AN ABSTRACT OF THE THESIS OF

Elamin Abdalla Elkhalifa for the degree of Master of Science

in Food Science and Technology presented on February 21, 1980

Title: THE EFFECT OF PRERIGOR ELECTRICAL STIMULATION ON pH DECLINE, PROTEIN SOLUBILITY AND WATER-HOLDING CAPACITY IN BEEF MUSCLES

Abstract approved: _____
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This study was designed to investigate the rate of pH decline, changes in protein solubility and in the water-holding capacity (WHC) of beef muscles subjected to pre-rigor electrical stimulation.

Samples for the experiment were obtained from animals slaughtered at the Meat Science Laboratory, Oregon State University. Nine good quality steers were slaughtered, split longitudinally into sides and the left sides were electrically stimulated for 1 min (600 volts, 7 amps, 60 cycles per sec) within 30-40 min postmortem. Samples were removed from the semitendinosus (ST) and longissimus dorsi (LD) muscles of each side for the appropriate analyses at 1-, 2-, 4- and 24-hr postmortem intervals.

Immediately following electrical stimulation, pH of the treated samples of both muscles was significantly lower ($P < 0.01$) than their respective controls. Similar differences were also noted at 2 and 4 hr postmortem. At 24 hr postmortem, the pH of the stimulated

and control samples of ST muscle were essentially equal, whereas the pH of the stimulated LD muscles was significantly lower ($P < 0.05$) than the controls.

WHC measurements revealed that electrical stimulation had no effect on the treated samples of ST muscles. The stimulated LD muscles had significantly lower ($P < 0.05$) WHC values than the controls during the first 2 hr postmortem. However, the differences were not significant at 4 and 24 hr postmortem.

Initially, the solubility values of the sarcoplasmic porteins of the stimulated samples of both muscles were significantly higher ($P < 0.05$) than their controls. At 24 hr postmortem, sarcoplasmic protein solubility values were equal for both the stimulated and control samples of the ST muscles, whereas those of the stimulated LD muscles were significantly lower ($P < 0.1$) than the controls.

The solubility values of the myofibrillar proteins of the stimulated samples of both the ST and LD muscles were significantly ($P < 0.05$) lower than the controls at 1 and 2 hr postmortem. At 4 and 24 hr postmortem, solubility of the myofibrillar proteins of the stimulated ST muscles had increased but not significantly. At 24 hr postmortem, myofibrillar protein solubility values of the stimulated LD muscle samples were significantly ($P < 0.1$) higher than the controls.

Solubility values of the salt-soluble proteins were lower in the stimulated samples than in the controls of both muscles during the 24 hr postmortem test period. At 4 and 24 hr postmortem, solubilities of the stimulated ST and LD muscles samples were significantly less, $P < 0.05$ and $P < 0.01$, than their respective controls.

Data of this study show that electrical stimulation of beef muscles results in an accelerated rate of pH decline and a substantial reduction in the solubility of the myofibrillar proteins during the first 2 hr postmortem. During this interval, however, the WHC and the solubility of the salt-soluble proteins of the stimulated muscles are at higher levels than at later postmortem times. In fact, solubility of the salt-souble proteins of the stimulated muscles was markedly reduced at the 4 and 24 hr sampling times. These data suggest that if electrically stimulated beef is to be used in the production of processed meat products it should be utilized within 1 to 2 hr post stimulation when the WHC and the solubility of the salt-soluble proteins are at their peak levels.

The Effect of Prerigor Electrical Stimulation on pH Decline, Protein Solubility and Water Holding Capacity in Beef Muscles

by

Elamin Abdalla Elkhalifa

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Commencement June 1980

APPROVED:

Professor of Food Science and Technology
in charge of major

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Date thesis is presented February 21, 1980

Typed by Sharon Mosley for Elamin Abdalla Elkhalifa

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. A. F. Anglemier, my advisor, for his guidance and assistance during the realization of this thesis.

Special thanks are extended to the members of my graduate committee, Dr. J. Krueger, Dr. R. Scanlan and Dr. W. Gamble. I extend my gratitude to Dr. W. H. Kennick for supplying the samples for the experiment.

I owe my gratitude to my mother, my great brother, El Tayeb, and the rest of my family for their letters of encouragement and support during the course of this study.

Further and special thanks are extended to the Sudan government who sponsored my scholarship.

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THE EFFECT OF PRERIGOR ELECTRICAL STIMULATION ON pH DECLINE, PROTEIN SOLUBILITY AND WATER HOLDING CAPACITY IN BEEF MUSCLES

INTRODUCTION

Tenderness is one of the more important attributes of meat affecting its acceptability as a food. The tenderness of meat is notoriously variable. It varies not only among anatomically different muscles but also among identical muscles from animals of the same species. Tenderness is influenced by both pre- and post-slaughter factors. Over the years considerable research has been directed towards establishing the effects of pre-slaughter factors such as species, breed, age, sex, nutrition and exercise, and of post-slaughter treatments such as aging (i.e., prolonged storage at temperatures above freezing) and freezing.

Initially, the connective tissue component of meat received the greatest research attention (Locker, 1976). However, there is now ample evidence that changes in myofibrillar component pre-rigor (i.e., during the period between slaughter and full development of rigor mortis) can markedly influence the tenderness of the resulting meat (Marsh, 1977).

Methods of handling carcasses immediately postmortem (until the onset of rigor mortis) have been shown to affect the ultimate tenderness of meat (Newbold and Harris, 1972). The most common problem associated with pre-rigor meat is "cold shortening" which is the major cause of meat toughness. Cold shortening results from the early exposure of carcasses to cold. Rapid chilling induces shortening of

muscles of 60-70% of their initial length. This shortening is accompanied by marked increases in toughness (Locker and Hagyard, 1963). Conclusions drawn from research on cold shortening have led to the realization that postmortem treatments far outweigh live-animal factors such as breed, age, and pre-slaughter state in determining eating quality. Different approaches have been investigated to restrict or prevent cold shortening by physical restraint (Herring et al., 1965; Hostetler et al., 1970, 1973; Quarrier et al., 1972, Davey and Gilbert, 1975; Smith et al., 1971) or by the use of high temperature conditioning during the development of rigor mortis (Parrish et al., 1969; Smith et al., 1976; Dutson et al., 1975; Fields et al., 1976). Although these procedures improved tenderness, certain problems arose which have deterred industrial application of either method. Changes in carcass conformation and muscle shape were encountered with the use of nonconventional methods of carcass suspension. The use of high temperature conditioning results in an undesirable, and somewhat costly, delay in processing. Moreover, storage facilities for this procedure must be equipped with sensitive environmental controls to prevent excessive carcass weight losses through evaporation, to retard microbial growth and to minimize subsequent spoilage.

With recent advances in meat science it now becomes possible to consider other less costly methods for increasing tenderness and/or preventing cold shortening. One method showing particular promise is electrical stimulation of the carcass soon after the death of the animal. The concept of electrical shock to improve tenderness is not new; its use for this purpose was first suggested by Benjamin

Franklin in 1749 to improve tenderness of turkeys (Lopez and Herbert, 1975). In 1951, Harsham and Deatherage patented (U.S. patent 2544681) a process for electrical stimulation of beef carcasses. A similar process is now used commercially in New Zealand for accelerated conditioning of lambs, to prevent toughening of lamb carcasses when rapidly chilled in a pre-rigor state (Carse, 1973; Chrystal and Hagyard, 1975, 1976, Davey et al., 1976).

Results of early research in the United States and New Zealand suggested that electrical stimulation accelerated postmortem pH decline, hastened rigor development and improved tenderness.

The mechanism(s) by which electrical stimulation improves tenderness has not been elucidated. Postulations include reduction in cold shortening, (Davey et al., 1976; Chrystall and Hagyard, 1976), increased proteolytic activity by natural enzymes (Cross, 1979; Parrish, 1977) and physical disruption of muscle fibers (Savell et al., 1978). The method has become of increasing interest to meat processors because (a) it requires little change in normal abattoir practice and (b) the removal of meat from the carcass pre-rigor (hot boning) could become a practical possibility.

This study was conducted to determine the changes occurring in certain characteristics of beef muscles subjected to pre-rigor electrical stimulation. Rate of pH decline, alterations in protein solubility and the water-holding capacity of the treated muscles were the particular properties investigated.

LITERATURE REVIEW

Muscular tissue is a major item of interest in any discussion on the subject of meat science. The present knowledge of muscle is vast and ranges from gross aspects to the molecular architecture. The molecular basis of muscle organization provides an explanation for many long-known characteristics of muscle.

Muscle Structure

Skeletal muscle is composed of long, narrow, multi-nucleated fibers (cells) ranging from a few to several centimeters in length and from 10-100 μm in diameter. The fibers are arranged in parallel fashion to form bundles, and groups of bundles make up a muscle. Each of the above units is surrounded by a sheath of connective tissue; the muscle itself is surrounded by the epimysium, the fiber bundle by the perimysium, and the fiber by the endomysium and sarcolemma.

The basic structural unit of the muscle fiber is the myofibril. Myofibrils are long, thread-like rods, composed of light bands (I-bands), dark bands (A-bands) and transverse Z-lines that divide the myofibrils into regular units called sarcomeres. The sarcomeres are the contractile units of the myofibrils. The origin of the alternating light and dark bands was explained in 1954 when H. E. Huxley and Jean Hanson (Huxley and Hanson, 1954), and independently,

A. F. Huxley and R. Niedergerke (Huxley and Niedergerke, 1954), proposed the interdigitating thick and thin filament structure for the myofibril (Figure 1). This interdigitating thick and thin filament array has enormous implications for all phases of meat science, and a strong case can be made for the proposal that discovery of this structure is the single most important finding thus far made in meat science.

Muscle Proteins

The muscle proteins are a specialized class of proteins. They include proteins capable of rapid contraction and relaxation which are surrounded by a connective protein tissue network to bind them into place and a complex system of enzymes which supply the muscle elements with energy (Bendall, 1964). Although the uninitiated may think that the term "muscle proteins" is used only in a generic sense to include all proteins in muscle, many biologists have come to accept the term "muscle proteins" as referring exclusively to the contractile or myofibrillar proteins and excluding the cytoplasmic, membrane, and connective tissue proteins also found in muscle. Use of the term "muscle proteins" should be reserved exclusively to refer to all proteins found in muscle cells (e.g., myoglobin is a noncontractile protein found exclusively in muscle cells), and the myofibrillar or contractile proteins should be designated specifically by name (Goll et al., 1977). Muscle proteins can be broadly divided into those which are soluble in water or dilute salt

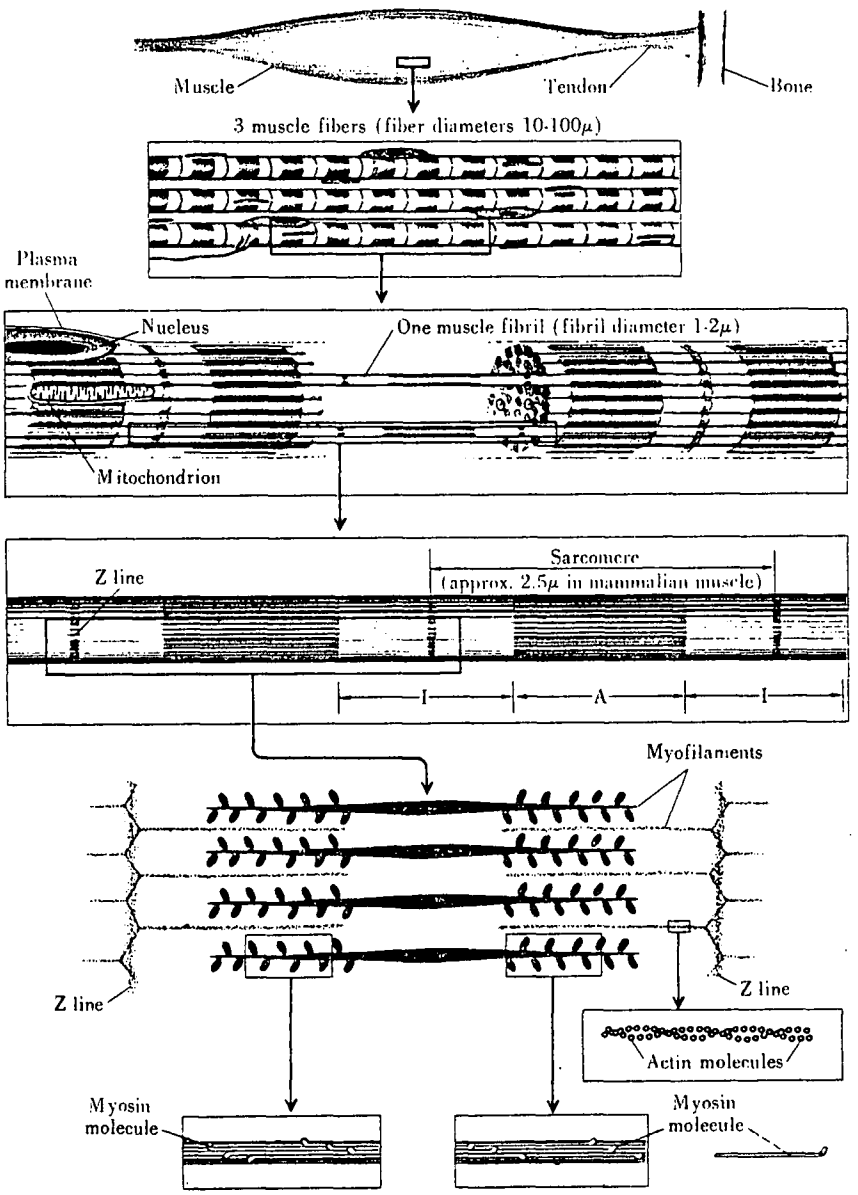

Figure 1. Schematic diagram showing structure of mature vertebrate skeletal muscle at several different levels of organization ranging from an entire muscle (top of figure) to the molecular architecture of myofilaments (bottom of figure). (From Novikoff and Holtzman, 1970)

solutions, those which are soluble in concentrated salt solutions and those which are insoluble (Lawrie, 1974). Differences in the extractibility of muscle proteins form a basis for their classification into the following three major categories: sarcoplasmic proteins, myofibrillar proteins, and stroma proteins (Goll et al., 1970, 1974). Distribution of these proteins and their abundance (% wet weight) in a typical mammalian muscle after rigor mortis, but before the onset of marked postmortem degradative changes, are shown in Table 1.

Sarcoplasmic Proteins

The sarcoplasmic protein fraction is the most soluble of the three classes of muscle proteins and generally includes those proteins found in the cytoplasm of the muscle cell. They can be brought into solution readily with either water or low ionic strength solutions of 0.1 or less at neutral pH. A solution thus obtained has low viscosity (Szent-Gyorgyi, 1960). Because of the ease with which these proteins are extracted, they are frequently mentioned as the "soluble proteins" of the muscle. They constitute a very significant portion of the proteins of the cell, usually from 30-35% of the total proteins in skeletal muscle. Exact protein composition of the sarcoplasmic protein fraction is influenced to a considerable extent by conditions used during extraction and may vary depending on speed and extent of homogenization of the tissue before extraction, pH of the extraction, nature of the extracting solvent, and centrifugal force used to

Table 1. Chemical composition of typical mammalian muscle after rigor mortis but before degradative changes postmortem.*

Components		% Wet Weight	
1. WATER			75.0
2. PROTEIN			19.0
(a) Myofibrillar		11.5	
myosin (H and L meromyosins, and proteins associated with them)	6.5		
actin	2.5		
tropomyosin	1.5		
troponins T, I and C	0.4		
α-and β-actinin	0.4		
M. proteins etc.	0.2		
(b) Sarcoplasmic		5.5	
glyceraldehyde phosphate dehydrogenase	1.2		
aldolase	0.6		
creatine kinase	0.5		
other glycolytic enzymes	2.2		
myoglobin	0.2		
haemoglobin and other extracellular proteins	0.4		
other unspecific proteins	0.4		
(c) Connective tissue and organelle		2.0	
collagen	1.0		
elastin	0.05		
mitochondrial etc.	0.95		
3. LIPID			2.5
4. CARBOHYDRATE			1.2
5. MISCELLANEOUS SOLUBLE NONPROTEIN SUBATANCES			2.3
(a) Nitrogenous		1.65	
(b) Inorganic		0.65	
6. VITAMINS			
Quantitatively minute amounts			

*Adapted from Lawrie (1975).

separate the soluble sarcoplasmic protein fraction from unsolubilized proteins and subcellular organelles (Goll et al., 1970). Homogenization is a critical factor in any protein extraction procedure. However, with the advent of high speed homogenizers, efficient cell dispersion and extraction can be accomplished without resorting to liquid nitrogen ($L\text{-}N_2$) pulverization techniques (Hwang et al., 1977). Under most extraction conditions used, the sarcoplasmic protein fraction contains all of the enzymes associated with glycolysis and most of the enzymes involved with carbohydrate and protein synthesis, because these processes occur largely in the cytoplasm of the muscle cell. These enzymes, in addition to being soluble at low ionic strength, are free and readily solubilized when the muscle cell is ruptured. Other soluble nonprotein nitrogenous substances and inorganic material are also present in the sarcoplasmic extract. In addition, myoglobin, a sarcoplasmic protein that is unique to muscle, is present in fairly large amounts in most muscle cells (Table 1).

The glycolytic and associated enzymes are not evenly distributed in quantity in the sarcoplasm. It has been reported that one enzyme alone, glyceraldehyde phosphate dehydrogenase, accounts for over 20% of the sarcoplasmic protein (Czok and Bucher, 1960). This, together with aldolase, enolase, kinases and lactate dehydrogenase make up about half of the sarcoplasmic protein. Several of these enzymes consist of more than one molecular species (Scopes, 1970). Nevertheless, some proteins have been isolated from the sarcoplasm of various species that have not been identified. According to Scopes (1966, 1968), there must be many uncharacterized proteins in muscle

sarcoplasm, mostly in minute quantity, to carry out the normal functions of cellular metabolism in addition to glycolysis. The specific term, myogen, used extensively in the older literature, refers to entire sarcoplasmic protein fraction containing at least 100-200 different proteins and not to a single homogeneous protein (Goll, et al., 1977).

The sarcoplasmic proteins do not contribute significantly to the filamentous organization of muscle. Their function is mainly concerned with the metabolic activities of the cell. It seems likely that the myofibrillar lattice in vertebrate striated muscle contains very little sarcoplasmic protein and is filled largely with water (84%), a considerable proportion of which is bound water. Many of the proteins of the sarcoplasm are important in affecting the quality and appearance of meat. Not only the major protein components, but also trace quantities of certain enzymes, can have beneficial or detrimental effects on various meat products.

Myofibrillar Proteins

The myofibrillar proteins make up 50-55% of the total muscle protein. They are generally defined as those proteins that are not extracted at low ionic strengths ($\mu < 0.2$) but are solubilized at high ionic strengths ($\mu > 0.4$). However, it is now known that some of the recently discovered myofibrillar proteins can be extracted at very low ionic strength ($\mu < 0.01$) upon disruption or maceration of the muscle tissue. Once extracted, many of the myofibrillar proteins are soluble throughout an ionic strength range of 0 to 1.5; thus the

myofibrillar proteins may be defined as those proteins that constitute the myofibril (Goll, et al., 1970).

For their extraction, neutral salt solutions of high ionic strength ($\mu > 0.5$) are required (Szent-Gyorgyi, 1960), even though after extraction, some of them as mentioned previously are highly soluble at lower ionic strength. Their resistance to extraction is partly the result of intimate associations and interactions between proteins within the myofilaments.

Many workers have shown that myosin is located exclusively in the thick filament, whereas actin, tropomyosin, troponin, and β-actinin reside in the thin filaments (Figure 1). The protein composition compiled from various sources is approximately 50-55% myosin, 15-20% actin, 5-8% tropomyosin, 5-8%troponin, 2-3% α-actinin, 0.5-1% β-actinin, and 5-8% of poorly identified proteins (Goll, et al., 1974).

A myosin molecule resembles a thin rod with two small globular "heads" at one end (Figure 1). It is built from two types of subunits, light (L) and heavy (H) meromyosins (Szent-Gyorgyi, 1953). Light meromyosin is insoluble in water, is soluble at ionic strengths above 0.3, has no ATP-ase activity or actin-binding ability, is almost 100% α-helical in nature, and under proper conditions will form filaments that resemble thick filaments without cross-bridges. Heavy meromyosin is soluble in water, has both ATP-ase activity and actin-binding ability, does not form filaments, is approximately 45% α-helical, and consists of two globular heads attached to a short

rod (Lowey et al., 1969). The properties depend upon free-SH groups in the molecule (Bailey, 1954).

Myosin can be extracted from a muscle mince with strong salt solutions of slightly alkaline conditions and then can be precipitated free of the various water soluble proteins upon reduction of ionic strength (Briskey, 1967). Solutions commonly used for extraction are 0.3 M KCL and 0.15 M phosphate at pH 6.5 (Guba, 1943), or 0.47 M KCL, 0.1 M phosphate, and 0.01 M pyrophosphate at pH 6.5 (Hasselbach, 1951). If a short period of time is used for extraction, a crude myosin, called myosin A, is produced. If longer periods of extraction (e.g., overnight) are used, then a crude actomyosin preparation, called myosin B, is obtained.

The other major protein of the myofibril is actin (Straub, 1942). It can exist in two forms, G-actin, which consists of relatively small globular units having a molecular weight of about 70,000, and F-actin, in which these golbular units are aggregated end to end to form a double chain of high molecular weight (Figure 1). G-actin polymerizes into F-actin in the presence of salts and small amounts of ATP. It is F-actin which combines with myosin to form the contractile actomyosin of active or pre-rigor muscle and the inextensible actomyosin of muscle in rigor mortis (Lawrie, 1974). The interaction of actin and myosin in the absence of ATP and ADP, and the plasticizing effect of these nucleotides when they are present, is a very important factor influencing the quality of meat.

Tropomyosin was isolated by Bailey in 1946, and when prepared according to his procedure, it is referred to as tropomyosin B. Tropomyosin A (paramyosin) is found in certain invertebrate muscles capable of prolonged tetanic contraction (Poglazov, 1966). Tropomyosin is resistant to denaturing effects of organic solvents such as alcohol, acetone, or ether (Bailey, 1948). It can be separated from muscle residue at a very low ionic strength (Perry and Corsi, 1958), but with difficulty at higher ionic strengths, unless actin is denatured.

Troponin is composed of three subunits which are intimately concerned in the contraction process. The largest subunit, troponin-T, binds strongly to tropomyosin. A smaller subunit, troponin-I, binds to actin and can inhibit the actin-myosin interaction. Troponin-C, which binds calcium, is the smallest subunit (Cohen, 1975). According to Perry (1967), troponin preparations usually contain tropomyosin. Schaub and Perry (1969) described a method for the preparation of troponin free of tropomyosin from low ionic strength extracts of myofibrils.

Two other porteins found in small quantities but which may have an important role in muscle function are α-actinin and β-actinin. α-Actinin was first isolated and studied by Ebashi and Ebashi (1964, 1965). The amino acid composition of α-actinin resembles that of actin; thus it has been suspected of being denatured actin. Ebashi (1966) indicated that α-actinin is located in the Z-band and perhaps in the H-band, while Goll et al. (1967) and Masaki et al. (1967) reported that it was present in the Z-band. β-actinin was discovered

by Maruyama (1965), who reported that this protein regulates the length of F-actin filaments (inhibits network formation in F-actin) at the length at which they occur *in vivo*. Ebashi (1966) suggested that β-actinin may function in muscle development rather than in muscle contraction. The amino acid composition of this protein is also similar to that of actin.

Other as yet unidentified proteins are probably also present within the contractile apparatus (Goll *et al.*, 1977).

Stroma Proteins

These proteins make up the connective tissues. They are often called the insoluble proteins because they are retained in the residue after exhaustive extraction of all salt-soluble muscle proteins. If prior extraction of myofibrillar proteins has not been done thoroughly or has been done at temperatures above 2° where myofibrillar proteins tend to become denatured and insoluble, the insoluble protein residue measured as stroma proteins may contain large amounts of unextracted myofibrillar proteins that may be erroneously included in the stroma protein fraction. Collagen, elastin and reticulin are the major stroma proteins. Because of their low content of charged and hydrophilic amino acid, stroma proteins reduce the water holding capacity of meat (Goll *et al.*, 1977).

Postmortem Changes

Rigor Mortis

Stiffening and the loss of extensibility are the most obvious physical changes that occur in postmortem muscle. These changes become pronounced with the onset of rigor mortis. In the rigor process, the sarcomere length decreases, cross links are formed between the actin and myosin filaments of the myofibril and the whole system becomes rigid and inextensible. Rigor mortis is frequently referred to as the "stiffness of death."

Stiffening and inextensibility are generally accompanied by the production of lactate and by marked acidification although neither of these changes is a prerequisite for the rigor process. The obligatory condition for the onset of rigor is the depletion of adenosine triphosphate (ATP), which acts as a plasticizer between the actin and myosin filaments to keep them in a flexible and easily extensible state. The time to the onset of rigor mortis (at a given temperature) is directly dependent on the supply of muscle ATP which is being slowly exhausted by the surviving sarcoplasmic ATP-ase activity. The latter operates under local control in an effort to maintain body heat and the structural integrity of the muscle cell (Lawrie, 1974). Theoretically, with a knowledge of the temperature, the initial store of glycogen and the initial levels of ATP and creatine phosphate (CP), the time to onset of rigor mortis can be predicted accurately (Bendall, 1951).

Postmortem Glycolysis

The chemistry of postmortem changes in muscle is primarily that of the high energy phosphate compounds and the mechanisms involved in their synthesis and degradation. Thus, glycolysis with all of its ramifications is involved and has extensive implications. Through a series of reactions, the potential chemical energy of glucose is utilized in the synthesis of ATP. The latter is the high energy compound that is the direct source of muscle energy (Pearson, 1971).

Postmortem glycolysis reflects the basic function of muscle when oxygen is permanently removed from the muscle at death. The sequence of chemical steps by which glycogen is converted to lactic acid is essentially the same postmortem as *in vivo* when the oxygen supply may become temporarily inadequate for the provision of energy in the muscle; but it preceeds further (Lawrie, 1974).

Muscle pH decreases from 7.2 to about 5.5 during rigor because muscle glycogen at the time of death is converted to lactic acid through the anaerobic glycolytic system. The process cannot proceed on through the citric acid cycle as *in vivo* because this cycle requires oxygen which is no longer available after death. Since the production of lactic acid is dependent on the supply of muscle glycogen, the level of this component at the time of death determines the ultimate pH. The conversion of glycogen to lactic acid will continue until a pH is reached which inactivates the glycolytic enzymes. The final pH obtained is referred to as the ultimate pH (Bendall, 1964).

Both the rate and the extent of the postmortem pH decline are affected by intrinsic factors such as species, type of muscle, and the variability between animals, and by extrinsic factors such as pre- or post-slaughter practices and the environmental temperature (Lawrie, 1974).

After death and during postmortem glycolysis, ATP is continuously utilized and continues to be rephosphorylated until the reserve energy supply, creatine phosphate, is exhausted. At this point, ATP is no longer resynthesized, the level drops and cross links between actin and myosin filaments become rigid (rigor mortis).

Water-Holding Capacity

Muscles contain about 75% water, of which perhaps 5% is bound water, and almost all of the remainder is entrapped bulk phase water (Hamm, 1975). The ability of muscle to immobilize its bulk phase water during the application of various stresses such as pressure, heating, grinding, etc. is referred to as water-holding capacity (WHC).

The myofibrils are well suited to hold water because of the three-dimensional network of the filaments, a lattice-type structure which persists after homogenization of the meat. The amount of water immobilized or held within the muscle fibers by purely osmotic forces depends on the space available between the actin and myosin filaments. If the muscle fibers are contracted so that the actin and myosin filaments overlap extensively, the lattice spacings diminish and the

WHC is reduced. Conversely, relaxation of the fibers allows for larger lattice spacings and, thus, greater immobilization of water (Bendall, 1964; Goll et al., 1977).

Like the swelling of any protein gel, the WHC of the muscle filaments is highly dependent on the pH, reaching a minimum at the isoelectric point and rising sharply on either side of it (Hamm, 1960). Muscles have the lowest WHC at about pH 5.0 which is the isoelectric point of actomyosin, the major protein component of postrigor muscle (Hamm and Deatherage, 1960).

A lowering in WHC accompanies the onset of rigor mortis. Even when rigor occurred at a high pH, a loss in WHC was noted (Marsh, 1952). This was attributed to the depletion of ATP coupled with the formation of actomyosin and a subsequent tightening of muscle structure.

Different muscles of cattle have different water-holding capacities (Empey and Howard, 1954; Howard and Lawrie, 1956). Even within the same muscle, differences of WHC have been noted. Paul and Bratzler (1955) found that steaks from the anterior portion of the longissimus dorsi muscle of beef carcasses showed lower cooking losses than steaks from the posterior area.

Water-holding capacity of meat is related to some important organoleptic properties such as juiciness and tenderness. Thus, WHC can serve as an index of palatability (Miller and Harrison, 1965) as well as an indicator of microbial activity (Jay, 1967) and manufacturing potential (Saffle, 1968).

Protein Solubility

Of all animal tissues, muscle undergoes possibly the most marked biochemical and physicochemical changes after death. Postmortem changes in the sarcoplasmic protein fraction have long interested medical researchers and those concerned with muscle as food, but it has been very difficult to obtain definitive data regarding these changes. This is largely due to the tremendous complexity of the sarcoplasmic protein fraction and to the fragility of the sarcolemma, which is a very labile membrane and may change rapidly after death (Onser, 1966; Reed, Houston and Todd, 1966).

Many studies have attempted to determine the gross effects of postmortem storage on sarcoplasmic proteins by measuring changes in their solubility. Because of the many different homogenization conditions and extracting solvents used, it is difficult to compare accurately the results of many of these studies. Moreover, many investigators used water to extract sarcoplasmic porteins from both at-death and postmortem muscle. Since the pH of postmortem muscle is usually below 6.0, whereas the pH of at-death muscle is near 7.0, use of unbuffered water for extraction of sarcoplasmic proteins results in extraction of the at-death fraction at a pH value near 7.0 while the postmortem fractions are extracted at some varying pH value, usually below 6.0. This difference in extraction pH alone may cause substantial differences in the type of protein components obtained, regardless of any postmortem alterations in the sarcoplasmic proteins themselves.

The rate and severity of any postmortem decrease in solubility depend critically on both rate of pH decline in the postmortem tissue and temperature. If muscle pH decreases below 6.0 while muscle temperature is still 35° or higher, sarcoplasmic protein solubility is decreased substantially (Sayre and Briskey, 1963; Scopes, 1964; Briskey and Sayre, 1963; Scopes and Lawrie, 1963). On the other hand, very rapid cooling (L N_2) of at-death muscle to temperature of 0-4° and subsequent storage at these temperatures allows the solubility of the myofibrillar and sarcoplasmic proteins to remain high, near the at-death level (Borchert and Briskey, 1965; Goll, Henderson, and Kline, 1964). Disney et al. (1967) investigated the biochemical changes resulting from glycolysis and ATP degradation in the semimembranosus and semitendinosus muscles of beef carcasses during the first 24 hours postmortem and from the effects of ice cooling compared with normal air-cooling at the slaughter plant. They found that a continuous loss of protein solubility occurred during the first 24 hours accompanied by a decrease in water-holding capacity.

Results of starch gel electrophoresis and analytical ultracentrifugation studies have indicated that decreased solubility of sarcoplasmic proteins after postmortem storage at 37° is due, at least in part, to denaturation and consequent insolubility of creatine kinase (Borchert, Powrie, and Briskey, 1969), lactic dehydrogenase (Kronman and Winterbottom, 1960), triosephosphate isomerase and F-protein (Borchert, Powrie and Briskey, 1969). On the other hand, with storage at temperatures of 5° or lower, gel electrophoresis patterns

of sarcoplasmic proteins extracted from at-death muscle were very similar to those extracted from postmortem muscle, even after 336 hours of storage (Aberle and Merkel, 1966).

Maxon and Marion (1969) studied the changes in protein solubility of turkey breast muscle taken at 0, 2, 4, 8, 24, 48 and 72 hours postmortem. They found protein solubility increased steadily up to 48 hours but decreased at 72 hours. The myofibrillar protein exerted a major influence on the pattern of the total protein extractability. However, alterations in myofibrillar proteins have been limited to measurements of their solubility in different solvent systems. Although the relatively small number of different myofibrillar proteins would seem to offer the possibility that postmortem changes in their solubility could be interpreted in terms of alterations in specific proteins, myofibrillar protein solubility is complicated by many strong and highly specific interactions which occur among these proteins. Moreover, certain substances such as ATP or KI have marked solubilizing effects on myofibrillar proteins. Recent ultrastructural and biochemical studies by many workers have produced evidence of the effect of postmortem storage on the myofibrillar proteins. They undergo at least two kinds of specific alterations: (1) a loss of Z-line structures, and (2) modification of the actin-myosin interaction.

Actin extraction is preceded by a break between the actin filaments and the Z-line structures. Moreover, since actin has strong affinity for myosin, the relative inextractability of actin may also have a marked effect on the extractability of myosin. Thus, in the absence of any agent to weaken the actin-myosin interaction,

actin will bind myosin and prevent its complete solubilization. In these circumstances, the rate of myofibrillar protein extraction will be controlled primarily by the rate of actin extraction (Haga et al., 1966). Davey and Gilbert (1968) characterized the protein solubilized from myofibrils by pyrophosphate-containing solutions. They showed only myosin was extracted from at-death myofibrils whereas extracts of postmortem myofibrils contained actin and a second water soluble protein in addition to myosin.

On the other hand, a weakening of the actin-myosin interaction was suggested by Penny (1968) who observed that 1M KCL solutions extracted larger amounts of protein from myofibrils stored for seven days at 4° than from freshly prepared myofibrils, and that this increased extractability was due almost entirely to increased solubilization of myosin. Since 1M KCL solutions do not cause dissociation of the actin-myosin complex, this increase in myosin extractability was interpreted to be indicative of a weakening of the actin-myosin interaction in stored myofibrils so that actin no longer bound myosin strongly enough to prevent its solubilization. Other factors, such as the rate, temperature and extent of postmortem pH decrease, reduced the solubility of both salt- and water-soluble protein fractions (Trautman, 1966). Sayre and Briskey (1963) reported that myofibrillar protein solubility ranged from no reduction during the first 24 hours after death, when pH remained high at rigor onset, to 75% reduction in muscle with low pH and high temperature at the onset of rigor mortis. They also reported that the 24-hour pH of the muscle appeared to have only a minor effect on protein solubility.

They further concluded protein solubility appeared to be one of the major factors affecting the juice-retaining properties of muscle. Brendl and Klein (1970) graphically demonstrated the relationship of postmortem changes in water-holding capacity, pH and the solubility of the myofibrillar proteins in Figure 2. These data indicate a continuous decrease during the first 24 hours postmortem.

Comparisons were made of the extractable salt-soluble protein content between pre- and post-rigor normal and low-pH muscles. Johnson and Henrickson (1970) found that pre-rigor normal pH muscle contained 69.9% more extractable salt-soluble protein than post-rigor normal-pH muscle, whereas pre-rigor low-pH muscle contained only 7.3% greater extractable salt-soluble protein that post-rigor low-pH muscle.

Attempts have been made to relate the solubility of different protein fractions of muscle to tenderness. Goll et al. (1964) reported that protein solubility did not appear to be related to tenderness. Their findings are contradictory to those of Hegarty et al. (1963), who found a positive relationship between myofibrillar protein solubility and tenderness.

Meat Tenderness

Considerable scientific evidence has accumulated in recent years that provides a much clearer understanding of meat tenderness. Two structural muscle components, collagen of connective tissue and the contractile protein complex, determine meat tenderness. For many years, collagen (the amount and quality) was regarded as the sole

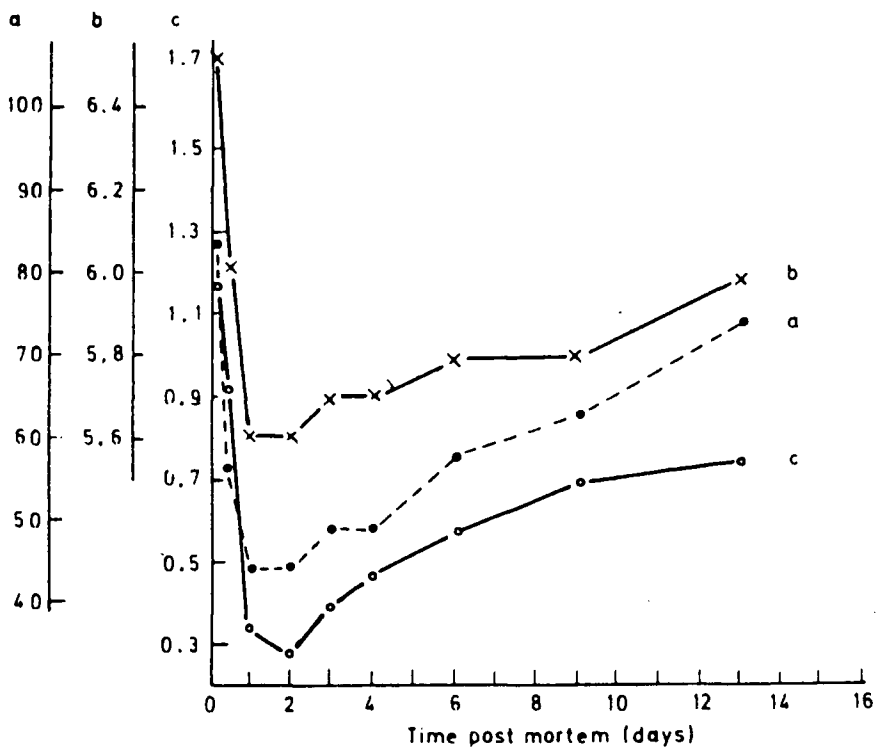

Figure 2. Changes in the water-holding capacity (a), pH (b) and soluble myofibrillar proteins (c) of beef muscle at different postmortem times.
(From Brendl and Klein, 1970)

cause of tenderness variation. During the past 20 years, however, the contractile protein complex has been found to be a significant contributor to toughness. Yet, its full relationship to meat quality is still not universally appreciated. This does not imply that collagen has only a minor role in tenderness but that the contractile protein complex is much more amenable to manipulation to control tenderness (Marsh, 1977; Newbold and Harris, 1972).

Tenderness or toughness due to the contractile proteins is determined by temperature conditions encountered during the initial postmortem period. Rapid chilling of beef or lamb in the early postmortem stage causes "cold shortening" of the muscles coupled with a marked toughening effect (Davey et al., 1976; Locker and Hagyard, 1963).

In cold shortening, the sarcoplasmic reticulum is stimulated by external temperatures lower than 15° to release calcium ions. Temperatures below 15° also appear to stimulate myofibrillar ATP-ase activity. These conditions in effect lead to a marked interdigitation of the myosin and actin filaments, i.e., shortening. Thus, cold shortening bears a strong resemblance to the contraction of living muscle because both are triggered by a release of calcium ions. Low temperatures (< 15°) in the early prerigor state apparently damage the sarcoplasmic reticulum and hinder its ability to sequester calcium ions. The latter condition allows the calcium ions to remain free to perpetuate the cold shortening effect (Lawrie, 1974; Marsh, 1977).

The state of muscle contraction at the time rigor mortis develops has a marked influence on tenderness. Muscles allowed to contract during rigor development are very tough. Toughening is maximal at about 40% shortening, and this cold shortening phenomenon occurs when muscle is chilled to about 15° or lower prior to the onset of rigor. Cold shortening and its accompanying toughening effect can be prevented by holding meat at 15-20° until rigor mortis develops. This is known as "conditioning." This procedure is effective, but it is wasteful of time, energy, space and labor (Marsh, 1977; Newbold and Harris, 1972).

Cold shortening of muscle decreases with postmortem time. It can be minimized if the actin and myosin filaments are allowed to "lock" into rigor before the muscle is chilled below 15°. Once crossbridging of the filaments has occurred, there is no cold shortening effect irrespective of temperature decline. With lamb, cold-induced toughening can be avoided by holding the carcasses at 15-20° for 16 to 20 hours until the incipient rigor bonds have locked the contractile structure into the appropriate configuration before moving it to colder temperatures (Bendall, 1976; Carse, 1973; Chrystall and Hagyard, 1976). Adaptation of this process to beef is not practical because the larger carcasses maintain higher internal temperatures for longer times. This would allow for development of deep spoilage and the possible proliferation of potentially pathogenic mesophiles. Thus, other methods of reducing the cold shortening effect are being sought.

One promising approach is to accelerate the rate of postmortem glycolysis to reduce the time for onset of rigor mortis. When the

onset of rigor is hastened, only a brief prechilling delay is needed to prevent cold shortening.

Electrical Stimulation

Postmortem electrical stimulation has recently received considerable attention as a possible means for improving meat quality. The results of the early American, British and New Zealand research suggest that electrical stimulation accelerated postmortem pH decline, hastened rigor development and improved tenderness (Bendall and Rhodes, 1976; Carse, 1973; Chrystall and Hagyard, 1976; Davey et al., 1976; Savell et al., 1977; Smith et al., 1977).

It is difficult to compare results of these studies directly because of the wide variations in experimental conditions employed, particularly in respect to voltage applied. Levels as high as 700V (Bendall and Rhodes, 1976) and 1600V (Davey et al., 1976) or even 3600V (Chrystall and Hagyard, 1976) have been used. High voltages present major safety problems, expecially in slaughter plants where strict safety precautions are essential.

Bendall (1976) investigated the effects of electrical stimulation (250V, 2 minutes) of lamb carcasses immediately after slaughter. He reported a rapid immediate decline of pH and a sustained rate of fall 2-3 times greater than normal. In addition, he found that the ATP level fell in parallel with the pH; at about pH 6.0, 50% of it had disappeared and at pH 5.7, more than 90%. Carse (1973) also stimulated lamb carcasses at 250V and observed that muscle pH fell from about seven to six in three hours as compared to 15.4 hours in the

controls. Data of other investigators (Bouton et al., 1978; Savell et al., 1977; Shaw and Walker, 1977) who used lower voltages (100-120V) to treat beef carcasses indicate that stimulation generally reduced pH by nearly 0.7 within one hour and by 1.0 at the end of four hours. Gilbert et al., (1976) found that the ultimate pH of the stimulated meat had been attained within five hours. By the end of 24 hours the pH of both stimualted and control carcasses was essentially the same, pH $\hat{}$ 5.5.

Several investigators have reported that postmortem electrical stimulation enhances the tenderness of the cooked muscle (Bouton et al., 1978, Carse, 1973; Chrystall and Hagyard, 1975; Davey et al., 1976; Savell et al., 1977, 1978; Smith et al., 1977). Tenderness was evaluated by both subjective (sensory panel) and objective (shear force) methods. Results of both types of testing showed that stimulation caused a marked increase in tenderness. Electrical stimulation appears to be more beneficial when applied to the lower quality beef carcasses which generally have less tender meat. Texas researchers (Savell et al., 1977; Smith et al., 1977) showed that stimulation resulted in a 55% improvement in sensory panel tenderness scores of low quality beef as compared to only a 12% improvement for beef from high quality carcasses.

Theories of the mechanisms by which electrical stimulation improves tenderness suggest (1) a reduction in cold shortening, (2) increased activity of the acid proteases, and (3) physical disruption of the myofibrils. The first was described previously in relation to the acceleration of postmortem glycolysis. The second suggests

that stimulation may cause enhanced activity of the autolytic enzymes of muscles in treated carcasses (Cross, 1979). The rapid decline in pH may hasten the rupture of lysosomal membranes to release the proteolytic enzymes while muscle temperature is still high; and these effects may increase the rate or duration of autolytic proteolysis. The third theory implies that physical disruption of muscle fibers resulting from the massive contractions during stimulation may result in the tenderness improvement associated with electrical shock (Savell et al., 1978).

Even though the exact mechanisms causing quality improvement of carcass beef and lamb subjected to prerigor electrical stimulation are not known, it is an effective procedure. This process is used commercially in New Zealand for the accelerated conditioning of lamb carcasses (Cross, 1979). It prevents or minimizes toughening of lamb carcasses that are rapidly chilled in the prerigor state for export trade.

MATERIALS AND METHODS

Carcass Preparation and Stimulation

Nine good quality steers (470-550 kg) were used for the experiment. The animals were slaughtered, dressed and split longitudinally into sides according to normal slaughter plant practices at the Oregon State University Meat Science Laboratory. At 30-40 min postmortem, the left side of each carcass was subjected to electrical stimulation for one min while the right side served as unstimulated control. Stimulation was performed by use of an "Electro-Sting" electrical stunner (Model No. 1015, Type SSR). The stunner was modified for use in electrical stimulation by connecting two cables to the end of the hog stunning attachment to facilitate extension of the stunning probes. One cable was divided into two subunits. A metal pin (approximately 0.6 x 10 cm) was attached to the end of each cable, one pin was placed in the muscles of the round near the achilles tendon, the second pin was inserted in the neck region and the third pin was inserted into the forelimb. The machine setting was 600 volts, 7 amps and 60 cycles per sec.

As soon as the stimulating power was turned on, all muscles of the carcass contracted. After about 35 sec of stimulation, a slow relaxation began which continued until the power was turned off. The entire carcass then sagged as the muscles relaxed completely. Both sides were then washed and transferred to a chiller (2°) according to normal commercial practice.

Sampling Procedure

Semitendinosus (ST) and longissimus dorsi (LD) muscles were investigated in this study. Samples were taken within 40-50 min postmortem from intact muscles from both the stimulated and nonstimulated sides. They were excised from the surface to a standard depth of 10-14 cm and 4-7 cm thick. Intramuscular fat, connective tissue and approximately one cm in depth of the exposed surfaces were trimmed off. Further samples were taken in similar manner at 2, 4 and 24 hour postmortem.

Half of each sample was used for the immediate determination of pH and water-holding capacity (WHC). The remainder was quickly cut into pieces (2 x 3 x 2 cm), frozen in liquid nitrogen (Borchert and Briskey, 1964), then enclosed in polyethylene bags and stored at -40° for subsequent analyses of water-soluble, myofibrillar and salt-soluble proteins.

Measurement of pH

For pH measurements, a technique similar to that of Bouton et al. (1971, 1978) was used. The pH of the two muscles (ST and LD) from stimulated and control sides was measured at 1, 2, 4 and 24 hours postmortem. Measurements were made with an Orion Research Model 801 Digital pH Meter with a probe type combined electrode (Model 605). The probe electrode was inserted into a superficial cut made in each muscle. The pH probe was rinsed with distilled water between muscles.

The pH meter was standardized against pH 4 and pH 7 buffers and was calibrated for ambient temperature at periodic intervals. pH values for individual muscles were taken as the mean of readings from three different sites.

Water-Holding Capacity (WHC)

The method used was essentially the filter paper press technique of Grau and Hamm (1953). A 0.4-0.6 g meat sample was placed on a 11 cm Whatman No. 1 filter paper, covered with a sheet of acetate paper, then placed between two plates of a Carver Laboratory Press (No. 10719-81) and pressed immediately at a constant pressure (5000 psi) for four min. Immediately after the pressing had been accomplished the meat film area was outlined with a pencil on the acetate paper. Similarly, water squeezed out onto the filter paper was marked. The areas (in square inches) of the outer ring (total area) and of the inner ring (meat film area) were measured by planimeter. The water-holding capacity was determined in triplicate for each sample and was calculated as the ratio of total area to the meat film area according to Hunt and Hedrick (1977); hence, large values indicate a lower actual WHC.

Protein Solubility

Samples of ST and LD muscles frozen in liquid nitrogen were used. These samples were held at -40°, cut into small pieces, weighed accurately and placed directly while frozen in the extraction solution to avoid thaw rigor. The samples were homogenized at 20,000 rpm in

a Super Dispax Tissue Mincer (SDT 182 N) for 20 sec at 4°. All extractions were conducted at 4° according to the method of Helander (1957). It should be emphasized that the analyses for protein solubility were completed within one week.

Sarcoplasmic Proteins

Duplicate 2-g frozen samples of minced muscle were homogenized in 10 ml of precooled 0.03 M potassium phosphate buffer, pH 7.4. The homogenizer blades were rinsed in 10 ml buffer four times. Each of the washings was added to the homogenate, which was then agitated continuously for 30 min. The homogenate was centrifuged for 20 min at 30,000 x G at 4°. The clear supernatants were held at 4° until the protein content was determined.

Myofibrillar Proteins

A buffer consisting of 1.1 M KI in 0.1M potassium phosphate, pH 7.4, was used to extract total soluble protein. Duplicate 2-g samples were homogenized in 10 ml of buffer solution. The blades were washed four times with a total volume of 40 ml buffer solution which was added to the homogenate. The homogenate was extracted for a period of one hour with continuous agitation and then centrifuged 20 min at 30,000 x G at 4°. The clear supernatants were held at 4° until the protein content was determined. The myofibrillar protein was calculated as the difference between total soluble protein and the sarcoplasmic protein.

Salt-Soluble Proteins

Duplicate 2-g frozen muscle samples were homogenized and extracted in 50 ml of precooled unbuffered 5% NaCl solution for one hour with continuous agitation. The homogenate was then centrifuged at 4° for 20 min at 30,000 x G. The supernatants were held at 4° until analyzed for protein.

Protein Determination

Soluble protein was determined in each of the protein extracts obtained above by the biuret method (Gornall et al., 1949). Absorbance was determined by a Beckman Model B Spectrophotometer at 540 nm after 40 min of color development. The biuret standard was a solution of bovine serum albumin (BSA). The calibration curve (containing five points) was linear to 0.55 absorbance units with a coefficient of determination (r^2) of 0.995. Each protein extract, containing 3-10 mg protein, was run in quadruplicate, and the results were reported as the mean.

Statistical Analysis

The data were analyzed by use of paired-t distribution analysis (Steel and Torrie, 1960) to determine the significance of differences between control and electrically stimulated samples.

RESULTS AND DISCUSSION

Muscle pH

Mean values and standard deviations of the pH measurements of the electrically stimulated and non-stimulated muscles are summarized in Table 2 and graphically depicted in Figure 3.

At the initial sampling which was completed approximately one hour postmortem, the mean pH of the electrically stimulated muscles, semitendinosus (ST) and longissimus dorsi (LD), was 6.48 as compared to 6.75 for the controls. The pH of the stimulated samples was significantly lower ($P < 0.01$) than the controls immediately after treatment. Although it would have been highly desirable to take these measurements immediately upon death of the animals, it was impossible to do so under the slaughter conditions employed.

The mean pH values of 6.16 and 5.86 of the stimulated muscles at the two and four hour sampling periods were significantly lower ($P < 0.01$) than those of 6.47 and 6.23 respectively for the controls. By four hours postmortem, the pH of both stimulated muscles had declined below 6.0 to a value within 0.34 of the ultimate pH. Conversely, the untreated ST and LD muscles at four hours postmortem had pH values above 6.0 and were 0.68 and 0.50 units higher than their ultimate levels respectively. At 24 hours postmortem, pH of the stimulated LD muscle was significantly lower ($P < 0.05$) than the control (pH 5.50 versus 5.60), whereas the difference between the

Table 2. Mean pH values obtained at 1, 2, 4, and 24 hour post slaughter for the semitendinosus and longissimus dorsi muscles from pre-rigor electrically stimulated and control beef sides.

Time After Slaughter (hr)	Muscle	Treatment of Paired Sides				Level of Probability[a]
		Control		Stimulated		
		Mean	S.D.[b]	Mean	S.D.[b]	
1	ST	6.78	0.08	6.49	0.09	$P < 0.01$
2	ST	6.49	0.09	6.12	0.20	$P < 0.01$
4	ST	6.26	0.23	5.88	0.21	$P < 0.01$
24	ST	5.58	0.04	5.53	0.05	N.S.
1	LD	6.72	0.15	6.47	0.20	$P < 0.01$
2	LD	6.45	0.08	6.19	0.15	$P < 0.01$
4	LD	6.19	0.16	5.84	0.21	$P < 0.01$
24	LD	5.60	0.07	5.50	0.10	$P < 0.05$

[a]The probability that the difference between treatments is statistically significant based on paired-t test analysis (Steel and Torrie, 1960). $P > 0.05$ was reported as nonsignificant (N.S.)

[b]Standard deviation.

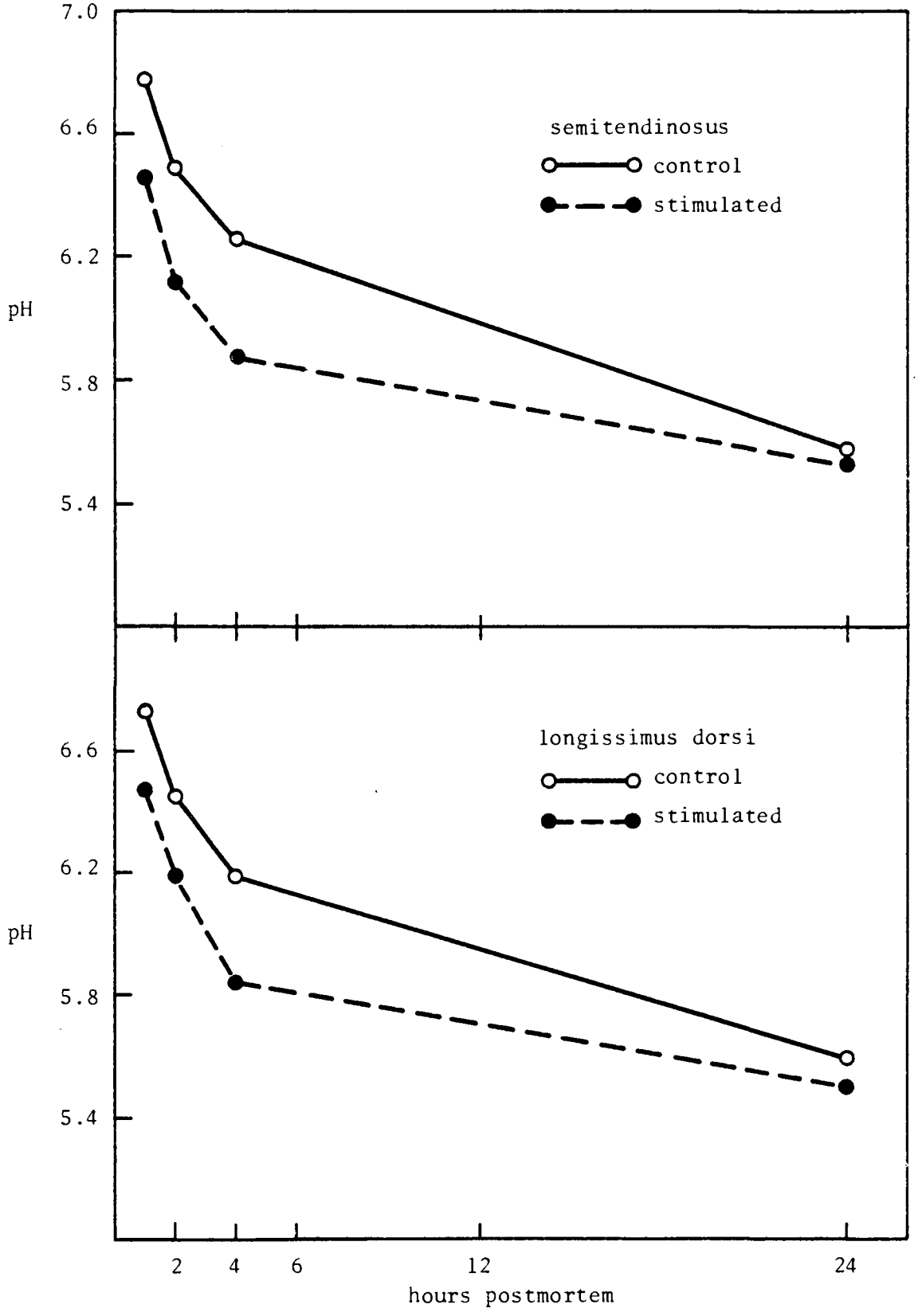

Figure 3. The effect of electrical stimulation of beef sides on the decline of pH in the semitendinosus and longissimus dorsi muscles.

the treated and untreated ST muscles was not statistically significant ($P > 0.05$).

These results support the general observation that electrical stimulation accelerates postmortem glycolysis, causing a rapid decline in muscle pH initially; however, it has much less effect upon the ultimate pH. Stimulation increased the rate of glycolysis, converting most of the glycogen stored in muscle to lactic acid and energy during the period of stimulation. Lactic acid brought about the immediate drop in pH, while the energy, which is in the form of ATP, was utilized by the muscle to power the massive contractions during stimulation.

The effect of electrical stimulation on acceleration of postmortem glycolysis has been studied by many workers (Bendall, 1976; Carse, 1973; Davey et al., 1976; Savell et al., 1976) who reported that stimulation increased the rate of postmortem glycolysis and hastened the onset of rigor mortis. Chrystal and Hagyard (1976) found that the longissimus dorsi muscle pH in stimulated lamb carcasses fell below six within one hour of slaughter compared to 14 hours required by unstimulated muscle. Similar results were reported by Bouton et al. (1978), who found that muscles from stimulated beef sides had significantly lower pH values at 1, 4 and 24 hours after slaughter than muscles from control sides.

Water-Holding Capacity

Data of water-holding capacity (WHC) determinations are presented in Table 3. WHC measurements were conducted on only six of the nine animals used. It is expressed as a ratio of total expressible juice

Table 3. Mean water-holding capacity values of the semitendinosus and longissimus dorsi muscles from electrically stimulated and control beef sides.[c]

Time After Slaughter (hr)	Muscle	Treatment of Paired Sides				Level of Probability[a]
		Control		Stimulated		
		Mean	S.D.[b]	Mean	S.D.[b]	
1	ST	2.21	0.25	2.30	0.31	N.S.
2	ST	2.32	0.26	2.40	0.33	N.S.
4	ST	2.52	0.24	2.50	0.33	N.S.
24	ST	2.52	0.46	2.70	0.54	N.S.
1	LD	1.86	0.28	2.03	0.26	$P < 0.05$
2	LD	2.05	0.27	2.17	0.30	$P < 0.05$
4	LD	2.20	0.22	2.38	0.43	N.S.
24	LD	2.31	0.28	2.30	0.35	N.S.

[a]The probability that the difference between treatments is statistically significant based on paired-t analysis (Steel and Torrie, 1960). $P > 0.1$ was reported as nonsignificant (N.S.).

[b]Standard deviation.

[c]Higher values mean lower actual water-holding capacity.

area to the meat film area; therefore, large values indicate a lower WHC. The WHC's of stimulated ST samples were not significantly different than the controls during the 24 hour test periods. On the other hand, stimulated samples of LD muscles retained significantly less ($P < 0.05$) water than the controls during the first two hours postmortem, but these differences were not significant at four and 24 hours postmortem. The reason for this is not apparent, although it might be construed that the onset of rigor mortis was more rapid in the LD than in the ST muscles during the first two hours postmortem. This condition would result in a tightening of the myofibrils and thus less lattice spacing for retaining water. The data indicate that stimulation had no effect on the WHC at the ultimate pH of the ST and LD muscles. WHC of muscle has been reported to be negatively related to rate and extent of postmortem glycolysis (Bendall and Wismer-Pedersen, 1962; Briskey, 1964; Disney et al., 1967).

Water-holding capacity within the individual treatments showed a considerable decrease with postmortem pH fall in both muscles. However, LD showed higher WHC values than ST muscles throughout the 24-hour postmortem period (Table 3). WHC has been found to be positively related to pH (Hamm, 1960; Miller et al., 1968, Bouton et al., 1971, 1972). The influence of pH on muscle hydration is very important because of the pH effects on protein net charge. Proteins have minimum WHC at their isoelectric points. At pH's on the acid or alkaline sides of the isoelectric points, proteins possess net positive or negative charges respectively which allow repulsion of filaments to leave more space for water molecules.

Protein Solubility

Sarcoplasmic Proteins

Changes in the extractability of sarcoplasmic proteins of the control and electrically stimulated ST and LD muscles during the 24-hour postmortem periods are given in Figure 4 and Tables 4 and 5.

Solubility of the sacroplasmic proteins of the control and stimulated ST muscles increased from the initial sampling 26.7 and 9.8% respectively by 24 hours postmortem. Sarcoplasmic protein solubility of the stimulated muscles was 15.2% higher than that of the control at one hour postmortem; at two and four hour postmortem sampling times, the increase was 12.6 and 5.3% respectively. By 24 hours postmortem, the sarcoplasmic protein extractability of the control and stimulated samples was essentially equal. Extractability of sarcoplasmic proteins from stimulated ST muscles was significantly higher ($P < 0.05$) than that of the controls at one and two hour postmortem sampling times, whereas differences thereafter were not.

Sarcoplasmic protein solubility of the control and stimulated LD muscles increased 15 and 1.6% respectively during the 24 hours postmortem period. The solubility of the sarcoplasmic proteins of the stimulated LD muscles was 7.1, 7.2 and 6.1% greater than the controls at one, two and four hour postmortem sampling times respectively. At 24 hour postmortem, the solubility of the stimulated muscle was 5.7% less than that of the control. Extractability of sarcoplasmic proteins of stimulated LD muscles was significantly greater ($P < 0.05$) than the controls at the initial sampling, although differences were not

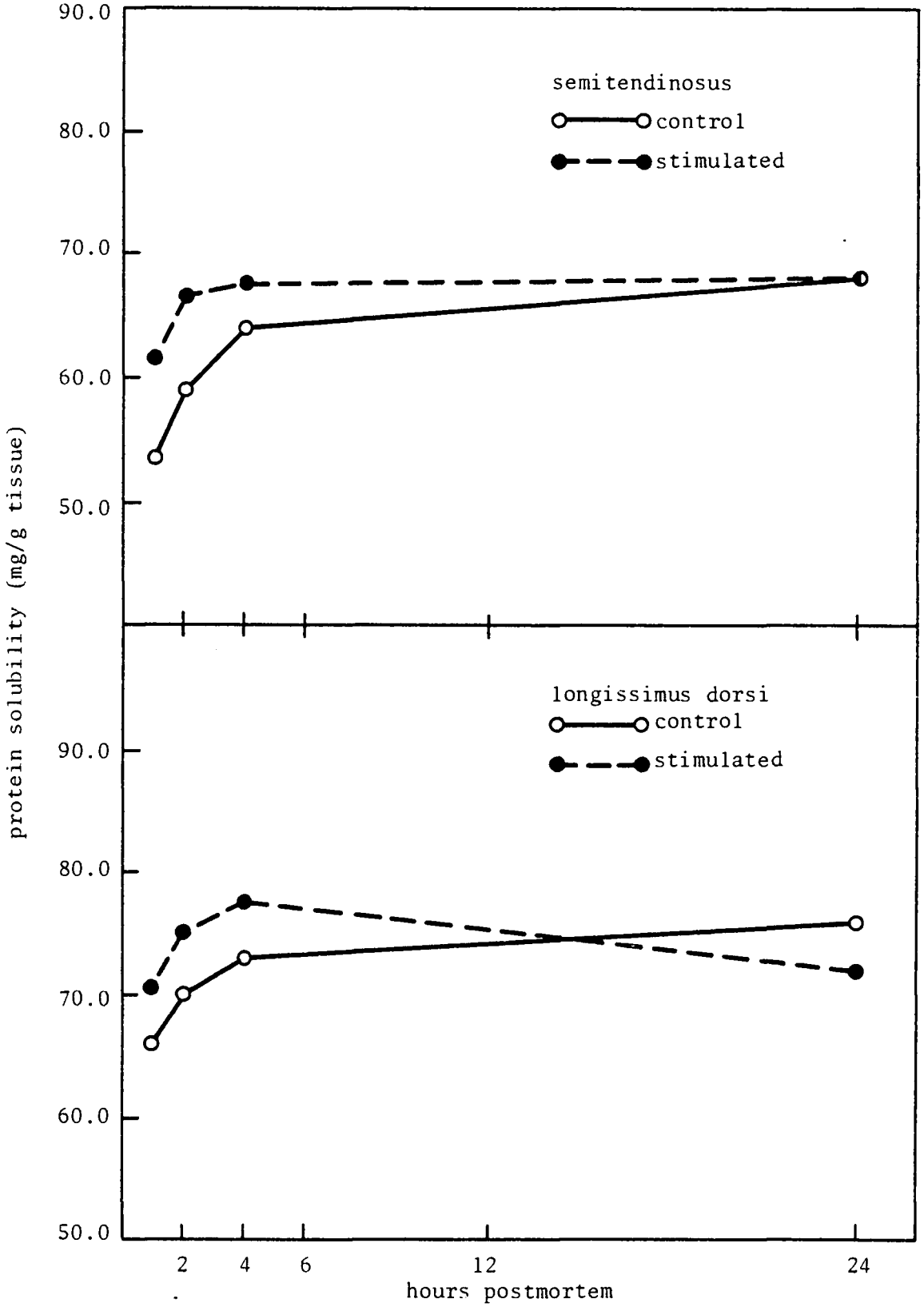

Figure 4. The effect of electrical stimulation of beef sides on the sarcoplasmic protein solubility of the semitendinosus and longissimus dorsi muscles.

significant at two and four hour postmortem. At 24 hours postmortem, sarcoplasmic protein solubility of the control muscles was significantly higher ($P < 0.1$) than that of the stimulated muscles.

In both muscles, stimulation resulted in an increase in the solubility of the sarcoplasmic proteins during the early postmortem sampling times. The reason for the increase is not readily apparent. However, research completed elsewhere (Cross, 1979) has indicated that electrical stimulation causes an early postmortem release of the lysosomal enzymes which tend to enhance the autolytic proteolysis of the myofibrillar proteins. Protein fragments resulting from the increased autolytic activity would have been extracted along with the sarcoplasmic proteins by the low ionic strength solution. Furthermore, Cross reported that a greater amount of enzymes was released into the cytoplasm from the lysosomes of the electrically stimulated samples than from those of the control samples. This would also help to increase the amount of sarcoplasmic proteins to be extracted from the stimulated muscles. At 24 hour postmortem sampling times, sarcoplasmic protein solubility values of the control and stimulated ST muscles were equal, while those of the stimulated LD muscles were slightly less than the controls.

The sarcoplasmic protein extractability values obtained in this study for both muscles (stimulated and non-stimulated) were higher than those reported by others (Sayre and Briskey, 1963; Scopes and Lawrie, 1963; Goll et al., 1964; Disney et al., 1967). The increase was most likely due to the improvement in the solubilization of the

Table 4. Mean protein solubility values of the semitendinosus muscle from electrically stimulated and control beef sides.[a]

Time After Slaughter (hr)	Sarcoplasmic Protein		Myofibrillar Protein		Salt-Soluble Protein	
	Control	Stimulated	Control	Stimulated	Control	Stimulated
1	53.81 (9.76)[b]	61.99 (6.80)*	130.59 (10.17)	121.88 (11.35)*	143.03 (5.11)	140.28 (6.83)
2	59.43 (8.05)	66.89 (9.86)*	122.76 (8.67)	116.31 (5.67)*	142.72 (4.88)	138.95 (7.50)
4	64.41 (10.28)	67.84 (7.05)	111.40 (5.19)	114.84 (6.33)	141.09 (4.34)	122.07 (20.1)*
24	68.16 (7.27)	68.06 (9.29)	109.26 (4.63)	111.85 (6.51)	95.80 (5.78)	91.18 (5.52)*

[a]Mean values expressed as mg/g wet tissue

[b]Parenthetical values are standard deviations

*$P < 0.05$

Table 5. Mean protein solubility values of the longissimus dorsi muscle from electrically stimulated and control beef sides.[a]

Time After Slaughter (hr)	Sarcoplasmic Protein		Myofibrillar Protein		Salt-Soluble Protein	
	Control	Stimulated	Control	Stimualted	Control	Stimulated
1	66.16 (8.22)[b]	70.88 (7.60)**	128.39 (9.71)	122.91 (2.97)*	160.49 (5.87)	156.28 (8.22)
2	70.00 (7.28)	75.03 (10.04)	124.47 (5.67)	115.63 (8.34)**	160.59 (5.53)	151.13 (9.43)**
4	73.11 (7.19)	77.58 (7.59)	118.25 (7.71)	111.35 (8.46)	156.44 (5.49)	133.69 (17.04)***
24	76.09 (7.14)	72.02 (9.07)*	110.35 (2.95)	115.29 (4.68)*	109.59 (4.19)	100.98 (7.31)***

[a]Mean values expressed as mg/g wet tissue

[b]Parenthetical values are standard deviations

*P < 0.1

**P < 0.05

***P < 0.01

of the sarcoplasmic proteins effected by the liquid nitrogen treatment (Borchert and Briskey, 1965) and by the use of a more efficient homogenization procedure (Hwang et al., 1977).

Throughout the sampling period, solubility of the sarcoplasmic proteins was higher in the LD than in the ST muscles. This can be explained by the fact that the LD muscles generally contain less connective tissue proteins and slightly higher levels of sarcoplasmic proteins than the ST muscles (Lawrie, 1974).

Myofibrillar Proteins

Data concerning the solubility of the myofibrillar proteins of the control and stimulated ST and LD muscles are presented in Tables 4 and 5 and Figure 5. These data show a general loss of myofibrillar protein solubility during the 24-hour postmortem period. Solubility of the ST control and stimulated muscles decreased 16.3 and 8.2% respectively. For the LD muscles, myofibrillar protein solubility of the control and stimulated samples decreased 14.1 and 6.2% respectively.

Myofibrillar protein solubility of the stimulated ST muscles was 6.7 and 5.3% less than that of the control at one and two hours postmortem. These differences were statistically significant at the five percent level of probability. At four and 24 hours postmortem, the stimulated ST had 3.1 and 2.4% higher solubility levels than the controls, although these differences were not significant ($P > 0.1$).

Myofibrillar protein solubility of the stimulated LD muscles was 4.3, 7.1 and 5.8% less than the controls at one, two and four hours

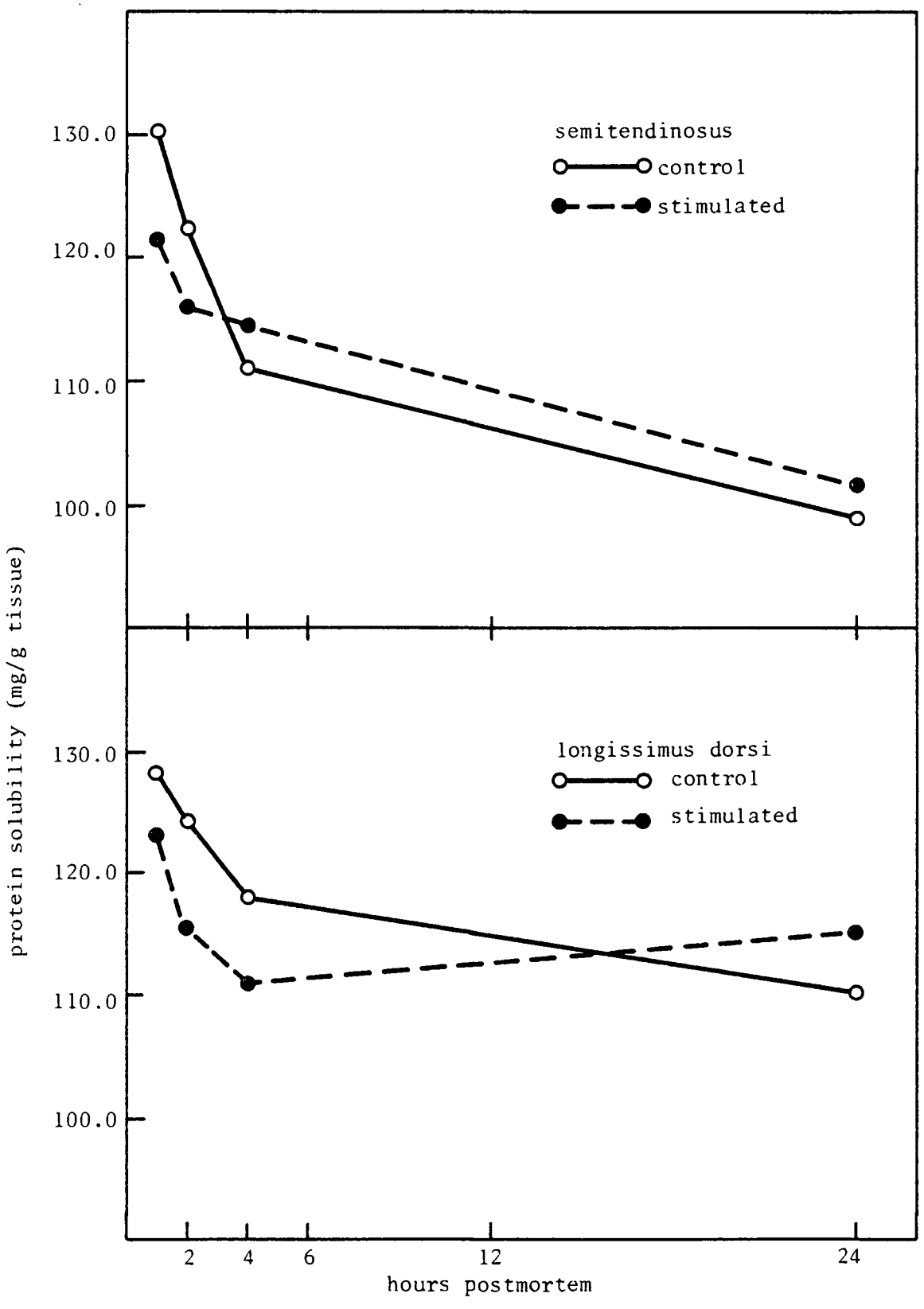

Figure 5. The effect of electrical stimulation of beef sides on the myofibrillar protein solubility of the semitendinosus and longissimus dorsi muscles.

postmortem respectively. At two hours postmortem the control samples had significantly higher ($P < 0.05$) solubility values than the stimulated LD samples but the difference was not significant ($P > 0.1$) at four hour postmortem. However, this trend was reversed at 24 hours postmortem wherein the stimulated samples had a 4.5% higher solubility level than the controls. This difference was statistically significant ($P < 0.1$).

During the early postmortem period (one and two hour sampling times), both the ST and LD stimulated muscles showed lower myofibrillar protein solubility values than the control muscles. This observation suggests that the depletion of ATP and the onset of rigor mortis occurred more rapidly in the stimulated muscles because myofibrillar protein solubility is directly related to the presence or absence of both ATP and actomyosin. The latter is the rigid protein complex formed in rigor. As this complex forms upon ATP depletion, protein solubility decreases because the actin and myosin filaments bind together chemically and it is more difficult to extract these proteins quantitatively in rigor than in pre- or post-rigor conditions.

Reasons are not readily available to explain why the myofibrillar protein solubility values of the stimulated ST muscles at four and 24 hours postmortem and those of the stimulated LD muscles at 24 hours postmortem were higher than their respective controls. These findings may be the result of physical disruption of the Z-line and other structural entities of the myofibrils as reported by Savell et al. (1978) to be an effect of the massive contractions caused by electrical stimulation. Although such events would tend to improve the

solubilization of the myofibrillar proteins, the same effects should also have been apparent at the earlier postmortem sampling times. However, effects of the more rapid onset of rigor mortis occurring in the stimulated samples may have masked this particular effect during the early postmortem periods.

Salt-Soluble Proteins

Results of the solubility of the salt-soluble proteins of the control and stimulated ST and LD muscles are given in Tables 4 and 5 and Figure 6.

Solubility of the salt-soluble proteins of the control ST muscles at 24 hours postmortem was 33% less than the initial value, while the stimulated samples decreased 35% in the same time interval. Salt-soluble protein solubility of the stimulated samples was 1.9, 2.6, 13.5 and 4.8% less than that of the control samples at 1, 2, 4 and 24 hours postmortem respectively. Only those differences at four and 24 hours postmortem between the control and stimulated samples were statistically significant ($P < 0.05$).

Extractability of salt-soluble proteins of the control LD muscles decreased 31.7% between the initial and 24 hours postmortem sampling times while that of the stimulated samples decreased 35.4%. Salt-soluble protein solubilities of the stimulated muscles were 2.6, 5.9, 14.6 and 7.9% lower than the control samples at 1, 2, 4 and 24 hours postmortem respectively. The difference at two hour postmortem was statistically significant at the five percent level of probability,

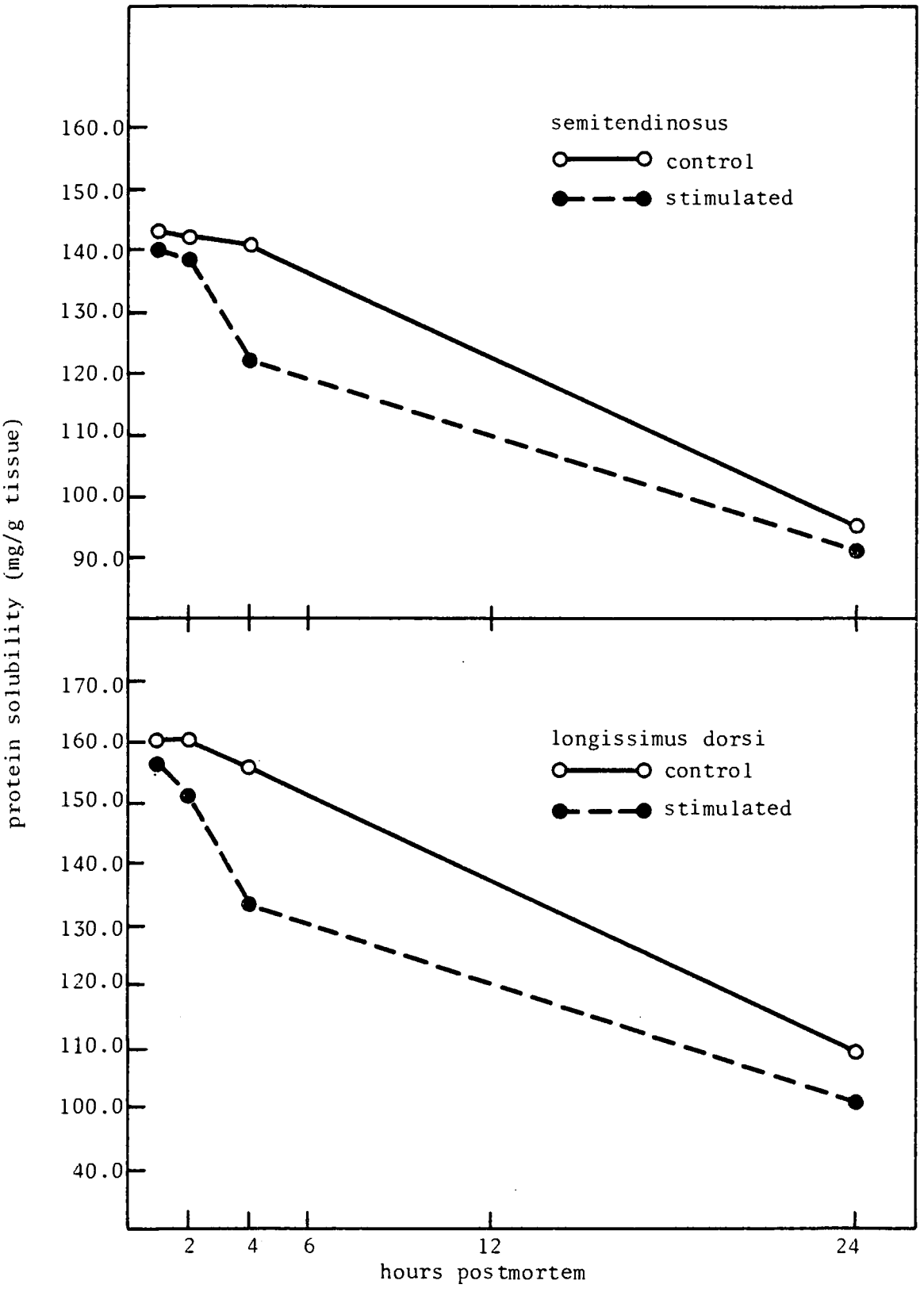

Figure 6. The effect of electrical stimulation of beef sides on the solubility of proteins in 5% NaCl (salt-soluble proteins) of the semitendinosus and longissimus dorsi muscles.

while differences at four and 24 hour sampling times were highly significant ($P < 0.01$).

The present results show that the extractability of salt-soluble proteins decreased substantially in the stimulated samples of both muscles. Differences in extractability between the control and stimulated samples were significant at different postmortem times. This may be partially explained by the fact that the pH of control samples was higher than that of stimulated samples during the 24 hour test periods. As mentioned previously throughout this discussion, protein extractability increases as muscle pH is increased. Another factor involved with the decreasing extractability of the salt-soluble proteins with time is that NaCl is a less potent solubilizer of actomyosin than KI or the polyphosphates. These compounds are more effective in dissociating the rigor complex than NaCl (Wismer-Pedersen, 1971). In addition, NaCl has a low buffering capacity and the pH of this extracting solution was not adjusted to pH 7.4 initially as were the extracting buffered solutions used to solubilize the sarcoplasmic and myofibrillar proteins.

The decrease in extractability of salt-soluble proteins from both muscles during the test periods is in general agreement with findings of other investigators. Saffle and Galbreath (1964) and Disney et al. (1967) working with beef, Johnson and Hendrickson (1970) and Trautman (1966) working with pork reported that the salt-soluble protein extractability decreased during the postmortem periods.

General Considerations

It is evident from the data collected in this study that the electrical stimulation of beef muscles resulted in an initial rapid pH decline, a lowering of WHC and generally less extractability of the myofibrillar and salt-soluble proteins during the 24 hour post-mortem test periods. These changes were undoubtedly caused by an initial accelerated rate of glycolysis and a rapid depletion of ATP induced by stimulation, which resulted in a low pH while the temperature of muscles was still relatively high. These conditions lead to a quicker onset of rigor mortis and to protein denaturation which, in turn, depress protein solubility.

These findings have commercial applicability in that early boning of beef muscles (e.g., hot-processing) is being employed increasingly in the meat industry. Meat derived from hot-processing may be used in the manufacture of emulsion-type processed meat products. Quality and stability of these products are dependent upon the solubilization and/or extractability of an adequate level of salt-soluble proteins.

It is a common meat industry practice to prepare meat emulsions with a 3 to 5% salt (NaCl) brine which extracts simultaneously both the sarcoplasmic and myofibrillar proteins. The latter class of proteins is more efficient and effective than the former in forming and stabilizing meat emulsions. Thus, if electrically stimulated meat is to be used in the manufacture of processed meat products, emulsions should be prepared within one hour (but no later than two hours) post-stimulation in order to take advantage of higher myofibrillar protein solubility.

SUMMARY AND CONCLUSIONS

An investigation was completed to determine the changes occurring in certain properties of beef muscles subjected to pre-rigor electrical stimulation. Rate of pH decline, changes in protein solubility and the water-hold capacity (WHC) of the stimulated muscles were the specific characteristics investigated.

Nine good quality steers were slaughtered and dressed, and the carcasses were split longitudinally into halves (sides). At 30-40 min postmortem, the left side of each carcass was electrically stimulated for one min at 600 volts (7 amps, 60 cycles per sec), while the right side served as the unstimulated control. Samples were removed from the semitendinosus (ST) and longissimus dorsi (LD) muscles of each side about 40-50 min postmortem and at 2, 4 and 24 hour postmortem intervals. A portion of each sample was used for the immediate determination of pH and WHC while the remainder was frozen in liquid nitrogen and stored at -40° for subsequent analyses of water-soluble, myofibrillar and salt-soluble proteins.

Immediately following electrical stimulation, pH of the treated samples of both muscles was significantly lower ($P < 0.01$) than that of the controls. Similar differences were also noted at two and four hour postmortem. At 24 hours postmortem, pH of the stimulated and control samples of ST muscles were essentially equal, whereas the pH of the stimulated LD muscles was significantly lower ($P < 0.05$) than that of the control. These data indicate that electrical stimulation

was effective in accelerating postmortem glycolysis to result in a more rapid, initial pH decline.

Only minor, non-significant differences were noted between the results of the WHC determinations of the stimulated and control ST muscles during the 24 hour test periods. The stimulated LD muscles had significantly lower ($P < 0.05$) WHC values than the controls during the first two hours postmortem; however, these differences were not significant at the later sampling times of 4 and 24 hour postmortem. These data suggest that the depletion of ATP and the onset of rigor mortis were more rapid in the LD than in the ST muscles.

At the time of the initial sampling, solubility values of the sarcoplasmic proteins of the stimulated samples of both muscles were significantly higher ($P < 0.05$) than those of the controls. At 24 hour postmortem, sarcoplasmic protein solubility values were equal for both the stimulated and control samples of the ST muscles, whereas those of stimulated LD muscle samples were significantly lower ($P < 0.1$) than the controls. The initial increase in the extractability of the sarcoplasmic proteins might be due to the early postmortem release of lysosomal enzymes into the cytoplasm of the electrically stimulated samples of both muscles.

During the first two hours postmortem, solubility values of the myofibrillar proteins of the stimulated samples of both the ST and LD muscles were significantly lower ($P < 0.05$) than the controls. These data suggest that electrical stimulation caused a more rapid depletion of ATP and a quicker onset of rigor mortis which, in effect, reduced

the solubility of these proteins. Although the solubility of the myofibrillar proteins of the stimulated ST muscles had increased by the 4 and 24 hour sampling times, the values were not significantly different than those of the controls. At the 24 hour sampling period, myofibrillar protein solubility of the stimulated LD muscle samples improved to levels that were significantly higher ($P < 0.1$) than those for the controls. In this case, electrical stimulation of the LD muscles may have resulted in a partial disruption and/or degradation of the Z-line structures of the myofibrils to allow for greater solubility to occur with time.

Solubility values of the salt-soluble (5% NaCl) proteins were substantially lower in the stimulated samples than in the controls for both muscles during the 24 hour test periods. These decreases were most pronounced at the 4 and 24 hour sampling times; solubilities of the stimulated ST and LD muscles samples were significantly less, $P < 0.05$ and $P < 0.01$ respectively, than their controls at the above mentioned time intervals. These decreases in solubility may be attributed to lower muscle pH of the stimulated samples and to the fact that 5% NaCl solution is not a particularly good extractant for solubilizing muscle proteins, especially when muscle is in rigor mortis.

Data of this study show that the pre-rigor electrical stimulation of beef muscles resulted in an accelerated rate of pH decline and altered the solubilities of the different protein fractions. These changes may have an important influence on the manner in which stimulated muscles are used in the manufacture of processed meat products.

Additional studies are needed to clarify the effects of pre-rigor electrical stimulation upon the specific muscle protein fractions.

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