline in ATP concentration which lowers the ability of the sarcoplasmic reticulum to accumulate calcium ions against a concentration gradient and the release of Ca^{++} ions is required to initiate tension development. Busch et al. (1967) and Jungk et al. (1967) reported separately that, after a given period of postmortem storage, both rabbit and bovine muscle strips gradually develop tension when suspended isometrically. The onset of isometric tension development, however, depends on environmental temperature of the strips and on ante-mortem condition of the animal (Jungk et al., 1967). For rabbit muscle Bendall and Davey (1957) reported that ATP levels dropped to 25 and 50% of initial level at 17° and 37°C respectively at the time of rapid rigor onset. Busch et al. (1972) found that in porcine and rabbit longissimus muscle, tension development was minimal and similar at 2°, 16° and 25°C, but increased greatly if the strips were incubated at 37°C. Bovine semitendinosus muscle strips developed most isometric tension at 16° to 25°C. Like rabbit and porcine muscle, isometric tension development in bovine muscle reached a maximum sooner after death at 37°C than it did at 2°, 16° or 25°C. Some workers (Busch et al., 1967; Goll et al., 1970; 1971) have reported that carcass softening or the dissipation

of rigidity results from the loss of ability to maintain this tension development. The period of isometric decline is defined as resolution of rigor mortis.

pH Changes. The rate and extent of pH changes vary with a number of factors. McLoughlin (1965) and McLoughlin and Davidson (1966) studied the effect of stunning on pH of the longissimus dorsi of pig muscle. They noted that stunning either electrically or with CO₂ increased the tendency for low pH (6.0) over that found in animals slaughtered without prior stunning measured at 30-45 mins postslaughter. Van der Wal (1978) reported that muscle contractions which occur as a consequence of stunning lead to significantly increased concentrations of lactate in the animal body. He had earlier suggested that the process of stunning may be responsible for the sudden increase in the catecholamine level of blood plasma and stated that this contributes to an improvement of pork quality depending on the level present in the blood.

The most-used criterion for rigor is the attainment of ultimate pH. Although pH fall is usually close to linear over most of the range, the rate declines in the last stages, and it is not easy to give a precise time for the end point (Locker, et al., 1975). Marsh (1954) showed that beef longissimus reached ultimate pH (5.80)

in 20 hours at 7°C and in 16 hours at 17°C ($\approx$ pH 5.6) and considered 36 hours in a chiller adequate for completion of rigor. Moeller et al (1977) observed an increased rate of pH decline for the muscles incubated at 37°C over those of 2°C. Cassens and Newbold (1967) found the rate of pH fall in beef sternomandibularis during the first 8 hours to be the same at 1°C and 15°C, and slightly less at 5°C. They considered the fall complete in 72 hours at 1°C or 5°C, and in 30 hours at 15°C. Marsh and Thompson (1958) had found a constant rate of pH fall over most temperature ranges in lamb longissimus muscle. They suggest 10 hours at 15°C to approach rigor and found only an 11% decrease in the rate of pH fall at temperatures between 17°C and 7°C. Scopes (1972) reported that the rate-temperature curve for lamb muscles has a shallow minimum at 12°C. The rate of pH fall at 0°C equalled that at 15°C. Wenham et al (1973) reported that the pH of ewe longissimus falls within 0.05 unit of ultimate (mean 5.77) in 13 hours at 15°C while biceps femoris takes 14 hours (S.D. $\approx$ 3).

Stimulation of carcass is known to accelerate the fall of pH (Defremery and Pool, 1960; Chrystall and Hagyard, 1976). Hallund and Bendall (1965) and McLoughlin (1970) found that, in pigs with a naturally slow glycolytic rate, 30 seconds of stimulation approximately doubled the rate of pH fall. Chrystal and Devine (1978) observed

that when prestimulation pH was about 7.0, the change in pH (pH) was high, but dropped to less than 0.2 pH units at a pre-stimulation pH of 6.5 (3-4 hr at 35°C) and approached zero at pH 6.3. They concluded that stimulation has little effect on muscles below this value, and that even with prolonged stimulation periods, muscle pH rarely dropped below 6.3.

Muscle Shortening. Locker and Hagyard (1963) were among the first to observe the fact that excised, unrestrained beef muscle shortened more rapidly at 0°C (32°F) than at any other temperature and that minimum shortening occurred at 14°C to 19°C. Earlier, Bendall (1951) working with rabbit muscle and Marsh (1954) with beef muscles, had reported that the shortening of excised muscle during onset of rigor increased with storage temperature from 17°C to 37°C. Cassens and Newbold (1967) reported that the delay phase of rigor mortis increased as the temperature decreased from 37°C to 15°C but was shorter with lower temperature in the range of 15° to 1°C. Marsh (1966) suggested that cold shortening may be due to inactivation of the relaxing factor by Ca^{++} and there is evidence (Buege and Marsh, 1975) to suggest that the calcium ions which trigger the shortening are released from the mitochondria responding to normal postmortem anoxia, and not from the sarcoplasmic reticulum reacting

to cold. The phenomenon of cold shortening occurred, not only in beef sternomandibularis muscle, but also in beef longissimus muscle and to a lesser extent for beef psaos major muscle. Ovine (Marsh, 1968), porcine (Henderson et al., 1970; Hendricks et al., 1971), rabbit red semitendinosus (Henderson et al., 1970) and avian (Smith et al., 1969) muscles, have also been shown to cold shorten.

The chemical changes involved in cold shortening indicate that shortening occurred while about 40% of the ATP still remained (Newbold, 1966). Bendall (1975) suggested that cold shortening was minimized by delaying exposure to cold temperatures until muscle pH reached a value below 6.0 and approximately 50% of the adenosine triphosphate (ATP) had been depleted. These observations confirmed findings of Locker and Hagyard (1963), and Marsh and Leet (1966) that cold shortening decreases as the period between slaughter and exposure to cold or freezing conditions is extended. Davey (1970) observed that rapid chilling of beef carcass sides produced an erratic toughening, with shear force values ranging from 10 to 20 - that is from very tender to very tough, and a large proportion of the contracted meat would not age. Butcher (1972); and Lee (1976) confirmed this in young bulls, cows and calves where there was progressive toughening in the loin as chilling rate increased.

Marsh et al (1968) evaluated the effectiveness of conditioning intact lamb carcasses at 18° to 24°C for 0.5 to 24 hrs before freezing. They found that there was little effect on tenderness if carcasses were conditioned less than 6 hrs, a rapid increase in tenderness as holding time increased from 6 to 16 hrs, and a plateau in tenderness values between 16 and 24 hrs of conditioning. Although the amount of conditioning time for prevention of cold shortening was similar at 7°, 10° or 18°C (Locker et al., 1975), an additional conditioning time was needed to prevent thaw rigor. Davey and Gilbert (1976) reported that even when very low levels of ATP (5 to 20% of initial concentration) remain in the muscle upon freezing, severe thaw contracture can be induced. There is therefore, a need to allow sufficient conditioning time to reduce muscle pH to near its ultimate value (Locker et al., 1975) or extended frozen storage in order to reduce ATP levels to near zero (Davey and Gilbert, 1976). This should prevent thaw contracture and the extreme toughness accompanying it.

Factors Affecting Muscle Tenderness

One quality of meat palatability is its tenderness. Consumer studies have shown that tenderness is the most important factor in the acceptance of beef and probably of other meats, including poultry and game (Bratzler, 1971).

Genetic Factors. Brackelsberg et al. (1971) have shown meat toughness to be moderately heritable and Carlo et al. (1970) reported differences in tenderness scores between roasts and steaks from the humped (Zebu/Brahman) cattle and humpless British breeds. Breed variations within a species have been found to influence such items as yield of cuts, lean-fat ratio, intramuscular fat distribution (marbling), firmness of fat, and color, tenderness, and juiciness of cooked meat (Paul, 1972). Bryce-Jones et al (1963) reported differences in tenderness, flavor, juiciness and iodine number of the fat due to sire. Paul (1962) listed variations in tenderness of beef muscle fibers among average size animals of similar heredity and management.

Sex influence. Variations in growth rate and musculature resulting from sex differences have been reported by many researchers. Locker and Hagyard (1963) reported that muscles from steer carcasses sustained greater shortening than samples from cow carcasses when stored at 19°C and 31°C, although when refrigerated at 2°C, the samples from

cow carcasses shortened to a greater extent than the muscle samples from steer carcasses. No significant differences were found in Warner-Bratzler shear values of steaks from bulls less than 16 months of age and steers and heifers of comparable chronological age (Hedrick et al., 1969), although shear values of steaks from more mature bulls were greater than those from steers or heifers at the same age. Bull meat appeared to be as acceptable as steer meat, although the cuts from steers were usually more tender, and in some cases had more flavor and juice; the differences becoming more marked with increasing age of animals (Bailey et al., 1966; Woodhams and Trower, 1965; and Field, et al., 1966). Champagne et al. (1969) and Warwick et al. (1970) reported non-significant differences in tenderness ratings between steaks from bull and steer carcasses.

Age Influence. Meat from very young animals would give cooked products differing in flavor, tenderness, juiciness and yield from mature animals (Paul, 1972), since the ratio of lean, and of fat to bone increases as the animal increases in age. Meat from older animals would therefore seem to be less tender due to increasing amounts of connective tissue. Helander (1966) reported a small increase in connective tissue protein during old age, although analytical studies have shown that the lean tissue of very young animals contains the largest percent

of connective tissue of any age group, and that after the animal is mature there is little quantitative change in percent connective tissue. In their study, Smith et al. (1978) found that yearling Angora goats produced chops and roasts which were ($P < .05$) juicier and more tender than those from young goats. Six-month-old spanish goats produced chops and roasts that were ($P < .05$) more tender than those from more youthful or more mature goats in a majority of such comparisons.

With increasing age, meat from bovines tends to be darker and redder in color, lower in pH, and less tender (Walter et al., 1965; Henrickson and Moore, 1965 and Webb et al., 1967).

Connective Tissue Effects. A major factor of muscle tenderness is the character and content of connective tissue. Herring et al. (1967) and Field et al. (1970) reported the existence of more collagen in some muscles than in others and that the amount of collagen in the various muscles had a negative relationship to tenderness. McClain et al. (1965) and Herring et al. (1967) have demonstrated that with decreased tenderness due to increased age of animal there was essentially no change in total amount of collagen present in the muscle, and that although tenderness of muscle increased with postmortem aging, there was little change in the total amount of collagen

over the aging period.

Some workers have investigated the possibility of increased cross-linking of collagen with increased age in a number of different species. Partington and Wood (1963) proposed possible structures for such cross-links, and Heikkinen et al. (1964) suggested the existence of varying strengths of cross-links. Goll (1962) reported that increased cross-linking of the collagenous fibers makes the tissue more resistant to the softening influence of heat, and may be the reason why meat from older animals is often less tender. There are changes in the molecular structure of collagen due to postmortem aging (Kruggel and Field, 1971; Pfeiffer et al., 1972; and Stanley and Brown, 1973). They noticed an increase in the amount of smaller molecular weight subunits and a decrease in the amount of larger molecular weight subunits that could be extracted from muscles with increased postmortem aging.

Shortening Effect. There is a well defined relationship between toughness and the degree to which a muscle has shortened (Marsh and Leet, 1966; Davey and Gilbert, 1975).

Earlier workers (Paul and Bratzler, 1955) had indicated that muscles which were cut or excised before the onset of rigor were tougher than comparable muscles still attached to the skeleton. Locker (1960) suggested that

the toughening which occurred might have been due to shortening of the muscle during the onset of rigor mortis. He noticed that the pre-rigor excision of psaos major muscle resulted in a 20-30% shortening and there was a corresponding decrease in sacomere length. The relaxed muscles were more tender than partly contracted muscles. Marsh and Carse (1974) attempted to explain the length/tenderness relationship in terms of the overlap of thick and thin filaments within the sacomere, but later studies (Rowe, 1974) suggested that this may not be so. Dransfield and Rhodes (1976) are of the opinion that there may be an interaction between the contractile filaments and the connective tissue, but the confirmation by Locker and Leet (1975, 1976) and Locker et al. (1976) of the presence of so-called "gap" filaments in the muscle has complicated the comparatively simple picture of a few years ago. Locker et al. (1977) believe this new component to be a major contributor to the tensile properties of meat.

Aging Effect. Muscle characteristics change significantly during the period of development and resolution of rigor. Experience has shown that tenderness, juiciness, and flavor of resulting meat is usually improved by a period of storage or aging especially in bovine and ovine species (Paul, 1972). The length and temperature of aging are known to influence tenderness of meat.

Doty and Pierce (1961) reported an improvement in both fat and lean flavor of broiled beef rib eye aged for two weeks. Taki (1965) found that beef longissimus dorsi steaks were more tender at 1 hour than at 24 or 48 hours, but less tender than at 192 hours post mortem.

Usually aging is done between 0-15°C to minimize bacterial growth. Stanley et al. (1974) identified some possible separate mechanisms taking place during postmortem aging to include specific chemical changes at the Z-line and probably at actin-myosin interaction sites, catheptic activity, degradation of collagen cross-links and general microbial action. The importance of aging temperature as an essential factor has been demonstrated by a number of workers. Busch et al. (1967) and West (1978) reported increases in tenderness with high temperature conditioning (HTC) of freshly slaughtered animal carcasses. Henderson et al. (1970) found that the Z-line and M-line degradation occurred more rapidly upon storage at 25°C or 37°C as against 2°C or 16°C in 24 hours postmortem in porcine muscles. Ultrastructural changes in myofibrils such as degradation of the Z-line and changes in the actin-myosin interaction might play very crucial roles in post-mortem tenderization of meat (Goll et al., 1970; Hay et al. 1973). Hegarty et al. (1973) reported structural changes or breakdown in myofibrils from "normally" aged and rigor-stretched turkey and porcine muscle. Goll et al. (1974)

and Penny et al. (1974) have indicated that a role of proteinases in tenderization of meat during post-rigor aging, since these enzymes are compartmentalized in most tissues and partial disruption, such as by freezing and thawing, expose them to their substrates and hence affect the rate of tenderization.

Johnson and Bowers (1976) suggested that fragmentation may result from weakening of bonds between the actin filaments and the Z-line material. Abbott et al. (1977) reported that the I-band was the area of the myofibril most susceptible to autolytic breakdown while the white fibers appeared to be slightly more labile to autolysis than red fibers.

Contrary to most findings, however, Wolfe and Samejima (1976) demonstrated that postmortem aging of muscle has no effect on the dissociation of actin and myosin, and these findings are incompatible with the hypothesis that the actin-myosin interaction undergoes a "weakening" during post-mortem aging.

Marbling Effect. It has been generally accepted that marbling is an indicator of desirable eating quality, especially of meat. McBee and Wiles (1967) found significant differences in tenderness, juiciness and flavor among carcass grades of prime, choice, good and standard. Dryden and Marchello (1970) found a significant correlation

between muscle fat content and taste panel tenderness. Some researchers (Henrickson and Moore, 1965; Norris et al. 1971; and Parrish et al., 1973) have, however, reported that tenderness was not significantly affected by marbling scores. Campion and Crouse (1975) reported low positive correlation between ether extract, marbling and tenderness.

The role of fatness in altering the rate of carcass chilling has been investigated. Pike (1974) observed that certain thinly finished and light-weight goat carcasses which produced very tough cooked meat had muscles with very short sacomeres. This suggests that carcass weight and/or fatness can affect tenderness via the cold shortening phenomenon. Cross et al. (1972) and Reagan (1974) reported significant ($P < 0.01$) correlations between intramuscular fat content and sacomere length in lamb and beef longissimus muscles, respectively, thus suggesting that marbling might also be related to tenderness via its insulatory effect in reducing the severity of cold shortening induced by low temperature or changes in postmortem chilling rate (Lee, 1976; Smith et al., 1976). There is now evidence (Meyer et al., 1977) that the beneficial effects of marbling and fat cover on quality may be little more than a reflection of the slower cooling rate (and hence reduced cold-shortening) of the larger and fatter carcass (Marsh, 1974).

Meat Tenderization

Newbold and Harris (1972) have shown that methods of handling carcasses immediately postmortem, until the onset of rigor mortis, affects the ultimate tenderness of meat. A number of procedures such as the suspension via the pelvic bone, mechanical restraint of muscles, cooler aging, high temperature conditioning (West, 1978), delayed chilling, blade or needle tenderization, use of tropical plant or fungal enzymes, have been developed for increasing meat tenderness (Smith et al., 1977).

It is generally known and accepted that pre-rigor meat is inherently more tender than its post-rigor counter-part (Paul et al., 1952; Pearson et al., 1973). Attempts have been made to maintain muscle relaxation during the contractile process of rigor mortis by injection of chemical muscle relaxants such as phosphates and magnesium chloride (Huffman et al., 1969; and Streitl et al., 1977). The use of sodium citrate to maintain muscle relaxation and tenderize pre-rigor beef has been suggested (Lardy, 1966). It is believed to retard the rate of postmortem glycolysis through the inhibition of the enzyme phosphofructokinase (Conn and Stumpf, 1972). This will prevent a pH decline, raise the water holding capacity and effect tenderization.

A wide variety of enzymes are contained in the muscle, some of which are hydrolytic (Randall and MacRae, 1967). These include cathepsins and leucine aminopeptidase.

Maximum activity of these enzymes at low pH and inactivation by heating to 65°C (Parish and Bailey, 1966) or aging at 15° to 20°C for 12 to 24 hours before chilling (West, 1978) has been known to cause or contribute to, the softening of muscle post-rigor. They are believed to act on the sarcoplasmic proteins (Bodwell and Pearson, 1964) and increase with storage (Suzuki and Fujimaki, 1968).

The position in which the carcass is suspended during cooling may stretch certain muscles while permitting others to shorten. The method of suspending intact carcasses through the obturator foramen has been described and evaluated by Orts et al. (1972) and Bouton and Harris (1972). Various other methods of carcass suspension have also been designed to place positive stress during rigor in order to optimize sarcomere length and thereby improve tenderness in as many muscles on the intact carcass as possible (Hostetler et al., 1972; Bouton et al., 1974). Okubanjo (1978) reported that pre-rigor leg-twisting of mutton carcasses lowers shear force values, yields longer sarcomeres and increases fragmentation of test muscles. Over the years, there has been increased use of blade or needle tenderizing machines to improve the palatability of retail cuts (Bowes, 1975). This method increased tenderness above that achieved by aging alone for strip beef loin steaks, but did not generally affect flavor, juiciness or overall palatability ratings.

Another method for increasing tenderness and/or preventing cold shortening may exist in the form of electrical stimulation of the carcasses shortly following slaughter. This idea was first suggested by Harsham and Deatherage (1951). Some workers (Carse, 1973; Davey et al., 1976; and Chrystall and Hagyard, 1976) have suggested that electrical stimulation could be used to prevent cold shortening or increase the rate of conditioning of carcasses. Chrystall and Hagyard (1975), Grusby et al. (1976), Savell et al. (1977), Savell et al. (1978), Sorinmade et al. (1978) and Cross (1978) reported substantial increases in tenderness of lamb, beef or goat meat by use of electrical stimulation. Investigations on electrical stimulation have documented its effect on the acceleration of postmortem glycolysis (McLaughlin, 1970; Carse, 1973; Chrystall and Hagyard, 1976; Grusby et al., 1976; Cross, 1978; Savell et al., 1978). Smith et al. (1977) found that electrical stimulation using low voltage (100 V, 5 amps) increased tenderness by 12 to 55 percent, decreased variability in tenderness in 5 of 6 test animal groups and did not negatively affect muscle color, condition or pH. They noted, however, that prevention of cold shortening, as determined by relative sarcomere lengths, did not explain all of the tenderization effects achieved by electrical stimulation, suggesting that enhanced autolytic proteolysis may also be involved. Dutson et al.

(1978) and Sorinmade et al (1978) inferred that the enhanced activity of the autolytic enzymes of muscles, in stimulated sides, may partly be responsible for some of the tenderization benefit. Chrystall and Hagyard (1975) suggested that electrical stimulation induced acceleration of post-mortem glycolysis, caused the muscle fibers to enter into rigor mortis before the effects of cold-shortening can take place.

Cassens and Newbold (1967), Moeller et al. (1976) and West (1978) reported a more rapid drop in pH with high temperature conditioning. With a lower pH at a high temperature, disruption of the lysosomal membrane and the concurrent release of acid hydrolases into the muscle tissue occurs. Savell et al. (1978) are, however, of the view that physical disruption of muscle fibers resulting from the massive contractions during stimulation may be a mechanism for the tenderness improvement associated with electrical shock rather than high temperature conditioning per se. Harsham and Deatherage (1951) had earlier investigated the influence of electrical stimulation on aging of beef and observed that application of voltages as high as 3000 volts also produced a fall in the pH of muscle to 6.1 in one hour. The meat was as tender, after 2 hours at 1°C, as that from unstimulated controls after 18 days at 1°C. Forrest and Briskey (1967), McLoughlin (1970) and Tarrant et al. (1972), reported that with approximately

30 seconds of electrical stimulation, the pH decline of naturally slow glycolytic rate pigs doubled. The glycolysis of freshly slaughtered lambs was accelerated by the application of high voltages (3600 V) of electrical stimulation (Carse, 1973; Chrystall and Hagyard, 1976), and the longissimus muscle pH in stimulated carcasses fell to below 6 within one hour of slaughter, compared with 14 hours required by unstimulated muscle. Shear force values for muscles from leg and loin area cuts of stimulated carcasses roasted from the frozen state were about half of those from unstimulated carcasses and there were no deleterious effects due to stimulation.

Davey et al. (1975) stimulated beef sides for 1 to 2 minutes period with high-voltage electrical stimulation immediately after carcass dressing and noted that the time for the development of rigor was reduced from 24 hours to about 5 hours. The stimulated carcasses, even though chilled rapidly, were still warm at rigor onset. Cold shortening or toughening would not develop under these conditions and the meat could be aged to a high degree of uniform tenderness. Although the electrical parameters chosen to hasten rigor in lamb carcasses (Carse, 1973; Chrystall and Hagyard, 1975, 1976) and beef sides (Davey et al., 1976) were empirically derived, they effectively reduced the time needed to achieve rigor to such a degree

that it was possible for all risk of toughening from cold and thaw shortening to be avoided and tenderness maintained.

Chrystall and Devine (1978) suggested a two-stage process involved in the accelerated onset of rigor in electrically stimulated muscles based on the pH changes. The first, occurring during stimulation, induces a remarkable 0.5 to 0.7 pH unit drop (ΔpH) in 120 seconds, representing a 100- to 150-fold increase in the rate of underlying biochemical reactions. In the second stage, occurring after cessation of stimulation, the rate is much slower, but is still almost twice as fast as in non-stimulated muscle over the same pH range. They observed that pH change (ΔpH) decreased with pre-stimulation pH, although Bendall et al. (1976) had claimed that rates of pH fall after stimulation do not differ from those in non-stimulated muscles if temperature corrections are applied.

MATERIALS AND METHOD

This experiment was a two-phased study for which 12 steers purchased on four different sale dates at a local auction (Howell, MI) and judged to be "grass-fed" by virtue of their condition and character and color of feces were selected. They were considered "Feeder" steers at the market place.

The first part was a study to determine the influence which the technique of slaughter of the beef animals, electrical stimulation of the carcasses and temperature storage conditions had on the tenderness of steaks obtained from the short-loin region. The second part was to ascertain the relationships between these treatments and the changes in muscle pH, adenosine triphosphate (ATP) levels and total microbial load.

Experimental Animals

These were thin, humpless (Bos taurus) breed of cattle averaging between 314.10 and 448.70 Kg live weight, with a dressed weight of between 152.56 and 259.90 Kg. A total of 12 animals in 3 replications of 4 animals per replication were involved. All the animals averaged between 18 and 24 months of age. Because of the distortion of the longissimus due to warm cutting of the loin, marbling

and other grade factors were not recorded. The quality grade range was estimated to be between mid point U.S. utility and end point U.S. standard.

Table 1. Group Distribution of Experimental Animals.

Animal	Live Weight (Kg)	Dressed Weight (Kg)	
		Left Side	Right Side
1	424.40	121.30	117.50
2	448.70	129.40	130.50
3	424.80	115.70	117.00
4	436.10	116.60	119.30
5	402.80	100.90	103.00
6	386.10	90.77	93.91
7	374.40	103.19	104.54
8	363.60	96.89	98.69
9	335.30	86.72	87.17
10	391.50	108.59	110.57
11	357.30	96.17	96.17
12	314.10	74.84	77.72

Slaughter Procedure

The twelve animals were slaughtered in the abbatoir at Michigan State University Meat Laboratory. Each was fasted for at least 24 hours prior to slaughter but were provided with drinking water ad lib. The throat slashed steers were held 4 days without feed. Two animals were slaughtered on each scheduled day. Six of the steers were stunned with a captive bolt pistol prior to bleeding. Each of the other

six was trapped in a steel bleeding chute and haltered. The animal's head was raised by the halter rope and then throat slashed with a sharp knife. They were exsanguinated and dressed within 50 minutes following death and split into right and left sides.

Electrical Stimulation

The right side of each carcass was subjected to electrical stimulation within 60 minutes postmortem. The source of electrical stimulation was a set-up consisting of a rheostat (a Powerstat variable autotransformer, Type 116B-50/60 Hz) ampmeter and a voltmeter to which were connected two cables which ended terminally in two metal probes (Figure 1). This modification was to facilitate the extension of these electrodes to the hind limb and the neck regions of the sides to be electrically stimulated. These probes were inserted into, and electricity was administered to:

- (i) Round and fore-arm (10 bursts at 5 secs/burst);
- (ii) Neck and Gluteus muscle (10 bursts at 5 secs/burst).

The current of the electrical impulse was single phase, 100 volts and 60 cycles similar to that used by Savell et al. (1977). However, the amperage of the current passing through the carcass was noted on the ammeter.

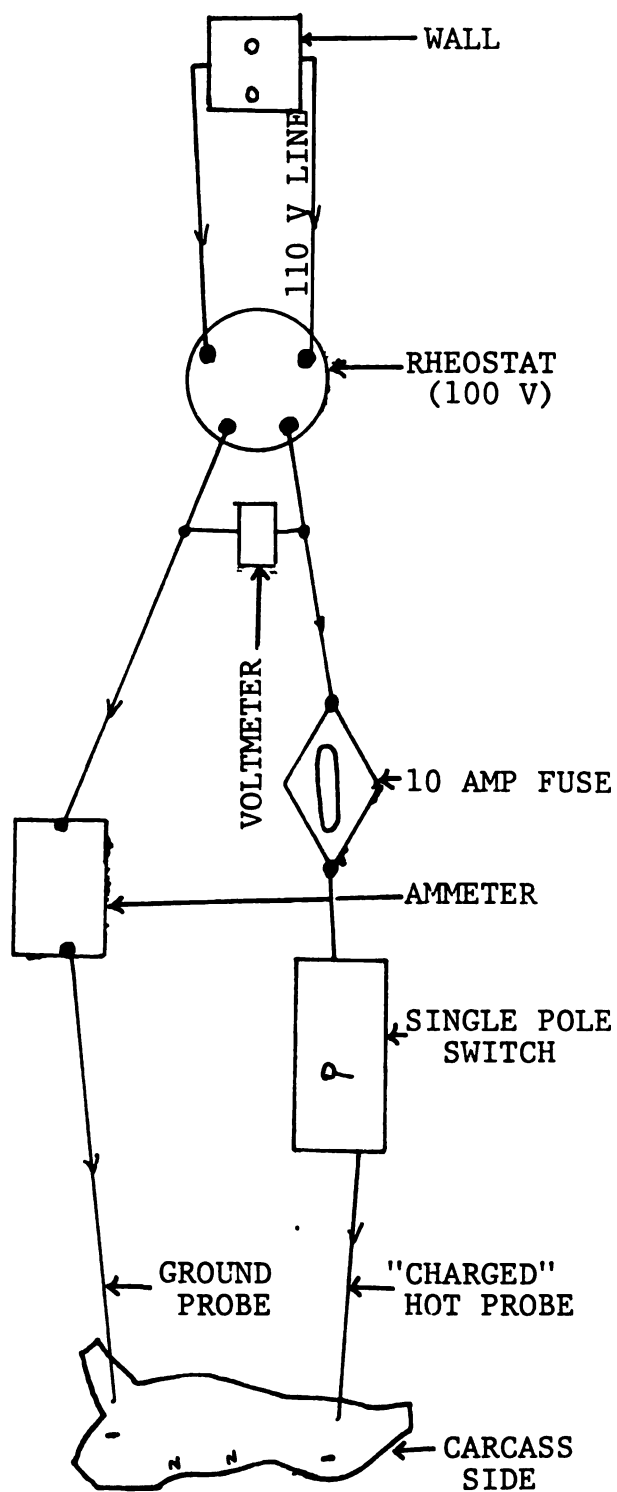


Figure 1. Diagram of the electrical set-up used for stimulating the carcasses.

Individual carcass sections received a grand total of 100 seconds of electrical stimulation. The amperage noted ranged from 0.6 to 1.0 amp at each burst.

Measurement of pH and Temperature

Initial temperature and pH of each carcass side were noted before and immediately after electrical stimulation. The left and right sides of the carcasses of one group were wheeled into the cooler corridor whose temperature had earlier been regulated to 20°C, while the left and right sides of the carcasses of the other group were wheeled into the cooler set at -1°C. Internal temperature and pH of the longissimus dorsi muscles, at a point opposite the 6th lumbar vertebra of each side, were monitored every hour for the first 6 hours and later at 24 and 96 hours post-mortem. The thermocouples were attached to a Honey-Well brand "Brown Electronic" potentiometer. A type 7GR 231/100L combination electrode, with a Type 36101 portable pH meter (Kerotest Manufacturing Corp., Pittsburg, PA) was used to measure pH.

Microbial Count

Standard plate counts were performed on all samples using the methods described by the American Public Health Association (1966) and Patterson (1971). Sterile cotton

swabs were used to obtain samples from each of three randomly selected 6.5 cm² areas described by a sterile template on the inner body cavity surfaces of each carcass side at 0, 5, 24, 96 and 168 hours postmortem. 0 hour samplings were from the rib cage (11-13th rib) and flank areas, while subsequent samplings were from the internal surfaces of the shortloin region. The tips of the cotton swabs were broken into tubes containing 10 ml citrate buffered distilled water (pH 7.0) and these were vigorously shaken. Serial dilutions of the samples were plated on Plate Count Agar and incubated at 32°C for 48±2 hours. Microbial counts were recorded as the mean of duplicate plates.

Holding and Sampling

For each treatment, the whole shortloin (13th thoracic through the 6th lumbar vertebra) was removed from each side of the carcasses at the end of 5 hours postmortem. In the first treatment, the shortloin was placed in a plastic container (approx. 32 gals. capacity) filled with cold (21°C) tap water, covered with a lid and left at room temperature (20°C) till the end of 24 hours postmortem before being placed inside the cooler (1°C) for the remaining period of the experiment. Shortloins from the rapidly chilled sides were left in cooler storage (1°C). Thermocouples were inserted into each of the shortloins and the

internal temperatures monitored by a potentiometer over a period of 24 hours. Two 3.8 cm thick steaks were cut out of each shortloin proceeding serially from the anterior end at the end of 5, 24, 96 and 168 hours postmortem, for cooking.

Cooking Method

The 3.8 cm thick steaks from the longissimus muscles were cooked in deep fat ("Crematex" - an all purpose shortening made from hydrogenated vegetable oils) with a deep fat fryer (Hotpoint Co., brand SER. B-58451, CAT 201 HK3) set at 144°C to 62°C internal temperature of the steaks. Cooked steaks were wrapped in aluminum foil and stored in the cooler at 4°C for 24 hours.

Measurement of Tenderness

At the end of 24 hours of storage, the cooked steaks were laid out for panel evaluation and physical measurements of tenderness.

Panel Evaluation. A 9-point hedonic scale was used by the twelve panel members which was made up of faculty, staff and graduate students of the Meat Laboratory at Michigan State University. They evaluated the tenderness of cubes taken from the steaks, obtained by cutting through sections.

Warner-Bratzler Shearforce Values. Six 1.25 cm cores were obtained parallel to the muscle fibers from each of the steaks. They were removed from the medial, central and lateral positions of the longissimus muscle. Each core was sheared 3 times by the Warner-Bratzler Shear instrument and the mean values calculated. 1.25 cm cores were chosen so as to facilitate the removal of 6 cores from each steak. Paul and Bratzler (1955) found that there was close agreement between shears of 1.25 and 2.54 cm in diameter and suggested that either size may be used to measure shear force.

Allo-Kramer (Texture Recorder) Shear Force. Sections measuring approximately 2.50 x 1.50 cm were obtained from each steak and weighed. Each of these was sheared with the flexible blunt multiple blades of the standard shear compressor cell using a 135 Kg (300 lb) force ring and a range of 20. The mean of six shears per steak was used for computation.

Adenosine Tri-phosphate (ATP) Determination

ATP determination was based on the Luciferase enzyme technique described by Strehler and Totter (1952). Samples were excised from portions of the fresh short-loins at 0 hr 10 mins, 5 hr and 24 hr poststimulation and frozen in liquid nitrogen. They were placed in Whirlpack

polyethylene bags and stored in a freezer at -36°C . The frozen muscle pieces were powdered by grinding with dry-ice in a Waring brand commercial blender in a -23 to -29°C atmosphere. 500 mg of each of the powdered samples were put in test-tubes each containing 15 ml of boiling distilled water and allowed to boil for 5 minutes. The test-tubes were immediately transferred into ice-cold water. 0.1 ml volumes of the meat ATP-extracts were mixed with 0.25 ml of the luciferase enzyme (a Commercial fire-fly extract - an Aldrich Chemical Co., Inc. product), which had been standardized with known concentrations of the ATP standard (from Equine Muscle - a Sigma Chemical Co. product) inside the dark compartment of the Aminco-Bowman Spectrophotofluorometer (An American Instrument Company, Inc. product). The emission wavelength was set at 550 and the degree of light emission from the mixture was recorded as percent of needle deflection in the light detecting portion of the Farrand photofluorometer. The values so obtained were expressed in terms of $\mu\text{Moles ATP per gm of meat tissue sample}$.

Statistical Analysis

The statistical analysis was done by a CDC 6500 computer at the Michigan State University (MSU) Computer Center using the MSU STAT Systems Group (1977). Simple correlation coefficients were determined as described

under the BASTAT program (STAT Systems Group, 1977) following Snedecor and Cochran (1973). Data were analyzed by a split plot analysis of variance using the STAT Systems program.

RESULTS AND DISCUSSION

Postmortem Changes of pH

The rates of pH decline for all treatments are presented in Table 2 and illustrated in Figures 2-4. These results show the electrically stimulated beef sides to have the most rapid rate of decline ($P < 0.01$) while the unstimulated (control) group had the slower decline rate. This is in line with the findings by Chrystall and Hagyard (1976), Chrystall and Devine (1978) and Sorinmade et al. (1978). Both stimulated and unstimulated groups had an average 0 hr (post-stimulation) pH value of 6.85. This falls within the range of 6.48-7.04 reported in literature (Moeller et al., 1976; McCollum and Henrickson, 1977; Tarrant and Mothersill, 1977; and Sorinmade et al., 1978). In both the stimulated and control treatments, the slope of the pH curves (Figure 2) decreased gradually after 5 hours post-stimulation storage until they attain their ultimate 96 hour values. The pattern of pH drop, however, shows an average of 0.54 pH units fall within the first 60 minutes of storage for the stimulated sides as against 0.26 units for the control, representing about 2 times the rate of pH decline of longissimus muscles from stimulated carcass sides over the control. This agrees with the findings of Bendall (1976) who reported a rate of fall 2-3 times greater than normal for electrically stimulated rabbit and lamb carcasses,

Table 2. Muscle pH* changes as affected by electrical stimulation, slaughter method and holding temperature.

Treatment	n	Post-Stimulation Aging Time (hrs)								
		0	1	2	3	4	5	6	24	96
Stimulated	12	6.84 ^a	6.30	6.08	5.95	5.83	5.73	5.68	5.59	5.57
Unstimulated	12	6.86 ^a	6.60	6.40	6.15	5.98	5.88	5.81	5.74	5.79
Stunned	12	6.95	6.52 ^b	6.33 ^b	6.13 ^b	5.96	5.83 ^b	5.79 ^b	5.73 ^b	5.69 ^b
Unstunned	12	6.75	6.47 ^b	6.14 ^b	5.99 ^b	5.85	5.75 ^b	5.73 ^b	5.63 ^b	5.60 ^b
1°C	12	6.85 ^c	6.71 ^c	6.48 ^c	6.32 ^c	6.13 ^c	6.03 ^c	5.92 ^c	5.88 ^c	5.70 ^c
20°C	12	6.85 ^c	6.45 ^c	6.20 ^c	6.00 ^c	5.85 ^c	5.78 ^c	5.73 ^c	5.61 ^c	5.60 ^c

*Values in the same column, in a given treatment, followed by the same letter are not significantly different from each other at $P < .05$.

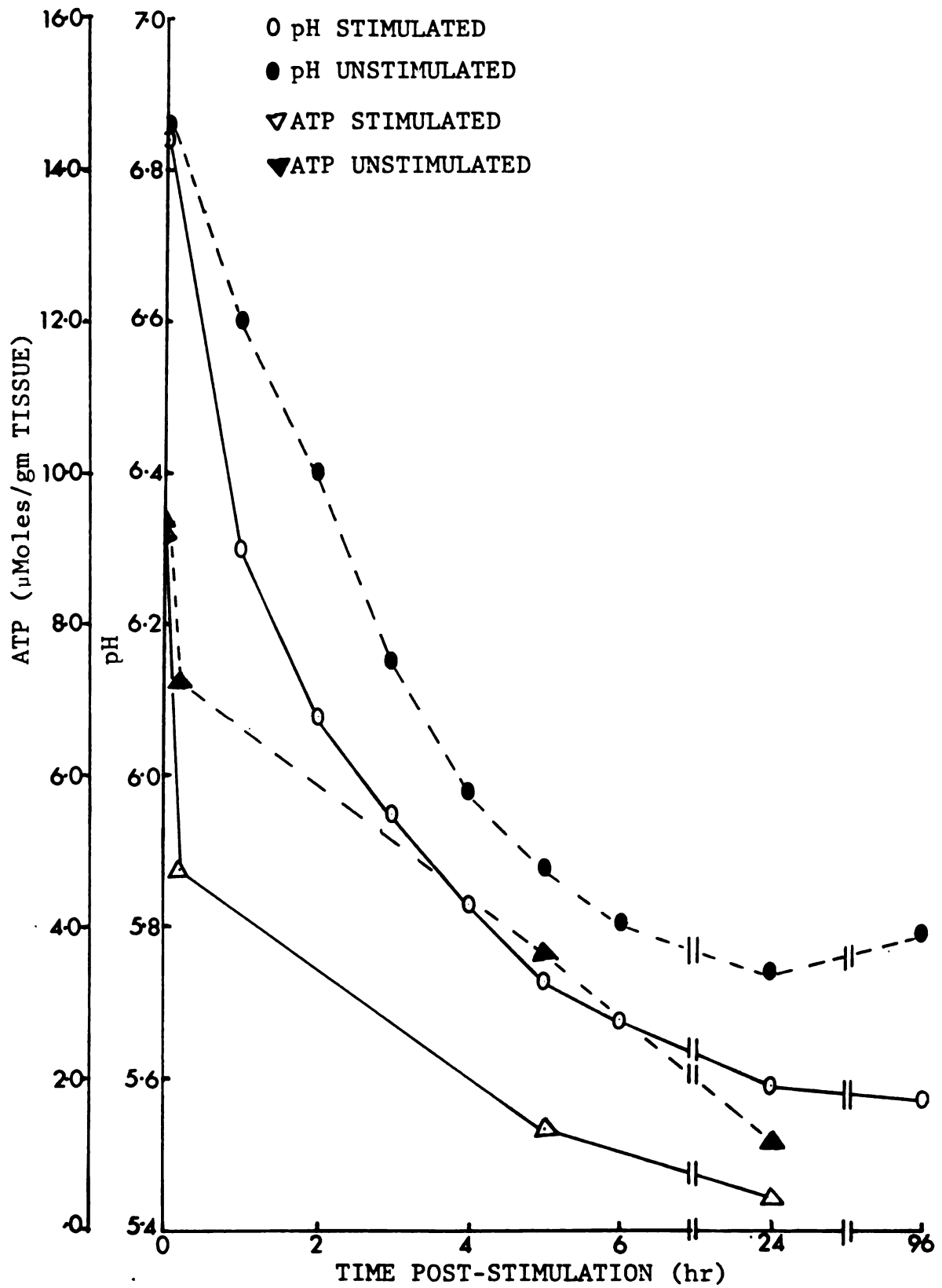


Figure 2. The effect of electrical stimulation on the rate of longissimus muscle pH and ATP decline.

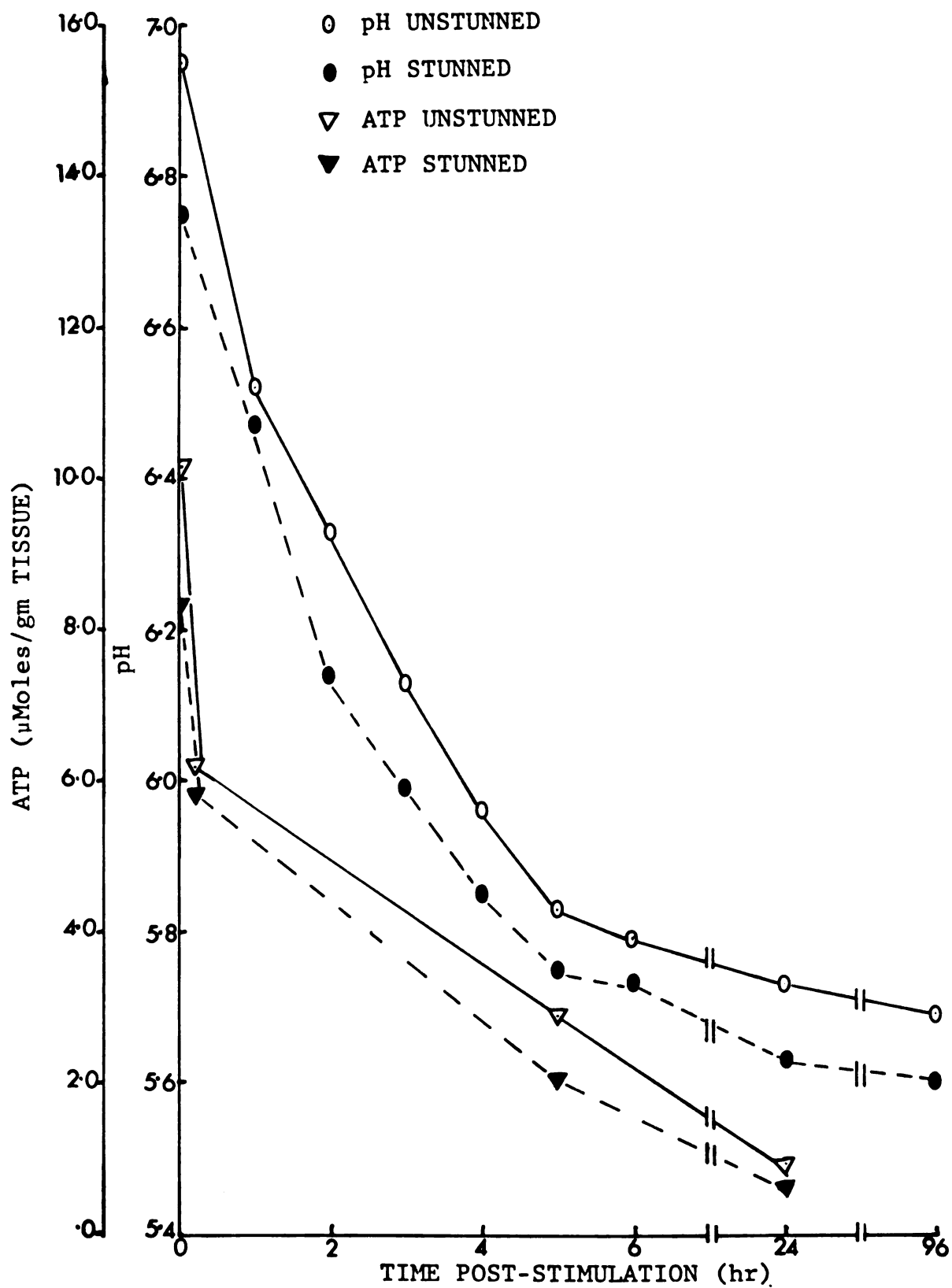


Figure 3. The effect of slaughter method on the rate of longissimus muscle pH and ATP decline,

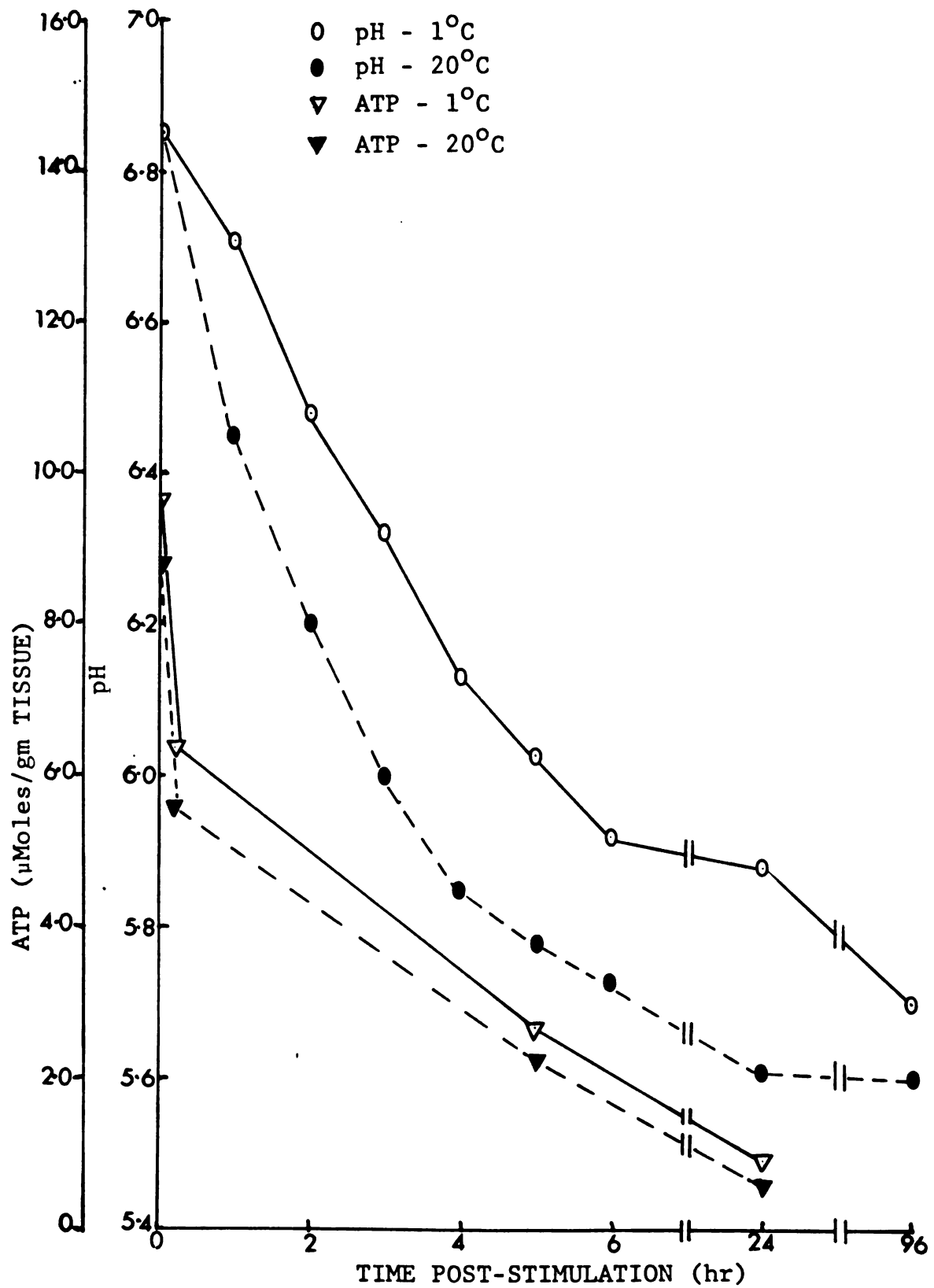


Figure 4. The effect of holding temperature on the rate of longissimus muscle pH and ATP decline.

and those of Chrystall and Devine (1978) who reported that the rate of fall in stimulated bovine sternomandibularis muscles is more than one and one-half times that in non-stimulated muscles over the same pH range. The value for the stimulated sides dropped to 5.68 within 6 hours while the control fell to 5.81 thus representing a highly significant ($P < 0.01$) difference due to electrical stimulation. Gilbert and Davey (1976) obtained a pH of 5.4 in 5 hours (stimulated) using a higher voltage (3600 volts) and Sorinmade et al. (1978) obtained a value of 5.45 within 5 hours post-stimulation. At 96 hours post-stimulation the pH of the unstimulated carcass sides (5.78) was significantly higher ($P < 0.05$) than that for the stimulated sides (5.57).

The unstunned steer longissimus muscles exhibited a significantly ($P < 0.05$) lower 0 hour (post-stimulation) pH as compared to the stunned steers (Table 2). This conflicts with the observations of McLoughlin (1965), and McLoughlin and Davidson (1966) working on porcine longissimus dorsi and Van der Wal (1978), but may be attributed to the fact that the unstunned steers, at time of slaughter, struggled furiously thus inducing a faster breakdown of ATP and/or glycogen present in the muscles and influencing a faster decline in pH levels (Bendall and Rhodes, 1976) due to lactate accumulation from accelerated glycolysis (Lister, 1970).

pH decline was slower in muscles held at 1°C when compared with the rate of decline for muscles held at 20°C (Figure 4). Ultimate pH of 5.70 was reached in 96 hours at 1°C while the value of 5.60 was attained in 24 hours at 20°C. These results are of identical pH change pattern as those reported by Marsh (1954), Moeller *et al.* (1977) and Jeacocke (1977) who observed that the rates of pH decline and ATP depletion occur faster as temperature increases above 10°C. This suggests that the higher the holding temperature, the more rapid the glycolytic rate, thus influencing the time of attainment of ultimate pH levels, although Cassens and Newbold (1967) reported that the rate of pH decline at 1°C was not slower than that at 15°C until several hours postmortem.

ATP Changes

The 0 hour post-stimulation (50 minutes postmortem) ATP levels and the extent of glycolysis are well reflected in the postmortem pH profile (Figures 2-4). They also illustrate the relationship between ATP degradation and pH decline during period of aging for all treatments. Positive correlations exist between pH decline rate and ATP disappearance (Table 3). This is particularly evident at 4 hour pH and 5 hour ATP ($r=.65$, $P<.01$). As with most postmortem changes, ATP depletion was precipitous (Figures 2-4) and although some residual ATP (≈ 1.14 $\mu\text{Moles/gm}$

Table 3. Simple correlation coefficients between various physical measurements, chemical and microbial analysis of beef steaks, a,b

	Panel tenderness 5 hr	Panel tenderness 96 hr	W-B Shear 5 hr	W-B Shear 96 hr	A-K Shear 5 hr	A-K Shear 96 hr	ATP 0 hr	ATP 5 hr	ATP 24 hrs	pH 0 hr	pH 4 hr	pH 5 hr	pH 24 hr	Temperature 5 hr	Temperature 24 hr	Microbial 5 hr	Microbial 24 hr
Panel tenderness 5 hr	1.00																
Panel tenderness 96 hr	.61	1.00															
W-B Shear 5 hr	-.62	-.65	1.00														
W-B Shear 96 hr	-.54	-.66	.76	1.00													
A-K Shear 5 hr	-.89	-.65	.83	.70	1.00												
A-K Shear 96 hr	-.47	-.60	.29	.08	.41	1.00											
ATP 0 hr	-.44	-.24	.12	.42	.44	-.17	1.00										
ATP 5 hr	-.53	-.56	.48	.66	.58	.33	.33	1.00									
ATP 24 hr	-.45	-.60	.70	.79	.59	.10	.27	.83	1.00								
pH 0 hr	-.69	-.51	.61	.25	.65	.43	.00	.19	.25	1.00							
pH 4 hr	-.76	-.66	.36	.50	.66	.60	.43	.65	.42	.42	1.00						
pH 5 hr	-.70	-.60	.37	.50	.63	.50	.36	.69	.46	.77	.91	1.00					
pH 24 hr	-.45	-.38	.11	.41	.26	.20	.21	.39	.31	.26	.65	.66	1.00				
Temp. 5 hr	.32	.52	-.51	-.40	-.45	-.50	-.16	-.26	-.37	-.15	-.27	-.21	.11	1.00			
Temp. 24 hr	.49	.45	-.56	-.61	-.55	-.28	-.36	-.35	-.47	-.17	-.39	-.35	-.10	.85	1.00		
Microbiol. 5 hr	.33	.37	-.45	-.48	-.39	-.30	-.12	-.15	-.25	-.26	-.18	-.11	-.03	.74	.74	1.00	
Microbiol. 24 hr	.25	.28	-.34	-.48	-.32	-.13	-.35	-.19	-.34	.02	-.28	-.25	-.15	.73	.80	.78	1.00

^an=24; ^bp < .05 = .39 - .49

P < .01 = .50

tissue) remained after 24 hours in the longissimus of the unstimulated carcass sides, practically all the ATP in the electrically stimulated carcasses had disappeared (0.45 Moles/gm tissue) and had dropped to about 1/3 of the 0 hour value at 5 hours post-stimulation. This relationship is in agreement with the results reports by Davey et al. (1976) and Sorinmade et al. (1978). At pH 5.88 (5 hours post-stimulation) about 60% of the ATP level in the muscles of unstimulated carcasses had disappeared reaching a residual low level of about 10% of 0 hour content at pH 5.78. Bendall and Rhodes (1976) reported that at pH 6, 50% of the resting content of ATP had disappeared, and at pH 5.7 more than 90%. Samples from stimulated sides showed an 86% ATP depletion at pH 5.73 post-stimulation and fell to a low residual level of 5% of the original at pH 5.57. These values were reached at 5 hour and 24 hour, respectively. The muscle samples from stimulated sides contain 44% less ATP than those from control samples at 10 minutes post-stimulation showing that electrical stimulation of carcasses immediately after slaughter is a very effective means of rapidly lowering ATP level and the pH of longissimus muscles of beef animals.

0 hour (post-stimulation) ATP levels for control carcasses were slightly higher (9.31 μ Moles/gm tissue) than previous observations on bovine longissimus by Scopes and Newbold (1968) and Sorinmade et al. (1978). The

differences in ATP may be due to the different procedures used for the estimation of ATP or to the rapidity with which the muscles were removed from the animal and prepared for analysis. There is a high correlation coefficient between the 5 hour ATP level and 5 hour pH ($r=0.69$, $P<0.01$) indicating that the simple measurement of pH would be sufficient to estimate the extent of glycolysis.

There exists a significant difference ($P < 0.05$) in initial ATP levels (0 hour post-stimulation) due to technique of slaughter of steers. The stunned steer carcass longissimus muscles contained a higher 0 hour (post-stimulation) ATP than the longissimus muscle from unstunned steers (Table 4). This is most likely due to the effect of struggling at slaughter by the unstunned steers which is also reflected in the difference in 0 hour pH (Figure 3) resulting from accelerated rate of post-mortem glycolysis. ATP levels drop significantly with time ($P < 0.01$) with the unstunned steer longissimus muscles attaining a lower residual ATP ($0.63 \mu\text{Moles/gm tissue}$) than the stunned steer longissimus muscle ($0.96 \mu\text{Moles/gm tissue}$) at 24 hour post-stimulation time.

Figure 4 illustrates the effect of a variation in holding temperature on the decline in ATP levels in longissimus muscles of steer carcasses held at 20°C and 1°C over a 24 hour period. The muscles held at an elevated temperature (20°C) generally had lower ATP levels than those of muscles

Table 4. Longissimus muscle ATP* (μ Moles/g tissue) changes as affected by electrical stimulation, slaughter method and holding temperature.

Treatment	n	Post-Stimulation Aging Time (Min)			
		0	10	300 (5 hr)	1440 (24 hr)
Stimulated	12	9.22 $\pm$ 1.53 ^a	4.77 $\pm$ 1.06	1.32 $\pm$ 0.95	0.45 $\pm$ 0.41
Unstimulated	12	9.31 $\pm$ 1.60 ^a	7.23 $\pm$ 1.02	3.70 $\pm$ 1.48	1.14 $\pm$ 0.62
Stunned	12	10.17 $\pm$ 1.16	6.15 $\pm$ 1.53 ^b	2.93 $\pm$ 1.42 ^b	0.96 $\pm$ 0.61 ^b
Unstunned	12	8.36 $\pm$ 1.33	5.85 $\pm$ 1.75 ^b	2.08 $\pm$ 1.94 ^b	0.63 $\pm$ 0.61 ^b
1°C	12	9.69 $\pm$ 1.49 ^c	6.39 $\pm$ 1.56 ^c	2.75 $\pm$ 1.75 ^c	0.90 $\pm$ 0.62 ^c
20°C	12	8.83 $\pm$ 1.50 ^c	5.61 $\pm$ 1.65 ^c	2.28 $\pm$ 1.73 ^c	0.69 $\pm$ 0.63 ^c

* Values in the same column, in a given treatment, followed by the same letter are not significantly different at $P < 0.05$.

held at 1°C although the differences were not statistically significant even at the end of 24 hours of holding time ($P \geq 0.481$). ATP level dropped to as low as 0.69 $\mu\text{Moles/gm}$ tissue in the 20°C held muscles while those kept in 1°C environment averaged levels of 0.90 $\mu\text{Moles/gm}$ tissue at the end of 24 hours of aging time. This observation may be due to the accelerated glycolytic process suggested by Moeller et al. (1977) and West (1978) which is enhanced at elevated temperatures in the range used in this experiment.

Post-mortem Temperature Changes

Changes in internal temperatures of the longissimus muscles were monitored during the first 24 hours of aging and are illustrated in Figures 5-7 for all treatments. These results indicate that no significant differences exist in internal temperatures of longissimus muscles due to electrical stimulation, method of slaughter or holding temperature within the first 3 hours of aging time. At the end of 3 hours of holding time, a significant difference ($P < 0.05$) was noted between muscles held at 1°C and 20°C, and becomes highly significant ($P < 0.01$) at the end of 24 hours where internal temperatures averaged 12.08 and 22.79 °C, respectively. The average initial temperature of carcasses for all treatments was 37.79°C (0 hour post-

Table 5. Longissimus muscle temperature* (°C) changes as affected by electrical stimulation, slaughter method and holding temperature.

Treatment	n	Post-Stimulation Aging Time (hr)						
		0	1	2	3	4	5	6
Stimulated	12	37.67 ^a	34.92 ^a	32.17 ^a	30.33 ^a	27.67 ^a	26.42 ^a	25.25 ^a
Unstimulated	12	37.96 ^a	35.25 ^a	32.29 ^a	30.29 ^a	27.96 ^a	26.17 ^a	25.00 ^a
Stunned	12	38.38 ^b	34.92 ^b	31.88 ^b	29.83 ^b	27.63 ^b	25.83 ^b	24.50 ^b
Unstunned	12	37.16 ^b	35.25 ^b	32.17 ^b	30.46 ^b	28.00 ^b	26.75 ^b	25.75 ^b
1°C	12	37.92 ^c	34.71 ^c	30.50 ^c	27.50	24.50	22.17	20.71
20°C	12	37.58 ^c	35.46 ^c	35.00 ^c	32.79	31.13	30.42	29.50

* Values in the same column in a given treatment, followed by the same letter are not significantly different at $P < .05$.

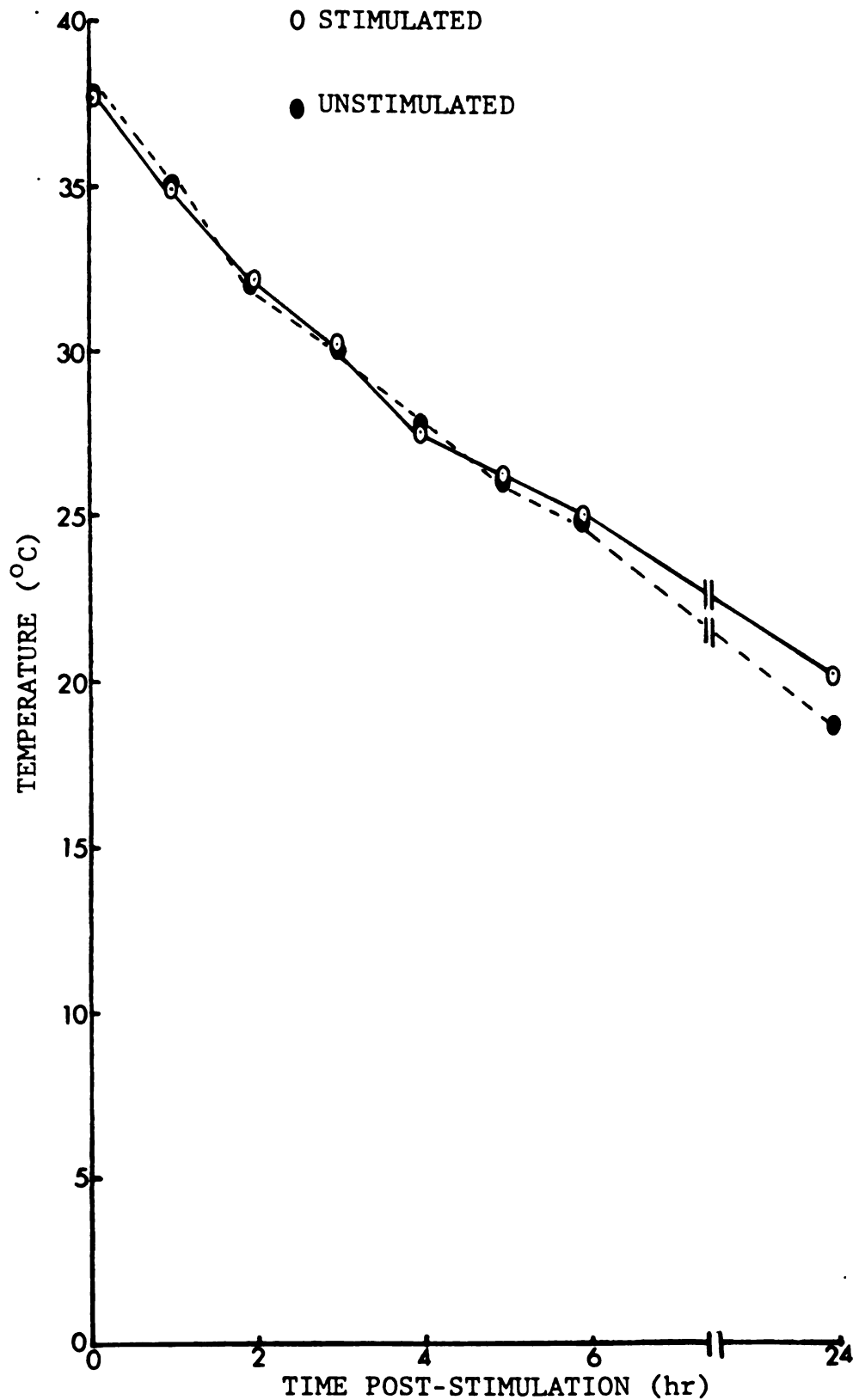


Figure 5. Rate of temperature decline in beef longissimus muscle of stimulated and control carcasses.

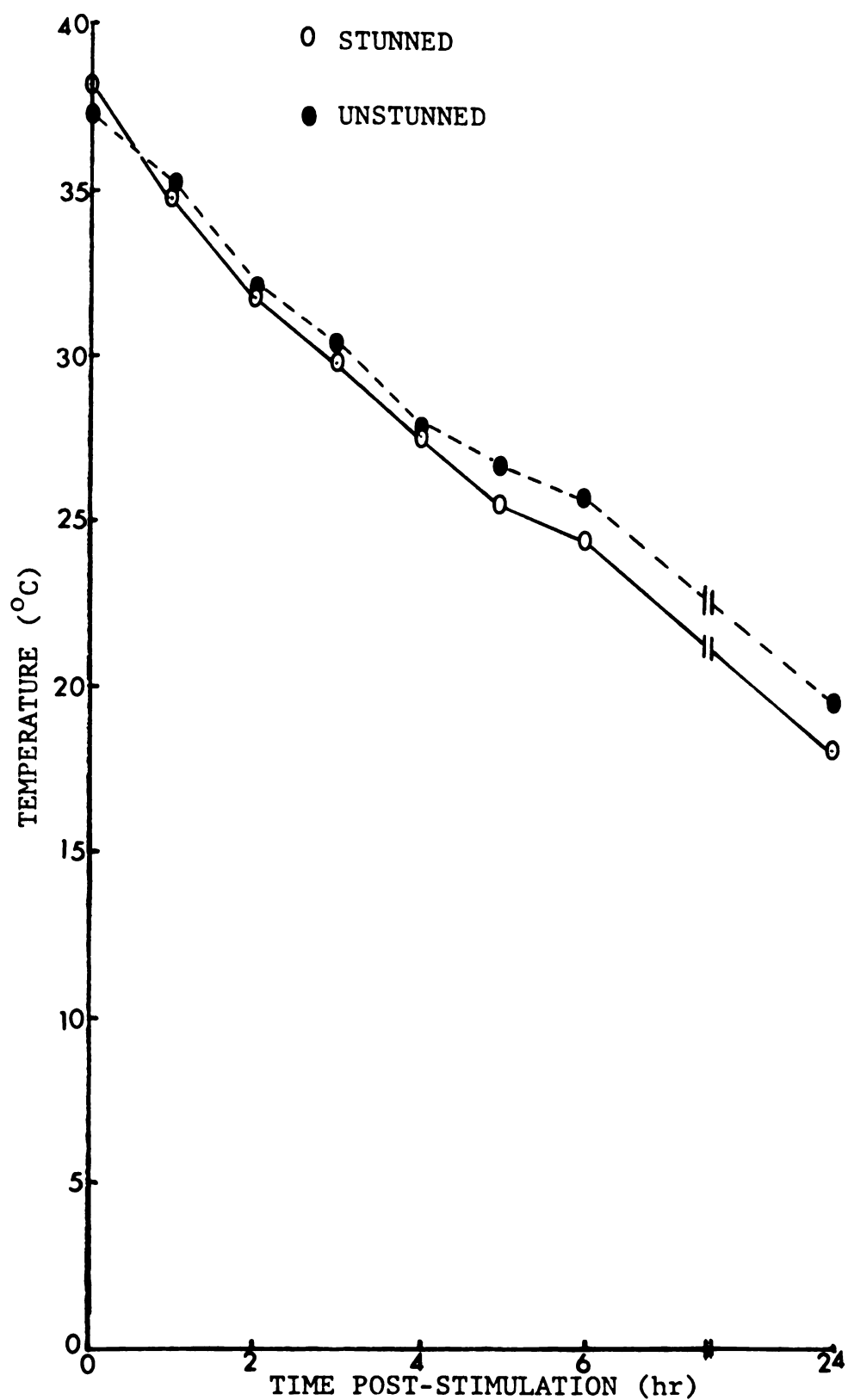


Figure 6. Rate of temperature decline in beef longissimus muscle of stunned and unstunned steers.

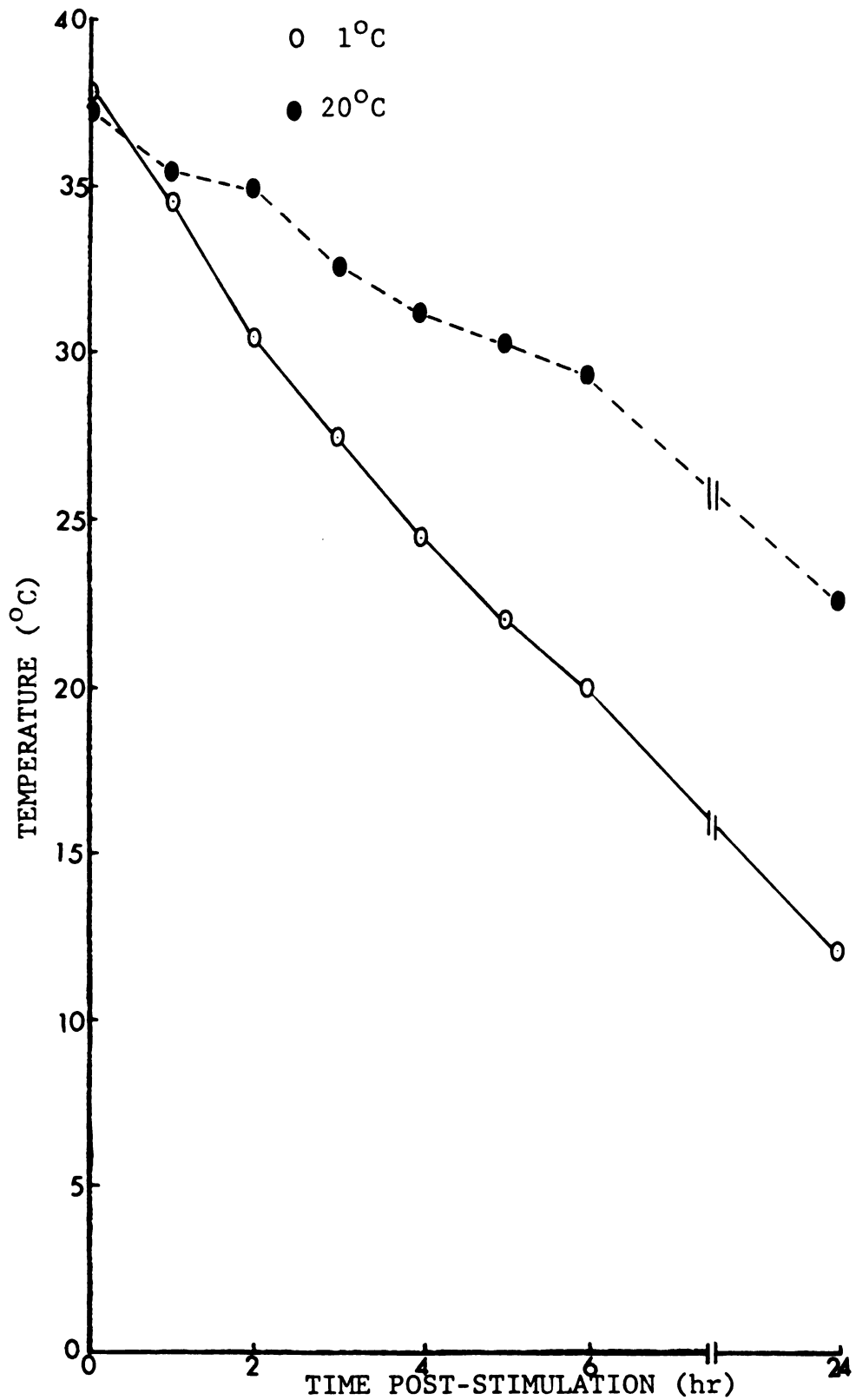


Figure 7. Rate of temperature decline in beef longissimus muscle of carcasses held at 1°C and 20°C.

stimulation). The normal cattle body temperature is approximately 38.3°C to 39.1°C (Hafez, 1968). The internal temperature of carcasses used for this study falls short of this range, although this value (37.79°C) does not vary much considering that the reading was obtained at 50 minutes postmortem. The 1°C stored carcasses showed a more rapid decline until the internal temperature of the longissimus at 24 hours post-stimulation reached 12.08°C. Muscle temperatures of carcasses that were held at 20°C dropped very gradually reaching an ultimate 24 hour average internal temperature of 22.79°C. Thus, the environmental temperature in which the beef carcass is held has a significant influence on the rate at which internal temperature of the muscle changes. There does not appear to be any influence on changes in internal temperatures due to either electrical stimulation or method of slaughter.

Tenderness Evaluation

Panel Scores. Panel data as related to muscle tenderness are presented in Table 6. These scores tend to indicate that the panelists were able to distinguish the steak samples that had been tenderized, as a result of the treatments, from the controls. Samples from electrically stimulated carcasses had significantly ($P < 0.01$) higher panel scores than the controls. This agrees with the findings of Savell et al. (1977) who reported significant

Table 6. Comparison of means and standard deviations of Panel Tenderness ratings^{1,2} for beef longissimus dorsi muscles.

Treatment	n	Post-Stimulation Aging Time (hrs)			
		5	24	96	168
Stimulated	12	3.00±0.69	5.23±1.30	6.03±0.92	6.97±0.79
Unstimulated	12	2.60±0.76	3.85±1.34	4.45±1.20	5.40±1.03
Stunned	12	2.30±0.65	3.67±1.31	4.63±1.41	5.93±1.49 ^b
Unstunned	12	3.46±0.73	5.41±1.07	5.85±0.91	6.45±0.82 ^b
1°C	12	2.62±0.92 ^c	4.08±1.39 ^c	4.72±1.37	5.89±1.14
20°C	12	3.14±0.84 ^c	5.01±1.45 ^c	5.75±1.09	6.69±1.06

¹Means based on a 9-point rating scale 1 = extremely tough; 9 = extremely tender.

²Values in the same column, in a given treatment, followed by the same letter are not significantly different at P < .05.

($P < 0.15$ to $P < 0.01$) improvement in light-weight beef steak tenderness as a result of stimulation with 25, 50 or 75 electrical impulses, and Sorinmade et al. (1978). Comparisons of panel tenderness for steaks aged for 168 hours indicate that tenderness difference ($P < 0.01$) could be detected as early as 5 hours post-stimulation. As expected, these panel scores show that improvement in tenderness progressed positively as aging time was prolonged. Panel scores also rate the steaks from carcasses of unstunned steers significantly ($P < 0.01$) more tender than those from stunned steer carcasses up to 96 hours post-mortem. At 168 hours of post-stimulation time any differences due to method of slaughter had significantly ($P < .15$) disappeared. The differences of scores between steaks obtained from 1°C and 20°C held carcasses showed a tendency for steaks from carcasses held at elevated temperature (20°C) to be more tender than those from carcasses held at 1°C especially at 96 hours aging time ($P < .05$). This relationship is in line with the results reported by Locker et al. (1975) and also by West (1978).

Shear Values. Mean values for shear force (Warner-Bratzler and Allo-Kramer) are presented in Tables 7 and 8. These values confirm the panel scores on the tenderness advantage of steaks from electrically stimulated carcass halves over those from unstimulated sides. They had

Table 7. Comparison of means and standard deviations of Warner-Bratzler shear force* values (Kg/cm²) for beef longissimus muscles.

Treatment	n	Post-Stimulation Aging Time (hrs)		
		5	24	96
Stimulated	12	7.51±2.63	5.44±1.08	4.50±1.12
Unstimulated	12	8.63±2.88	6.82±1.66	5.87±1.43
Stunned	12	9.44±3.23 ^b	6.98±1.84 ^b	5.77±1.54 ^b
Unstunned	12	6.71±1.20 ^b	5.52±1.16 ^b	4.69±1.27 ^b
1°C	12	8.66±3.10 ^c	6.98±1.69 ^c	5.73±1.49 ^c
20°C	12	7.49±2.36 ^c	5.45±1.41 ^c	4.31±0.99 ^c

* Values in the same column, in a given treatment, followed by the same letter are not significantly different at P < .05.

Table 8. Comparison of means and standard deviations of Allo-Kramer shear force* values (Kg/gm) for beef longissimus muscles.

Treatment	n	Post-Stimulation Aging Time (hrs)			
		5	24	96	168
Stimulated	12	21.60±4.10	12.53±3.19	9.32±2.74	8.11±2.27
Unstimulated	12	23.48±5.73	15.41±3.78	12.27±3.85	10.70±3.66
Stunned	12	26.01±4.53 ^b	14.36±4.68	11.17±3.91 ^b	9.86±3.61 ^b
Unstunned	12	19.07±2.14 ^b	13.58±2.61	10.42±3.38 ^b	8.95±2.96 ^b
1°C	12	23.81±5.78 ^c	15.34±3.79 ^c	12.12±3.76 ^c	10.61±3.57 ^c
20°C	12	21.68±4.42 ^c	12.60±3.25 ^c	9.30±2.71 ^c	7.20±1.54 ^c

* Values in the same column, in a given treatment, followed by the same letter are not significantly different at $P < 0.05$.

significantly ($P < 0.01$) lower shear force values than the controls, and as with panel scores, the tenderness differences were detectable as early as 5 hours post-stimulation (5 hours) of differences in tenderness tends to give further credence to the theory by Savell et al. (1978) that physical disruption of muscle fibers is a major contributor to muscle tenderness by electrical stimulation rather than the lysosomal enzyme theory proposed by Dutson et al. (1978).

dence to the theory by Savell et al. (1978) that physical disruption of muscle fibers is a major contributor to muscle tenderness by electrical stimulation rather than the lysonsomal enzyme theory proposed by Dutson et al. (1978).

Also in Tables 7 and 8 are shear mean values for comparisons between steaks obtained from carcasses held at 1°C and 20°C. Detectable differences ($P < 0.065$) were observed for Warner-Bratzler shear at the end of 24 hours post-stimulation, but Allo-Kramer shears found no significant ($P \geq 0.215$) differences for same samples. Shear values show that steaks from 24 hour samples from stimulated sides were almost as tender as those obtained at 96 or 168 hours post-stimulation aging time from the control sides. Similar observations were reported by Parrish et al. (1973) who demonstrated that longissimus samples from choice carcasses conditioned 24 hours at 16° before subsequent chilling were

as tender as samples from control carcasses chilled and aged 7 days at 2°C, and also by Smith et al. (1971) using 12 to 18 month old steer carcasses. One reason for the tenderness advantage due to high temperature conditioning may be due to the fact that the technique accelerates the rate of postmortem glycolysis and hastens the onset of rigor mortis, thus preventing toughening due to cold shortening. At the resultant low pH and elevated temperature conditions there is increased autolytic activity of the endogeneous proteolytic enzymes on the myofibrillar structures (Henderson et al., 1970 and Olson et al., 1976).

Shear values did not identify any significant differences in tenderness of steaks due to method of slaughter. In this experiment, the simple correlations between panel tenderness and shear force values gave highly negative relationships as evidenced at 5 hours for Allo-Kramer ($r = -.89$, $P < 0.01$) or Warner-Bratzler ($r = -.62$, $P < .01$) and at 96 hours for Allo-Kramer ($r = -.60$, $P < .01$) or Warner-Bratzler ($r = -.66$, $P < .01$). These results therefore, tend to confirm the suggestion that either of these three methods could be used to estimate the tenderness of cooked steak samples.

Microbial Count

Results of microbial count ($\log_{10}$) per cm^2 of the surface of the short loin and standard deviations, for all treatments, are presented in Table 9 and illustrated in

Table 9. Microbiological* count ($\log_{10}$) of longissimus muscle surface as affected by electrical stimulation, slaughter method and holding temperatures.

Treatment	n	Post-Stimulation Aging Time (hr)			
		0	5	24	96
Stimulated	12	2.30±0.81 ^a	2.82±0.92 ^a	4.58±1.79 ^a	4.82±1.90 ^a
Unstimulated	12	2.33±1.03 ^a	2.99±1.01 ^a	4.77±1.56 ^a	4.88±1.81 ^a
Stunned	12	2.32±0.84 ^b	2.89±0.93	4.76±1.68 ^b	4.95±1.73 ^b
Unstunned	12	2.31±1.89 ^b	2.93±1.02 ^b	4.59±1.67 ^b	4.74±1.96 ^b
1°C	12	2.11±0.74 ^c	2.19±0.47	3.34±0.75	3.30±0.93
20°C	12	2.52±1.05 ^c	3.63±0.75	6.02±1.08	6.39±0.89
					5.29±1.48
					5.04±1.69
					5.14±1.84 ^b
					5.20±1.30 ^b
					3.90±0.72
					6.44±1.02

* Values in the same column, in a given treatment, followed by the same letter are not significantly different at $P < 0.05$.

Figures 10-12. Statistical analysis show no significant difference in microbial load as a result of either electrical stimulation ($P \geq 0.940$) or method of slaughter ($P \geq 0.760$). Differences due to aging time were similarly insignificant for both treatments. Analysis of variance for counts obtained at 0 hour poststimulation also showed insignificant differences due to slaughter method ($P \geq 0.975$), electrical stimulation ($P \geq 0.935$) or holding temperature ($P \geq 0.431$). Counts ($\log_{10}$) per cm^2 obtained at 5, 24, 96 and 168 hours (post-stimulation from samples held at 1° and 20°C showed highly significant differences ($P < 0.01$) in microbial load of samples held between these two temperatures. Maximum count ($\log_{10}$) at 168 hours was 3.90 for samples held at 1°C , but this value is far below the recommended bacteriological limits that have been suggested by the USDA (1968) and Kotula (1970). That for samples held at 20°C (6.40) also falls below the recommended limit, indicating that the holding conditions employed in this experiment were within reasonable sanitary levels. Highly positive correlation coefficients were observed between microbial load and holding temperature at 5 hours ($r=.74$, $P<.01$) and 24 hours ($r=.80$, $P<.01$).

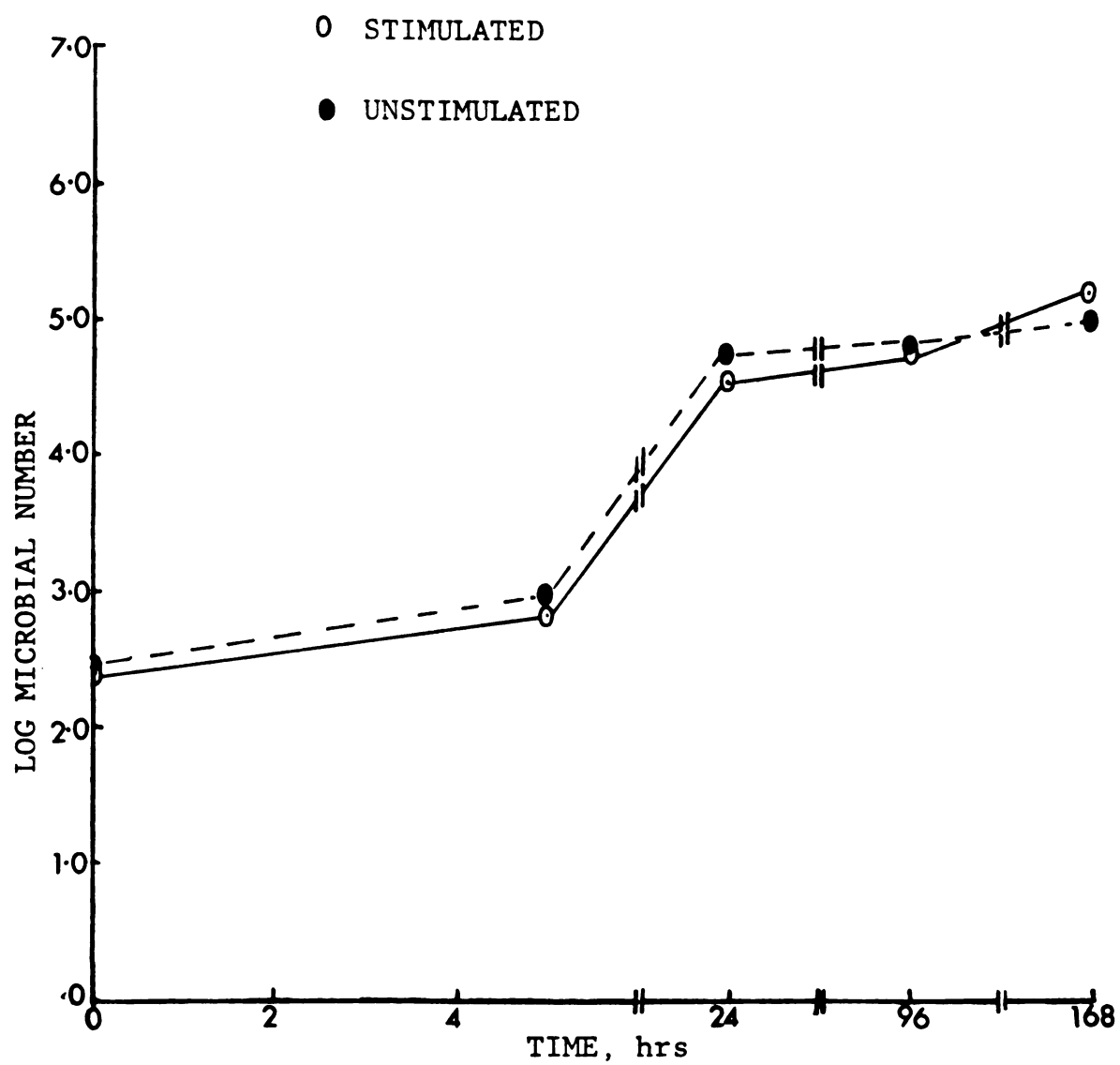


Figure 8. Microbial growth rate on loin surfaces of stimulated and control beef carcasses.

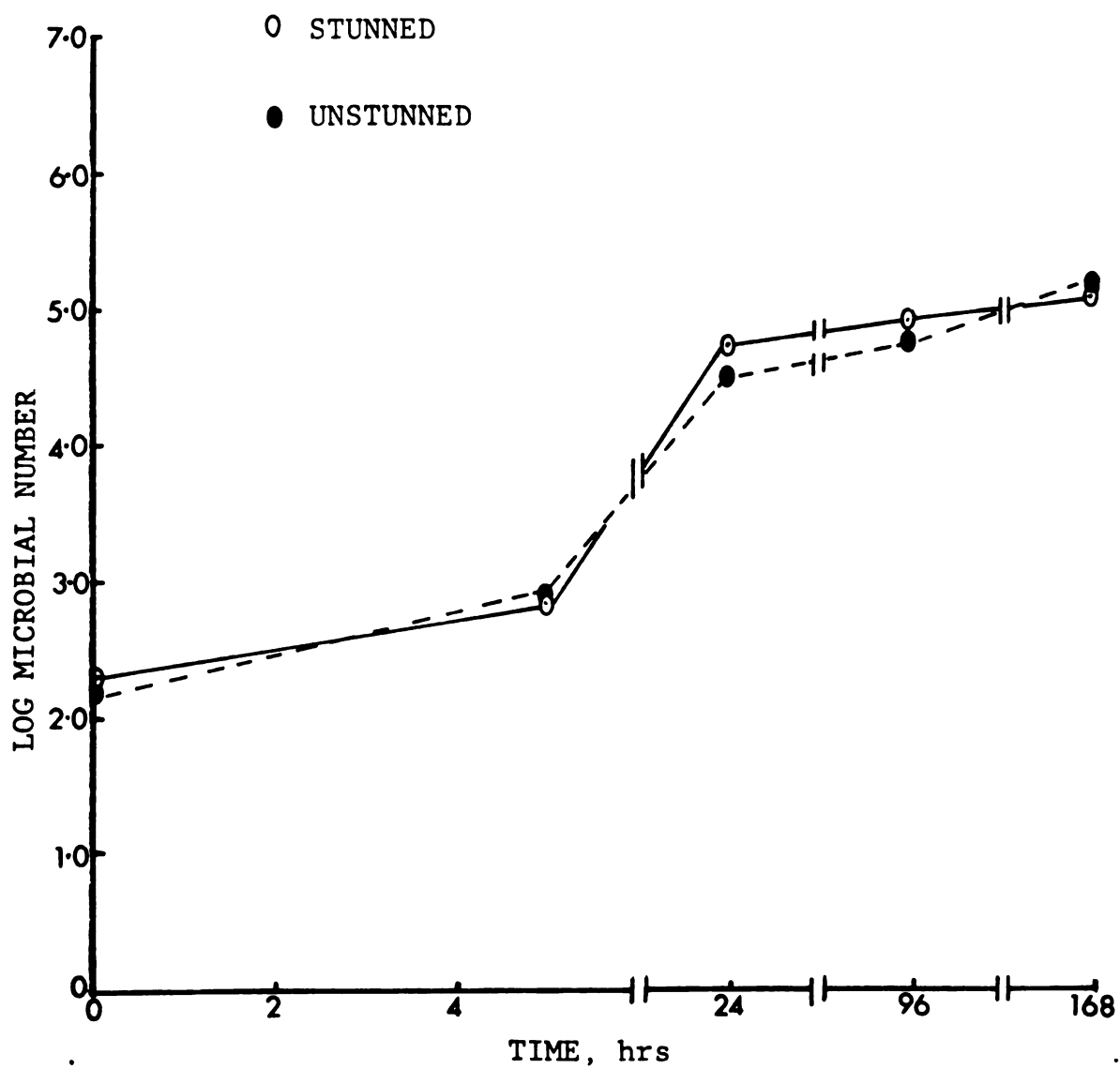


Figure 9. Microbial growth rate on loin surfaces of stunned and unstunned steer carcasses.

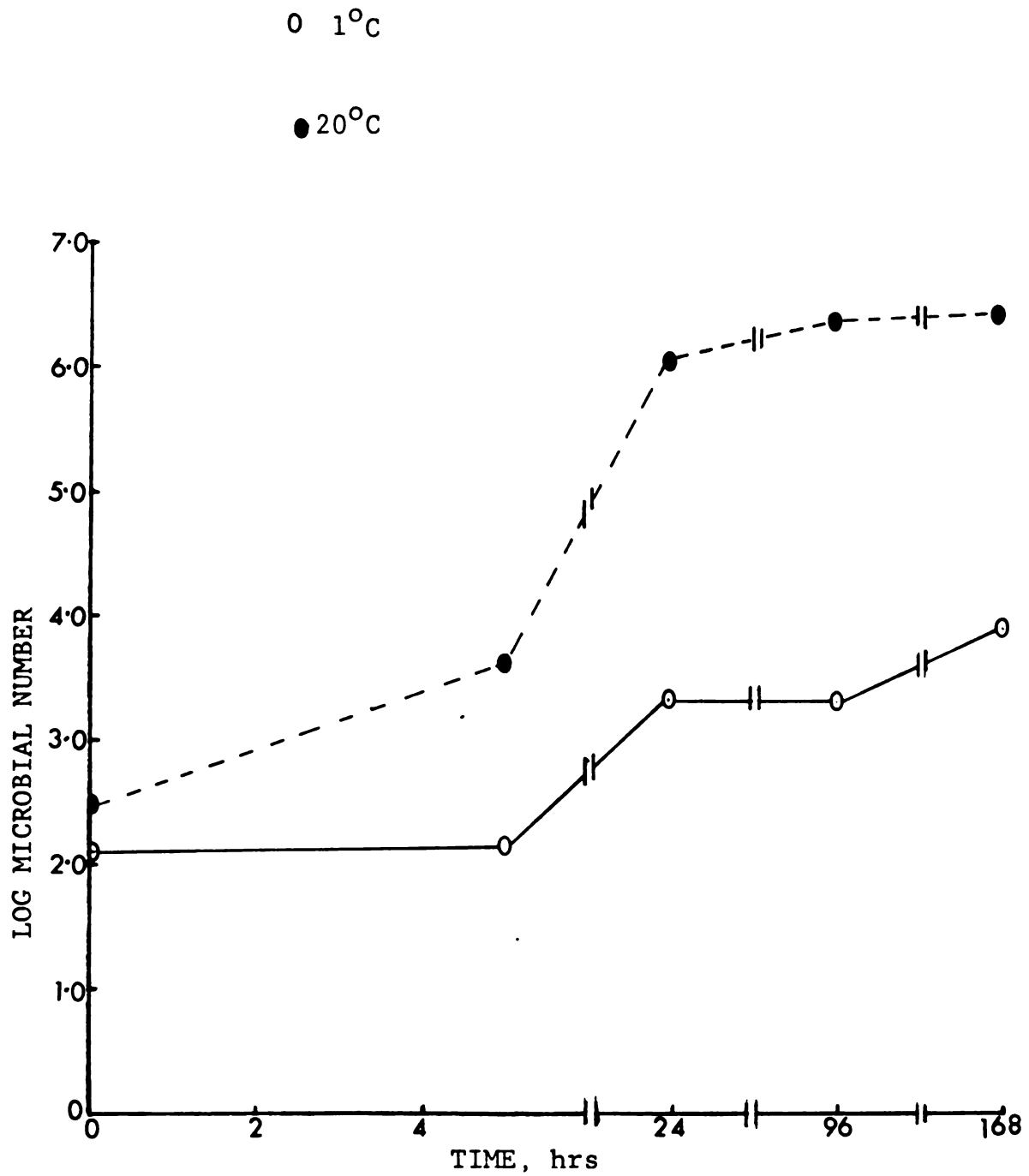


Figure 10. Microbial growth rate on loin surfaces of carcasses held at 1°C and 20°C.

SUMMARY

Twelve grass-fed steers were used to conduct experiments to investigate the influence of slaughter technique, electrical stimulation and holding temperature on the tenderness of beef steaks from the longissimus muscles, and the influence of the treatments on changes in muscle pH, adenosine triphosphate (ATP), internal temperature and microbial load. Six steers were slaughtered conventionally while the other 6 were confined in a steel frame cattle chute, haltered and throat slashed with a sharp knife. All the steer carcasses were split into right and left sides, and the right sides were subjected to 100 seconds of electrical stimulation (100 volts) each. Twelve steer halves (6 steer carcasses) were placed in a 1°C chilling cooler and the others in a 21°C storage atmosphere. Internal temperatures and pH of the longissimus muscle were monitored. ATP levels and total microbial count were also determined. At 5, 24, 96 and 168 hours (post-stimulation), samples were obtained from the longissimus muscle in the lumbar region for tenderness determinations. Tenderness was determined using Warner-Bratzler and Allo-Kramer shear devices and by taste panels. ATP determination was based on the luciferase enzyme technique read spectrophotometrically as percent deflection and converted to μ Moles ATP per gram tissue.

Panel evaluation and Warner-Bratzler and Allo-Kramer shear values indicate that longissimus muscle samples from electrically stimulated sides of all steers were significantly ($P < 0.01$) more tender than samples from the untreated sides at each sampling time. All the 24, 96 and 168 hour samples had higher panel tenderness scores and lower shear values than did the 5 hour steaks. There appears to be a tenderness advantage of steaks from the unconventionally slaughtered steers over the conventional method as judged by the panelists although this was not significantly detected by the shear measurements. Elevated holding temperature (20°C) did improve the tenderness of steaks after 96 hours of post-stimulation aging as reflected in the panel scores, although this was not significantly so with the shear values.

Changes in internal temperatures of the longissimus muscles were dependent on the holding temperatures. There were no significant differences in initial post-stimulation temperatures due to treatments within the first 3 hours. Highly significant difference ($P < .01$) after 5 hours of holding time were observed. Temperature decline in the 1°C held carcasses was faster than in those held at 20°C . Rates of temperature drop due to slaughter method or electrical stimulation showed no significant difference throughout 168 hours.

Electrically stimulated sides had a faster pH decline than

did the controls, and 20°C holding temperatures caused pH to drop much faster in carcasses than did 1°C. There were no significant differences in 0 hour pH levels, although a slight difference ($P < .02$) exists as a result of method of slaughter with the conventional technique having a higher value. The ultimate pH reached in the electrically stimulated muscles were highly significantly ($P < 0.01$) different from the controls but showed no significant difference for either holding temperature ($P \geq 0.833$) or slaughter method ($P \geq 0.531$). ATP content and pH levels of muscles had identical change pattern through 24 hours post-stimulation for all treatments. ATP levels dropped rapidly as a result of electrical stimulation, and slaughter by unconventional method hastened ATP depletion in longissimus muscles of steers so treated thus precipitating a significant difference ($P < 0.05$) in initial (0 hour) ATP content.

Meat microbial count ($\log_{10}$) per cm^2 of the surface of the shortloin at 0 hour showed no significant differences due to treatments. Counts at 168 hour post-stimulation were 6.44 and 3.90 for carcass sides held at 20° and 1°C, respectively. This represents a highly significant ($P < .0005$) difference as a result of holding temperature. Count difference due to either electrical stimulation or slaughter technique were consistently insignificant throughout postmortem aging time.

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LITERATURE CITED

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APPENDIX

Appendix I. Raw data of panel* scores between stimulated and unstimulated beef langissimus muscles.

Post-Stimulation Aging Time (Hrs)							
Stimulated Sides				Unstimulated Sides			
5	24	96	168	5	24	96	168
2.75	6.25	6.36	7.45	2.50	4.27	5.36	5.27
1.75	4.45	5.45	6.60	1.10	2.18	3.00	6.27
3.36	6.82	7.00	7.25	3.55	5.27	5.45	6.25
4.27	4.73	5.33	6.17	3.36	3.64	4.00	5.83
3.15	5.13	6.40	7.57	2.60	3.01	3.56	3.80
2.78	4.90	6.20	7.00	2.96	2.99	3.10	3.50
3.65	7.01	6.50	7.62	3.20	6.14	6.25	6.60
8.10	6.29	6.31	6.26	3.00	5.59	5.75	5.95
3.41	5.79	7.21	7.84	2.85	4.19	5.10	5.49
3.25	5.24	6.24	6.91	2.62	4.20	5.04	5.21
2.57	3.31	5.60	7.81	2.11	2.92	4.20	6.31
2.01	2.84	3.70	5.23	1.29	1.85	2.63	4.30

*(Scale - 1, extremely tough - 9, extremely tender).

Appendix II. Raw data of Warner-Bratzler shear force (Kg/cm^2) between stimulated and unstimulated beef langissimus muscles.

Post-Stimulation Aging Time (Hrs)							
Stimulated Sides				Unstimulated Sides			
5	24	96	168	5	24	96	168
5.44	4.26	3.45	2.60	6.97	5.52	4.92	4.51
5.80	4.93	3.37	3.07	8.63	6.93	6.51	5.46
4.43	3.51	2.59	2.03	4.93	3.69	2.77	3.59
5.29	4.57	3.34	2.57	8.30	6.98	4.85	4.07
8.05	5.46	4.73	4.07	8.31	6.78	6.60	5.22
8.25	6.64	5.25	4.25	8.69	8.04	6.97	5.49
6.67	5.16	5.11	4.05	7.00	5.82	5.63	5.27
7.16	6.06	5.85	4.59	7.22	6.84	6.74	5.49
6.96	5.08	4.01	3.53	7.82	5.76	4.98	4.30
7.61	6.05	5.09	4.72	7.14	6.76	5.30	5.08
10.24	6.08	5.01	4.21	13.06	8.24	6.94	5.32
14.24	7.46	6.20	5.76	15.52	10.47	8.24	7.03

Appendix III. Raw data of Allo-Kramer shear force (Kg/gm) between stimulated and unstimulated beef longissimus muscles.

Post-Stimulation Aging Time (Hrs)							
Stimulated Sides				Unstimulated Sides			
5	24	96	168	5	24	96	168
22.26	11.21	9.57	9.21	24.53	16.86	13.71	13.74
23.63	15.66	11.55	10.92	30.86	22.09	18.50	17.41
17.65	10.69	9.18	7.38	15.62	14.31	15.12	11.46
16.60	14.61	13.32	11.13	17.45	16.50	15.18	14.64
21.20	7.28	5.61	5.58	22.32	9.10	7.47	6.44
22.91	8.82	7.50	6.16	23.13	12.13	8.44	7.51
20.21	9.96	5.51	5.11	20.59	11.14	7.49	6.30
20.29	11.24	7.22	5.90	21.03	12.13	7.86	6.76
17.02	12.46	8.24	7.34	19.24	16.27	10.19	9.04
20.46	16.62	11.34	10.21	22.63	17.02	14.40	12.15
25.62	14.51	9.04	7.37	28.94	17.36	12.48	9.48
31.37	17.24	13.80	11.03	35.46	20.06	16.36	13.42

Appendix IV. Raw data of pH decline in beef longissimus muscles of stimulated and un-stimulated carcasses.

Reading Time (Hrs)																	
Stimulated Sides									Unstimulated Sides								
0	1	2	3	4	5	6	24	96	0	1	2	3	4	5	6	24	96
6.9	6.4	6.1	6.0	5.9	5.8	5.8	5.6	5.6	6.8	6.7	6.5	6.2	6.1	5.9	5.8	5.7	5.7
7.0	6.4	6.2	6.1	6.0	5.9	5.8	5.8	5.6	7.0	6.8	6.6	6.2	6.2	6.0	5.9	5.8	5.9
6.7	6.3	6.1	5.8	5.7	5.6	5.6	5.5	5.5	6.8	6.6	6.4	6.0	5.9	5.9	5.8	5.7	5.8
6.7	6.3	6.0	6.1	5.8	5.7	5.6	5.5	5.5	6.8	6.5	6.3	6.1	5.8	5.6	5.6	5.5	5.6
6.9	6.2	6.1	6.0	5.8	5.7	5.7	5.6	5.6	7.0	6.5	6.3	6.1	6.0	5.9	5.8	5.9	5.9
6.8	6.0	6.0	5.8	5.9	5.7	5.6	5.6	5.6	6.8	6.4	6.2	6.0	5.9	5.8	5.7	5.7	5.7
6.6	6.0	5.8	5.8	5.7	5.7	5.6	5.6	5.6	6.7	6.5	6.3	6.0	5.9	5.8	5.8	5.7	5.7
6.6	6.1	6.0	5.9	5.9	5.8	5.7	5.6	5.6	6.6	6.4	6.2	6.1	5.9	5.9	5.8	5.7	5.8
6.9	6.4	5.9	5.8	5.7	5.6	5.6	5.6	5.6	6.8	6.5	6.2	6.1	5.9	5.8	5.8	5.8	5.8
6.9	6.3	6.1	5.9	5.8	5.7	5.8	5.8	5.7	6.9	6.7	6.5	6.3	6.1	6.0	5.9	5.8	5.8
7.0	6.5	6.1	5.9	5.7	5.7	5.7	5.4	5.4	7.1	6.8	6.6	6.3	6.0	5.9	5.9	5.8	5.9
7.1	6.7	6.5	6.3	6.0	5.9	5.7	5.5	5.5	7.0	6.8	6.7	6.4	6.1	6.0	5.9	5.8	5.9

Appendix V. Raw data of adenosine triphosphate (ATP-
 μ Moles/gm tissue) in beef longissimus muscles
of stimulated and unstimulated carcasses.

Sampling Time (Mins)-Post-stimulation							
Stimulated				Unstimulated			
0	10	300 (5 hr)	1440 (24 hr)	0	10	300 (5 hr)	1440 (24 hr)
10.85	5.77	2.29	0.21	10.88	6.85	3.53	0.53
11.16	6.17	1.55	0.50	10.88	7.75	4.09	0.53
7.19	3.81	0.90	0.09	7.16	6.08	1.83	0.09
7.56	4.22	1.18	0.06	7.97	6.57	2.08	0.93
9.70	3.39	0.67	0.25	10.04	7.38	3.26	1.18
11.22	4.62	1.58	0.78	11.54	7.84	2.98	1.52
10.01	5.89	0.56	0.56	9.67	8.74	6.11	2.02
10.11	6.23	0.90	0.31	10.57	8.62	5.52	1.30
7.29	3.47	0.34	0.09	7.04	5.46	1.83	0.81
8.06	4.09	0.43	0.22	7.66	6.13	3.29	1.05
7.91	4.22	1.76	0.81	8.25	6.29	4.25	1.61
9.58	5.38	3.69	1.46	10.01	8.09	5.67	2.14

Appendix VI. Raw data of temperature ($^{\circ}\text{C}$) decline in
beef longissimus muscles of stimulated and
unstimulated carcasses.

Reading Time (Hrs)								
Treatment	0	1	2	3	4	5	6	24
<u>Stimulated Sides</u>								
	39.0	36.0	35.0	32.0	32.0	31.0	31.0	27.0
	40.0	37.0	34.0	28.0	28.0	24.0	23.0	16.0
	36.0	35.0	33.0	32.0	30.0	30.0	28.0	28.0
	35.0	32.0	29.0	25.0	23.0	22.0	21.0	28.0
	40.0	38.0	37.0	37.0	35.0	32.0	30.0	27.0
	39.0	35.0	31.0	30.0	25.0	23.0	21.0	12.0
	36.0	35.0	33.0	33.0	31.0	30.0	30.0	27.0
	40.0	37.0	35.0	34.0	26.0	25.0	23.0	10.0
	38.0	36.0	34.0	33.0	31.0	31.0	30.0	28.0
	38.0	35.0	31.0	29.0	25.0	24.0	23.0	13.0
	36.0	31.0	29.0	28.0	26.0	27.0	26.0	20.0
	35.0	32.0	25.0	23.0	20.0	18.0	17.0	8.0
<u>Unstimulated Sides</u>								
	40.0	36.5	35.0	33.0	31.5	31.0	30.0	26.5
	40.5	36.5	33.5	28.0	26.0	24.0	21.0	15.0
	35.0	34.0	33.0	31.5	31.0	30.0	30.5	28.0
	35.0	33.0	29.0	25.0	23.0	20.0	20.5	15.0
	41.0	40.0	39.0	38.0	36.0	34.0	32.5	29.0
	40.0	35.0	31.0	30.0	26.0	22.0	20.0	11.0
	38.0	37.0	35.0	34.0	32.0	31.0	30.0	25.0
	40.0	36.0	34.0	31.0	28.0	23.0	21.0	10.0
	37.0	37.0	35.0	34.0	32.0	31.0	30.0	26.0
	39.0	36.0	30.0	28.0	24.0	24.0	22.0	14.0
	35.0	30.0	28.0	28.0	26.0	27.0	26.0	19.0
	35.0	32.0	25.0	23.0	20.0	17.0	16.5	7.0

Table VII. Raw data of microbial count ($\log_{10}$) of the surface of loin muscle of stimulated and unstimulated carcasses.

Sampling Time (Hrs)									
Stimulated Sides					Unstimulated Sides				
0	5	24	96	168	0	5	24	96	168
2.48	3.62	6.20	6.93	6.79	3.74	4.43	6.59	6.96	6.91
2.92	2.72	3.36	4.53	4.41	1.48	1.51	3.85	4.75	4.18
3.61	4.36	7.93	7.84	7.28	4.15	4.83	7.15	7.40	6.80
3.18	2.53	4.49	3.32	4.36	3.48	2.08	4.34	2.15	3.56
3.36	4.20	5.52	5.79	4.82	2.11	3.30	6.45	5.80	5.40
2.38	2.72	3.87	2.43	2.71	1.52	2.66	3.53	2.15	2.38
1.52	3.54	4.48	5.61	5.53	1.49	3.48	4.26	4.51	5.23
1.49	2.32	2.56	3.41	4.63	2.08	2.18	3.38	3.49	3.43
1.48	2.23	5.63	6.40	6.78	1.48	3.60	5.04	6.46	6.08
2.23	1.48	2.49	2.15	4.32	1.48	2.51	3.36	4.15	4.34
1.49	2.63	6.30	6.23	7.53	3.30	3.26	6.66	6.77	8.11
1.48	1.60	2.15	3.11	4.34	1.60	2.00	2.66	3.96	4.08

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