Postmortem physical and chemical events related to bovine skeletal muscle tenderization

Dennis Gene Olson

Iowa State University

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RELATED TO BOVINE SKELETAL MUSCLE
TENDERIZATION.

Iowa State University, Ph.D., 1975
Food Technology

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Postmortem physical and chemical events related to bovine skeletal muscle tenderization

by

Dennis Gene Olson

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Meat Science

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa

1975
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INTRODUCTION

Shortly after the exsanguination of an animal, a series of chemical and physical events are initiated in the first 24-48 hours post-mortem. During this time skeletal muscle transforms from a physical state of being elastic and pliable to a state of being stiff and rigid, termed rigor mortis, to one of being pliable again. Energy stores in muscle are depleted and muscle pH falls due to anaerobic glycolysis during rigor mortis. The significance of these chemical and physical changes is that almost all meat quality attributes are established at this time. Rigor mortis and its associated events and relationships to meat tenderness have intrigued meats researchers for many years. However, most researchers have been unsuccessful in elucidating and relating these changes to tenderness.

Tenderness is one of the meat quality factors that is considered to be very important in meat palatability. It is well known that a number of variables affect tenderness, but the one that is still not understood is the large increase that occurs in tenderness during post-mortem storage. For example, it has been shown that muscles left attached to the carcass skeleton are least tender immediately after death and become progressively more tender with increasing time post-mortem. The causes for the increase in meat tenderness during storage have remained obscure even though many postmortem
biochemical and structural muscle changes have been well characterized.

It is known, for example, that myofibril structure weakens during postmortem storage, but it is unknown what causes this structural weakening or whether it is related to meat tenderness. It is also known that postmortem muscle tenderization is influenced primarily by alterations in myofibrillar proteins and not sarcoplasmic or stroma proteins, but it is unknown what myofibrillar protein alterations directly influence meat tenderness or what causes those alterations. It has been hypothesized that postmortem muscle tenderization results from proteolysis by enzymes which have been isolated from muscle, but it has not been shown that muscle proteases directly affect muscle tenderness. While many postmortem muscle characteristics have been thoroughly investigated and well established, the association of these characteristics to postmortem muscle tenderization has not been clearly made.

Z-line degradation in myofibrils has been shown to occur during postmortem muscle storage which causes myofibrils to fragment into small pieces. Z-line degradation clearly has an influence on the strength of the muscle fiber and hence should be related to meat tenderness. Also a calcium-activated-sarcoplasmic protease (CAF) has been shown to selectively degrade Z-lines in myofibrils. It seems
probable that CAF may cause a weakening in the myofibril structure during postmortem storage which would be reflected in an increase in meat tenderness.

The objectives of this study were to 1) develop a quantitative method to determine the extent of Z-line degradation by measuring myofibril fragmentation, 2) characterize specific changes in myofibrillar proteins during postmortem storage since sarcoplasmic and stroma proteins have little influence on postmortem tenderness changes, 3) determine relationships of CAF activity to postmortem myofibrillar protein changes and myofibril fragmentation and 4) correlate postmortem changes in myofibrillar proteins and myofibril fragmentation to postmortem changes in meat tenderness.

**Abbreviations**

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<td>a</td>
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<td>ATP</td>
<td>adenosine triphosphate</td>
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<td>C</td>
<td>celsius</td>
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<td>Ca²⁺</td>
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<td>calcium-activated-factor</td>
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<td>USDA</td>
<td>United States Department of Agriculture</td>
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LITERATURE REVIEW

Muscle Biology

Skeletal muscle is a highly specialized tissue used for skeletal support and locomotion. A discussion on the intricacies of muscle and its relationship to meat requires a review of some of the fundamental structural features of skeletal muscle. The following brief discussion of skeletal muscle structure is based on reviews of the microstructure of muscle and muscle components by Briskey and Fukazawa (1971), Gould (1973) and Huxley (1972). From a gross anatomical viewpoint, skeletal muscles are usually conical in shape, tapering at both ends where they are attached by tendons to the skeleton. Microstructurally a connective tissue sheath surrounds an entire muscle, termed the epimysium, and it protrudes into the body of the muscle at various locations to separate the muscle into bundles or fasciculi. The layer (or sheath) of connective tissue surrounding the fasciculi is termed the perimysium. Another connective tissue sheath anastomoses and surrounds the individual muscle fibers (cells) called the endomysium, and it lies just outside the muscle cell membrane or sarcolemma. Hence the functions of the entire connective tissue system are to maintain the organization of the muscle, provide greater efficiency of movement during muscle contraction and support the vast network of branching blood vessels and nerves throughout the muscle sys-
The muscle fiber is a complete cell which is nonbranching, cylindrical and multinucleated with tapering conical ends and having variable dimensions. The length of the muscle fiber averages 2 to 3 cm and has a diameter that ranges from 10 to 100 μm. Within the muscle cell there are long, nonbranching threads of proteins, 1 to 3 μm in diameter lying parallel to one another along the long axis of the cell. These threads of proteins which travel the entire length of the cell and have no membranes surrounding them are termed myofibrils. They contain the contractile apparatus of the muscle cell. Myofibrils exist as structural entities of the cell because they are insoluble at the ionic strength of the cell. Myofibrils are bathed in a solution termed the sarcoplasm containing many soluble proteins. The muscle cell also contains mitochondria (sarcosomes), sarcotubular system, numerous nuclei, Golgi apparatus and lipid droplets.

Myofibrils have sequential light and dark bands with the direction of banding perpendicular to the long axis of the myofibril. Because myofibrils lie in register so that their light and dark bands are aligned at right angles to the long axis of the fiber, a banded appearance is conferred upon the entire muscle cell. These banding patterns take their names from their appearance under polarized light; the light bands, or I-bands are weakly birefringent (isotropic) and the dark
bands, or A-bands, are strongly birefringent (anisotropic). Bisecting the center of the I-band is a dark line, the Z-line, and in the center of the A-band is a light zone, the H-zone. Bisecting the H-zone is a thin line of protein, called the M-line. The region from one Z-line to the next is defined as a sarcomere and this represents the contractile unit of the myofibril.

Myofibrils are comprised of fine, long filaments of proteins seen only in the electron microscope, lying side by side and extending longitudinally parallel with the long axis of the myofibril. Myofilaments do not continue through the sarcomere but are divided into two kinds of filaments, termed thick and thin filaments. Huxley (1953) described the striated muscle myofibril as a double array of interdigitating thick and thin filaments. The thin filaments are attached at one end to the Z-line and extend into the A-band between adjacent thick filaments whereas the thick filaments comprise the A-band and are not attached to the Z-line. The I-band contains only the thin filaments and the Z-line while the A-band contains the thick filaments, H-zone, M-line and part of the thin filaments. Thick filaments contain the protein myosin and are approximately 110 A in diameter and 1.5um long, while the thin filaments contain several proteins, actin, tropomyosin and troponin and are approximately 65 A in diameter and 1.0um long. A sarcomere is approximately 2 to
A 3μm long. During contraction thin filaments slide into the A-band so that a contracted myofibril will have a narrower I-band than in a relaxed myofibril while the A-band remains at a constant length.

A review of the sarcotubular system of the muscle cell has been made by Franzini-Armstrong (1973). The sarcotubular system consists of two parts: 1) the T-system or T-tubules and 2) the sarcoplasmic reticulum (SR) or L-system. The T-tubules are a series of membrane-limited tubules that pass through the muscle cell generally perpendicular to its long axis. These tubules are invaginations of the sarcolemma so the lumen of the tubule is extracellular. T-tubules occur at regular intervals along the muscle cell at every A-I band junction or at every Z-line. The function of the T-tubules is to spread nerve impulses very quickly from the sarcolemma to myofibrils. The SR consists of an extensive network of membrane-limited tubules which extend laterally from the center of each sarcomere to meet the T-tubule at either the A-I band junction or at the Z-line. As the SR membranes approach the T-tubule, they form a large sac, termed the lateral cisternae adjacent to the T-tubule. The T-tubule has lateral cisternae on either side of it which appears as three closely adhering tubules in cross-section termed the triad. The SR membranes have an enormous ability to accumulate Ca^{2+} against a concentration gradient and whenever a nerve impulse
occurs, the membrane of the lateral cisternae is depolarized, releasing Ca^{2+} into the cell near the myofibrils. When membranes are repolarized, the free intracellular Ca^{2+} is reaccumulated in the lateral cisternae. The release and reaccumulation of Ca^{2+} serves as the switch to initiate or stop contraction.

Muscle proteins have been classified into three groups, sarcoplasmic, myofibrillar and stroma proteins, based on their solubility in salt solutions of various ionic strengths (Briskey, 1967; Goll et al., 1969b; Szent-Gyorgi, 1960).

The sarcoplasmic protein fraction is soluble in a neutral salt solution having an ionic strength below 0.2. This class of muscle proteins makes up 34% of the total muscle proteins (Hamm, 1960). Through differential centrifugation, inhomogeneous fractions of the sarcoplasm can be isolated. These fractions include the sarcoplasmic supernatant, the nuclear fraction, the mitochondrial fraction and the microsomal fraction. The sarcoplasmic supernatant contains the sarcoplasmic proteins which are comprised of over 100 different proteins. Out of a total of 55mg of sarcoplasmic proteins approximately 40mg are glycolytic enzymes, 5mg creatine kinase, 0.2 to 2mg myoglobin, 3 to 6mg extracellular proteins and 8 to 12mg of other proteins (Scopes, 1970). Obviously glycolytic and associated enzymes are not in equal abundance in the sarcoplasm. Glyceraldehyde phosphate dehydrogenase
(GAPDH) alone accounts for over 20% of the sarcoplasmic proteins in rabbit muscle (Czok and Bucher, 1960). GAPDH, phosphorylase b, aldolase, triose phosphate isomerase, enolase, pyruvate kinase and lactate dehydrogenase make up over half of the sarcoplasmic proteins with probably over 100 other proteins in minute quantities making up the remainder of the sarcoplasmic proteins. The nuclear fraction contains ribonucleic acids, deoxyribose nucleic acids and lipoproteins. The mitochondrial fraction consists of 30% lipoprotein and the various enzymes and constituents of the tricarboxylic acid cycle and the electron transport system, including flavin nucleotides, various hemes and cytochromes. The microsomal fraction consisting of lysosomes containing acid hydrolases, proteolytic enzymes, cathepsins, acid phosphatase, and ribonuclease (Laakkonen, 1973).

The myofibrillar proteins, which are soluble in salt solutions having an ionic strength of 0.4 to 1.5, constitute about 50-55% of the total muscle proteins and are therefore the largest class of muscle proteins (Briskey, 1967). In this protein fraction there are at least nine myofibrillar proteins, namely, myosin, actin, tropomyosin, troponin, alpha-actinin, beta-actinin, component C and two M-line proteins (Goll et al., 1974). These proteins are insoluble at the ionic strength within the cell so that they exist as structural entities making up the myofibril.
Myosin represents 50-55% of the myofibrillar proteins and is found in the thick filament of the myofibril. Myosin has a molecular weight of approximately 468,000 daltons (Lowey et al., 1969). Myosin has two classes of subunits: 1) those naturally existing in the molecule, and 2) those produced by brief proteolytic digestion. The natural subunits of myosin consist of two similar molecular weight (MW) chains of 200,000 to 210,000 daltons each and four small subunits (light chains) of various weights (Lowey et al., 1969). The different kinds of small subunits are: 1) the 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) subunits, MW = 19,000 daltons; 2) the alkali-1 subunits, MW = 21,000 daltons; and 3) the alkali-2 subunits, MW = 17,000 daltons. Each myosin molecule has two of the DTNB subunits and two of the alkali-1 or alkali-2 subunits in addition to the two large subunits.

On the other hand, a 1 to 5 minute digestion with trypsin, chymotrypsin or subtilisin at enzyme to myosin ratios of 1:300 (by weight) will cleave the myosin molecule at its center to produce two fragments called light meromyosin (LMM) and heavy meromyosin (HMM). The LMM is the tail part of the molecule and the HMM contains part of the tail and the bulbous head of the molecule. All of the ATPase activity, actin-binding ability and the light chains of the myosin molecule are found in the HMM fragment. The tails of adjacent myosin molecules aggregate to form the shaft of the
thick filament and the heads of the myosin molecules are bent outward to form the cross-bridges.

Actin represents 15-20% of the total myofibrillar proteins and is found in the thin filaments. Actin exists in two forms in vitro: globular (G) actin and fibrous (F) actin. Only F-actin is found in vivo and it consists of a double-stranded fiber of G-actin subunits. G-actin has a molecular weight of 42,000-44,000 daltons and contains one molecule of Ca\(^{2+}\) and one molecule of ATP per G-actin molecule and it is polymerized to F-actin by the addition of 0.1M KCl or 1mM Mg\(^{2+}\) (Laki, 1971). Actin and myosin together are both necessary and sufficient for a contractile response if Mg\(^{2+}\) and ATP are present. The contractile response continues until the ATP is depleted. This contractile response can not be controlled (i.e. started and stopped at will).

Troponin (TN) makes up 5-8% of the total myofibrillar protein and is found in thin filaments. The TN molecule contains two subunits of approximately 33,000 and 36,000 daltons (Cohen et al., 1973). TM lies along the length of the thin filament in grooves of the actin helix.

Troponin (TN) constitutes 5-8% of the total myofibrillar protein and is found in thin filaments. The troponin molecule is a slightly asymmetrical spherical molecule having a molecular weight of 80,000 daltons. The troponin molecule consists of three polypeptide chains each having a specific
function (Hartshorne and Dreizen, 1973): 1) TN-T, having a molecular weight of 37,000-39,000 daltons, binds tightly to TM but not actin, 2) TN-C, having a molecular weight of 18,500 daltons, binds to TN-T but not actin and has four high-affinity binding sites for Ca$^{2+}$ and 3) TN-I, having a molecular weight of 24,000 daltons, binds to the TN-T/TN-C complex and also weakly binds to TM. TN-I strongly inhibits the Mg$^{2+}$-modified ATPase of actomyosin but this inhibition can be derepressed by TN-C in the presence of Ca$^{2+}$. Cohen et al. (1973), Drabikowski et al. (1973) and Greaser et al. (1973) have found that during the preparation and purification of troponin, a neutral protease in muscle partially degrades troponin yielding a 30,000-dalton molecular weight protein. Moreover Dayton et al. (1974a, b) have found a calcium-activated neutral protease isolated from muscle sarcoplasm that degrades troponin after brief treatment to yield a 30,000-dalton molecular weight protein. The troponin complex is distributed periodically along the thin filament at 385 Å intervals and it is bound to a specific site on TM (Ohtsuki et al., 1957). The control of the Mg$^{2+}$-modified ATPase of actomyosin by troponin is therefore located in the contraction regions of the myofibril by tropomyosin. The TM-TN complex therefore regulates muscle contraction (Ebashi et al., 1967, 1968).
Alpha-actinin makes up approximately 1% of the total myofibrillar protein and has a molecular weight of 200,000 daltons with two identical subunits of 100,000 daltons. Schollmeyer et al. (1973) have shown that alpha-actinin constitutes the dense, amorphous material of the Z-line and Singh (1974), in a review of alpha-actinin, suggests that one role of alpha-actinin in muscle is to enhance the structure and stability of the Z-line. Dayton et al. (1974a, b) have shown that a neutral, calcium-activated-factor (CAF) isolated from the sarcoplasm of muscle removes alpha-actinin from myofibrils. Purified alpha-actinin, however, is not proteolytically degraded by CAF.

The stromal proteins, which are insoluble in neutral aqueous solvents, comprise the connective tissue located in the epimysium, perimysium and endomysium. Goll (1962, 1965a, b) has described the fibrous connective tissue proteins to be principally collagen, elastin and reticulin. Of these, collagen is the principal protein found in connective tissue; it makes up 1-2% of muscle (Goll, 1965a). The following brief discussion on collagen structure is based on reviews by Piez (1966) and Veis (1970). The basic structural unit of a collagen fiber is the needle-like tropocollagen (TC) molecule which consists of a group of three polypeptide chains. The rigidity of the TC molecule is due to a triple helix structure which makes possible the formation of maximal amounts of
hydrogen bonds. The imino acids, proline and hydroxyproline make up about one-fourth of the linkages in the primary structure of the collagen molecule. With greater quantities of proline and hydroxyproline, resistance of the molecule to heat or chemical denaturation is increased, but the collagen fiber is easily converted to a soluble form, gelatin, by heating in aqueous solution. The temperatures required for this conversion varies with 1) the nature of the solution, over 60°C being required with water but lower temperatures sufficing in the presence of acids or bases and 2) advancing animal age. Heat breaks hydrogen bonds in the TC molecule which results in the separation of the peptide chains. One TC molecule having no covalent bonds will give up three alpha-chains. Some TC molecules have covalent intramolecular cross linkages binding together two alpha-chains, called the beta-component, or binding all three alpha-chains together called, the gamma-component. A formation of intermolecular covalent bonds between adjacent alpha-subunit chains is a characteristic of the maturation of collagen. Mature collagen fibrils may be represented as assemblies of tropocollagen molecules joined together by a variety of intermolecular covalent cross-linkages to form vast asymmetrical polymer networks. No significant increase in the amount of collagen occurs as animal age increases (Goll, 1965a; Hill, 1965). Bailey and Lister (1968) have shown that a proportion of the
covalent intermolecular cross-links in native collagen are in a thermal labile form which gradually become stabilized with increase in animal age. Stabilization of such a bond would account both for the decrease in salt solubility and for the increase in shrinkage temperature with animal age.

Native undenatured collagen fibers are relatively resistant to enzymic digestion by enzymes except for collagenase. Gelatin or denatured collagen, however, is susceptible to the action of many proteolytic enzymes including papain, elastase, ficin and trypsin. Furthermore elastin is the second most abundant protein in connective tissue, however, there is less than one-third as much elastin as collagen (Wilson et al., 1954) in the muscles where elastin is most abundant. Elastin fibers are smaller than collagen fibers and branch freely to form networks (Partridge, 1966). Elastin is resistant to aqueous extractions and not greatly affected by dilute weak acids or dilute bases at 100°C for several hours. The insolubility of elastin seems to be due to the presence of the desmosine cross-links among adjacent peptide chains. Elastin is not hydrolyzed by trypsin or collagenase but it is affected by ficin and elastase (Partridge, 1966).

Reticulin is found in almost all connective tissue but in only small amounts. It is morphologically similar to collagen and may possibly be a precursor of collagen; however, the fibers are smaller than collagen, branch freely, and con-
tain considerable amounts of lipid and carbohydrates not possessed by collagen. Reticulin is also resistant to aqueous extraction at 100°C, pH 7 for 2-3 hr.

Because muscle contraction has an important bearing and relationship to postmortem muscle shortening and meat tenderness, it seems appropriate to present a brief description of the events and components associated with muscle contraction (Bendall, 1960, 1966; Huxley, 1960, 1964, 1972; Taylor, 1972). A nerve impulse transmitted to the muscle cell causes the external membrane to depolarize. This wave of depolarization travels along the T-tubules to the interior of the cell to the lateral cisternae of the SR where a release of the Ca^{2+}, bound to the SR, occurs and causes the free intracellular Ca^{2+} concentration to increase from about 1 x 10^{-7} to 1 x 10^{-4} M. Free Ca^{2+} which is bound to troponin and held at the actin site by tropomyosin causes a release of the suppression of Mg^{2+}-activated ATPase of actin-myosin. The released suppression permits the ATPase site on one of the myosin heads to be activated by Mg^{2+} to hydrolyze ATP. As ATP is hydrolyzed, the actin-combining site on myosin and the myosin-combining site on actin interact to generate a relative sliding movement of one set of filaments past the other. Contraction of myofibrils is the result of repetitive cyclic changes at the sites of interaction between actin and myosin. During contraction, the sarcomere length decreases but the
length of the thick and thin filaments remain constant. When repolarization of the sarcolemma occurs and free intracellular Ca\(^{2+}\) is bound back to the SR leaving a level of 1 X 10\(^{-7}\)M or less free Ca\(^{2+}\) in the cell, the Mg\(^{2+}\)-activated ATPase of the actin-myosin is suppressed by troponin. If the ATP concentration is 8-10mM the actin-myosin complex is dissociated and relaxation occurs.

Energy is used by muscle in sustaining the life processes of the cell and during muscle contraction. Hence the reactions and events of muscle metabolism are very important and these events have been reviewed by Kastenschmidt (1970), Lardy (1966) and Lawrie (1966b). Glucose, a product of digestion, is carried to the muscle cell by the blood stream. Glucose is stored in the muscle cell as glycogen when the demand for energy is low. During high muscle activity, however, glycogen is catabolized back to glucose and ATP either by aerobic or anaerobic mechanisms. Aerobic glycolysis yields a 14-fold greater amount of ATP than does anaerobic glycolysis, hence aerobic glycolysis is the preferential pathway of glucose breakdown. However, some muscles do not have sufficient oxygen stores (due to a lower amount of myoglobin in the cell) to meet the needs of aerobic glycolysis during vigorous activity. Such muscle cells, when stimulated for heavy activity, rapidly incur an oxygen debt and must resort to anaerobic glycolysis to supply the remainder
of their needs. These muscle cells usually store large amounts of glycogen and produce large amounts of lactic acid during activity. Also aerobic glycolysis occurs exclusively in the muscle sarcosomes (mitochondria) so the extent of aerobic glycolysis is also limited somewhat by the number of mitochondria in the cell.

Postmortem Muscle

After the exsanguination of an animal, the muscle cell attempts to continue its life processes and therefore continues to use ATP as a source of energy. Since the glucose supply to the muscle cell ceases immediately with exsanguination, the muscle cell begins to degrade glycogen to meet its energy needs. Aerobic glycolysis continues until all oxygen stored in the myoglobin is exhausted after which anaerobic glycolysis begins (Lardy, 1966). Since there is no longer any blood for transport of lactic acid away from the cell, it accumulates and pH begins to decrease. The amount of lactic acid produced (and therefore, the pH of the muscle) depends on the amount of glycogen in the cell at death, on the amount of glycogen used by aerobic glycolysis and on the rate at which the glycolytic enzymes catalyze glycolysis (Kastenschmidt, 1970). In starved or exhausted animals, glycogen levels at death may be low and very little acid is produced. Glycogen levels can also be depleted by antemortem injection of adrenaline and insulin (Bouton et al., 1971; de
Premery and Pool, 1963; Khan and Nakamura, 1970; Klose et al., 1970; Penny et al., 1963). In extreme situations of glycogen depletion, muscle pH may not even fall below 7.0, this is called alkaline rigor. Normally, pH decreases from 7.0 to 7.2 at death to 5.3 to 5.6 24 hours after death (Lawrie, 1966a). As lactic acid accumulates and pH falls, activity of some of the glycolytic enzymes decreases, and glycolysis may stop before all glycogen is completely catabolized (Lardy, 1966). Hence the amount of glycogen in muscle immediately after death is not necessarily directly proportional to the final postmortem pH of that muscle. The final postmortem pH of muscle is called the ultimate pH and is usually about pH 5.3 to 5.6 which is near the isoelectric point of actomyosin (Lawrie, 1966a). Ultimate pH of postmortem muscle is dependent upon the initial pH of at-death muscle, the glycogen content of meat at the moment of death, the intracellular oxygen available for aerobic glycolysis at the moment of death and the buffering capacity of the muscle proteins (Kastenschmidt, 1970).

When glycolysis ceases, either because of depletion of muscle glycogen or because of inactivation of some glycolytic enzymes, no more ATP is produced. The muscle cell has a reservoir of high energy phosphate in creatine phosphate (CP) which is used to very quickly replenish any ATP used in muscle cells (Lardy, 1966). Creatine kinase rephosphorylates
ADP to ATP faster than ADP can be produced, even under tremendous energy utilization (Kastenscheidt, 1970). The ATP level of living muscle cells never drops below the normal physiological levels until CP concentration drops or until the acid pH begins to denature creatine kinase. As creatine phosphate is depleted or creatine kinase becomes inactivated by low pH, ADP cannot be rephosphorylated at the same rate as it is produced by dephosphorylation of ATP. Consequently, ATP concentration begins to fall and may be completely depleted or if the ATP-utilizing mechanisms are inactivated, the postmortem decline in ATP concentration may stop when muscle ATP concentration is still 10-20% of what it was in living muscle (Bendall, 1951).

The rate of pH fall has been shown to be greatly influenced by postmortem storage temperature, but the effect of temperature can vary among different muscles from the same animal and among corresponding muscles of different species (Bate-Smith and Bendall, 1949; Bendall, 1951; 1960; Busch et al., 1967; Cassens and Newbold, 1966; 1966a, b; Cook and Langsworth, 1966; de Frenery and Pool, 1960; Laurie, 1966a; Marsh, 1954; Marsh and Thompson, 1958). As the storage temperature declined from 37°C to 0°C, the rate of pH fall declined in rabbit psoas major muscle (Bendall, 1960) and in bovine psoas major and semitendinosus muscle (Busch et al., 1967). However, in bovine sternomandibularis muscle the rate
of pH fall is greater at 1°C than at 5°C during the first few hours post-mortem (Cassens and Newbold, 1967a; Newbold and Scopes, 1967).

As long as sufficient quantities of ATP are present in postmortem muscle, the muscle will contract (when stimulated) and relax in a manner similar to physiological muscle contraction and relaxation (Bendall, 1960). A muscle strip excised from a muscle immediately after death will contract or shorten because of the stimulatory effects of excision. The muscle strip can also be easily extended by an applied force (simulating relaxation). When ATP is greatly diminished, the muscle becomes fixed in whatever degree of contraction it happens to be at the time of ATP depletion (Newbold and Harris, 1972). Huxley and Brown (1967) have shown that during muscle contraction only about 20% of the cross-bridges on the thick filaments were actually attached to the thin filaments at any one time. When ATP was depleted, however, 100% of the cross-bridges were attached to the thin filaments causing the muscle to be inextensible (Huxley, 1968) since the thick and thin filaments are locked into position and can no longer slide past one another. Sarcomere length in myofibrils has been shown to be a measure of the contraction state (degree of shortening) of the muscle (Herring et al., 1967b). The extent of postmortem shortening is influenced by storage temperature, although not all mus-
cles show the same temperature dependence. Bate-Smith and Bendall (1949) first showed this temperature effect when they found that rabbit muscle stored at 37°C shortened 2.5 times more than muscle stored at 17°C. Marsh (1954) showed the amount of work done in terms of shortening against a load in bovine muscle strips increased progressively as storage temperature was increased from 7°C to 37°C. Locker and Hagyard (1963) showed rabbit psoas major muscle, excised pre-rigor, shortened 30% at 37°C but only 9% at 2°C. They also found that postmortem bovine muscle shortened maximally at 2°C, termed cold shortened muscle, minimally in the range of 14-19°C, and intermediately in the range of 19-37°C. The extensive and dramatic shortening in bovine muscle at 2°C, termed cold-shortening (Locker and Hagyard, 1963) differed from normal shortening in two respects: 1) it occurred while ATP concentration was 1-2mM or more (which is higher than in normal rigor mortis) and 2) it was reversible for a short time after death. Ovine (Cook and Langworth, 1965) and porcine (Galloway and Goll, 1967; Hendricks et al., 1971) muscles have also been shown to exhibit some cold-shortening characteristics.

Furthermore, Stromer and Goll (1967a, b) have shown that myofibrils from bovine semitendinosus and psoas major muscles excised at-death, were relaxed, having wide I-bands and H-zones in the sarcomere. After these muscles were allowed to
shorten at 2°C, the sarcomeres were greatly contracted having no H zone and a very narrow I band. Henderson et al. (1970) showed that sarcomeres were 1.6 to 1.7um in length at 24 hr post-mortem in rabbit and porcine muscle stored at 2°C and 16°C and in bovine muscle stored at 16°C and 25°C. Sarcomeres were 1.3um in length in bovine muscle stored at 2°C for 24 hr. Sarcomeres were 1.5 to 1.6um in length in rabbit, porcine and bovine muscles stored at 37°C for 24 hr. Even greater shortening can occur in muscles frozen pre-rigor and then thawed and stored at 2°C called thaw shortening. Thaw shortening in excess of 70% of at-death length has been reported in rabbit psoas major muscle (Lawrie, 1968), bovine sternomandibularis muscle (Marsh and Leet, 1966b; Scopes and Newbold, 1968) and ovine longissimus muscle (Marsh and Thompson, 1958). The amounts of thaw-shortening and cold-shortening decrease as the period between death and exposure to cold or freezing conditions is extended (Locker and Hagyard, 1963; Marsh and Leet, 1966b; Marsh and Thompson, 1958).

At some point after death, rigor mortis develops in the muscles of the carcass. The term "rigor mortis" literally means "stiffness of death" and refers to stiffness or rigidity of muscles that occurs at some varying time after death. Most of the early work on rigor mortis originated during the 1930's from the Low Temperature Research Station at
Cambridge, England where E. C. Bate-Smith, J. R. Bendall, R. A. Lawrie, B. B. Marsh, and co-workers found that muscle became inextensible during the onset of rigor mortis (Bate-Smith, 1939). A kymograph was used to record the time-course of postmortem muscle extensibility changes during the development of rigor mortis. This method consisted of periodic loading and unloading of a 50gm weight on a muscle strip excised immediately post-mortem and recording the resultant amount of extension which occurred in the strip after loading. It was shown that as rigor mortis developed, the degree of muscle extension decreased to a point where the muscle strip was no longer extensible. At this point the muscle strip was considered to be in full rigor. The muscle extensibility measurement was described in three different phases—delay phase, rapid phase and postrigor phase. The delay phase was the period of time during which extensibility does not change and ATP, being continually resynthesized by glycolysis, was maintained at antemortem levels. The rapid phase was the period during which extensibility rapidly decreases, creatine phosphate becomes depleted and ATP concentration falls to the point that all possible cross-links were formed between the thick and thin filaments. The postrigor phase was a continuous phase in which little or no change in extensibility occurred. This was an irreversible phase since the energy stores needed for muscle extensibility
were depleted and could not be replenished. The method of measuring muscle extensibility was later improved by Bate-Smith and Bendall (1949), who developed a procedure for automatically loading and unloading the muscle strip by electrical means. This instrument was further improved by Briskey et al. (1962), who added sealed chambers in which humidity, temperature and gaseous atmosphere could be controlled or changed. Because of the availability and widespread use of these instruments, rigor mortis has been almost universally defined in terms of extensibility; a muscle which has become inextensible is said to be in rigor mortis.

Muscle inextensibility, caused by the formation of cross-links between the thick and thin filaments, occurs at the same time as rigor mortis but does not cause muscle rigidity. Muscle rigidity is caused by attempted shortening (tension development) by two muscles on opposing sides of the same bone (Goll, 1968). Since neither of these two muscles were able to shorten substantially, this attempted shortening amounts to an isometric contraction. The stiffness or rigidity of an isometric contraction is directly responsible for rigor mortis. Only recently has rigor mortis development been measured by the amount of isometric tension generated by postmortem muscle. Busch et al. (1967), Jungk and Marion (1970) and Jungk et al. (1967) measured the amount of tension developed in muscle strips held at a fixed length as it
passed into rigor mortis. These investigators showed that maximum isometric tension development occurred at approximately the same time as muscle inextensibility occurred. However, isometric tension gradually decreased after maximum tension development even though the muscle remained inextensible. Gradually increasing isometric tension development corresponded to the onset of rigor, maximum isometric tension development corresponds to maximum rigor and gradually declining isometric tension corresponded to the resolution of rigor mortis.

During the course of rigor mortis it is relatively well recognized that muscles in a carcass, become less rigid. This loss of rigidity is measured by a decline in isometric tension, however, the postmortem measure of muscle extensibility counterdicts any resolution of rigor mortis. Development of muscle inextensibility and maximum isometric tension occur at about the same postmortem time, but each develops by different causes. The loss of muscle extensibility is directly related to ATP depletion in that postmortem muscle loses its extensibility only after the ATP level has declined to less than 20% of its antemortem level (Davies, 1967). With such a low level of ATP, the cross-links between the thick and thin filaments will not split, making the muscle inextensible. Postmortem isometric tension develops as the muscle attempts to shorten. This shortening requires ATP
(Bendall, 1951), so that shortening and isometric tension must precede the loss of extensibility in postmortem muscle.

The onset of rigor mortis occurs as the muscle attempts to shorten post-mortem. This postmortem contraction is caused by the increase in free, intracellular Ca\(^{2+}\) concentrations, just as an increase in free Ca\(^{2+}\) initiates contraction in living muscle. The increase in free Ca\(^{2+}\) in postmortem muscle is due to the loss of Ca\(^{2+}\) binding ability of the SR (Greaser et al., 1967). The SR loses the ability to bind Ca\(^{2+}\) by: 1) the loss of ATP because the energy of ATP is needed for Ca\(^{2+}\) accumulation by the SR, 2) a low pH inhibits Ca\(^{2+}\) binding by the SR and 3) possibly, proteolysis which would destroy the ability of the SR to bind Ca\(^{2+}\).

The resolution of rigor mortis was proposed by Goll (Goll, 1968; Goll et al., 1969b) to result from two alterations in the myofibril. These alterations are: 1) a modification of the actin-myosin bond resulting in some subtle increase in postmortem muscle sarcomere length and 2) a degradation of the Z-line structure with a weakening and possible rupture of the bonds between the thin filaments and Z-filaments.

The modification of the actin-myosin bond may be caused by traces of residual ATP, ADP or other agents in the muscle cell resulting in some slippage or partial dissociation at the points where myosin cross-bridges interact with the actin
filament. This slippage would cause slight lengthening of the sarcomere and result in a lessening of tension in the muscle (Goll et al., 1971a). Fujimaki et al. (1965a, b) have shown that in the presence of 1mM Mg\(^{2+}\) and 600mM KCl, myosin B prepared from rabbit muscle after 7 days post-mortem was dissociated to actin and myosin by only 0.1mM ATP, while 0.6mM ATP was required to cause the same dissociation in myosin B prepared from at-death muscle. Goll and Robson (1967) and Robson et al. (1967) found that the Mg\(^{2+}\)- or Ca\(^{2+}\)-modified ATPase activities of myofibrils or myosin B prepared from bovine psoas major and semitendinosus muscles at 24 hr post-mortem at 20\(^\circ\)C or 16\(^\circ\)C are 20-50\% higher than the corresponding activities from at-death muscles. Okitani et al. (1967) observed that storage of myosin B in 0.6M KCl at pH 6.0 and 25\(^\circ\)C resulted in a 50-100\% increase in Mg\(^{2+}\)-modified ATPase activity. Hay et al. (1972) and Jones (1972) have shown that the Ca\(^{2+}\)- and Mg\(^{2+}\)-modified ATPase activities of chicken actomyosin increases during postmortem storage indicating a change in the actin-myosin interaction. Strandberg et al. (1973) have reported that alteration of sulfhydryl groups in at-death actomyosin produced many of the same changes in nucleoside triphosphatase activity as that caused by postmortem storage.

Many investigators (Gothard et al., 1966; Stromer et al. 1967; Takahashi et al., 1967) have shown, ultrastructurally,
a tendency of rigor-shortened sarcomeres to lengthen between 12 and 30 hr post-mortem in the absence of ATP. Stromer et al. (1967) have shown that with bovine muscle, allowed to cold-shorten, the sarcomeres will lengthen post-rigor by a sliding of interdigitating filaments past one another caused by a slippage of the interaction of the myosin cross-bridges and actin filaments. These studies, however, were conducted on myofibrils that were supercontracted to sarcomere lengths of approximately 1.0μm, and such shortening does not occur normally in muscles attached to the carcass during rigor development.

Degradation of Z-line structures would cause breaks to occur in the myofibrils and as a result the tension held by the muscle would necessarily lessen. Postmortem Z-line degradation has been observed by Davey and Dickson (1970), Davey and Gilbert (1967; 1969), Henderson et al. (1970), Penny (1970) and Takahashi et al. (1967). Haga et al. (1966) have shown that a break between the actin filaments and the Z-line must occur before actin was extracted. Myosin can be solubilized in one hour by extraction with 0.5M KCl solutions at pH 7.0, but actin requires a considerably longer time for extraction. Since myosin cross-bridges are bound to actin during rigor mortis, actin extractability will affect myosin extraction. If the actin-myosin bonds are not weakened or dissociated, the rate of actomyosin extraction is dependent
on the rate of actin extraction or the rate of dissociation of the thin filaments from the Z-lines (Goll et al., 1969a). Complete extraction of myosin without actin is possible when pyrophosphate or ATP is present to dissociate and prevent further interaction of myosin and actin (Haga, et al., 1965). However, if the bonds between the thin filaments and Z-line are weakened or disrupted actin will be extracted along with myosin in the pyrophosphate or ATP-containing solutions (Mihalyi and Rowe, 1966).

The amount of actomyosin extracted has been shown to increase during postmortem storage (Chaudhry et al., 1969; Davey and Gilbert, 1968a; Valin, 1968). Davey and Gilbert (1968b) found, by extracting myofibrillar proteins with a 0.6M KCl solution at pH 9.2, that extraction was complete in 60 minutes from myofibrils stored 2 days post-mortem, but only 10 minutes was required to yield the same amount of myofibrillar protein from myofibrils stored 17 days post-mortem. They also found that by adding magnesium and pyrophosphate to the extraction solution the total myofibrillar proteins extracted from myofibrils increased from 54% at 1 day to 75% at 17 days of postmortem storage. Myosin was almost completely extracted at 1 day post-mortem while at 17 days post-mortem myosin, actin plus other myofibrillar proteins were extracted (Davey and Gilbert, 1968a). These results suggest that during postmortem storage a weakening of the Z-line
occurs so that actin becomes soluble in pyrophosphate extractions.

Penny (1968), using the Ca$^{2+}$-modified ATPase activity to determine the amount of myosin, found that pyrophosphate-containing solutions quantitatively extracted myosin from at-death myofibrils, but extraction of myofibrils stored for 4 days at 15-18°C or for 14 days at 4°C caused solubilization of myosin and tropomyosin. Chaudhry et al. (1969) found that increased postmortem storage time at 2°C resulted in increased amounts of actin extracted.

A weakening or rupture of the Z-line would also result in a decrease of the tensile strength of the muscle fiber. Stanley et al. (1971) found that bovine longissimus muscle strips at 1 day post-mortem required 15% greater force to break the strips by opposing longitudinal forces than was required for muscle strips 7 days post-mortem. Bouton and Harris (1972b) also found a significant reduction in fiber tensile strength in bovine longissimus muscles from 2 to 14 days post-mortem. Additionally, during the preparation of myofibrils by homogenization, fewer sarcomeres per myofibril (myofibril fragmentation) would result if the number of weakened or ruptured Z-lines were greater.

Numerous workers (Darey and Gilbert, 1967, 1969; Fukazawa et al., 1969; Hay et al., 1973b; Henderson et al., 1970; Parrish et al., 1973b; Sayre, 1970; Takahashi et al.,
1967) have observed that during postmortem storage greater fragmentation of myofibrils, breakage of the myofibril at the Z-line into smaller segments, occurs after homogenization of muscle tissue. Davey and Gilbert (1969) found that as postmortem storage time proceeded, muscle fiber pieces lose the precise register of the myofibrils and finally break down to single myofibrils. Since fragmentation of the myofibril reflects the loss of strength or weakness of the Z-line, increased myofibril fragmentation during postmortem storage indicates a weakening of the Z-line during storage.

The postmortem changes in sarcoplasmic proteins primarily involve the proteins which have an integral role in muscle metabolism. The energy stores, ATP and CP, are utilized resulting in the products adenosine monophosphate (AMP) and creatine (Scopes, 1970). Several sarcoplasmic proteins such as creatine kinase are particularly susceptible to precipitation at a low pH (about pH 5.5) (Scopes, 1964). Because of the vast number of different proteins in the sarcoplasmic protein fraction, protein solubility studies have been made on this fraction to determine gross effects of postmortem storage. Sarcoplasmic protein solubility is normally greatest immediately after death and then either remains unchanged or decreases during postmortem storage (Chaudhry et al., 1969; Scopes and Lavrie, 1963). If muscle pH falls below 6.0 while the muscle temperature is 35°C or higher,
however, sarcoplasmic protein solubility decreases substantially (Sayre and Briskey, 1963; Scopes, 1964). Chaudhry et al. (1969) have shown that a large decrease in sarcoplasmic protein solubility occurred in bovine and rabbit muscle during postmortem storage above 30°C. On the other hand, rapid cooling and storage of at-death muscle to 4°C resulted in sarcoplasmic protein solubility to remain near the at-death level (Borchert and Briskey, 1965; Goll et al., 1964). Under normal postmortem storage conditions 10-30% of at-death sarcoplasmic protein solubility is lost. Numerous investigators (Aberle and Merkel, 1966; Borchert et al., 1969; Hay et al., 1973a; Lawrie et al., 1963; Maier and Fischer, 1966; Neelin and Rose, 1964) have shown, however, that gel electrophoretic patterns of sarcoplasmic proteins extracted from at-death muscle were very similar to those extracted from postmortem muscle stored at 5°C or lower. Even when sarcoplasmic protein solubility of postmortem muscle was lower than at-death muscle, few qualitative differences were shown (Borchert et al., 1969).

Neealin and Ecobichon (1966) have suggested that during the preparation of sarcoplasmic proteins in hypotonic salt solutions cytoplasmic structures such as the sarcolemma or the sarcoplasmic reticulum are released into the sarcoplasm. Furthermore, these proteins were increasingly released during postmortem storage even when the sarcoplasmic proteins were
prepared in hypertonic sucrose solutions, which supposedly preserve the cytoplasmic structure in at-death muscle. Eason (1969), Greaser et al. (1967, 1969a, b, c) and Nakamura (1973) have also found an alteration in the cytoplasmic structure during postmortem muscle storage. These investigators have shown that the sarcoplasmic reticular membranes very quickly lost their calcium sequestering ability after death, even though their ATPase activity and ultrastructure did not undergo large postmortem changes. Osner (1966) and Reed et al. (1966) have also shown that the sarcolemma is a very labile system that may change rapidly after death.

Postmortem changes in the stroma protein fraction has been studied by numerous investigators. Winegarden et al. (1952) reported that strips of collagenous connective tissue from bovine skeletal muscle required a slightly lower shear-force to shear them at 35 days of postmortem storage at 2°C than at 10 days of postmortem storage at 2°C. Husaini et al. (1950) and Hershberger et al. (1951) found that collagen and elastin content, measured by alkali-insoluble nitrogen, of bovine longissimus muscle decreased by 10-30% after 15 days of postmortem storage at 30°C. Prudent (1947) and Wierbicki et al. (1954), however, reported no change in the alkali-insoluble protein content of bovine muscle up to 15 days of postmortem storage. Khan and van den Berg (1964) and Sayre (1968) found the alkali-insoluble protein content of chicken
muscle remained constant during postmortem storage at 0°C for 4 days and, in some instances, for several months.

While these studies did not investigate any ultrastructural changes in the fibrous connective tissue proteins, they demonstrated that no large change in alkali-insoluble protein content appears to occur during postmortem storage.

Sharp (1963) reported no change in the extractability of hydroxyproline (a measure of soluble collagen content) during postmortem storage of bovine muscle for 172 days at 37°C. However, Herring et al. (1967a) reported that the percentage of total muscle hydroxyproline that was solubilized by heating at 77°C for 10 minutes increased significantly after post-mortem storage at 4°C for 10 days. These studies suggested that a subtle change in collagen occurred during postmortem storage and this change was not detected during storage at 37°C but it was detectable when heated to 50-80°C.

McClain et al. (1965) reported there was no change in total collagen of bovine longissimus muscle during 7 days of postmortem storage at 4°C, but that there was a gradual conversion of neutral-soluble collagen to acid-soluble collagen during this storage period. McClain (1969) and McClain and Pearson (1969) have also shown that a rapid drop in pH immediately after death in porcine muscle can alter the solubility of collagen after heating. These results suggested that postmortem storage affected the structure of the collagen by
the alteration of the number and strength of cross-linkages between collagen molecules. Clayson et al. (1966) have suggested that postmortem changes in cross-linkages between collagen molecules were probably not due to proteolytic enzymes but rather caused by traces of oxidized nitrogen in a highly active form.

These studies indicated that postmortem storage caused some weakening or rupture of the collagen cross-linkages resulting in increased collagen solubility after heating to about 50°C. De Fremery and Streeter (1969) found no increase in susceptibility of collagen solubilization by heating during postmortem storage at 2°C for 24 hr in poultry muscle nor was any change found in the amount of alkali-insoluble protein during postmortem storage at 2°C for periods up to 8 days. It is not clear to what extent postmortem storage influences connective tissue proteins, but it is indicated that these influences are subtle and probably confined to changes in the number and strength of collagen cross-linkages.

McClain et al. (1970) suggested that the postmortem pH fall, more than prolonged storage, altered the collagen structure. Furthermore, Kruggel and Field (1971) and Pfeiffer et al. (1972) suggested that the number of covalent cross-linkages in collagen could be reduced by pre-rigor muscle stretching. Herring et al. (1967a) noted, however, that the amount of postmortem shortening did not significantly
affect collagen content nor solubility of bovine longissimus muscle.

Almost sixty years ago Hoagland reported (Hoagland et al., 1917) that proteolysis was an important factor contributing to postmortem changes in muscle proteins. Whitaker (1964) suggested that the postmortem changes in muscle were due to proteolytic activity affecting the myofibrillar proteins. Goll (1968) proposed that the alteration of the actin-myosin interaction and the degradation of the Z-line during postmortem storage may have been due to very limited and specific proteolysis. Parrish and Bailey (1966, 1967) showed the presence of a proteolytic enzyme, cathepsin D, in bovine muscle and presented evidence that it was located in the lysosomes. Iodice et al. (1966), Parrish and Bailey (1967) and Suzuki and Fujimaki (1968) found cathepsin D was the active protease in skeletal muscle although Caldwell and Grosjean (1971) found cathepsin A was more active in chicken skeletal muscle. De Duve (1959, 1963) demonstrated that lysosomes contained a number of acid hydrolases including cathepsin D and that the function of lysosomes was that of acid digestion under physiological and pathological conditions (de Duve and Wattiaux, 1966), such as an increase in catheptic activity in dystrophic muscle (Tappel et al., 1962; Weinstock et al., 1955; Zalkin et al., 1962). Pellegrino and Franzini (1963) showed that lysosomes appeared only during muscle
atrophy and that no lysosomes were present in normal muscle. Price et al. (1964) and Smith (1964) indicated that lysosomes of apparent muscle origins were confined to blood vessels and macrophages. Canonico and Bird (1970), however, demonstrated the existence of two locations for two distinct groups of lysosomes; one group originated from macrophages and connective tissue cells and the other group originated in the muscle cells. Those lysosomes in muscle cells contained cathepsin D and acid phosphatase. Stauber and Bird (1974) have shown that lysosomes in skeletal muscles are part of the sarcotubular system of the cells.

Classically, the extent of proteolysis in postmortem muscle is determined by an increase in free amino acids and non-protein nitrogen (Parrish, 1971). Increases in free amino acids and non-protein nitrogen have been observed in postmortem beef muscle (Davey and Gilbert, 1966; Field and Chang, 1969; Field et al., 1971; Gardner and Stewart, 1966; Locker, 1960b; Parrish et al., 1969b) poultry muscle (Khan and van den Berg, 1964; Miller et al., 1965) and rabbit muscle (Suzuki et al., 1967). Parrish et al. (1969b) showed a four-to-six fold increase in several amino acids but the protein source of these amino acids could not be determined, although it was suggested that the myofibrillar proteins were an unlikely source. Additionally, most of the increase in free amino acids and non-protein nitrogen occurred from 7 to
28 days post-mortem which is the period after which most of the myofibril structural change had already occurred (Parrish et al., 1969b). Bodwell and Pearson (1964) and Sharp (1963) concluded that the increase in free amino acids largely originated from the sarcoplasmic protein fraction which has little influence on meat tenderness. Locker (1960b) using N-terminal group analysis, found no evidence that myofibrillar proteins were proteolytically cleaved during postmortem storage. Friedman et al. (1969), Fukazawa and Yasui (1967) and Park and Pennington (1967) have shown no proteolysis of intact myofibrils or thick and thin filaments. From the studies of several investigators (Clayson et al., 1966; Etherington, 1971; Goll, 1965b) it was concluded that there was no significant proteolysis during postmortem storage in the sarcoplasmic and stroma proteins.

Goll et al. (1971b) and Stromer et al. (1967) treated supercontracted myofibrils briefly with trypsin in the absence of ATP and found that sarcomeres lengthened from 1.0um to 1.7um. Tryptic digestion of at-death myofibrils also caused a 50-80% increase in the Mg²⁺- and Ca²⁺- modified ATPase activities, which is similar to ATPase activities of myofibrils from postmortem muscle. The close similarity between the effects of trypsin on myofibrils and the effects of postmortem storage on myofibrils suggests that the increased ATPase activity and the lengthening observed in postmortem
muscle may be due to a very limited and specific proteolysis (Goll, 1968). Busch et al. (1972) and Suzuki and Goll (1974) have shown that many of the characteristic alterations in Mg\textsuperscript{2+}-modified ATPase activity of actomyosin by postmortem storage can be duplicated by brief incubation of at-death actomyosin with a calcium-activated factor (CAF), isolated from muscle tissue, which will also selectively degrade Z-lines. Goll et al. (1969b), in a review, discussed three sites in the myofibril which are labile to proteolysis, based on known vulnerability to trypsin. The first site is myosin, which is rapidly cleaved into light and heavy meromyosin by trypsin (Mihalyi and Szent-Gyorgi, 1953) or chymotrypsin (Gergely et al., 1955). Both Bodwell and Pearson (1964) and Martins and Whitaker (1968) have shown that neither actomyosin nor myosin is affected by partially purified muscle cathepsins. Also, myosin ATPase has not been found to change appreciably during postmortem storage (Goll and Robson, 1967; Penny, 1968; Robson et al., 1967; Hu and Sayre, 1971) as would be expected if myosin had been proteolytically degraded. However, Suzuki et al. (1969) have shown some hydrolysis of the myofibrillar proteins by purified cathepsin D from rabbit muscle. Dayton et al. (1974a, b) have shown that purified CAF does not proteolytically degrade myosin even though the intact myofibril has been degraded. On the other hand, Suzuki and Goll (1974) have shown a very slight increase in
myosin ATPase after treatment of myofibrils with CAF.

All work considered, it can be stated that the functional activity of myosin may be slightly modified by CAF, but no significant proteolysis of myosin occurs due to CAF or catheptic action.

The second proteolytically-labile site in the myofibril is the tropomyosin-troponin complex. This complex has been shown to be rapidly degraded to small peptides by trypsin (Laki, 1957; Ebashi and Kodama, 1966). However, biochemical evidence on calcium-sensitivity of the ATPase of postmortem myofibrils has shown that the tropomyosin-troponin complex was not proteolytically destroyed, even after 13 days post-mortem at 16°C (Galloway and Goll, 1967; Goll and Robson, 1967). Davey and Gilbert (1966) observed that tropomyosin remains difficult to extract even after 21 days post-mortem at 20°C indicating no extensive degradation of tropomyosin. Arakawa et al. (1970a, b) showed that yields of alpha-actinin and the tropomyosin-troponin fraction were not changed by postmortem storage and that these two protein fractions prepared from postmortem muscle stored for 8 days at 25°C were functionally active and exhibited normal sedimentation patterns. These findings indicated that no extensive degradation of alpha-actinin, tropomyosin or troponin occurred during postmortem storage. However, Arakawa et al. (1970a) acknowledged that some weakening in the interaction between
the tropomyosin-troponin complex and actin had occurred.
Robson (1971) and Robson and Cohen (1971) found a significant decrease in the calcium-sensitive response of myofibrils even after 1 day post-mortem at 2°C and this decrease continued to decrease up to 7 days post-mortem in both bovine and porcine muscles. Additionally, Suzuki and Goll (1974) have shown that brief treatments with CAF increased the Mg²⁺-modified ATPase activity of at-death myofibrils indicating that this sarcoplasmic enzyme diminishes the inhibition of troponin-I on the Mg²⁺-modified ATPase activity of actomyosin (Ebashi and Kodama, 1966). Furthermore, Dayton et al. (1974a, b) found that CAF degraded troponin and tropomyosin into several fragments ranging in molecular weight from 10,000 daltons to 30,000 daltons, but did not degrade myosin, actin or alpha-actinin even after prolonged incubation periods. Alpha-actinin was shown to be released from the Z-line without being proteolytically degraded. Hay et al. (1973a) observed in chicken breast myofibrils stored up to 168 hr post-mortem that little change occurred in the migration of the proteins on SDS-polyacrylamide gels except for the obvious appearance of a 30,000-dalton molecular weight protein appearing after 48 hr post-mortem. They suggested that this protein may be a proteolytic breakdown product of myosin or troponin B. Dayton et al. (1974b) reported that very brief treatment of purified troponin with CAF yields a 30,000-dalton molecular
weight protein from troponin-T. These findings indicate that CAP, which is proteolytically active under normal postmortem muscle conditions (Dayton et al., 1974a, b; Suzuki and Goll, 1974) may cause a very limited and specific proteolytic degradation of the troponin-tropomyosin complex which may not, however, inhibit its functional activity.

The third possible site for postmortem proteolysis of myofibrils is at or near the Z-line. Goll et al. (1969) and Stromer et al. (1967) have shown that brief treatments of at-death myofibrils with trypsin quickly removes the Z-lines and releases alpha-actinin. Furthermore, Stromer and Goll (1967a) have shown that at 13 days of postmortem storage at either 20°C or 16°C, bovine myofibrils are more extensively fragmented into myofibrils of three or four sarcomeres in length than immediately after death. The fragmentation of the myofibril occurred due to breaks at the Z-line. Increased fragmentation of the myofibril occurred during postmortem storage of chicken muscle after 4 days at 20°C (Takahashi et al., 1967) and after 2 hr at 15°C post-mortem plus 22 hr at 30°C (Sayre, 1970). Fukazawa and Yasui (1967) have reported complete loss of Z-line structure in chicken muscle after 24 hr at 20°C, however, incubation of at-death myofibrils, having intact Z-lines, with cathepsins did not produce any loss of Z-line structure. While Z-line degradation of myofibrils was a very dramatic postmortem observa-
tion, cathepsins apparently did not influence this degradation.

Busch et al. (1972) found that a muscle strip held isometrically in a saline solution containing Ca\(^{2+}\) had a faster decline in post rigor isometric tension and considerably greater myofibril fragmentation than a muscle strip held in only saline or saline plus EDTA solution. Davey and Gilbert (1969) prepared myofibrils from bovine *sternomandibularis* muscles at 30 hr post-mortem and stored those myofibrils in a KCl-phosphate, KCl-phosphate-EDTA or clarified sarcoplasmic extract solution for up to 20 days. This study showed that 1) EDTA-containing solution inhibited any change in fragmentation for 20 days of storage, 2) myofibrils in the KCl-phosphate solution were very fragmented after 10 days of storage and 3) myofibrils in the sarcoplasmic extract were extremely fragmented before 10 days of storage. This study indicated that some factor in the sarcoplasmic extract, which is inhibited by EDTA, enhances myofibril fragmentation.

Henderson et al. (1970) found Z-line degradation occurred within 24 hr after death in bovine, porcine and rabbit muscle with bovine muscle exhibiting the least degradation. These muscles were stored at 20°C, 16°C, 25°C or 37°C during the first 24 hr post-mortem with Z-line degradation being greater at the higher storage temperatures. This study indicated that some factor in muscle, activated by higher temperatures,
enhanced Z-line degradation causing greater myofibril fragmentation. Busch et al. (1972) were successful in isolating a crude calcium-activated-sarcoplasmic factor (CAF) which selectively degraded Z-lines. Dayton et al. (1974a, b) have purified this enzyme and have shown that it completely removed the Z-line of the myofibril in 5 minutes at 25°C. Suzuki and Goll (1974) have shown that crude CAF was active at pH 5.5, at low temperatures (5°C) and at 1mM Ca²⁺ levels (Dayton et al., 1974a); these conditions are typically found in post-rigor muscle. It is highly possible that Z-line degradation observed in postmortem muscle is caused by CAF action on the myofibril.

Influences on Postmortem Muscle Tenderness

It has been universally recognized for over a century that the storage of muscle post-mortem above 0°C for a period of several days improves the tenderness of that muscle. The complexities of the postmortem tenderization of muscle have been studied by numerous investigators for the past several decades and it was not until now that these complexities have been unraveled to a more understandable level.

Both the rate and extent (ultimate pH) of the postmortem fall in pH have been associated with the tenderness of meat. Treatments which increase the rate of pH and ATP fall have been shown to increase poultry meat toughness (de Fremery and Pool, 1960; Khan and Nakamura, 1970). Busch et al. (1967),
however, did not find a parallel increase in tenderness with
the rate of pH drop in bovine semitendinosus and psoas major
muscles stored at 20°C, 16°C or 37°C. Bouton et al. (1957)
found that bovine muscle with an ultimate pH of 6.0 was least
tender whereas tenderness was higher when the ultimate pH was
either higher or lower than 6.0. In contrast, tenderness of
rabbit muscle (Miles and Lawrie, 1970), ovine muscle (Bouton
et al., 1971) and fish muscle (Kelly et al., 1966) has been
shown to be greater with higher ultimate pH.

While these studies indicate some possible relationships
between the rate and extent of pH fall in postmortem muscle
and meat tenderness, no adequate explanation has been given
for a causal effect of pH on structural changes in the myofi­
bril that would directly influence meat tenderness. The cir­
cumstances surrounding the pH fall probably indicates other
events or conditions in the muscle cell that more directly
influences meat tenderness. Bouton et al. (1973a) have shown
that the influence of muscle contraction on meat tenderness
was diminished as the ultimate pH of bovine muscle increased.
The rate and extent of pH fall in postmortem muscle may in­
dicate the rate and extent of biochemical reactions occurring
without pH directly affecting postmortem muscle tenderiza­
tion.

Locker (1960a) attributed meat toughness to two
components: background and actomyosin toughness. Connective
tissues constitute the background toughness and the myofibrillar proteins constitute the actomyosin toughness. The relationships of background toughness and actomyosin toughness to the total meat toughness is not constant since some muscles have different amounts of connective tissue than others (Vognarova et al., 1968). Because no objective method exists for measuring background and actomyosin toughness accurately and independently, the relative contributions of these two components of tenderness to total toughness is difficult to determine in any given sample of meat. Estimates of the relative contributions of connective tissue to total meat toughness can be made by fiber adhesion measurements (Bouton and Harris, 1972a, c; Pool, 1967) which measure the strength or weakness of the connective tissue between muscle fibers. W-B shear-force values (Bouton and Harris, 1972b; Bouton et al., 1973b) or fiber tensile strength measurements (Bouton and Harris, 1972b, c; Stanley et al., 1972), which are estimates of actomyosin toughness, measure the strength or weakness of the myofibril. Purchas (1972) has used such measurements together with an analysis designed to express all measurements on the same relative scale and has concluded that a 1% change in actomyosin toughness produced the same tenderness change as a 10% change in background toughness. Paul et al. (1973) also determined that myofibrillar proteins influenced muscle texture considerably more than collagen.
While connective tissue establishes the primary degree of meat tenderness, it is clear that actomyosin toughness, hence myofibrillar proteins, have extremely important effects on postmortem muscle tenderization.

The relationship between the changes in the muscle proteins during postmortem storage and the change in meat tenderness has been studied by numerous investigators. Thompson et al. (1968) found that the solubility of sarcoplasmic proteins of bovine longissimus muscle from a carcass side stored at 30°C for the first 24 hr post-mortem was slightly higher than from the carcass side stored at 30°C for 3 days post-mortem. However, no significant qualitative protein changes were found to be related to the tenderness differences found between the treatments. McClain and Mullins (1969) found no relationship between the amount of extracted sarcoplasmic proteins of bovine muscle and the muscle tenderness. Dikeman et al. (1971) found a correlation coefficient of -0.70 between sarcoplasmic protein solubility and muscle tenderness using bovine longissimus muscles of animals of A and E maturity. However, they also found sarcoplasmic protein solubility increased with animal age indicating that protein solubility and tenderness may both be directly related to animal age and only indirectly related to each other.

From the results of these studies and because the sarcoplasmic proteins do not constitute a part of the muscle
structure, it is doubtful that the sarcoplasmic protein fraction directly affects meat tenderness. The active role that this protein fraction plays in the tenderness of meat may be due to the proteolytic enzymes found in this fraction (Busch et al., 1972; Dayton et al., 1974a, b; Randall and MacRae, 1967).

As mentioned previously, connective tissue is important in that it establishes the basal level of meat tenderness. Connective tissue is thus important in the tenderness differences in muscles from animals of different maturities. Goll (1965a), Hill (1966) and Shimokomaki et al. (1972) have shown that the number of intermolecular covalent bonds in collagen increases as an animal matures and grows older. Giffee (1962) and Kim et al. (1967) have shown that the increase in these covalent bonds of collagen is reflected in lower muscle tenderness of older animals. Field et al. (1969) found bovine longissimus muscle epimysium to be less tender in older animals, however, neither hydroxyproline content nor epimysium thickness was significantly related to tenderness measured by W-B shear. Cross et al. (1973) found a significant correlation between the amount of collagen and elastin and the evaluation by a sensory panel of the amount of connective tissue residue remaining at the end of mastication. However, no significant correlation was found between the amount of collagen and elastin and the tenderness the sensory panel at-
tributed to the muscle fiber component. Kruggel et al. (1970) found more aldehyde-type covalent cross-linkages in acid soluble collagen in less tender bovine longissimus muscles than in more tender longissimus muscles.

Elastin comprises less than one-third of the connective tissue proteins (Goll et al., 1963) and it is considered to have only minimal influence on muscle tenderness (Cross et al., 1973; Partridge, 1966).

These results indicate that background toughness of meat is an important part of the total tenderness of meat, however, the influence that postmortem storage has on the improvement of the connective tissue component of muscle tenderness is not completely clear.

On a macromolecular level, intramuscular fat (marbling) has been associated with meat tenderness. Marbling in beef longissimus muscle has been used in U.S.D.A. Beef Quality Grades to indicate the quality level (of which tenderness is a principal part) of beef carcasses. Parrish (1974), in a review, has found that, in only a few selectively controlled studies, marbling accounted for as high as 36% of the variation in meat tenderness, but in most studies marbling accounted for less than 10% of the variation in meat tenderness. It was concluded that intramuscular fat has little influence on meat tenderness especially on the increase in meat tenderness that occurs during postmortem storage. Parrish et
al (1973a) have shown that the degree of internal doneness of broiled steaks from bovine longissimus was a much more important factor of meat tenderness than was marbling. Draudt (1972) and Hamm (1966), in reviews, have described the predominant changes in the two major structural components of muscle, the collagenous and myofibrillar proteins, during heating. Heating to an internal temperature of 60°C causes shrinkage and partial solubilization of collagen and minimum drying, hardening and coagulation of the myofibrillar proteins resulting in maximum beef tenderness. Further heating causes greater collagen solubilization, however, this is offset by the toughening effect from coagulation of the myofibrillar proteins. At high internal meat temperatures (80°C-90°C), the meat becomes very tough due to the coagulation of the myofibrillar proteins (Schmidt and Parrish, 1971). Cooking temperature can thus have extremely large influences on meat tenderness making comparisons between tenderness measures difficult when different cooking temperatures are used.

Because the myofibrillar proteins play an integral role in meat tenderness, (actomyosin toughness), the postmortem changes in these proteins have been studied in their relationship to the postmortem tenderization of meat. Haga et al. (1965), Sayre (1968) and Weinberg and Rose (1960) suggested that the increase in postmortem actomyosin extraction,
caused by detachment of thin filaments from Z-line, was related to the increase in postmortem meat tenderness. Correlation coefficients of -0.69 (Hegarty et al., 1963) and -0.77 (Aberle and Merkel, 1966) have been found between the amount of myofibrillar protein extracted post-mortem and W-B shear-force measurements of bovine longissimus muscles. Dikeman et al. (1971) found a correlation coefficient of 0.66 between myofibrillar protein extracted and sensory panel tenderness of bovine longissimus muscle stored 4 days post-mortem. However, Goll et al. (1964) found no significant correlation coefficients between myofibrillar protein solubility and tenderness for muscles removed from the carcass at-death or for those left attached to the carcass. Chaudhry et al. (1969) found no significant differences in the solubility of myofibrillar proteins up to 4 days of postmortem storage between bovine psoas major and semitendinosus muscles, both of these muscles being extremely different in their tenderness properties (Dumont, 1971; Herring et al., 1965b). McClain and Hullins (1969) found no significant difference between myofibrillar proteins extracted from tender and less tender muscles of bovine longissimus muscles. It appears that the solubility of myofibrillar proteins is not an accurate and dependable indicator of meat tenderness, however, the change in solubility during postmortem storage may indicate a weakening of the Z-line-thin filament attachment which is proba-
bly related to postmortem muscle tenderization. Apparently, however, myofibrillar protein solubility is not a precise enough measure of that weakening to form a direct relationship with muscle tenderness.

The onset of rigor mortis is caused by the attempted shortening of the carcass muscles on opposing sides of the skeleton. The degree to which the muscles actually shorten could have important effects on the tenderness of those muscles. Locker (1960a) concluded that there was a direct relationship between postmortem muscle shortening and muscle tenderness. Numerous investigators have examined the extent and significance of postmortem shortening as it influences meat tenderness (Buck and Black, 1967; Buck et al., 1970; Busch et al., 1967; Cooper et al., 1968; Davey et al., 1967; Herring et al., 1965a, b; 1967b; Howard and Judge, 1968; Marsh and Leet, 1966a, b; Harsh et al., 1968; McCrae et al., 1971; Smith et al., 1971; Weidemann et al., 1967; Welbourn et al., 1968).

Using bovine sternomandibularis muscles excised soon after death, Behnke et al. (1973), Davey et al. (1967), Marsh (1972), Marsh and Carse (1974, and Marsh and Leet (1966a, b) have shown that muscle shortening by up to 20% of the excised length only slightly affects tenderness whereas muscle shortening from 20-40% causes a four-to five-fold decrease in tenderness. Further muscle shortening from 40-55% causes a
progressive increase in meat tenderness until it finally approximates the same level of tenderness found in muscles shortened by less than 20%. Muscle shortening has also been shown to similarly influence tenderness of ovine muscles (McCrae et al., 1971). The increase in toughness with shortening is considered to be due to the increased density of myofilaments per fiber area caused by contraction of the sarcomere. This increased density results in a greater number of myofilaments which must be sheared across per fiber area during mastication (Marsh and Leet, 1966b).

Herring et al. (1965a, b) and Wiskus et al. (1973) reported a highly significant relationship between the amount of muscle contraction, as evaluated by sarcomere length, and tenderness in bovine muscles. Herring et al. (1967b) showed that when a muscle was excised and permitted to severely shorten during the development of rigor mortis, the muscle did not have an acceptable sensory panel tenderness level even after ten days of postmortem storage.

Goll et al. (1964) showed that excising a muscle from a carcass immediately after death stimulated a greater degree of shortening of the muscle than in muscles left intact in the carcass. These excised muscles had increased W-B shear values (less tender) and after postmortem storage became more tender, but steaks from these excised muscles never did become as tender as steaks from muscles left in the carcass.
This was explained on the basis of the effect of cold shortening on unrestrained muscles when stored under cold (2-4°C) conditions. Muscles left attached to the carcass, however, became more tender during postmortem storage with most of this increase in tenderness occurring the first 24 hr of postmortem storage. Herring et al. (1974b) have also shown that bovine semitendinosus muscle excised at death which shortened 36% of its excised length still improved in tenderness during postmortem storage.

Marsh and Leet (1966b) investigated the effect of muscle shortening on meat tenderness and found that the excised pre-rigor muscle stored at 1°C or 5°C had sarcomere lengths 50% shorter than muscle excised post-rigor. The post-rigor excised muscle was found to always be more tender than muscle excised pre-rigor. Additionally, Reville et al. (1971) reported that post-rigor bovine psoas major muscle had considerably longer sarcomere lengths than post-rigor semitendinosus muscle, which is almost always less tender than the psoas major muscle (Dumont, 1971). These studies indicate that postmortem muscle shortening is detrimental to post-rigor muscle tenderness, thus it is clearly desirable to minimize or prevent postmortem muscle shortening.

Muscle restrained from shortening pre-rigor will not shorten post-rigor (Marsh and Thompson, 1958) nor will it become tougher by pre-rigor exposure to cold-shortening
conditions (Harsh and Leet, 1966b; Taylor et al., 1972). Muscles on a suspended carcass are not all restrained to the same extent because the amount by which a muscle can shorten depends on the hanging position of the carcass during the development of rigor mortis. Herring et al. (1965b) have shown that the psoas major, latissimus dorsi and rectus femoris muscles from a bovine carcass side suspended by the Achilles' tendon were more tender than the corresponding muscles from a side suspended horizontally; semimembranosus, semitendinosus, longissimus and biceps femoris muscles were less tender and serratus ventralis and triceps brachii muscles were unchanged. Arango et al. (1970), Harris and Macfarlane (1971) and Hostetler et al. (1970, 1972, 1973) have investigated the effects of hanging bovine carcass sides in different ways and have found that, compared with the conventional method of hanging by the Achilles' tendon, hanging a carcass side by the obturator foramen improves the tenderness of the longissimus, semimembranosus and biceps femoris muscles. Bouton and Harris (1972c) have shown that the tenderness of ovine longissimus, semimembranosus and biceps femoris muscles can be improved by hanging the whole carcass from the pelvis instead of the Achilles' tendon. Davey and Gilbert (1974) have shown that placing ovine carcasses in a standing position pre-rigor prevents toughening due to shortening of most carcass muscles. The improvement in tenderness results from
the reduction in or prevention of shortening in the affected muscles.

Not only will the method of carcass suspension improve meat tenderness but postmortem storage temperature also influences meat tenderness. Postmortem storage of a carcass at 16°C or higher immediately after death for at least 12 hr compared to storage at 2-4°C has been shown to stimulate a greater increase in postmortem muscle tenderization (Culp et al., 1973; Fields et al., 1971; Judge et al., 1971; Martin et al., 1971; Parrish et al., 1969a, 1973b; Semelsk and Riley, 1974). The longissimus muscles from intact ovine carcasses held for various periods at 20°C before being exposed to freezing conditions have been shown by Marsh et al. (1968) to be more tender when the storage period exceeds 16 hr than when it is less than 16 hr. McCrae et al. (1971) have shown that ovine muscle tenderness is greatly improved if the carcass is stored at 18°C from 10-16 hr before freezing than if held at a lower temperature for 16 hr. Smith et al. (1971) concluded that storing a bovine carcass at 15°C for the first 16-20 hr post-mortem produces as much improvement in longissimus muscle tenderness as hanging the carcass at 2°C by the obturator foramen to prevent shortening. Bouton et al. (1974) have shown that postmortem storage of ovine carcasses at 7-8°C will prevent the muscle toughness that is caused by muscle shortening that occurs when the carcass was stored at
Schmidt and Gilbert (1970) have compared the tenderness of bovine longissimus and biceps femoris muscles excised pre-rigor and stored at 15°C for 24 or 48 hr with the tenderness of corresponding muscles left attached to the other side of the carcass and stored at 9°C for 24 hr. The biceps femoris and longissimus muscles stored at 15°C for 24 hr were equivalent in tenderness to the corresponding muscles restrained on the carcass, stored at 9°C for 24 hr. The excised muscles stored at 15°C for 48 hr were significantly more tender than the excised muscles stored at 15°C for 24 hr or the muscles left attached to the carcass and stored at 9°C for 48 hr.

Besides reducing toughness by preventing or reducing shortening by immediate postmortem storage at high temperature or by unconventional hanging of carcasses to provide greater muscle restraint, Herring et al. (1964) suggested that tenderness may have resulted from increased tension placed on the muscle in the carcass. Gillis and Henrickson (1967, 1969) studied the influence of tension on the sarcomere length of bovine semimembranosus and semitendinosus muscle strips excised at death and weighted with 1000 to 5000gm weights at 2°C. The increased tension resulted in increased sarcomere length, increased muscle tenderness and decreased fiber diameter. Buck et al. (1970) have shown that stretching muscles in a rabbit carcass by draping the carcass over a
hook and tying the feet together resulted in longer sarcomeres of the longissimus muscle myofibrils. Buck and Black (1967, 1968) indicated that the improvement in tenderness, from stretching bovine longissimus muscle, resulted from a weakening or thinning of the perimysial connective tissue causing more denaturation during heating. Kruggel and Field (1971) and Kruggel et al. (1970) found that stretching bovine longissimus muscles reduced the number of aldehyde-type cross-linkages of collagen. Buege and Stouffer (1974a, b) and Quarrier et al. (1972) stretched excised ovine and bovine longissimus muscles with weights and found that only a 5% stretch (increase over excised length) produced most of the tenderness improvement that can be obtained from any applied degree of muscle tension. These studies also showed that even the stretched muscles continued to improve in tenderness during postmortem storage. The rate of postmortem muscle tenderization was not significantly different between the stretched and intact muscles.

The following conclusions can be drawn from the studies on shortening by the aforementioned investigators: 1) muscle shortening during rigor development is detrimental to muscle tenderness, 2) extreme cold-shortening or thaw-shortening causes an extreme and almost irreversible toughening effect on muscle, however, these effects do not normally occur under conventional carcass handling, 3) normal muscle shortening in
the carcass can be avoided or reduced by storage of the carcass immediately post-mortem at 8-25°C for 8-20 hr (depending on species and muscles) or by hanging the carcass in an unconventional manner, such as by the obturator foramen, to apply increased tension on some particular muscles of the carcass and 4) shortened muscles, except those extremely shortened by cold-shortening or thaw-shortening, to slightly stretched muscles still undergo the process of postmortem muscle tenderization. While shortening or stretching muscle pre-rigor may influence the post-rigor muscle tenderness, a post-rigor storage period will normally improve the muscle tenderness.

As previously discussed, one possible cause of the resolution of rigor mortis is the degradation of the Z-line. A degradation or weakening of the Z-line in postmortem muscle would also cause a decrease in the muscle tensile strength. Nakamura (1972) found that muscle fiber tensile strength declined during postmortem storage. Bouton and Harris (1972a) and Stanley et al. (1972) found significant correlation coefficients of 0.4 to 0.8 between fiber tensile strength and W-B shear-force values. These studies indicate that the degradation of the Z-line was reflected in the reduction of fiber tensile strength which is correlated with meat tenderness. A degradation or weakening of the Z-lines in postmortem muscle would also cause shorter myofibril fragments, due to breakage
at the Z-line during myofibril preparation and should improve
the tenderness of the muscle. Comissiong (1974) found that
muscle fibers, treated with dithiothreitol which removed the
Z-lines, fragmented to almost all single sarcomeres during
homogenization indicating that the Z-line is essential for
the structural integrity of the myofibril. Since the length
of the myofibril fragment should reflect the extent of Z-line
degradation, myofibril preparations were characterized ac­
cording to numbers of myofibrils having specific lengths,
numbers of sarcomeres per myofibril fragment, by Fukazawa et
ured fragmentation by using a scale from A to E, which com­
bined length of the myofibril fragment with state of contrac­
tion. The results of this study showed that fragmentation
was not a reliable index of tenderness, but it was noted that
widely distributed breaks in the myofibrils at the Z-line may
cause appreciable tenderization that might not be detected by
microscopic examination. Parrish et al. (1973b) observed
that smaller myofibril fragments originated from the more
tender meat samples and suggested that myofibril fragmenta­
tion was a much more important factor in meat tenderness than
was muscle shortening although the degree of fragmentation
was not quantified. Takahashi et al. (1967) and Fukazawa et
al. (1969) used a ratio of the number of myofibril fragments
having 1-4 sarcomeres to the total number of myofibril
fragments. Although fragmentation as estimated by this procedure changed with post-mortem time and with different pH values and temperatures, no relationship to tenderness was found. Berry et al. (1974) found a significant but low correlation coefficient (0.20) between shear-force values and the number of sarcomeres per myofibril of bovine *longissimus* muscle. High correlation coefficients were found between myofibril fragmentation and W-B shear values in bovine *longissimus* muscle (-0.75) (Muller et al., 1974) and chicken pectoralis muscle (-0.58) at 6 days of postmortem storage (Whiting and Richards, 1971).

Davey and Gilbert (1969) who acknowledged that microscopic measurements of myofibril fragmentation involves some subjective judgements and was subject to inherent sampling errors, used turbidity of a myofibril suspension as a measure of fragmentation. Turbidity results from light scattering from myofibrils and its value will be related in large part to the number of fiber pieces in suspension. An increase in turbidity value should indicate an increase in the number of myofibrils in suspension. If equal protein concentrations are used, an increase in turbidity should therefore, indicate an increase in fragmentation, which results in a larger number of shorter myofibrils. The advantage of a turbidometric measure of fragmentation is that it allows a larger and more representative sample than can be used microscopically.
Davey and Gilbert (1969) confirmed that turbidometric measures on myofibril suspensions paralleled microscopic observations of myofibril fragmentation, but the relationships of these measures to meat tenderness were not investigated.

Holler et al. (1973), using the procedure developed by Davey and Gilbert (1969), showed that fragmentation, as measured by percentage emission of a myofibril suspension on a spectrophotometer, gave a correlation coefficient of -0.78 with W-B shear-force values. This was one of the first reports that Z-line degradation as measured by myofibril fragmentation was highly correlated with bovine muscle tenderness. Love and Mackay (1962) and Kelly et al. (1966) found a close relationship between fish muscle tenderness and the optical density of a whole muscle homogenate without separating sarcoplasmic or connective tissue from the homogenate. Holler et al. (1973), however, showed that microscopic measurements of myofibril fragmentation (average number of sarcomeres per myofibril of 100 myofibrils measured) were not as highly correlated with tenderness (0.61) as were turbidity measurements of fragmentation (-0.78). This study did not, however, investigate the changes in fragmentation with increasing time of postmortem storage to determine whether increasing fragmentation during postmortem storage could be related to increasing tenderness during this period.
Laakkonen (1973) has stated that the presence of a collagen proteolytic enzyme in skeletal muscle has not been confirmed. Clayson et al. (1966) and Etherington (1971) have shown that any change in collagen during post-mortem storage can not be attributed to enzymatic action on collagen. However, numerous investigators (El-Gharbawi and Whitaker, 1963; Dawson and Wells, 1969; Gottschall and Kies, 1943; Miyada and Tappel, 1956; Tappel et al., 1956; Tsen and Tappel, 1959; Wang et al., 1958) have shown that application of proteolytic enzymes of plant, animal and microbial origin exogenous to muscle tissue improves meat tenderness. Wang et al. (1957) have microscopically characterized the effect of twelve enzymes on muscle components of freeze dried steaks. The amount of proteolysis on the muscle components varied depending upon enzyme specificity. Plant and pancreatic enzymes caused both collagen and myofibrillar degradation whereas the microbial enzymes exhibited primarily myofibrillar degradation. Several investigators (Gooser, 1961; Huffman et al. 1967a, b; Robinson and Gooser, 1962) have shown that antemortem injection of papain in bovine animals improves the tenderness of postmortem muscles. Kang and Rice (1970) and Kang and Warner (1974) have shown that trypsin, bromelin and collagenase were stronger solubilizing agents of the connective tissue proteins while papain and ficin were more effective in solubilizing the myofibrillar proteins. While collagenolysis
would obviously increase meat tenderness, there appears to be no significant postmortem proteolysis by endogenous proteases of the connective tissue proteins (Clayson et al., 1966; Etherington, 1971; Goll et al. 1969). While the above studies have shown that proteolysis of the muscle proteins improves meat tenderness, these proteases are exogenous to the muscle tissue, however, and can not therefore contribute to natural postmortem meat tenderization.

Recently, Penny et al. (1974) have shown that freeze-dried steaks from bovine *semitendinosus* muscle, reconstituted in a solution containing CAF, were considerably more tender than steaks reconstituted in solutions without CAF. The Z-lines of the myofibrils from the steaks reconstituted in CAF-containing solutions were also extensively degraded indicating that the tenderness improvement of CAF was due to the structural weakening of the Z-line. This study has shown that CAF, which is an enzyme endogenous to the muscle cell (Dayton et al., 1974a, b), improves meat tenderness in a similar manner as has been shown to occur during postmortem storage.
METHODS AND MATERIALS

Source of Muscle Tissue

Muscle samples and steaks were obtained from bovine animals weighing 400-500 kg, 12-18 months of age and on similar feeding regimes at the Iowa State University Beef Nutrition Farm. At-death samples were obtained from the anterior portion of the M. longissimus, M. semitendinosus and M. psoas major within one hour after exsanguination of the animal at the Iowa State University Meat Laboratory, taken to the Food Research Laboratory and used immediately for myofibril, CAF or troponin preparation. Subsequent samples of the longissimus, semitendinosus and psoas major muscles were removed from the companion side of the carcass at 1, 2, 3, 6, 7, 10 or 13 days post-mortem (not all postmortem times were used in each experiment). Samples for each postmortem time, selected at random locations within each muscle, were used immediately for myofibril fragmentation determination, CAF activity assay or Instron fiber tensile strength determination. Steaks, 3.1 cm thick, were removed from the muscle, packaged in freezer paper 24 hr post-mortem and stored at 2°C. The packaged steaks were randomly selected for each postmortem storage period. On each particular postmortem day, the selected steaks were frozen and stored at -20°C for 2-4 months for subsequent sensory panel evaluation or Warner-Bratzler (W-B) shear-force determination.
Wholesale short loins from thirty-five A-maturity carcasses grading U.S.D.A. Choice and twelve C-maturity carcasses were randomly selected 24-30 hr after death (1 day post-mortem) from a commercial packing company (Wilson and Company, Cedar Rapids, Iowa). The wholesale short loins were taken to the Iowa State University Meat Laboratory where five 3.1cm steaks were removed from the anterior end of the loin and packaged in freezer paper. Two steaks (one for sensory panel and one for W-B shear) were randomly selected, frozen and stored at -20°C. Two other steaks were stored at 2-4°C for 7 days post-mortem and then frozen and stored at -20°C. The frozen steaks were stored for 14-40 days for subsequent sensory panel evaluation and W-B shear-force determination. A four-gram sample was removed from the fifth steak at 1 and 7 days post-mortem and used immediately for myofibril fragmentation determination.

Bovine longissimus muscles from the wholesale short loin of six veal were obtained from animals originating from the Iowa State University Dairy Farm and slaughtered at the Iowa State University Meat Laboratory. Muscle samples and steaks were removed at 1 and 7 days post-mortem and used in the same manner as previously described for A- and C-maturity bovine carcasses in the preceding paragraph.
Myofibril Preparation

Myofibrils were isolated from bovine longissimus, psoas major and semitendinosus muscles. Four grams of scissor-minced muscle were homogenized in a Waring Blender for 30 seconds in 10 volumes (v/w) of a 2°C isolating medium containing 100 mM KCl, 20 mM K phosphate (pH 7.0), 1 mM EDTA and 1 mM sodium azide. The homogenate was sedimented at 1000 x g for 15 minutes and the supernatant decanted. The sediment was resuspended in 5 volumes (v/w) of the same isolating medium using a stir rod, sedimented again at 1000 x g for 15 minutes and the supernatant decanted. The sediment was resuspended in 10 volumes (v/w) of the same isolating medium and passed through a polyethylene strainer to remove the connective tissue and debris. Ten volumes (v/w) of the isolating medium were used to facilitate passage through the strainer. The suspension was sedimented at 1000 x g for 15 minutes and the supernatant decanted. The sediments were washed three times more by suspending in 5 volumes (v/w) of the same isolating medium and sedimented at 1000 x g for 15 minutes. Finally the sedimented myofibrils were resuspended in 5 volumes (v/w) of the same isolating medium and the protein concentration determined by the biuret procedure of Gornall et al. (1949).
Myofibril Fragmentation Determination

An aliquot of the myofibril suspensions were diluted, with the same isolating medium in which the myofibrils were suspended, to 0.5mg/ml protein concentration. Protein concentration of the diluted myofibril suspension was determined by the biuret method of Gornall et al. (1949). If the protein concentration of the diluted myofibril suspension was not within the range of 0.45 to 0.55mg/ml, a redilution and protein concentration determination of the myofibril sample was made. A 10ml aliquot of the diluted myofibril suspension was stirred and absorbance of this suspension was measured at 540nm with a Spectronic 20 Bausch & Lomb Colorimeter. Myofibril fragmentation values were recorded as absorbance units per 0.5mg/ml myofibril protein concentration multiplied by 200. The constant, 200, expanded the absorbance units to range from 30 to 100. The absorbance units, expanded by the constant, is called the myofibril fragmentation index.

Light Microscopy

Myofibrils were examined with a Zeiss Photomicroscope equipped for phase and polarized light optics. For phase optics, a 100x neofluar, a 40x neofluar and a 25x phase objectives were used with a green interference filter in the light path. For polarized light optics a 10x objective was used with polarizing filters in the light path. Results were
recorded on Kodak Panatomic X film.

Troponin Preparation

Troponin was prepared from 100gm of at-death bovine longissimus muscle according to the procedure described by Arakawa et al. (1970a, b, c). Myofibrils, swollen by a series of water washes, were centrifuged at 15,000 x g for 30 minutes. The supernatant was salted out at 30% to 75% ammonium sulfate saturation and centrifuged at 15,000 x g for 10 minutes. The precipitate was dissolved in 1mM K bicarbonate and dialyzed for 30 hr against 1mM K bicarbonate. Enough 3M KCl was added to the dialyzed solution to make the final solution 1M KCl. This solution was clarified at 105,000 x g for 1 hr and the pH of the supernatant adjusted to 4.6 and held at that pH for 1 hr at 20C. The solution was then centrifuged at 15,000 x g for 10 minutes and the supernatant adjusted to pH 7.5. The pH adjusted supernatant was salted out at 40% to 60% ammonium sulfate saturation and centrifuged at 15,000 x g for 10 minutes. The precipitate was dissolved in 1mM K bicarbonate, 0.5% 2-MCE and dialyzed for 60 hr against 1mM K bicarbonate. The dialyzed solution was clarified at 105,000 x g for 1 hr to yield a partially purified troponin preparation. Troponin was further purified by diethylaminoethyl (DEAE)-cellulose column chromatography as described by van Berd and Kawasaki (1973). A 1.6 x 20cm column was packed with DEAE-cellulose, equilibrated with 20mM Tris-
HCl (pH 7.5) and eluted with a linear KCl gradient (from 0 to 0.4M) in the same buffer solution. Troponin was eluted at a single peak at 3.18M KCl. DEAE-cellulose column chromatography was performed at 2°C. Troponin purity was monitored before and after column chromatography by SDS-polyacrylamide gel electrophoresis.

Calcium-Activated-Factor (CAF) Preparation and Assays

Crude CAF was prepared from 100gm of at-death muscle and at 1, 3 and 6 days postmortem muscle according to the procedure described by Busch et al. (1972). Ground muscle was suspended in 6 volumes (v/w) of 4mM EDTA, pH 7.0-7.6, by use of a Waring Blender for 90 seconds. Additional Tris was added to the postmortem muscle homogenate to adjust the pH to 7.0-7.6. The suspension was centrifuged at 15,000 x g for 20 minutes and the supernatant filtered through glass wool. The supernatant was then adjusted to pH 6.2 with acetic acid, left at 0°C for 30 minutes and then centrifuged at 15,000 x g for 20 minutes. The pH 6.2 supernatant was adjusted to pH 4.9 with acetic acid, left at 0°C for 30 minutes and centrifuged at 15,000 x g for 20 minutes. The pH 4.9 sediment was suspended in 7ml of 100mM Tris-HCl, pH 8.2, 4mM EDTA and the pH adjusted to 7.0. The suspension was diluted with water to 20ml final volume and clarified at 105,000 x g for 1 hr. The clarified solution was dialyzed against 1mM K bicarbonate, 5mM EDTA, 5mM 2-mercaptoethanol for 12-18 hours. After dialysis, the so-
olution was clarified at 105,000 x g for 60 minutes. The supernatant from this clarification was designated crude CAP.

Assay conditions were as follows: 100mM KCl, 100mM Tris-acetate (pH 7.5), 10mM 2-MCE, 5mM Ca²⁺, 5mg/ml casein and 0.25mg/ml CAP (CAP to casein ratio of 1:20) in a 2ml total assay volume incubated at 25°C for 30 minutes. The reaction was stopped by precipitating the protein with 2ml of 5% TCA. It was centrifuged at 1000 x g for 20 minutes and the optical density (OD) of the supernatant was measured at 278nm. CAP activity was calculated as total OD units per 100gm of muscle.

Bovine myofibrils and purified troponin were treated with purified porcine CAP (prepared by William Dayton, Dayton et al., 1974a, b). Myofibrils were treated with porcine CAP (CAP to myofibril ratio of 1:200 by protein concentration) under the same conditions described in the CAP-casein assay except casein was replaced with 10mg of myofibrillar protein. The reaction was stopped by addition of 0.22mls of 0.1M EDTA. Myofibrils were washed with 100mM NaCl three times at 2°C to remove the Tris, KCl, HCE and Ca²⁺.

Myofibrils were then observed with light microscopy and also run on SDS-polyacrylamide gel electrophoresis. Purified troponin was also treated with porcine CAP (CAP to troponin ratio of 1:1000 by protein concentration) for 5, 10, 20 and
40 minutes. Incubation conditions were the same as those described for CAF-casein assay and terminated in the same way as described for myofibril treatment. CAF-treated troponin was dialyzed after incubation against 1mM K bicarbonate for 3 hr and then run on SDS-polyacrylamide gel electrophoresis.

Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis

Myofibrils and purified troponin were run on SDS-polyacrylamide gel electrophoresis according to the method of Weber and Osborn (1969) to determine troponin purity and alternations of the myofibrillar proteins and troponin by postmortem storage time and temperature and CAF treatments.

Both 7 1/2% acrylamide and bis-acrylamide in a 75:1 weight ratio and 10% acrylamide and bis-acrylamide in a 37:1 weight ratio were used in gels which were run in 5mm (inner diameter) X 120mm tubes. The gels and the upper and lower reservoirs contained 100mM Na phosphate (pH 7.1) and 0.1% SDS. SDS-protein complexes were prepared by incubating myofibrils and troponin in the SDS-tracking dye solution consisting of 1.75% SDS, 1.5M 2-MCE, 20mM Na phosphate (pH 7.0), 0.01% bromophenol blue (tracking dye) and 6.75% glycerol (to increase the density of the solution) at 100°C for 15 minutes. The applied loads to the gels were 25 to 35ug of myofibrillar proteins and 5 to 30ug of troponin. Electrophoresis was performed at 20-25°C in gels 8cm long and run at a
constant current of 6 mamp per gel until the tracking dye had traveled to approximately 1.5cm from the bottom of the tube. The gels were stained in a solution of 0.2% Coomassie Brilliant Blue in 279ml of 50% methanol and 21ml of glacial acetic acid for 12-16 hr and destained in a quick gel destainer (Canalco Co.) for 20 minutes containing a solution of 7.5% (v/v) acetic acid and 5% (v/v) methanol. After destaining, the gels were stored in destaining solution and after the background gel stain had been completely destained (usually 10-21 days after destaining) the gels were photographed with a Nikon single-lens reflex camera on Kodak Panatomic X film. The molecular weights of the protein components were determined by comparison of their mobilities with proteins of known molecular weight as described by Weber and Osborn (1969).

Myofibrils, in preparation for SDS-polyacrylamide gel electrophoresis, were washed in 100mM NaCl three times to remove the KCl and finally suspended in 100mM NaCl to a minimum protein concentration of 5mg/ml. An aliquot of this suspension was diluted with water to a myofibrillar protein concentration of 1.5mg/ml and 15-30mM NaCl. To 1ml of the diluted myofibrils was added 0.5ml of the SDS-tracking dye solution just prior to incubation at 100°C. Troponin was dialyzed against 1mM K bicarbonate to remove all salt and then 50ul of the SDS-tracking dye solution was added to 100ul
of troponin (1.5ug/ul) just prior to incubation at 100°C.

Muscle Fiber Tensile Strength Measurement

A TT-B floor model Instron Universal Testing Machine was used to determine the tensile strength of strips from six bovine semitendinosus and six psoas major muscles at 1, 3, 7 and 13 days post-mortem stored at 2°C. Strips were removed from both raw muscles and muscles cooked in plastic bags submerged in a 60°C water bath for 1 hr and then cooled to 2°C. The muscle strips were dissected from the muscles by cutting parallel to the fiber axis. The sample strips were clamped to a type B load cell and crosshead with type B clamps. Sample strip dimensions were: length-6.0cm, length available for stretching after clamping-3.0cm, strip weight-1.0 to 2.4gm. All samples were weighed on an analytical balance prior to testing. Instrument parameters were: crosshead speed-10cm/min., chart speed-10cm/min., full scale deflection-0 to 1000gm. The average breaking strength value of six strips was used as the measure of fiber tensile strength for each sample. Results were recorded as grams of force per gram of muscle strip.

Warner-Bratzler (W-B) Shear-Force and Sensory Panel Determinations

Steaks, 3.1cm thick, from fresh, or frozen and then thawed for 24 hr at 2°C, of bovine longissimus, semitendinosus and psoas major muscles of various postmortem times were
evaluated for shear-force on a Warner-Bratzler (W-B) shear apparatus. Raw steaks, not previously frozen, were cooked in plastic bags submerged in a 60°C water bath for 1 hr and the frozen and thawed steaks were broiled to 65°C in an electric oven. Steaks were placed 10cm from the heating coil and oven broiled to half the final temperature (determined by a thermometer placed in the geometric center of the steak) on one side, turned and broiled to the final temperature on the other side. All steaks were allowed to cool to room temperature (approximately 25°C) before three 1.27cm diameter cores were removed and each sheared twice. The average of six shear values were used as the shear-force for each steak and recorded as Kg of force per cm².

A ten-member sensory panel, consisting of 5 men and 5 women, having a mean age of 26, were selected for their repeatability of detecting broiled beef steak tenderness. They were subsequently trained to improve their sensitivity to detect tenderness differences.

Steaks were randomly selected, from all steaks frozen, thawed for 24 hr at 2°C and broiled, in the same manner as described for the W-B shear-force determinations, to an internal temperature of 65°C. Panelists sat in separate cubicles isolated from the preparation area and lighted by a red fluorescent light. Panelists received six different samples (1.27cm x 2.5cm x 2.5cm) from the same location in each steak
per testing day. Panelists evaluated each meat sample for tenderness, flavor and juiciness based on a hedonic system from 8 to 1 with 8 being extremely tender, desirable flavor or juicy to 1 being extremely tough, undesirable flavor or dry. The average of the ten panelists' values was used as the tenderness, flavor and juiciness score for each sample.

Data Analysis

The data from the various experiments were analyzed for means, standard deviations, standard errors, simple correlations and significant mean differences by the analysis of variance and students' "t" test according to methods described by Snedecor (1967) and Steel and Torrie (1960). The Statistical Analysis System of the Iowa State Computation Center were used for data computations.
RESULTS

Myofibrils were prepared from bovine longissimus semitendinosus and psoas major muscles at 1, 3, 6 and 10 days of postmortem storage at 2°C for microscopic observations. Figure 1 shows polarized-light and phase-contrast micrographs of myofibrils from longissimus muscle. Polarized-light micrographs (Figs. 1a-d) show that the muscle sample separates into fiber segments or pieces and single myofibrils during homogenization. The size of the fiber piece become smaller with postmortem time (Fig. 1d). At higher magnification and under phase-contrast microscopy, myofibrils at 1 day postmortem (Fig. 1e) are long having many (over 20) sarcomeres per myofibril. As postmortem time increases myofibrils (Figs. 1f-h) become shorter and thus become more fragmented. This increase in myofibril fragmentation during postmortem storage agrees with the observations of Davey and Gilbert (1967, 1969), Fukazawa et al. (1969), Hay et al. (1973b), Henderson et al. (1970), Parrish et al. (1973b), Sayre (1970) and Takahashi et al. (1967). Even at higher magnification, phase-contrast of myofibrils (Figs. 1i-l) show that single myofibrils become shorter with postmortem time. The Z-lines in myofibrils at 1 day post-mortem (Fig. 1i) are very distinct and prominent whereas Z-lines become slightly less distinct as postmortem time increases. Z-lines, however, are faint, but detectable even at 10 days post-mortem (Fig. 1l).
Figure 1: Polarized-light and phase micrographs of myofibrils prepared from bovine longissimus muscle at different times of postmortem storage at 2°C

a. Polarized-light micrograph of myofibrils at 1 day post-mortem. Both single myofibrils and fiber pieces are present. X200

b. Polarized-light micrograph of myofibrils at 3 days post-mortem. Fiber pieces and myofibrils are more fragmented than those in Fig. 1a. X200

c. Polarized-light micrograph of myofibrils at 6 days post-mortem. Greater myofibril fragmentation is shown than in Fig. 1a and 1b. X200

d. Polarized-light micrograph of myofibrils at 10 days post-mortem. Myofibrils are highly fragmented. X200

e. Phase micrograph of myofibrils at 1 day post-mortem. X800

f. Phase micrograph of myofibrils at 3 days post-mortem. Myofibrils are fragmented more than myofibrils in Fig. 1e. X800

g. Phase micrograph of myofibrils at 6 days post-mortem. Myofibrils are more fragmented than those in Fig. 1e and 1f. X800

h. Phase micrograph of highly fragmented myofibrils at 10 days post-mortem. X500

i. Phase micrograph of myofibril at 1 day post-mortem. Sarcomeres are relaxed and Z-lines are prominent. X2000

j. Phase micrograph of myofibrils at 3 days post-mortem. Some fragmentation is observed and Z-lines are prominent. X2000

k. Phase micrograph of myofibrils at 6 days post-mortem. Myofibrils are more fragmented and Z-lines are less prominent. X2000

l. Phase micrograph of myofibrils at 10 days post-mortem. Myofibrils are highly fragmented and Z-lines are very faint. X2000
Henderson et al. (1970), on the other hand, have shown that Z-lines become amorphous and lose their prominent appearance during postmortem storage. Myofibrils prepared from bovine semitendinosus muscles at 1, 3, 6 and 10 days of postmortem storage at 20°C are shown in Figure 2. The polarized light micrographs of myofibrils (Figs. 2a-d) show that fiber pieces were similar to those found for longissimus muscle (Figs. 1a-d). Also, size of the fiber pieces greatly decreased with postmortem time. At higher magnification, the phase-contrast micrographs (Figs. 2e-h) show a similar increase in myofibril fragmentation with postmortem time as did myofibrils from longissimus muscle (Figs. 1e-h). At 2000X magnification, the phase-contrast micrographs of myofibrils (Figs. 2i-l) show that myofibrils become shorter with postmortem time, Z-lines appear to become slightly less prominent and more degraded. Myofibrils were also prepared from bovine psoas major muscles at 1, 3, 6 and 10 days of postmortem storage at 20°C. Figure 3 shows polarized light micrographs of these myofibrils. Myofibrils at 1 day postmortem have extremely large fiber pieces (larger than shown for the longissimus (Fig. 1a) and semitendinosus (Fig 2a) muscles) and a few single myofibrils. While the size of these fiber pieces decrease as postmortem time increased, the fiber pieces at 10 days post-mortem (Fig. 3d) are larger than those shown at 1 day post-mortem for the longissimus (Fig. 1a) and semitendinosus (Fig. 2a) muscles.
Figure 2. Polarized-light and phase micrographs of myofibrils prepared from *semitendinosus* muscle at different times of postmortem storage at 2°C

a. Polarized-light micrograph of myofibrils at 1 day post-mortem. X200

b. Polarized-light micrograph of myofibrils at 3 days post-mortem. X200

c. Polarized-light micrograph of myofibrils at 6 days post-mortem. Myofibrils are more fragmented than those shown in Fig. 2a and 2b. X200

d. Polarized-light micrograph of myofibrils at 10 days post-mortem. Myofibrils are highly fragmented. X200

e. Phase micrograph of myofibrils at 1 day post-mortem. X500

f. Phase micrograph of myofibrils at 3 days post-mortem. Myofibrils are more fragmented than in Fig. 2e. X500

g. Phase micrograph of myofibrils at 6 days post-mortem. Myofibrils are very fragmented. X500

h. Phase micrograph of myofibrils at 10 days post-mortem. Myofibrils are very highly fragmented. X500

i. Phase micrograph of myofibrils at 1 day post-mortem. X2000

j. Phase micrograph of myofibril at 3 days post-mortem. Myofibrils are more fragmented than those in Fig. 2i. X2000

k. Phase micrograph of myofibrils 6 days post-mortem. Z-lines are prominent and myofibrils are very fragmented than in Fig. 2i and 2j. X2000

l. Phase micrograph of myofibrils at 10 days post-mortem. Z-lines are faint and myofibrils are very highly fragmented. X2000
Phase-contrast micrographs of myofibrils from the psoas major muscles are shown in Figure 4 and these micrographs further exemplify their nature not to fragment. At low magnification, the micrograph of myofibrils (Figs. 4a-d) show a slight increase in myofibril fragmentation from 1 to 10 days postmortem. Figures 4e-h, at 2000X magnification, show no change in myofibril length or appearance of the Z-line as postmortem time increases. The increase in myofibril fragmentation during postmortem storage at 2°C, shown in the myofibrils of longissimus (Fig. 1) and semitendinosus (Fig. 2) muscles, was considerably greater than was shown for myofibrils of psoas major muscles (Fig. 3 and 4).

Several investigators (Fukazawa et al., 1969; Sayre, 1970; Takahashi et al., 1967) have attempted to measure the myofibril fragmentation observed in the light microscope and relate it to meat tenderness. While these investigators acknowledged that myofibril fragmentation increased as meat tenderness increased during postmortem storage, they were unsuccessful in developing a method to quantitate myofibril fragmentation. Davey and Gilbert (1969) found that the microscopic method of measuring myofibril fragmentation was limited by the sample size that could be measured and inherent sampling errors existed due to the limited sample size. These investigators found that a turbidometric measure of myofibril fragmentation was a more accurate and objective
Figure 3. Polarized-light micrographs of myofibrils prepared from bovine *psoas major* muscle at different times of post-mortem storage at 2°C

a. Myofibrils prepared at 1 day post-mortem. Arrow points to a muscle fiber piece which did not separate into single myofibrils. X200

b. Myofibrils prepared at 3 days post-mortem. Large fiber pieces are more fragmented than at 1 day post-mortem (Fig. 3a). X200

c. Myofibrils prepared at 6 days post-mortem. Note that large fiber pieces are more fragmented than those at 1 day post-mortem (Fig. 3a and 3b). X200

d. Myofibrils prepared at 10 days post-mortem. These show somewhat more fragmentation than myofibrils in Fig. 3c. X200
Figure 4. Phase micrographs of myofibrils prepared from bovine *psosas major* muscles at different times of postmortem storage at 2°C

a. Myofibrils at 1 day post-mortem. X800

b. Myofibrils at 3 days post-mortem. Myofibrils are somewhat more fragmented than shown in Fig. 4a. X800

c. Myofibrils at 6 days post-mortem. X500

d. Myofibrils at 10 days post-mortem. Myofibrils are slightly fragmented. X500

e. Myofibrils at 1 day post-mortem. Sarcomeres are relaxed and Z-lines are prominent. X2000

f. Myofibrils at 3 days post-mortem. Sarcomeres are relaxed and Z-lines prominent. X2000

g. Myofibrils at 6 days post-mortem. Z-line integrity appears similar to Fig. 4e and 4f. X2000

h. Myofibrils at 10 days post-mortem. Z-lines remain prominent. X2000
measure of fragmentation. Möller et al. (1973) adapted the turbidometric method of measuring fragmentation (Davey and Gilbert (1969) and found it to be highly correlated to meat tenderness. The following is a study made by this author to utilize a similar method of turbidometric measure of myofibril fragmentation. Myofibrils were prepared from bovine longissimus muscles at 1, 3, and 7 days of postmortem storage at 20°C by homogenizing a 4gm, scissor-minced muscle sample in a Waring Blender for 5, 10, 15, 20, 30, 45, 60, 75, and 90 seconds. Absorbance of the myofibril suspensions (0.5mg myofibrillar protein per ml) measured at each homogenizing time for three postmortem storage periods are shown in Figure 5. As homogenizing time increased the absorbance of myofibril suspensions increased. Also absorbance measures of myofibril fragmentation were higher with increased postmortem storage time. The 30-second homogenizing time was chosen because it gave the greatest difference in absorbance measures between the three postmortem storage periods and this homogenizing time was close to the maximum absorbance measure of the 7 days postmortem storage period. All subsequent myofibrils were prepared according to the procedure as described in the METHODS AND MATERIALS section of this dissertation using a 30-second homogenizing time. Because the turbidometric method measures not only the relative sizes of myofibrils but also the relative sizes of fiber pieces, the absorbance val-
Figure 5. Effect of homogenizing (Waring Blender) and post-mortem storage times on the fragmentation index (absorbance at 540nm x 200) of myofibrils from bovine longissimus muscle. Day 1, 3, and 7 refers to 1, 3, and 7 days of postmortem storage of the carcass at 2°C.
MYOFIBRIL FRAGMENTATION INDEX (ABSORBANCE AT 540nm x 200)

HOMOGENIZING TIME (SEC)

DAY 1

DAY 3

DAY 7
ues are transformed (as described in the METHODS AND MATERIALS section) to myofibril fragmentation index values.

An attempt was also made to develop a procedure for measuring myofibril fragmentation of cooked meat. Myofibrils prepared from cooked meat are, surprisingly, altered only slightly as seen with phase-contrast microscopy (Schmidt and Parrish, 1971). However, heating causes the denaturation of many sarcoplasmic proteins even at 50°C (Hamm, 1966) which appear to encapsulate and clump to the myofibrils. These denatured proteins are impossible to remove from myofibrils using routine myofibril isolation techniques. Because these denatured proteins are present in myofibril suspensions, the suspensions are so cloudy that a turbidometric measure does not accurately reflect the relative sizes of the myofibrils and fiber pieces. Hence, the method used to isolate myofibrils and measure myofibril fragmentation in fresh muscle tissue is inadequate for cooked meat.

Myofibril fragmentation index was determined on bovine semitendinosus and psoas major muscles at 1, 3, 7 and 13 days of postmortem storage at 2°C and these results are illustrated in Figure 6. The means and standard errors of the myofibril fragmentation index values of semitendinosus and psoas major muscles at various postmortem storage times are contained in Table 1. In the semitendinosus muscle, myofibril fragmentation index mean values were significantly different
Figure 6. Effect of postmortem storage time on the fragmentation index of myofibrils from bovine *semitendinosus* and *psoas major* muscles at different times of postmortem storage at 2°C.
Table 1. Effect of postmortem storage (2°C) on myofibril fragmentation index (PI), Warner-Bratzler (W-B) shear-force and fiber tensile strength (TS) (raw and cooked) of semitendinosus (ST) and psoas major (PM) muscles.

<table>
<thead>
<tr>
<th>Days of postmortem storage</th>
<th>PI²</th>
<th>W-B³</th>
<th>TS* (raw)</th>
<th>TS* (cooked)</th>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ST</td>
<td></td>
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<tr>
<td>48.8±3.0</td>
<td>68.8±2.3</td>
<td>80.0±2.6</td>
<td>82.8±2.1</td>
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<tr>
<td>PM</td>
<td>43.6±1.2</td>
<td>52.1±1.0</td>
<td>54.4±1.1</td>
<td>56.4±1.3</td>
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<td>3</td>
<td></td>
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<tr>
<td>68.8±2.3</td>
<td>80.0±2.6</td>
<td>82.8±2.1</td>
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<tr>
<td>52.1±1.0</td>
<td>54.4±1.1</td>
<td>56.4±1.3</td>
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<td>7</td>
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<tr>
<td>80.0±2.6</td>
<td>82.8±2.1</td>
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<tr>
<td>54.4±1.1</td>
<td>56.4±1.3</td>
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<td>13</td>
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<td>82.8±2.1</td>
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<td>56.4±1.3</td>
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</table>

Means ± standard errors of five carcasses. Means not underscored by the same dotted line are significantly different (P<0.05).

²Absorbance per 0.5mg myofibril protein x100.

³Kg of shear-force per cm.

⁴Gm of breaking force per gm of muscle strip.
between all postmortem times except between 7 and 13 days post-mortem. In the *psoas major* muscle, myofibril fragmentation index mean values were not significantly different (P<0.05). In the *semitendinosus* muscle, myofibril fragmentation index values increased from 1 to 13 days post-mortem with the greatest increase occurring between 1 and 3 days post-mortem (Fig. 6). In the *psoas major* muscle, myofibril fragmentation index values increased only slightly from 1 to 13 days post-mortem when compared to the increase found in *semitendinosus* muscle. The increase in the myofibril fragmentation index of the *semitendinosus* muscle appears to coincide with the observed increases in fragmentation of myofibrils during postmortem storage shown in Figure 2. The slight increase in the myofibril fragmentation index of *psoas major* muscle also appears to parallel the slight increase in myofibril fragmentation during postmortem storage observed in Figures 3 and 4. Additionally, differences in myofibril fragmentation index during postmortem storage between *semitendinosus* and *psoas major* muscles (Fig. 6 and Table 1) appear to agree with the observed fragmentation differences in myofibrils of these two muscles during postmortem storage shown in Figures 2, 3 and 4.

In another experiment, myofibril fragmentation index was determined on bovine *longissimus, semitendinosus* and *psoas major* muscle stored at 1, 3 and 6 days post-mortem. The
means and standard errors of myofibril fragmentation index values for all three muscles are shown in Table 2. Similar to that found in the previous experiment, the myofibril fragmentation index of longissimus and semitendinosus muscles increased from 1 to 6 days post-mortem with the greatest increase occurring between 1 and 3 days post-mortem and the means of the fragmentation index for each postmortem storage period were found to be significantly different (P<0.05) from each other. On the other hand, in psoas major muscle, the myofibril fragmentation index increased only slightly from 1 to 6 days post-mortem with only the means between 1 and 6 days post-mortem being significantly different (P<0.05). Fragmentation index results of the three muscles agree with the increases in myofibril fragmentation determined by microscopic observation during postmortem storage (Figs. 1-4). In summary, myofibrils from longissimus and semitendinosus muscles showed almost a doubling in fragmentation index values whereas myofibrils from psoas major muscles showed only a slight increase during postmortem storage at 2°C.

In another experiment, myofibrils were prepared from bovine longissimus, semitendinosus and psoas major muscles stored at 1, 2 or 3 days postmortem at 2°C and 25°C and the results of this experiment are illustrated in Figure 7. In all three muscles, myofibril fragmentation index increased from 1 to 3 days postmortem at both 2°C and 25°C treatments.
Table 2. Effect of postmortem storage (2°C) on myofibril fragmentation index (FI) and Warner-Bratzler (W-B) shear-force of longissimus (L), semitendinosus (ST) and psoas major (PM) muscles.

<table>
<thead>
<tr>
<th>Days of postmortem storage</th>
<th>FI</th>
<th>W-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 3 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 49.6±1.3 69.8±1.1 76.3±0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST 48.8±0.8 68.2±1.1 77.6±1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM 47.1±0.9 49.3±1.1 54.7±1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^1Means ± standard errors of five carcasses. Means not underscored by the same dotted line are significantly different (P<0.05).

^2Absorbance per 0.5mg myofibril protein x 200.

^3kg of shear-force per cm.
Figure 7. Effect of postmortem storage time and temperature treatments on the fragmentation index of myofibrils prepared from bovine *longissimus*, *semitendinosus* and *psoas major* muscles at different times of postmortem storage at 2°C and 25°C.
MYOFIBRIL FRAGMENTATION INDEX
(ABSORBANCE AT 540nm X 200)
Additionally, muscles stored at 25°C had a higher myofibril fragmentation index than muscles stored at 2°C. However, myofibril fragmentation index was not as high in the *psoas major* muscle as in the *longissimus* and *semitendinosus* muscles. Increase in myofibril fragmentation index, due to temperature treatment, was also much less in *psoas major* muscle than it was in *longissimus* and *semitendinosus* muscles. High postmortem storage temperatures (16°C) have been shown to produce accelerated tenderness increases in bovine *longissimus* and *semitendinosus* muscle (Parrish et al., 1973b). The 25°C storage temperature in the present study resulted in a much more rapid increase in myofibril fragmentation index value in *longissimus* and *semitendinosus* muscle with myofibril fragmentation index value being approximately the same at 3 days postmortem (25°C) as was found in these muscles stored for 6-13 days postmortem (2°C) (Tables 1 and 2). The factor that causes myofibril fragmentation index to be greater with increased storage temperature apparently acts similarly in *longissimus* and *semitendinosus* muscles but is either not present in similar quantities or is not as active in *psoas major* muscle.

Warner-Bratzler (W-B) shear-force values were determined on bovine *semitendinosus* and *psoas major* at 1, 3, 7 and 13 days of postmortem storage at 2°C and cooked to 65°C. The effect of postmortem storage on W-B shear-force values for
the *semitendinosus* and *psoas major* muscles are shown in Figure 8 and the W-B shear-force means and standard errors are shown in Table 1. In the *semitendinosus* muscle, the mean W-B shear-force at 1 day post-mortem was found to be significantly different (P<0.05) from the means of 3, 7 and 13 days post-mortem with the latter three postmortem days not significantly different (P<0.05) from each other. In the *psoas major* muscle, no significant difference (P<0.05) in mean W-B shear-force between days post-mortem was found. The decline in W-B shear-force of *semitendinosus* muscle corresponded to the increase in the myofibril fragmentation index of *semitendinosus* muscle (Fig. 6). This suggests that the increase in myofibril fragmentation may be associated to the decrease in the W-B shear-force value of the muscle. In contrast to the *psoas major* muscle (Fig. 8), the W-B shear-force only slightly decreased from 1 to 13 days post-mortem. The slight change in W-B shear-force during postmortem storage in the *psoas major* muscle also appears to be associated to the slight change found in myofibril fragmentation during post-mortem storage (Fig. 6).

In another experiment, W-B shear-force was determined on bovine *longissimus*, *semitendinosus* and *psoas major* muscle at 1, 3 and 6 days of postmortem storage at 2°C. Results from this experiment are shown in Table 2. In the *longissimus*, *semitendinosus* and *psoas major* muscles, W-B shear-force de-
Figure 8. Effect of postmortem storage time on Warner-Batzler (W-B) shear-force values of bovine *semitendinosus* and *psoas major* muscles at different times of postmortem storage at 2°C.
WARNER-BRATZLER SHEAR-FORCE VALUES (Kg/cm²)

POSTMORTEM STORAGE (DAYS)

1.00
2.00
3.00
4.00

0
1
2
3
4
5
6
7
8
9
10
11
12
13

PSOAS
SEMITENDINOSUS
creased from 1 to 6 days post-mortem. The mean W-B shear-force, for semitendinosus muscle, was significantly (P<0.05) higher at 1 day than at 3 and 6 days post-mortem; the means of the later two days were found to not be significantly (P<0.05) different. Also, no significant (P<0.05) differences between means of W-B shear-force at 1, 3 and 6 days post-mortem were found for longissimus and psoas major muscles. Parrish et al. (1973b), however, found W-B shear-force to be significantly (P<0.05) higher in longissimus muscle at 1 day than at 6 days post-mortem.

W-B shear-force was also determined on longissimus, semitendinosus and psoas major muscles stored for 1, 2 and 3 days post-mortem at 2°C and 25°C (Fig. 9). In all three muscles, W-B shear-force values were lower at 1, 2 and 3 days post-mortem at 25°C storage temperature than at 2°C. W-B shear-force decreased from 1 to 3 days post-mortem in all three muscles at both 2°C and 25°C storage but the least change in W-B shear-force, due to either storage time or temperature, was found for psoas major muscle. In both the longissimus and semitendinosus muscles, the decrease in W-B shear-force from 1 to 3 days post-mortem was greater at 2°C than at 25°C storage, however, the W-B shear-force was considerably lower at 1 day post-mortem at 25°C than at 2°C storage. Since the W-B shear-force at 1 day post-mortem, 25°C, for longissimus and semitendinosus muscles appeared to
Figure 9. Effect of postmortem storage time and temperature on Warner-Bratzler (W-B) shear-force values of bovine longissimus, semitendinosus and psoas major muscles at different times of postmortem storage at 2°C and 25°C.
be approaching a similar value as at 3 days postmortem, 20°C, it appears that a larger decrease in W-B shear-force had already occurred by 1 day post-mortem at 25°C storage. Busch et al. (1967) found W-B shear-force of semitendinosus muscle to decrease during postmortem storage and found that at 7 days post-mortem at 20°C the W-B shear-force was approximately the same as at 2 days post-mortem at 37°C with most of the decrease in W-B shear-force in the muscle stored at 37°C occurred by 6 hours post-mortem. They also found a large decrease in W-B shear-force in the psoas major muscle during postmortem storage, however, the muscle had been excised from the carcass at death.

In a comparative evaluation of W-B shear-force (Fig. 9) and myofibril fragmentation index (Fig. 7) of longissimus, semitendinosus and psoas major muscles at 1, 2 and 3 days of postmortem storage at 20°C and 25°C, three observations can be made. 1) In the psoas major muscle the change in myofibril fragmentation index and W-B shear-force are very slight during postmortem storage compared to the longissimus and semitendinosus muscle and only slightly affected by storage temperature. 2) In the longissimus and semitendinosus muscles, the change in myofibril fragmentation index and W-B shear-force from 1 to 3 days post-mortem is greater at 20°C than at 25°C. 3) The differences in myofibril fragmentation index and W-B shear-force at 1 day post-mortem between 25°C and 20°C
storage is similar between longissimus and semitendinosus muscles. These observations indicate: 1) that some factor in muscle is influencing both myofibril fragmentation index and W-B shear-force, 2) that this factor is in similar quantities or has similar activity levels in the longissimus and semitendinosus muscles but it is lower in quantity or activity in the psoas major muscle, and 3) that this factor is more active at higher storage temperatures.

Muscle fiber tensile strength was used to measure the strength or weakness of the myofibril structure. Muscle fiber tensile strength was measured by Instron breaking strength of muscle strips from raw and cooked semitendinosus and psoas major muscles at 1, 3, 7 and 13 days of postmortem storage at 2°C. The fiber breaking strength decreased in both raw and cooked muscle from 1 to 13 days post-mortem with the greatest decrease occurring from 1 to 3 days post-mortem for both the semitendinosus and psoas major muscles (Fig. 10). Cooked muscle strips from both muscles required almost twice the force to break the muscle strip compared with the force necessary to break raw muscle strips. Also to be noted is that cooked semitendinosus muscle strip required less breaking force than did the cooked psoas major muscle strip. It appears that the decrease in muscle fiber breaking strength during postmortem storage (Fig 10) occurs in a manner similar to the decrease in W-B shear-force during post-
Figure 10. Effect of postmortem storage time on the fiber breaking strength of raw and water-bath cooked (60°C) bovine semitendinosus and psoas major muscles at different times of postmortem storage at 2°C
mortem storage (Fig. 8) for the *semitendinosus* muscle. The means and standard errors of fiber tensile strength of raw and cooked *semitendinosus* and *psoas major* muscles at 1, 3, 7 and 13 days post-mortem are shown in Table 1. The means of fiber tensile strength for both raw and cooked *semitendinosus* muscle and raw *psoas major* muscle at 1 day post-mortem were found to be significantly (P<0.05) higher than the means at 7 and 13 days post-mortem, but they were not significantly different from the means at 3 days post-mortem. No significant (P<0.05) differences were found among the means at 3, 7 and 13 days post-mortem in raw and cooked *semitendinosus* muscle and raw *psoas major* muscle. In the cooked *psoas major* muscle, no significant (P<0.05) differences were found among the means of fiber tensile strength at 1, 3, 7 and 13 days post-mortem. These results (Fig. 10 and Table 1) indicate that the fiber tensile strength of the *semitendinosus* and *psoas major* muscles decrease during post-mortem storage and that the decrease in fiber tensile strength is greater in *semitendinosus* muscle than in *psoas major* muscle. These results also suggest that cooking in a water bath at 60°C causes a decided increase in force necessary to break fibers. It is puzzling that the *psoas major* muscle strips required such a high breaking force because it is a much more tender muscle than the *semitendinosus* muscle. Since breaking strength measures primarily strength or weakness of myofibrils, these results
substantiate that \textit{psoas major} muscle myofibril does not fragment as much as \textit{semitendinosus} muscle myofibrils.

The changes in myofibril fragmentation and W-B shear-force during postmortem storage as previously noted, indicate changes occur in myofibrillar proteins during postmortem storage. Myofibrils, prepared from bovine \textit{longissimus} muscle at death and at 1, 3, 6 and 10 days of postmortem storage at 2°C, were electrophoresed on sodium dodecyl sulfate (SDS) 7 1/2% and 10% polyacrylamide gels. Figures 11a-e show the SDS-7 1/2% polyacrylamide gels at 0, 1, 3, 6 and 10 days post-mortem. From 0 to 10 days post-mortem, no major changes (appearance or disappearance of protein bands) occurred in the bands above actin (myosin and alpha-actinin); however, obvious changes in protein bands occurred below actin. A gradual decrease and disappearance of the troponin-T protein band and a gradual appearance of the protein band in the 30,000-dalton molecular weight region occurred from 0 to 10 days post-mortem. While these changes in myofibrillar proteins of \textit{longissimus} muscle myofibrils appeared very limited and selective, it agreed with that reported for chicken breast muscle (Hay \textit{et al.}, 1973a). Goll \textit{et al.} (1969) have discussed two possible modifications of the myofibril after rigor development; one being a modification of the actin-myosin interaction and the other a degradation of the Z-line. No degradative changes in myosin, actin or alpha-actinin
Figure 11. SDS-7 1/2% and 10% polyacrylamide gels of myofibrils prepared from bovine *longissimus* muscle at death and at different times of postmortem storage at 2°C

a-e. 7 1/2% gels of myofibrils from 0(a), 1(b), 3(c), 6(d), and 10(e) days post-mortem. Note the gradual decrease of the troponin-T band and the gradual increase of the 30,000-dalton band from 0 to 10 days post-mortem. No other major changes of other bands are noted.

f-j. 10% gels of myofibrils from 0(f), 1(g), 3(h), 6(i), and 10(j) days post-mortem. Note the decrease of the troponin-T band and the increase of the 30,000-dalton band from 0 to 10 days post-mortem.
have been shown to account for these myofibril modifications. SDS-7 1/2% gels separate the heavy molecular weight proteins above actin but the lighter proteins below actin have better resolution in SDS-10% polyacrylamide gels. The SDS-10% polyacrylamide gels (Figs. 11f-j) more clearly show the gradual decrease and disappearance of the troponin-T protein band and the gradual appearance of the 30,000-dalton protein band from 0 to 10 days post-mortem. Figure 12 shows the SDS-7 1/2% and 10% polyacrylamide gels of myofibrils from bovine semitendinosus muscle at death and at 1, 3, 6 and 10 days of post-mortem storage at 2°C. The SDS-7 1/2% (Figs. 12a-e) and 10% (Figs. 12f-j) polyacrylamide gels show nearly identical changes in the myofibrillar proteins of the semitendinosus muscle myofibrils as found in myofibrils from the longissimus muscle (Fig. 11). The troponin-T band gradually becomes less intense and disappears at the same time as a 30,000-dalton protein appears from 0 to 10 days post-mortem while no other major changes occur in the other myofibrillar proteins.

Figure 13 shows the SDS-7 1/2% and 10% polyacrylamide gels of myofibrils from bovine psoas major muscle at death and at 1, 3, 6 and 10 days of post-mortem storage at 2°C. Both SDS-7 1/2% (Figs. 13a-e) and 10% (Figs. 13f-j) polyacrylamide gels show only extremely slight changes in the myofibrillar proteins from 0 to 10 days post-mortem. The troponin-T protein band, which appears very faintly in these
Figure 12. SDS-7 1/2% and 10% polyacrylamide gels of myofibrils prepared from bovine semitendinosus muscle at death and at different times of postmortem storage at 2°C

a-e. 7 1/2% gels of myofibrils at 0(a), 1(b), 3(c), 6(d), and 10(e) days post-mortem. Note the gradual decrease of the troponin-T band and the gradual increase of the 30,000-dalton band during postmortem storage. No other major changes of other bands are noted.

f-j. 10% gels of myofibrils at 0(f), 1(g), 3(h), 6(i), and 10(j) days post-mortem. Note the decrease of the troponin-T band and the increase of the 30,000-dalton band during postmortem storage.
Figure 13. SDS-7 1/2% and 10% polyacrylamide gels of myofibrils prepared from bovine psoas major muscles at death and at different times of postmortem storage at 2°C.

a-e. 7 1/2% gels of myofibrils at 0(a), 1(b), 3(c), 6(d), and 10(e) days post-mortem. No major changes occurred in the bands during postmortem storage.

f-g. 10% gels of myofibrils at 0(f), 1(g), 3(h), 6(i), and 10(j) days post-mortem. Note only a slight increase occurred in the 30,000-dalton band (j) during postmortem storage.
gels, does not completely disappear in either the SDS-7 1/2% or 10% polyacrylamide gels from 0 to 10 days of postmortem storage. Likewise only a very faint 30,000-dalton protein band appears at 10 days post-mortem (Figs. 11e and j). These slight changes in the myofibrillar protein in the myofibrils of the psoas major muscle during postmortem storage are in sharp contrast to the obvious changes in the troponin-T protein band and the 30,000-dalton protein band in myofibrils from longissimus and semitendinosus muscles during postmortem storage. Postmortem muscle storage of longissimus and semitendinosus muscles produce striking changes and similarities in myofibril fragmentation, as observed in the light microscope (Figs. 1 and 2), myofibril fragmentation index (Fig. 7 and Table 2), W-B shear-force (Fig. 9 and Table 2) and protein changes on SDS-polyacrylamide gels (Figs. 11 and 12). In contrast, postmortem stored psoas major muscle does not change substantially; consequently it differs consistently from the longissimus and semitendinosus muscles with the same measures. While no direct relationship can be established between the degradative protein changes occurring in muscle during postmortem storage, shown in SDS-polyacrylamide gels, and the changes in the myofibril fragmentation index or the W-B shear-force, it is probable that a factor, or group of factors, causes all of these measured postmortem muscle changes to occur simultaneously.
To determine if the 25°C postmortem storage temperature treatment affected the postmortem myofibrillar protein changes in a manner similar to myofibril fragmentation index (Fig. 7 and Table 2) and W-B shear-force (Fig. 9 and Table 2), myofibrils from bovine *longissimus* muscle stored for 1, 2 and 3 days post-mortem at 20°C and 25°C were electrophoresed on SDS-10% polyacrylamide gels. No changes in the proteins bands during postmortem storage are shown in the gels (Figs. 14a, c and e) of myofibrils from the *longissimus* muscle stored at 20°C. However, gels (Figs. 14b, d and f) of myofibrils from muscle stored at 25°C, show a 30,000-dalton protein band appearing slightly at 2 and 3 days post-mortem. Troponin-T is not clearly resolved in these gels (Fig. 14), however, it appears that troponin-T is present in myofibrils from muscle stored for 3 days post-mortem at 20°C but not at 25°C. The higher postmortem storage temperature (25°C) has no apparent effect on the other myofibrillar proteins (Fig. 14). The gel in Figure 14(f) (3 days post-mortem at 25°C) shows the same protein changes as in myofibrils from *longissimus* muscle stored for 10 days post-mortem at 20°C (Fig. 11j). The higher storage temperature appeared to only accelerate the changes in the myofibrillar proteins and not cause additional degradative changes. The same high temperature acceleration of postmortem changes was also found in myofibril fragmentation index (Fig. 7 and Table 2) and in W-B shear-force (Fig. 9 and
Figure 14. SDS-10% polyacrylamide gels of myofibrils prepared from bovine *longissimus* muscle at different times of postmortem storage at 2°C and 25°C

a. Gel of myofibrils at 1 day post-mortem, 2°C

b. Gel of myofibrils at 1 day post-mortem, 25°C. Note the decreased intensity of the troponin-T band compared with Fig. 14a

c. Gel of myofibrils at 2 days post-mortem, 2°C

d. Gel of myofibrils at 2 days post-mortem, 25°C. Note the slight appearance of the 30,000-dalton band and the decreased intensity of the troponin-T band

e. Gel of myofibrils at 3 days post-mortem, 2°C

f. Gel of myofibrils at 3 days post-mortem, 25°C. Note the absence of the troponin-T band and the presence of the 30,000-dalton band
<table>
<thead>
<tr>
<th>POSTMORTEM TIME</th>
<th>DAY 1</th>
<th>DAY 2</th>
<th>DAY 3</th>
</tr>
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<tr>
<td>TEMPERATURE</td>
<td>2°C</td>
<td>2°C</td>
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</tr>
<tr>
<td></td>
<td>25°C</td>
<td>25°C</td>
<td>25°C</td>
</tr>
</tbody>
</table>

- α-ACTININ
- ACTIN
- TRO Ponin-T
- 30,000 DALTONS
- TRO Ponin-I

a-b c-d e-f
Figure 15. SDS-10% polyacrylamide gels of myofibrils prepared from bovine semitendinosus muscle at different times of postmortem storage at 2°C and 25°C

a. Gel of myofibrils at 1 day post-mortem, 2°C. Note the presence of the troponin-T band and the absence of the 30,000-dalton band

b. Gel of myofibrils at 1 day post-mortem, 25°C. Note the decreased intensity of the troponin-T band and the presence of the 30,000-dalton band

c. Gel of myofibrils at 2 days post-mortem, 2°C. Note the slightly visible 30,000-dalton band

d. Gel of myofibrils at 2 days post-mortem, 25°C. Note the absence of the troponin-T band and the greater intensity of the 30,000-dalton band compared with the gel in Fig. 15b

e. Gel of myofibrils at 3 days post-mortem, 2°C. Note the decreased intensity of the troponin-T band and the increased intensity of the 30,000-dalton band compared with the gel in Fig. 15c

f. Gel of myofibrils at 3 days post-mortem, 25°C. Note the absence of the troponin-T band and the decreased intensities of the troponin-I band compared with the gel in Fig. 15d or 15e
POSTMORTEM TIME

<table>
<thead>
<tr>
<th>TEMPERATURE</th>
<th>DAY 1</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
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<td></td>
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</table>

α- ACTININ

ACTIN

TROPONIN-T

30,000 DALTONS

TROPONIN-I
Figure 15 shows the SDS-10% polyacrylamide gels of myofibrils from bovine *semitendinosus* muscle at 1, 2 and 3 days of postmortem storage at 2°C and 25°C. The gels of myofibrils from muscles stored at 2°C (Figs. 15a, c and e) show that the intensity of the troponin-T band (which is not clearly resolved) decreased slightly and the 30,000-dalton band increased slightly from 1 to 3 days post-mortem. However, the gels of myofibrils from *semitendinosus* muscle stored at 25°C (Figs. 15b, d and f) show that the troponin-T band decreased in intensity and then disappeared while the 30,000-dalton band increased in intensity from 1 to 3 days post-mortem. The 25°C storage temperature treatment caused similar myofibrillar protein degradation as the 2°C storage temperature treatment from 1 to 3 days post-mortem but the higher storage temperature caused the effects to occur sooner in both the *longissimus* and *semitendinosus* muscles. Gels of myofibrils from *semitendinosus* muscle stored at 3 days post-mortem at 25°C appeared to have the same degree of protein degradation as shown in the gels of myofibrils from the *semitendinosus* muscle stored at 10 days post-mortem at 2°C (Fig. 12j).

Figure 16 shows the SDS-10% polyacrylamide gels of myofibrils from bovine *psoas major* muscles at 1, 2 and 3 days of postmortem storage at 2°C and 25°C. No major differences are
Figure 16. SDS-10% polyacrylamide gels of myofibrils prepared from bovine psoas major muscle at different times of postmortem storage at 2°C and 25°C

a-b. Gels of myofibrils at 1 day post-mortem, 2°C (a) and 25°C (b). No difference between gels appear (a and b)

c-d. Gels of myofibrils at 2 days post-mortem, 2°C (c) and 25°C (d). Note that no major difference in the protein bands occur between gels (c and d)

e-f. Gels of myofibrils at 3 days post-mortem, 2°C (e) and 25°C (f). Note that no major difference in the protein bands occur between gels (e and f)
<table>
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</tr>
<tr>
<td>Protein</td>
<td>$\alpha$-Actinin</td>
<td>Actin</td>
<td>Troponin-T</td>
</tr>
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</table>

Diagram showing protein bands at different temperatures and time periods.
shown between the gels of myofibrils either among days of post-mortem storage or between storage temperatures at each postmortem period. The apparent lack of significant changes in the myofibrillar proteins due to either postmortem storage time or temperature is similar to the relatively slight change in myofibril fragmentation index (Fig. 7 and Table 2) and W-B shear-force (Fig. 9 and Table 2) during postmortem storage at 2°C or 25°C storage temperature. These results on SDS-polyacrylamide gels indicate that some factor, or factors, causing postmortem muscle changes in the longissimus and semitendinosus muscles has a greater activity at higher storage temperatures and that this factor is apparently lacking or suppressed in the psoas major muscle.

A calcium-activated-factor (CAF), a protease endogenous to muscle tissue, has been shown to selectively remove Z-lines and degrade the myofibrillar proteins troponia, tropomyosin and component-C (Dayton et al., 1974a, b). CAF was prepared from bovine longissimus, psoas major and semitendinosus muscles at death and at 1, 3 and 6 days of post-mortem storage at 2°C and its activity was measured on the proteolytic degradation of denatured casein (Fig. 17). CAF activity is highest at-death for all three muscles and its activity decreases from 0 to 6 days post-mortem. CAF activities of longissimus and semitendinosus muscles were nearly identical at-death and decreased at about the same rate by 6
Figure 17. Effect of postmortem storage on CAF activity of bovine longissimus, psoas major, and semitendinosus muscles at different times of postmortem storage at 2°C.
CAF ACTIVITY (ABSORBANCE UNITS AT 278nm/100g MUSCLE)

POSTMORTEM STORAGE (DAYS)

PSOAS

LONGISSIMUS

SEMITENDINOSUS
days post-mortem to less than half of the at-death level with the greatest decrease occurring between 0 and 1 day post-mortem. In the \textit{psoas major} muscle, the CAF activity at-death is less than half the at-death CAF activity of the \textit{longissimus} and \textit{semitendinosus} muscles. CAF activity in the \textit{psoas major} muscle decreases to near 0 at 1 day post-mortem and remains at that level from 1 to 6 days post-mortem. No direct relationship can be made between postmortem CAF activity and the postmortem changes in myofibril fragmentation index and W-B shear-force because the proteolytic action of CAF should increase if it caused the increase in postmortem myofibril fragmentation index (Figs. 6 and 7 and Tables 1 and 2) and the decrease in postmortem W-B shear-force (Figs. 8 and 9 and Tables 1 and 2). The causal effect of CAF proteolytic activity on increasing myofibril fragmentation index and decreasing W-B shear-force can not, however, be discounted even though no direct linear relationship can be made, because an active CAF fraction can be prepared from muscle stored 6 days post-mortem in the \textit{longissimus} and \textit{semitendinosus} muscles and 1 day post-mortem in the \textit{psoas major} muscle (Fig. 17). It is possible that even a postmortem CAF activity level, lower than the at-death level, is sufficient to cause the observed postmortem muscle changes. It is also possible that the CAF proteolytic activity from 0 to 1 day post-mortem causes most of the degradation of the myofibrillar proteins at that time.
but the effects on myofibril fragmentation index and W-B shear-force are not completely revealed until a later postmortem time. It is also interesting to note that differences in postmortem CAF activity between the *psoas major* muscle and that of the *longissimus* and *semitendinosus* muscles (Fig. 17) follows the pattern of differences in these muscles for the postmortem myofibril fragmentation index (Figs. 6 and 7 and Tables 1 and 2) and W-B shear-force (Figs. 8 and 9 and Tables 1 and 2) measurements. The coincidental occurrence of the similar changes in CAF activity, myofibril fragmentation index and W-B shear-force during postmortem storage of the *longissimus* and *semitendinosus* muscles while the changes of these measures are considerably less in the *psoas major* muscle indicates an integral relationship among these three characteristics of postmortem muscle exists.

To determine the effect of CAF on myofibrils, purified CAF from porcine muscle (prepared by W. Dayton, Dayton et al., 1974a, b) was incubated with myofibrils from at-death bovine *longissimus*, *semitendinosus* and *psoas major* muscles (Fig. 18). Myofibrils from the *longissimus* muscle incubated in the absence of CAF (Fig. 18a) had relaxed sarcomeres with Z-lines present, however, myofibrils incubated in the presence of CAF (Fig. 18b) show that Z-lines were completely removed with little other apparent change occurring in the myofibrils. The myofibrils from *semitendinosus* (Figs. 18c and
Figure 18. Phase micrographs of at-death (control) and CAF-treated at-death myofibrils of bovine longissimus, semitendinosus, and psoas major muscles

a. Control longissimus myofibrils. Note the presence of Z-lines. X2000

b. CAF-treated at-death longissimus myofibrils. Note the absence of Z-lines. X2000

c. Control semitendinosus myofibrils. Note the presence of prominent Z-lines. X2000

d. CAF-treated at-death semitendinosus myofibrils. Myofibrils are fragmented and Z-lines are absent. X2000

e. Control psoas major myofibrils. Note the presence of prominent Z-lines. X2000

f. CAF-treated at-death psoas major myofibrils. Note absence of Z-lines. X2000
d) and *psoas major* muscle (Figs. 18e and f) also show that CAF apparently only selectively removes the Z-lines from myofibrils. The ability of CAF to remove the Z-lines from myofibrils (Fig. 18) implicates CAF as the causal factor influencing a weakening of the Z-line in postmortem muscle; consequently, greater postmortem myofibril fragmentation resulted (Figs. 1 and 2). To test this implication, at-death myofibrils from the *longissimus*, *semitendinosus* and *psoas major* muscles were incubated in the absence and presence of CAF and electrophoresed on SDS-7 1/2% and 10% polyacrylamide gels (Figs. 19-21). The SDS-7 1/2% polyacrylamide gels (Figs. 19a and b) demonstrated that CAF-treated at-death *longissimus* muscle myofibrils removed alpha-actinin, troponin-T and troponin-I bands and a 30,000-dalton protein band appears. The SDS-10% polyacrylamide gels (Figs. 19c and d) showed more clearly the removal of the troponin-T and troponin-I bands and the appearance of the 30,000-dalton band in the CAF-treated myofibrils. These results agree with those reported by Dayton et al. (1974a, b) in that purified porcine CAF selectively removes, after brief incubation with CAF, alpha-actinin, troponin-T and troponin-I while a 30,000-dalton band appears from porcine myofibrils. The removal of alpha-actinin from CAF-treated myofibrils accounts for the removal of Z-lines in the myofibril (Fig. 18b). Also, since only the troponin subunits (troponin-T and I) were proteolytically de-
Figure 19. SDS-7 1/2% and 10% polyacrylamide gels of at-death bovine *longissimus* myofibrils incubated in the absence and presence of CAF

a. 7 1/2% gel of control myofibrils. Note the presence of alpha-actinin, troponin-T and troponin-I bands

b. 7 1/2% gel of CAF-treated myofibrils. Note the absence or decrease intensity of alpha-actinin, troponin-T and troponin-I bands and the presence of bands in the 30,000-dalton region

c. 10% gel of control myofibrils. Note the presence of alpha-actinin, troponin-T and troponin-I bands

d. 10% gel of CAF-treated myofibrils. Note the absence or decreased intensity of alpha-actinin, troponin-T and troponin-I bands and the presence of bands in the 30,000-dalton region
Figure 20. SDS-7 1/2% and 10% polyacrylamide gels of at-death bovine *semitendinosus* myofibrils incubated in the absence and presence of CAF

a. 7 1/2% gel of control myofibrils. Note the presence of alpha-actinin, troponin-T and troponin-I bands

b. 7 1/2% gel of CAF-treated myofibrils. Note the absence or decreased intensity of alpha-actinin, troponin-T and troponin-I bands and the presence of bands in the 30,000-dalton region

c. 10% gel of control myofibrils. Note the presence of alpha-actinin, troponin-T and troponin-I bands

d. 10% gel of CAF-treated myofibrils. Note the absence or decreased intensity of alpha-actinin, troponin-T and troponin-I bands and the presence of bands in the 30,000-dalton region
Figure 21. SDS-7 1/2% and 10% polyacrylamide gels of at-death bovine psoas major myofibrils incubated in the absence and presence of CAF

a. 7 1/2% gel of control myofibrils. Note the presence of alpha-actinin, troponin-T and troponin-I bands

b. 7 1/2% gel of CAF-treated myofibrils. Note the absence or decreased intensity of alpha-actinin, troponin-T and troponin-I bands and the presence of the bands in the 30,000-dalton region

c. 10% gel of control myofibrils. Note the presence of the alpha-actinin, troponin-T and troponin-I bands

d. 10% gel of CAF-treated myofibrils. Note the absence or decreased intensity of the alpha-actinin, troponin-T and troponin-I bands and the presence of bands in the 30,000-dalton region
7 1/2%
CAF-CONTROL TREATED

α-ACTININ

ACTIN
TROPONIN-T
30,000 DALTONS
TROPONIN-I
TROPONIN-C

10%
CAF-CONTROL TREATED

α-ACTININ

ACTIN
TROPONIN-T
30,000 DALTONS
TROPONIN-I
TROPONIN-C

a b c d
graded, the myofibril (other than the Z-line) remained apparently unaffected by CAF treatment. SDS-7 1/2% and 10% polyacrylamide gels of myofibrils incubated in the absence and presence of CAF from *semitendinosus* (Fig. 20) and *psoas major* (Fig. 21) muscles show the same CAF proteolytic effects as shown in *longissimus* muscle (Fig. 19). SDS-10% polyacrylamide gels of myofibrils from *longissimus, semitendinosus* and *psoas major* muscles incubated in the presence of CAF (Figs. 19, 20 and 21) show similar results as those shown in gels of myofibrils from postmortem muscle (Figs. 11, 12 and 13) except that alpha-actinin was present in the gels of myofibrils from postmortem muscle. Dayton *et al.* (1974a, b) have shown that while CAF removes the Z-lines from myofibrils and alpha-actinin in SDS-polyacrylamide gels of CAF-treated myofibrils, CAF does not proteolytically degrade purified alpha-actinin. Alpha-actinin is thus apparently released from the myofibril but is not itself proteolytically degraded by CAF. The close similarities of gels of CAF-treated myofibrils (Figs. 19, 20 and 21) and gels of myofibrils from muscle stored over 3 days post-mortem (Figs. 11, 12 and 13) clearly implicate CAF as the causal factor producing the changes in the myofibrillar proteins in postmortem muscle.

The gels of myofibrils from postmortem muscle and from CAF-treated myofibrils have all shown the common alterations in myofibrils to be primarily removal of the troponin-T band
appearance of the 30,000-dalton band. To determine whether the 30,000-dalton band originated from troponin-T, purified troponin prepared from at-death bovine longissimus muscle was incubated with purified CAF from porcine muscle for 0, 5, 10, 20 and 40 minutes. SDS-10% polyacrylamide gels of control and CAF-treated troponin are shown in Figure 22. After 5 minutes incubation with CAF, troponin showed some apparent proteolytic degradation by the appearance of bands below the troponin-T band, in the 30,000-dalton region, and the bands below the troponin-C subunits. As incubation time increased from 5 to 40 minutes (Figs. 22b-e), the troponin-T band decreased in intensity and almost disappeared at 40 minutes incubation. The troponin-I band decreased slightly as incubation time with CAF increased, but the troponin-C band appeared to be unaffected by CAF treatment. The 30,000-dalton band increased in intensity up to 10 minutes incubation and then decreased in intensity as incubation time increases beyond 10 minutes. The bands below troponin-C became increasingly greater in intensity during the 40-minute CAF treatment. The 30,000-dalton band apparently originated from the degradation of troponin-T by CAF treatment, however, the 30,000-dalton protein is not a final product of troponin-T degradation because it apparently is further degraded by CAF into smaller molecular weight proteins. Troponin, before and after CAF treatment, was electrophoresed along with myo-
Figure 22. SDS-10% polyacrylamide gels of purified troponin prepared from the at-death bovine longissimus muscle and incubated with CAF for 0, 5, 10, 20 and 40 minutes

a. Troponin with no CAF treatment. Note the troponin subunits T, I and C

b. Troponin after 5 minutes incubation with CAF. Note the proteolytic breakdown products in the 30,000-dalton band region and below the I and C subunits

c. Troponin after 10 minutes incubation with CAF. Note the increased intensity of the proteolytic breakdown products and the decreased intensity of the troponin-T band

d. Troponin after 20 minutes incubation with CAF. Note the increase in intensity of the bands below the I and C subunits and the decrease in the troponin-T band and the bands in the 30,000-dalton region

e. Troponin after 40 minutes incubation with CAF. Note the faint bands in troponin-T and in the 30,000-dalton region and the increased intensity in the bands below the I and C subunits. The troponin-I band has also decreased compared to the gel in Fig. 17b
TIME OF CAF TREATMENT (MIN) 0 5 10 20 40

TROPININ-T
30,000 DALTONS
TROPONIN-I
TROPONIN-C
fibrils from at-death bovine *longissimus* muscle and at 10
days of postmortem storage on SDS-10% polyacrylamide gels
(Fig. 23). The gel of at-death (control) myofibrils (Fig.
23a) showed the presence of troponin-T and the absence of the
30,000-dalton band. When troponin (with no CAF treatment)
was electrophoresed along with the at-death myofibrils, (Fig.
23b) troponin subunit bands showed increased intensity but
the 30,000-dalton band was not present. When CAF-treated
troponin was electrophoresed with the at-death myofibrils
(Fig. 23c), the troponin subunit bands showed greater inten­sity and the 30,000-dalton band appeared. The gel of myofi­brils from muscle stored 10 days post-mortem (Fig. 23d)
showed the absence of the troponin-T band and the presence of
the 30,000-dalton band. When troponin (with no CAF treat­ment) was electrophoresed with myofibrils from postmortem
muscle (Fig. 23e), the troponin-T band appeared in the same
location as that shown in the gel of at-death myofibrils
(Fig. 23a), the troponin-I and C bands were of greater inten­sity and the 30,000 dalton band was of similar intensity as
shown in Figure 23d. When CAF-treated troponin was electro­phoresed with myofibrils from postmortem muscle (Fig. 23f),
the troponin-T band appeared and the troponin-I, troponin-C
and 30,000-dalton bands increased in intensity. It is clear
that the band in at-death myofibrils (Fig. 23a) that
disappears from the gel of myofibrils after postmortem muscle
Figure 23. SDS-10% polyacrylamide gels of myofibrils prepared from at-death bovine longissimus muscle and at 10 days of postmortem storage in the absence (control) and presence of troponin (TN) and CAF-treated troponin (CAF-TN)

a. Gel of at-death control myofibrils

b. Gel of at-death myofibrils combined with troponin. Note the increased intensities in the troponin subunit bands

c. Gel of at-death myofibrils combined with CAF-treated troponin. Note the presence of the 30,000-dalton band

d. Gel of myofibrils at 10 days post-mortem (control). Note the absence of troponin-T and the presence of the 30,000-dalton band

e. Gel of myofibrils at 10 days post-mortem combined with troponin. Note the troponin-T band is in the same location as shown in the at-death myofibrils (Fig. 23a)

f. Gel of myofibrils at 10 days post-mortem combined with CAF-treated troponin. Note the 30,000-dalton band and the bands below troponin-C are of greater intensity than when CAF-treated troponin was not combined with myofibrils (Fig. 23d and 23e)
storage (Fig. 23d) is troponin-T and the 30,000-dalton band that appears in the gel of myofibrils from postmortem muscle (Fig. 23d) originates from the degradation of troponin-T.

Figures 24a and b show the SDS-10% polyacrylamide gels of myofibrils from at-death bovine longissimus muscle and at 10 days of postmortem storage at 2°C. The major changes in the myofibrillar proteins during postmortem storage is the selective degradation of troponin-T yielding a 30,000-dalton molecular weight protein. Figure 24c and d show the SDS-10% polyacrylamide gels of troponin from at-death bovine longissimus muscle with and without CAF treatment. CAF treatment of troponin shows troponin-T degraded partially to a 30,000-dalton molecular weight protein and troponin-I being also degraded. It is interesting to note that the major change occurring naturally in postmortem muscle (Figs. 24a and b) can be manufactured by CAF treatment of purified troponin (Figs. 24c and d).

Since the myofibrillar proteins constitute the major portion of the muscle structure, myofibril fragmentation should play an integral role in meat tenderness and quality. To test this, loin steaks from longissimus muscles from veal, A-maturity and C-maturity were examined at 1 and 7 days postmortem. Table 3 shows the means and standard errors of myofibril fragmentation index, W-B shear-force and sensory panel tenderness, flavor and juiciness of loin steaks from longis-
Figure 24. SDS-10% polyacrylamide gels of myofibrils prepared from at-death (control) bovine longissimus muscle and at 10 days of postmortem storage and gels of purified troponin (TN) and troponin incubated with CAF (CAF-TN)

a. Gel of at-death myofibrils (control). Note presence of troponin-T bands and absence of 30,000-dalton band

b. Gel of myofibrils 10 days post-mortem. Note absence of troponin-T band and presence of 30,000-dalton band

c. Gel of CAF-treated troponin (CAF-TN). Note presence of bands in 30,000-dalton region

d. Gel of purified troponin (TN). Note absence of 30,000-dalton band
muscle from six veal carcasses at 1 and 7 days of post-mortem storage at 20°C. Myofibril fragmentation index was significantly (P<0.05) higher at 7 days than at 1 day post-mortem. However, W-B shear-force and sensory panel tenderness, flavor and juiciness were not significantly (P<0.05) different between 1 and 7 days post-mortem. Even though tenderness (measured by W-B shear-force and sensory panel) was considerably higher at 7 days than at 1 day post-mortem, the standard errors were too large to make these differences significant.

Table 4 contains the means and standard errors of myofibril fragmentation index, W-B shear-force and sensory panel tenderness, flavor and juiciness of bovine longissimus muscle loin steaks from thirty-five A-maturity carcasses at 1 and 7 days of post-mortem storage at 20°C. Myofibril fragmentation index was significantly (P<0.05) higher at 7 days than at 1 day post-mortem, whereas sensory panel flavor was significantly (P<0.05) but only slightly lower at 7 days than at 1 day post-mortem. W-B shear-force and sensory panel tenderness and juiciness were not significantly (P<0.05) different between 1 and 7 days post-mortem. In comparison with results of a previous experiment, the mean myofibril fragmentation index of 64.8 at 1 day post-mortem (Table 4) was nearly the same as the mean myofibril fragmentation index of 69.8 at 3 days post-mortem (Table 2) measured on longissimus muscle of
Table 3. Effect of postmortem storage (20°C) on myofibril fragmentation index (PI), Warner-Bratzler (W-B) shear-force and sensory panel tenderness (TEND), flavor (FLA) and juiciness (JU) of longissimus muscle from six veal carcasses1

<table>
<thead>
<tr>
<th>Days of postmortem storage</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>PI²</td>
<td>44.4±1.1</td>
<td>58.6±1.2</td>
</tr>
<tr>
<td>W-B³</td>
<td>4.53±0.55</td>
<td>3.62±0.49</td>
</tr>
<tr>
<td>TEND*</td>
<td>3.91±0.54</td>
<td>5.17±0.55</td>
</tr>
<tr>
<td>FLA*</td>
<td>3.36±0.18</td>
<td>3.22±0.17</td>
</tr>
<tr>
<td>JU*</td>
<td>5.32±0.27</td>
<td>5.34±0.23</td>
</tr>
</tbody>
</table>

1 Means ± standard errors. Means not underscored by the same dotted line are significantly different (P<0.05).

2 Absorbance per 0.5mg myofibril protein X 200.

3 Kg of shear-force per cm.

4 Hedonic scale of 1 to 8 with 8 being extremely tender, flavorful or juicy.
Table 4. Effect of postmortem storage (20°C) on myofibril fragmentation index (FI), Warner-Bratzler (W-B) shear-force and sensory panel tenderness (TEND), flavor (FLA) and juiciness (JU) of longissimus muscles from thirty-five A-maturity carcasses

<table>
<thead>
<tr>
<th>Days of postmortem storage</th>
<th>1</th>
<th>7</th>
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<tbody>
<tr>
<td>PI$^2$</td>
<td>64.8±0.7</td>
<td>71.4±0.6</td>
</tr>
<tr>
<td>W-B$^3$</td>
<td>2.85±0.08</td>
<td>2.72±0.07</td>
</tr>
<tr>
<td>TEND$^4$</td>
<td>5.29±0.20</td>
<td>5.52±0.15</td>
</tr>
<tr>
<td>FLA$^4$</td>
<td>5.86±0.08</td>
<td>5.62±0.08</td>
</tr>
<tr>
<td>JU$^4$</td>
<td>5.94±0.09</td>
<td>5.78±0.09</td>
</tr>
</tbody>
</table>

1Means ± standard errors. Means not underscored by the same dotted line are significantly different (P<0.05).

2Absorbance per 0.5mg myofibril protein x 200.

3Kg of shear-force per cm.

4Hedonic scale of 1 to 9 with 9 being extremely tender, flavorful or juicy.
animals having similar maturities as those reported in Table 4. The bovine carcasses in the two separate experiments originated from different sources and samples were handled differently (See METHODS AND MATERIALS section) which may account for part of the differences in the postmortem myofibril fragmentation index means. The W-B shear-force mean of 2.72kg/cm² (Table 4) at 7 days post-mortem was also higher than that shown for the longissimus muscle in Table 2 of 2.60kg/cm² at 1 day post-mortem.

Table 5 shows the means and standard errors of myofibril fragmentation index, W-B shear-force and sensory panel tenderness, flavor and juiciness of the bovine longissimus muscle from twelve C-maturity carcasses at 1 and 7 days of post-mortem storage at 2°C. Myofibril fragmentation index was significantly (P<0.05) higher at 7 days than at 1 day post-mortem, but W-B shear-force and sensory panel tenderness, flavor and juiciness were not significantly (P<0.05) different between 1 and 7 days post-mortem. A larger difference in myofibril fragmentation index means between 1 and 7 days post-mortem was found in the longissimus muscle of the C-maturity carcasses (Table 5) than was found in the A-maturity (Table 4) or veal (Table 3) carcasses. However, the tenderness of the longissimus muscle at respective days post-mortem was lower in the C-maturity carcasses than in the A-maturity carcasses, but higher than in the veal carcasses.
Table 5. Effect of postmortem storage (20°C) on myofibril fragmentation index (FI), Warner-Bratzler (W-B) shear-force and sensory panel tenderness (TEND), flavor (FLA) and juiciness (JU) of *longissimus* muscles from twelve C-maturity carcasses.  

<table>
<thead>
<tr>
<th>Days of postmortem storage</th>
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<tbody>
<tr>
<td>FI²</td>
<td>54.9±0.9</td>
<td>74.2±0.8</td>
</tr>
<tr>
<td>W-B³</td>
<td>3.36±0.16</td>
<td>2.94±0.13</td>
</tr>
<tr>
<td>TEND*</td>
<td>4.72±0.34</td>
<td>5.42±0.27</td>
</tr>
<tr>
<td>FLA*</td>
<td>5.57±0.18</td>
<td>5.42±0.17</td>
</tr>
<tr>
<td>JU*</td>
<td>5.73±0.20</td>
<td>5.56±0.18</td>
</tr>
</tbody>
</table>

1 Means ± standard errors. Means not underscored by the same dotted line are significantly different (P<0.05).

2 Absorbance per 0.5mg myofibril protein × 200.

3 Kg of shear-force per cm.

4 Hedonic scale of 1 to 8 with 8 being extremely tender, flavorful or juicy.
Simple correlation coefficients (Table 6) were determined among myofibril fragmentation index, W-B shear-force and sensory panel tenderness, flavor and juiciness of longissimus muscles from veal, A-maturity and C-maturity bovine carcasses at 1 and 7 days of postmortem storage at 2°C. The correlation coefficients between myofibril fragmentation index and W-B shear-force were significant (P<0.05, P<0.01) for both 1 and 7 days post-mortem for all three maturity groups with the lowest coefficient being -0.85 for the A-maturity group at 1 day post-mortem. Likewise, the correlation coefficient between myofibril fragmentation index and sensory panel tenderness was significant (P<0.05, P<0.01) for both 1 and 7 days post-mortem for all three maturity groups with the lowest coefficient being 0.65 for the C-maturity group at 7 days post-mortem. In addition, the correlation coefficient between W-B shear-force and sensory panel tenderness for veal steak was significant (P<0.01) at 7 days post-mortem (-0.94) but not at 1 day post-mortem (-0.76). Also the correlation coefficient between sensory panel tenderness and juiciness for veal was significant (P<0.05) at 7 days post-mortem (0.82) but not at 1 day post-mortem (0.75). All other correlation coefficients for veal carcasses were not significant (P<0.05). The correlation coefficients between sensory tenderness and sensory flavor and juiciness were moderately high (0.62-0.69) for the longissimus muscle of A-maturity carcass-
Table 6. Effect of postmortem storage (2°C) on correlation coefficients among myofibril fragmentation index (FI), Warner-Bratzler (W-B) shear-force and sensory panel tenderness (TEND), flavor (FLA) and juiciness (JU) of *longissimus* muscles from veal, A-maturity and C-maturity carcasses

<table>
<thead>
<tr>
<th></th>
<th>Veal&lt;sup&gt;1&lt;/sup&gt;</th>
<th>A-maturity&lt;sup&gt;2&lt;/sup&gt;</th>
<th>C-maturity&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td><strong>Days of postmortem storage</strong></td>
<td></td>
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<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

|                  | 1                | 7                      | 1                      | 7                      |
|------------------|------------------|------------------------|------------------------|
| FI vs W-B        | -0.95**          | -0.97**                | -0.65**                | -0.75**                |
| FI vs TEND       | 0.88*            | 0.95**                 | 0.67**                 | 0.73**                 |
| FI vs FLA        | 0.52             | 0.06                   | 0.43**                 | 0.54**                 |
| FI vs JU         | 0.58             | 0.78                   | 0.50**                 | 0.57**                 |
| W-B vs TEND      | -0.75            | -0.94**                | -0.86**                | -0.62**                |
| W-B vs FLA       | -0.54            | -0.01                  | -0.50**                | -0.49**                |
| W-B vs JU        | -0.59            | -0.79                  | -0.47**                | -0.31                  |
| TEND vs FLA      | 0.55             | 0.06                   | 0.63**                 | 0.69**                 |
| TEND vs JU       | 0.75             | 0.82*                  | 0.64**                 | 0.62**                 |
| FLA vs JU        | 0.22             | -0.27                  | 0.49**                 | 0.49**                 |

---

<sup>1</sup>Correlation coefficients of six veal carcasses.

<sup>2</sup>Correlation coefficients of thirty-five A-maturity carcasses.

<sup>3</sup>Correlation coefficients of twelve C-maturity carcasses.

*Significant at the 5% level.

**Significant at the 1% level.
ses. Sensory flavor and juiciness were also moderately correlated to W-B shear-force and myofibril fragmentation index, however, these correlations were probably not direct correlations, but resulted from their mutual relationship to sensory tenderness. The correlation coefficients between sensory tenderness and flavor, sensory tenderness and juiciness and sensory juiciness and flavor were higher in the longissimus muscle of the C-maturity carcasses than in either the A-maturity or veal carcasses.

Because myofibril fragmentation index is related to the degradation of the myofibrillar proteins during postmortem storage, myofibrils were selected from two longissius muscles of veal, A-maturity and C-maturity carcasses at 1 and 7 days postmortem at 2°C which had extreme differences in myofibril fragmentation index, W-B shear-force and sensory tenderness scores. These selected myofibrils were electrophoresed on SDS-10% polyacrylamide gels and are shown in Figures 25-27. The gels of the myofibrils of longissimus muscles of both veal carcasses (Fig. 25) show that at 1 day post-mortem the troponin-T band is present but the 30,000-dalton band is absent which coincided with a low myofibril fragmentation index and muscle tenderness (measured by W-B shear-force and sensory panel evaluation). The gels of the myofibrils form the longissimus muscle at 7 days post-mortem show the absence of the troponin-T band and the presence of the 30,000-dalton
Figure 25. SDS-10% polyacrylamide gels of myofibrils prepared from longissimus muscles of two veal carcasses at 1 and 7 days of postmortem storage at 2°C having different Warner-Bratzler (W-B) shear-force, sensory tenderness and myofibril fragmentation index values

a. Gel of myofibrils at 1 day post-mortem having a W-B shear-force of 4.42 and a fragmentation index of 60 (carcass 1). Note the faint 30,000-dalton band

b. Gel of myofibrils at 7 days post-mortem having a W-B shear-force of 2.85 and a fragmentation index of 74 (carcass 1). Note the intense 30,000-dalton band

c. Gel of myofibrils at 1 day post-mortem having a W-B shear-force of 3.42 and a fragmentation index of 63 (carcass 2). Note the faint 30,000-dalton band

d. Gel of myofibrils at 7 days post-mortem having a W-B shear-force of 3.01 and a fragmentation index of 70 (carcass 2). Note the intense 30,000-dalton band
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ACTIN
TROTONIN-T
30,000 DALTONS
Figure 26. SDS-10% polyacrylamide gels of myofibrils prepared from longissimus muscles of two A-maturity bovine carcasses at 1 and 7 days of postmortem storage at 2°C having different Warner-Bratzler (W-B) shear-force, sensory tenderness and myofibril fragmentation index values

a. Gel of myofibrils at 1 day post-mortem having a W-B shear-force of 2.90 and a fragmentation index of 58 (carcass 1). Note the faint 30,000-dalton band

b. Gel of myofibrils at 7 days post-mortem having a W-B shear-force of 2.74 and a fragmentation index of 62 (carcass 1). Note the faint 30,000-dalton band

c. Gel of myofibrils at 1 day post-mortem having a W-B shear-force of 2.83 and a fragmentation index of 64 (carcass 2). Note the slightly intense 30,000-dalton band

d. Gel of myofibrils at 7 days post-mortem having a W-B shear-force of 2.51 and a fragmentation index of 68 (carcass 2). Note the intense 30,000-dalton band
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![ACTIN TROTONIN-T 30,000 DALTONS](image)
Figure 27. SDS-10% polyacrylamide gels of myofibrils prepared from longissimus muscles of two C-maturity bovine carcasses at 1 and 7 days of postmortem storage at 2°C having different Warner-Bratzler (W-B) shear-force, sensory tenderness and myofibril fragmentation index values

a. Gel of myofibrils at 1 day post-mortem having a W-B shear-force of 3.79 and a fragmentation index of 46 (carcass 1). Note the absence of the 30,000-dalton band.

b. Gel of myofibrils at 7 days post-mortem having a W-B shear-force of 3.35 and a fragmentation index of 66 (carcass 1). Note the presence of the 30,000-dalton band.

c. Gel of myofibrils at 1 day post-mortem having a W-B shear-force of 3.56 and a fragmentation index of 54 (carcass 2). Note the very faint 30,000-dalton band.

d. Gel of myofibrils at 7 days post-mortem having a W-B shear-force of 2.96 and a fragmentation index of 74 (carcass 2). Note the intense 30,000-dalton band.
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![Diagram of protein bands](image)

- ACTIN
- TROPOGIN-T
- 30,000 DALTONS

**Legend:**
- a: Sample 1, Day 1
- b: Sample 1, Day 7
- c: Sample 2, Day 1
- d: Sample 2, Day 7
band, which coincided with higher myofibril fragmentation and muscle tenderness. These results in the veal carcass (Fig. 25) further indicate the relationships between the myofibril fragmentation index, W-B shear-force, sensory tenderness and the postmortem changes in the myofibrillar proteins.

The gels of the myofibrils from the two A-maturity carcasses at 1 and 7 days post-mortem are shown in Figure 26. Longissimus muscles of both carcasses have large differences in myofibril fragmentation index, W-B shear-force, sensory tenderness between only carcass 1, 1 day post-mortem and carcass 2, 7 days post-mortem. The gels of myofibrils from the longissimus muscle show that troponin-T is not degraded in myofibrils from carcass 1, 1 day post-mortem, but, in carcass 2, 7 days post-mortem, troponin-T is degraded. These results show that troponin-T degradation coincides with greater myofibril fragmentation index and muscle tenderness.

The gels of myofibrils, tenderness measures and myofibril fragmentation index of longissimus muscles from two C-maturity carcasses are shown in Figure 27. As has been previously observed in the veal and A-maturity longissimus muscles (Figs. 25 and 26), the presence of the troponin-T band in the gels generally parallels high W-B shear-force and low sensory tenderness and myofibril fragmentation index, while absence of troponin-T and the presence of the 30,000-dalton protein parallels the opposite of these measures in the lon-
gissimus muscle of the two C-maturity carcasses. From the data shown in Figures 25-27 of longissimus muscles of veal, A-maturity and C-maturity carcasses, it appears that inter­relationships exist between muscle tenderness, myofibril fragmentation and myofibrillar protein degradation.
DISCUSSION

The objective of this investigation was to provide understanding about some of the important cellular and subcellular events occurring in postmortem bovine skeletal muscle and to show how these events relate and contribute to postmortem muscle tenderization. To elucidate these events and determine something about their relationships two relatively new techniques were used to characterize some of these postmortem muscle events. The techniques, turbidometric measure of myofibril fragmentation and SDS-polyacrylamide gel electrophoresis, yielded significant results and gave impetus to the direction of this investigation.

The method of determining myofibril fragmentation by measuring the absorbance of myofibril suspensions was valuable in quantitating the postmortem changes in the fragmentation of the myofibril and associating those changes with postmortem muscle tenderization. The turbidometric measure of myofibril fragmentation is based on the premise that the amount of light absorbance of a myofibril suspension will indicate the relative size of myofibrils and fiber pieces in the suspension. For example, in two myofibril suspensions having the same protein concentration, the suspension having shorter myofibrils and smaller fiber pieces, will have a more turbid suspension and a higher absorbance value than the other suspension with longer myofibrils and larger fiber
pieces. Several investigators (Berry et al., 1974; Fukazawa et al., 1969; Sayre, 1969, 1970; Takahashi et al., 1967) have used microscopic methods, for counting sarcomeres per myofibril or determining myofibril lengths, as a measure of myofibril fragmentation. While these investigators found myofibril fragmentation changed during postmortem muscle storage similar to the change in muscle tenderness, a high relationship to tenderness was not found. Parrish et al. (1973b) followed both changes in sarcomere lengths and myofibril fragmentation during postmortem storage and they reported that improved tenderness seemed more a function of fragmentation of myofibrils than a change in sarcomere length of longissimus muscle stored in the carcass. These results suggested that an objective method of measuring myofibril fragmentation would be a potentially useful means of determining tenderness. An objective measure of myofibril fragmentation as opposed to microscopic methods provides the advantage of a larger and more representative sample and removes personal bias and subjectivity. Measurement of a suspension of myofibrils and fiber pieces is recorded in optical density units, but to give it a more descriptive expression the optical density value was multiplied by 200 and termed myofibril fragmentation index. Hence, the myofibril fragmentation index is not an absolute value of myofibril lengths but a relative value of the degree of disintegration of muscle fibers by ho-
mogenization.

The technique of SDS-polyacrylamide gel electrophoresis was valuable in separating and identifying myofibrillar proteins because of its sensitivity to small molecular subunit changes. The capability of preparing intact myofibrils from muscles after postmortem storage or other treatments and then identifying the proteins from the myofibrils was particularly useful. Changes in the myofibrils after postmortem muscle storage (to be discussed later) were found to be principally the degradation of one myofibrillar protein subunit. Also, the degradation of myofibrillar proteins in the presence of CAF (to be discussed later) was found to be similar to the natural degradation of myofibrillar proteins in postmortem muscle. These two results which were easily discernible on SDS-polyacrylamide gels, would probably have gone undetected if this technique had not been utilized. A distinct advantage of SDS-polyacrylamide gel electrophoresis over biochemical measures is that the myofibrillar proteins can be visually seen and degradative changes (disappearance or appearance of proteins bands) can be viewed and identified. These visual observations greatly assisted in interpretation of the changes occurring in the myofibril and muscle.

The disintegration of myofibrils into shorter myofibrils during postmortem muscle storage has been observed by many investigators with the light microscope but few have attempt-
ed to address the more important practical issues of 1) quantifying and identifying the changes which occur in myofibrils, 2) investigating possible causative factors responsible for fragmentation and 3) relating these changes to meat tenderness. Use of a system to objectively measure fragmentation must be carefully controlled and some understanding of what homogenization does to muscle tissue is imperative if it is to be effectively related to tenderness. Brief homogenization of muscle tissue does not separate it into homogeneous units and size, but muscle disintegrates into short, heterogeneous units moderate and long single myofibrils and small, moderate and large fiber pieces. Observing myofibrils under the light microscope at high magnifications (i.e. using 100 X objective) prohibits accurate appraisal of other than single myofibrils or very small fiber pieces. With low magnifications (i.e. using 10 X objective) fiber pieces of almost all sizes are discernible, however, sarcomeres in myofibrils cannot be clearly distinguished. The degree to which the postmortem muscle tissue is broken (severity of grinding and homogenizing) will have a tremendous impact on the size of myofibrils and fiber pieces. Extremes in grinding and homogenizing will result in very small fiber pieces and short single myofibrils, while minimal breaking of the muscle tissue will yield large fiber pieces and mostly long single myofibrils. To determine differences in the fragmentation of
muscle fibers and myofibrils between different muscles or treatments on the same muscle, the myofibril preparation procedure must be closely controlled. Severe methods of rupturing the muscle tissue may eliminate or mask the tissue differences while gentle rupturing techniques make the tissue differences undetectable. In this study, it was found that a 30-second homogenization time in a Waring Blender of a 4gm, scissor-minced muscle sample was an adequate compromise to sufficiently rupture the muscle tissue without eliminating the tissue differences. In all muscles measured for myofibril fragmentation, the control sample was taken from 1 day post-mortem muscle.

It was noted that myofibrils and fiber pieces from bovine longissimus and semitendinosus muscles broke into smaller sizes during homogenization from 1 to 10 days post-mortem at 2°C. Myofibrils appear to break at or near the Z-line indicating that post-mortem storage results in a weakening of the myofibril structure at or near the Z-line. However, even at 10 days of post-mortem storage, the Z-line appears prominent although less distinct than at 1 day post-mortem. These observations are substantiated by the work of Davey and Gilbert (1967, 1969), Fukazawa et al. (1969), Hay et al. (1973b), Parrish et al. (1973b), Sayre (1970) and Takahashi et al. (1967). Myofibrils and fiber pieces from the psoas major muscle appear to break in only slightly smaller sizes
during homogenization from 1 to 10 days postmortem. Obviously, muscle makes a difference in the way its myofibrils fragment during postmortem storage. Although there was a difference in amount of fragmentation due to muscle, myofibril fragmentation index, determined by absorbance of a myofibril suspension closely agreed with the myofibril changes observed microscopically in longissimus, semitendinosus and psoas major muscles. The major increase in myofibril fragmentation occurred between 1 and 3 days post-mortem with lesser increases being observed at 6 days post-mortem and only slight increase after 6 days postmortem in longissimus and semitendinosus muscles. In addition to following myofibril fragmentation index to determine weakening of the myofibril structure, possibly at the Z-line, two mechanical devices were used, the W-B shear-force apparatus and the Instron Universal Testing Machine. W-B shear-force is highly correlated with meat tenderness (correlation coefficients of -0.5 to -0.8 (Parrish et al., 1973a)) and measures primarily the toughness of meat associated with the myofibrillar proteins (Purchas, 1972). W-B shear-force, which measures myofibril resistance to shearing force, decreased during postmortem storage in longissimus and semitendinosus but not in psoas major muscle. The decrease in W-B shear-force coincided with the increase in myofibril fragmentation index in longissimus and semitendinosus muscle. The Instron Universal Testing Machine mea-
sures the force required to break a muscle strip (opposing forces are applied longitudinally to the muscle strip). The breaking force or strength indicates the strength or weakness of myofibrils in muscle strips to resist the opposing forces (Bouton and Harris, 1972b; Stanley et al., 1971, 1972). Breaking strength was much greater in strips from cooked muscle (60°C) than in strips from raw muscle in both semitendinosus and psoas major muscle, probably due to the coagulation and hardening of the muscle proteins (Bouton and Harris, 1972a). In strips from both cooked and raw semitendinosus and psoas major muscles, breaking strength decreased during postmortem storage with the greatest decrease occurring in semitendinosus muscle. The postmortem decrease in breaking strength in semitendinosus muscle coincided with postmortem changes in myofibril fragmentation index and W-B shear-force indicating that significant change or weakness in the myofibril occurs during postmortem storage.

Longissimus, semitendinosus and psoas major muscles were also stored for 3 days post-mortem at 25°C. High temperature (25°C) storage of the three muscles was implemented in this study to further determine what occurs to muscle structure at higher temperatures than at conventional postmortem storage temperatures and to ascertain how higher temperatures effect these changes. Myofibril fragmentation index greatly increased from 1 to 3 days post-mortem, with the greatest in-
crease occurring from 1 to 2 days post-mortem in longissimus and semitendinosus muscles stored at 25°C. Myofibril fragmentation index, in the longissimus and semitendinosus muscles at 3 days post-mortem at 25°C was nearly the same as that at 10 days post-mortem at 2°C. This indicates that higher storage temperature accelerates post-mortem increase in myofibril fragmentation index. Moreover, at 1 day post-mortem, in longissimus and semitendinosus muscle, myofibril fragmentation index was higher in muscles stored at 25°C than at 2°C indicating that an accelerated increase in myofibril fragmentation had started even before 1 day post-mortem. In psoas major muscle, however, higher storage temperature (25°C) only slightly increased myofibril fragmentation compared with 2°C storage temperature. Hence, this study is the first to quantitatively show that high post-mortem storage temperature (25°C) accelerates myofibril fragmentation and that myofibers and myofibrils from different muscles do not fragment in a similar manner. High post-mortem storage temperature (25°C) results in lower W-B shear-force from 1 to 3 days post-mortem more than 2°C post-mortem storage temperature and W-B shear-force at 3 days post-mortem, 25°C, was nearly the same as at 10 days post-mortem, 2°C, in longissimus and semitendinosus muscles which agreed with that reported for longissimus and semitendinosus muscles (Parrish et al., 1973b). Again, psoas major muscle reacted differently to
shear-force in that it decreased only slightly more during post-mortem storage at 25°C than at 20°C. These observations further substantiate the view that myofibril fragmentation is an important modifier of meat tenderness.

Although sample numbers were too small in these studies to determine meaningful correlation coefficients between the measures, it is obvious that weakening of the Z-line during post-mortem storage measured by myofibril fragmentation index and fiber tensile strength, is directly related to muscle tenderness. Similar changes in myofibril fragmentation index and W-B shear-force during post-mortem storage at both 20°C and 25°C and consistent differences in these measures between longissimus and semitendinosus muscles and the psoas major muscle practically exclude the possibility of an unrelated coincidence of these results. The accelerated changes in fragmentation and tenderness at 25°C storage temperature seems to imply that a specific and limited proteolysis, more active at higher temperatures, may be causing the weakening of the Z-lines and increased myofibril fragmentation.

SDS-polyacrylamide gel electrophoresis was a most useful tool to characterize the changes in myofibrils of longissimus, semitendinosus and psoas major muscles during postmortem storage at 20°C and 25°C. Through the use of this highly sensitive technique to protein changes, it was shown that the only major alteration occurring in myofibrils of longissimus
and semitendinosus muscles from 1 to 10 days of post-mortem storage at 2°C was the disappearance of troponin-T and appearance of a 30,000-dalton molecular weight protein. This agrees with that found during postmortem storage of chicken breast muscle (Hay et al., 1973a). Alpha-actinin, which is found exclusively in the Z-line (Schollmeyer et al., 1973), does not appear to decrease in intensity during post-mortem storage of longissimus and semitendinosus muscles, even though myofibrils from these muscles appear to increasingly break into shorter segments at the Z-line during postmortem storage. However, even though myofibril fragmentation index increased during postmortem storage, Z-lines did not disappear indicating that the Z-line may be weakened without alpha-actinin being degraded or released from the myofibril. In myofibrils from psoas major muscle, very little change occurs in myofibrillar proteins during postmortem storage at 2°C with only a subtle decrease in troponin-T and a faint appearance of a 30,000-dalton protein at 10 days post-mortem. At 25°C postmortem longissimus and semitendinosus muscle storage, disappearance of troponin-T and appearance of a 30,000-dalton protein are still the only major changes occurring in myofibrils, but these changes occur more quickly post-mortem at 25°C than at 2°C. No major changes occur in myofibrils from psoas major muscles during postmortem storage even at 25°C. Disappearance of troponin-T and appearance of
a 30,000-dalton protein during postmortem storage is a very limited and selective alteration of the myofibril. Because higher storage temperature (25°C) appears to only accelerate these alterations and not produce other alterations, suggests that a very specific protease is responsible for these alterations in the myofibril. Also, because these alterations in the myofibril are much greater in semitendinosus and longissimus muscles than in psoas major muscle, suggests that the alterations are related to postmortem myofibril fragmentation index increase (Z-line weakening and breakage to yield shorten myofibrils) and W-B shear-force decrease. Because the location of troponin is on tropomyosin in thin filaments of the myofibril and not in the Z-line (Ebashi et al., 1968) precludes a conclusion that Z-line weakening is caused by degradation of troponin-T. However, proteolytic degradation of Z-lines may coincide with proteolytic degradation of troponin-T. It may be speculated that bonds holding thin filaments to the Z-line may be cleaved proteolytically without degrading proteins at or in the Z-line. It is also possible that troponin-T may have some stabilizing effect on the thin filament which when degraded weakens the myofibril structure. No evidence, however, is presented in this investigation to substantiate either of these speculations.

Hay et al. (1973a, b) have shown in chicken breast muscle that myofibrils become more fragmented during postmortem
storage, a 30,000-dalton protein appears and an unspecified protein, which appears to be troponin-T, disappears on SDS-polyacrylamide gels during postmortem storage. In chicken leg muscle, however, no apparent increase in myofibril fragmentation, decrease in troponin-T or appearance of a 30,000-dalton protein was found during postmortem storage. While the precise relationship between myofibril fragmentation and alterations in myofibrillar proteins during postmortem storage is not clear, they occur at the same relative time postmortem and to the same relatively high degree in longissimus and semitendinosus muscles but consistently to a lesser degree in psoas major muscle. It appears very likely that both postmortem myofibril fragmentation and myofibrillar protein alteration is caused by the same factor, a protease.

A calcium-activated protease (CAF) has been isolated from skeletal muscle and has been found to selectively remove Z-lines from myofibrils (Busch et al., 1972). These findings led to studies to determine possible roles CAF may have as a causal factor in postmortem changes in muscle. First, CAF was isolated from longissimus, semitendinosus and psoas major muscle at 0, 1, 3 and 6 days post-mortem to determine if an active enzyme fraction existed in postmortem muscle. In longissimus and semitendinosus muscles, similar CAF activity levels were found with the highest activity being found in preparations from at-death muscle, but total activity of CAF
diminished during postmortem storage. This is the first study to show that an active CAF preparation can be made from postmortem bovine muscle. The postmortem decline in CAF activity indicates that it is not released from some cellular organelle, such as lysosomes, during postmortem storage. CAF may also gradually be denatured by low postmortem muscle pH or it may be autolytic and gradually degrade itself. In psoas major muscle, however, at-death CAF activity level was about half of the at-death level in longissimus and semitendinosus muscles, and CAF activity decreased to near zero at 1 day post-mortem. It is unknown why CAF activity is lower in preparations from psoas major muscle than semitendinosus and longissimus muscles, however, it may be related to the different physiological functions these muscles perform. Because an active CAF fraction can be isolated from postmortem muscle, it seems reasonable to conclude that CAF is the protease involved with postmortem muscle tenderization. Furthermore, Dayton et al. (1974a, b) and Suzuki and Goll (1974) have shown that CAF is active in conditions which normally exist in postmortem muscle (i.e. pH 5.5-5.7, 0-4°C, 1mM Ca²⁺). More importantly the postmortem CAF activity results show that CAF activity in longissimus and semitendinosus muscles are high while CAF activity is considerably lower in psoas major muscle. Because differences in CAF activity levels between longissimus and semitendinosus muscles and psoas major
muscle are of the same magnitude as differences in postmortem myofibril fragmentation index, W-B shear-force and myofibrillar protein alteration between these muscles, it is clear that CAF is the likely causal factor affecting these post-mortem muscle characteristics. To test this further, myofibrils from at-death longissimus, semitendinosus and psoas major muscles were briefly treated with CAF to see if Z-lines would be removed and if myofibril fragmentation occurred. Microscopic observation showed that CAF-treated myofibrils from all three muscles had Z-lines removed and were more fragmented than controls but had no other apparent disruption. In addition, SDS-polyacrylamide gels of CAF treated myofibrils from all three muscles showed the absence of alpha-actinin and troponin-T, decreased intensity of troponin-I and the presence of a 30,000-dalton protein. Dayton et al. (1972a, b) have also shown that alpha-actinin is released from myofibrils (porcine muscle) by CAF and also that purified alpha-actinin is not degraded by CAF treatment. Degradation of troponin-T and appearance of a 30,000-dalton protein after CAF treatment closely resembles changes in myofibrils from postmortem longissimus and semitendinosus muscles.

It appeared obvious that the 30,000-dalton protein originated from troponin-T degradation, however, to substantiate this, purified troponin from longissimus muscle was mildly
treated with C&F for 5 to 40 minutes and electrophoresed on SDS-polyacrylamide gels. Troponin-T was quickly degraded to a 30,000-dalton protein and other low molecular weight proteins. Troponin-I was slightly degraded at longer incubation times, but troponin-C appeared to not be degraded. The 30,000-dalton protein, originating from troponin-T degradation, was also eventually degraded so that at the end of the 40 minutes incubation period almost no protein appeared larger than troponin-I. During postmortem muscle storage of longissimus and semitendinosus muscles, gels of myofibrils show the 30,000-dalton protein increases in intensity and alpha-actinin appears unaltered. Thus, effects observed in these myofibrils from post-mortem muscles can result from only very brief and diluted C&F activity, otherwise more extensive degradation in gels of myofibrils would be observed and Z-lines in myofibrils would all be completely removed.

Myofibrils from longissimus muscle at-death and at 10 days post-mortem at 20°C were electrophoresed along with purified troponin and C&F-treated troponin on SDS-polyacrylamide gels. These gels clearly demonstrated that the protein thought to be troponin-T was in fact troponin-T which disappeared from 0 to 10 days post-mortem and that the 30,000-dalton protein appearing at 10 days post-mortem did in fact originate from troponin-T degradation. It is very interesting to note that alterations in myofibrillar proteins
on gels of myofibrils from at-death and at 10 days post-mortem *longissimus* muscle can be almost duplicated by very brief treatment of troponin with C&F. It is clear that C&F, while active in postmortem muscle, probably weakens the Z-line (as evidenced by its ability to remove alpha-actinin from myofibrils) and at the same time degrades troponin-T. Proteolysis by C&F in muscle appears to be very limited and specific and is probably responsible for postmortem changes of the muscle characteristics: myofibril fragmentation index increase, W-B shear-force decrease and troponin-T degradation. The subtle postmortem changes in these characteristics in *psoas major* muscle is probably due to a considerably lower level of C&F activity than that found in *longissimus* and *semitendinosus* muscles.

Myofibrillar protein change, increase in myofibril fragmentation index and decrease in W-B shear-force during post-mortem muscle storage have been suggested to be caused by C&F and have also been shown to be integrally related to the postmortem change in meat tenderness. Because the number of samples and animals in previous experiments were too few to make adequate correlations, an experiment was conducted to determine the relationship between myofibril fragmentation index and meat tenderness and quality (W-B shear-force and sensory tenderness, flavor and juiciness) on *longissimus* muscles of thirty-five A-maturity bovine carcasses. In addi-
tion, six veal and twelve C-maturity carcasses were used in the same experiment to determine effect of animal maturity on postmortem muscle attributes. Means of myofibril fragmentation index were significantly ($P<0.05$) higher at 7 days postmortem than at 1 day post-mortem for all maturity groups. Longissimus muscles from veal carcasses had the lowest myofibril fragmentation index mean and muscles from A-maturity carcasses had the highest means at 1 day post-mortem. At 7 days post-mortem, longissimus muscles from veal carcasses had the lowest myofibril fragmentation index means and muscles from A and C-maturity groups had similarly higher means. Evidently longissimus muscles from immature animals (veal) are more resistant to rupture and breaking during preparation of myofibrils than from mature animals. The explanation for this observation, however, has not been investigated and no reasons can thus be given. Differences in myofibril fragmentation index means of longissimus muscles from A-maturity carcasses between 1 and 7 days post-mortem was about 5-fold greater in previous experiments than in this experiment. Also myofibril fragmentation index means at 1 day post-mortem for A-maturity longissimus muscles in this experiment were closer to means found at 3 days post-mortem in previous experiments. Muscles in this experiment were handled differently than in previous experiments (see METHODS AND MATERIALS section) resulting, evidently, in some fragmentation changes
that had occurred after 1 day post-mortem in previous experiments, occurred before the 1 day post-mortem sample could be measured in this experiment. However, significant (P<0.05) differences in myofibril fragmentation index of muscles between 1 and 7 days post-mortem could be measured. On the other hand, differences in W-B shear-force and sensory tenderness in longissimus muscles from veal, A-maturity and C-maturity carcasses between 1 and 7 days post-mortem were not large enough to be significantly (P<0.05) different. Evidently, some of the postmortem increase in tenderness had occurred before the 1 day post-mortem sample could be evaluated. Myofibril fragmentation index was sensitive enough to detect the differences between 1 and 7 days post-mortem, while W-B shear-force and sensory tenderness could not detect the differences.

Simple correlation coefficients were determined among myofibril fragmentation index, W-B shear-force and sensory tenderness, flavor and juiciness of longissimus muscles of veal, A-maturity and C-maturity carcasses at 1 and 7 days post-mortem at 2°C. Significant correlation coefficients between myofibril fragmentation index and W-B shear-force ranged from -0.65 to -0.97 and between myofibril fragmentation index and sensory tenderness ranged from 0.65 to 0.95 in all three maturity groups and at 1 and 7 days post-mortem. The highest correlation coefficients were found in muscles
from the veal maturity group which may be misleading because only 6 carcasses from this maturity group were used in this experiment. Significant correlation coefficients between myofibril fragmentation index and W-B shear-force ranged from -0.65 to -0.75 and between myofibril fragmentation index and sensory tenderness ranged from 0.65 to 0.75 in the A and C-maturity groups at 1 and 7 days post-mortem. The postmortem increase in tenderness is therefore highly related to the increase in myofibril fragmentation. These results indicate that structural weakening of the myofibril enhances meat tenderness. Because more carcasses were used in the A and C-maturity groups, these correlations are probably closer to the true correlations of the population. Additionally, immature animals (veal) may not reflect the same conditions existing in the population of mature carcasses. These results closely agree with Moiler et al. (1973) who found, using a similar method of measuring myofibril fragmentation as was used in this investigation, a correlation coefficient of -0.78 between myofibril fragmentation and W-B shear-force of longissimus muscles from A-maturity bovine carcasses at 7 days post-mortem at 20°C. Correlation coefficients between W-B shear-force and sensory tenderness ranged from -0.48 to -0.94 in the three maturity groups at 1 and 7 days and these correlations were all significant except in the veal at 1 day post-mortem and C-maturity group at 7 days post-mortem.
These results clearly show that myofibrils isolated from postmortem muscle and determined as a fragmentation index is a good indicator of cooked muscle tenderness. Obviously, myofibril fragmentation index is one of the most highly correlated measures on raw muscle attributes that indicates cooked meat tenderness and it accounts for approximately 45% of the tenderness of meat broiled to 65°C internally. Evidently, the changes in the myofibril during postmortem muscle storage is not greatly altered during heating and enhances the tenderness of cooked meat.

Myofibrils from longissimus muscles of veal, A-maturity and C-maturity carcasses at 1 and 7 days post-mortem at 2°C were selected on the basis of extremes in myofibril fragmentation index, W-B shear-force and sensory tenderness of the muscles and electrophoresed on SDS-polyacrylamide gels. Gels of myofibrils from all three maturity groups at 1 and 7 days post-mortem show a general relationship between high muscle tenderness (high myofibril fragmentation index and sensory tenderness and low W-B shear-force) and the degradation of myofibrillar proteins (absence of troponin-T and appearance of 30,000-dalton protein). Conversely, less tender muscles showed less myofibril degradation. Comparisons between maturity groups in muscle tenderness and myofibril degradation do not show similar changes in magnitude in that veal myofibrils show as much myofibril degradation in the gels at 7
days post-mortem as shown in gels of A and C-maturity myofibrils even though veal muscles are less tender. Immature muscle (veal), however, may impart influences on muscle tenderness different from mature muscle (A and C-maturity).

Clearly, myofibril degradation is integrally related to muscle tenderness. As previously discussed, CAF activity in postmortem muscle appears to be responsible for degradative changes in myofibrils which apparently result in increased muscle tenderness during postmortem storage. Penny et al. (1974) has shown that freeze-dried steaks, rehydrated with a CAF-containing solution was considerably more tender than controls and that this tenderness increase was due to a structural weakening of the myofibril at or near the Z-line. It thus follows that CAF is the probable factor in muscle responsible for the postmortem degradation of myofibrils and increased meat tenderness.
SUMMARY

Measurements to detect postmortem physical and chemical events have been made on bovine longissimus, semitendinosus and psoas major muscles during postmortem storage at 20°C and 25°C. Myofibril fragmentation from these muscles was determined by a method that measured the absorbance of a myofibril suspension. This method utilized a larger and more representative sample size and afforded greater objectivity than could have resulted in a microscopic method of myofibril fragmentation determination. Myofibril fragmentation index (turbidimetric myofibril fragmentation measure) closely agreed to microscopically observed changes in myofibril fragmentation. Myofibril fragmentation index greatly increased from 1 to 10 days post-mortem at 20°C in longissimus and semitendinosus muscles but only slightly increased in psoas major muscle. Z-lines in myofibrils remained intact during post-mortem storage in all three muscles. W-B shear-force greatly decreased from 1 to 10 days post-mortem at 20°C in longissimus and semitendinosus muscles but only slightly decreased in psoas major muscles. Postmortem storage at 25°C resulted in accelerated changes in myofibril fragmentation index and W-B shear-force in longissimus and semitendinosus muscles but these changes were only slightly increased in psoas major muscle compared to 20°C postmortem storage. Myofibril Z-line weakening during postmortem storage, indicated by myofibril
fragmentation index, was greatest in longissimus and semitendinosus muscles and least in psoas major muscle and appeared to be associated with the postmortem decrease in W-B shear-force of these muscles.

Myofibrils from muscles stored at 20°C and 25°C for various time periods were electrophoresed on SDS-polyacrylamide gels to determine postmortem changes in myofibrillar proteins. Postmortem storage causes a very limited and specific change in the myofibrillar proteins. Troponin-T is degraded to a 30,000-dalton protein but all other myofibrillar proteins appear unaffected by post-mortem storage in longissimus and semitendinosus muscles. Only a very slight degradation of troponin-T occurs at 10 days postmortem in psoas major muscles. The greatest postmortem changes in myofibril fragmentation index, W-B shear-force and troponin-T degradation occur in longissimus and semitendinosus while the least changes occur in psoas major muscle. The higher storage temperature (25°C) causes troponin-T degradation to occur sooner post-mortem but does not cause other degradative changes in the myofibril in longissimus and semitendinosus muscles. Higher storage temperature (25°C) has little effect on myofibrils of psoas major muscle.

Active CAP (calcium-activated muscle protease) fractions were found in muscles at 0, 1, 3 and 6 days post-mortem. CAP fractions in longissimus and semitendinosus muscle were simi-
larly active but were considerably higher at-death and at all postmortem periods than in \textit{psoas major} muscle. At-death myofibrils treated with CAF had fewer Z-lines and greater fragmentation than controls. SDS-polyacrylamide gels of CAF-treated myofibrils showed the absence of alpha-actinin, degradation of troponin-T to a 30,000-dalton protein and decreased intensity of troponin-I. Absence of alpha-actinin in gels corresponded to Z-line removal in myofibrils. Degradation of troponin-T by CAF is the same as degradation of troponin-T by post-mortem storage. Because CAF affects the Z-line, causes the same degradation of troponin-T as occurs in postmortem muscle and has considerably higher activity in \textit{longissimus} and \textit{semitendinosus} muscles than in \textit{psoas major} muscle, it is likely that CAF is directly responsible for the postmortem increase in myofibril fragmentation index in \textit{longissimus} and \textit{semitendinosus} muscles.

\textit{Longissimus} muscles from veal, A-maturity and C-maturity carcasses were investigated for myofibril fragmentation index, W-B shear-force and sensory tenderness, flavor and juiciness at 1 and 7 days post-mortem at 20°C. Significant correlation coefficients between myofibril fragmentation index and W-B shear-force were found to be for muscles in veal carcasses at 1 day post-mortem -0.95 and at 7 days post-mortem -0.97, in A-maturity carcasses at 1 day post-mortem -0.65 and at 7 days post-mortem -0.75 and in C-maturity carcasses at 1
day post-mortem -0.68 and at 7 days post-mortem -0.72. Significant correlation coefficients between myofibril fragmentation index and sensory tenderness were found to be for muscles in veal carcasses at 1 day post-mortem 0.88 and at 7 days post-mortem 0.95, in A-maturity carcasses at 1 day post-mortem 0.67 and at 7 days post-mortem 0.73 and in C-maturity carcasses at 1 day post-mortem 0.68 and at 7 days post-mortem 0.65. Myofibril fragmentation index is one of the highest correlated measures of raw muscle found with cooked meat tenderness. Myofibrils from selected longissimus muscles from veal, A-maturity and C-maturity carcasses were electrophoresed on SDS-polyacrylamide gels. Within each maturity group, high myofibril fragmentation index and muscle tenderness accompanied troponin-T degradation while low index and tenderness values accompanied no troponin-T degradation. Myofibrils from muscles in immature (veal) animals showed similar myofibril degradation as in the mature animal groups, but myofibril fragmentation index and muscle tenderness was lower in the immature (veal) animals.

The increase in muscle tenderness during postmortem storage has been shown to be highly correlated with myofibril fragmentation index. As discussed previously, proteolytic CAF activity is probably responsible for postmortem increase in myofibril fragmentation index. Since postmortem increase in myofibril fragmentation index is highly correlated to
postmortem increase in muscle tenderness, it is also likely that CAP is responsible for postmortem muscle tenderization.
CONCLUSIONS

1. A measure of a myofibril suspension by light absorbance (myofibril fragmentation index) was found to closely parallel the degree of fragmentation of myofibrils observed in the light microscope.

2. Myofibril fragmentation index is muscle and storage temperature dependent. Myofibril fragmentation index increased considerably during postmortem storage at 20°C and 25°C in longissimus and semitendinosus muscles with the higher storage temperature (25°C) resulting in a greater increase in myofibril fragmentation index. Only a slight increase in myofibril fragmentation index during postmortem storage at either 20°C or 25°C occurred in psoas major muscle.

3. W-B shear-force is muscle and storage temperature dependent. W-B shear-force was highest in semitendinosus muscle, lowest in psoas major muscle and intermediate in longissimus muscle. W-B shear-force decreased considerably during postmortem storage at 20°C and 25°C in longissimus and semitendinosus muscles with the higher storage temperature (25°C) resulting in a greater decrease in W-B shear-force. Only a slight decrease in W-B shear-force during postmortem storage at either 20°C or 25°C occurred in psoas major muscle.

4. A very limited and specific proteolytic degradation of
myofibrillar proteins occurred during postmortem muscle storage at 2°C and 25°C. Disappearance of troponin-T and appearance of a 30,000-dalton molecular weight protein were the only major changes in myofibrils from longissimus and semitendinosus muscles during postmortem storage at 2°C and 25°C with the higher storage temperature (25°C) resulting in more rapid changes in the myofibrils. In the psoas major muscle, a 30,000-dalton protein did not appear and troponin-T appeared to not be degraded, although troponin-T was not clearly separated, during postmortem storage at 2°C or 25°C.

5. CAF activity is muscle dependent and decreases with postmortem time. Similar CAF activity was found in at-death longissimus and semitendinosus muscles, but less than half of that CAF activity was found in at-death psoas major muscle. CAF activity in longissimus and semitendinosus muscles decreased to less than half of at-death level at 6 days post-mortem, but CAF activity in psoas major muscle decreased to near zero level at 1 day post-mortem and stayed at that level.

6. Myofibrils after mild treatment with CAF, had Z-lines removed and greater fragmentation than control myofibrils. SDS-polyacrylamide gels of CAF-treated myofibrils showed alpha-actinin and troponin-T removed, troponin-I slightly degraded and a 30,000-dalton protein appeared.
7. Purified troponin from bovine longissimus muscle and treated with CAF showed on SDS-polyacrylamide gels rapid degradation of troponin-T yielding a 30,000-dalton protein which was also degraded by CAO after longer incubation and slight degradation of troponin-I after prolonged incubation.

8. Longissimus and semitendinosus muscles were shown to have similar myofibril fragmentation index, myofibrillar protein degradation and CAO activity levels at all postmortem times. W-B shear-force values were higher in semitendinosus muscle than in longissimus muscle, however, both showed similar changes in W-B shear-force during postmortem storage. Lower myofibril fragmentation index, W-B shear-force, extent of myofibrillar protein degradation and CAO activity level were found in psoas major muscle than found in longissimus and semitendinosus muscles and only a slight change in these measures occurred during postmortem storage in psoas major muscle.

9. Correlation coefficients between myofibril fragmentation index and W-B shear-force ranged from -0.65 to -0.97 and between myofibril fragmentation index and sensory tenderness ranged from 0.63 to 0.94 for longissimus muscles from veal, A-maturity and C-maturity carcasses at 1 and 7 days post-mortem.

10. Greater degradation of troponin-T in myofibrils from lon-
gissimus muscles which were more tender and had greater myofibril fragmentation index was found in selected veal, A-maturity and C-maturity carcasses.

11. Longissimus muscles from mature (A-maturity and C-maturity) and immature (veal) carcasses showed similar myofibril degradation and changes in myofibril fragmentation index during postmortem storage but lower means in W-B shear-force, sensory tenderness and myofibril fragmentation index were found in the immature (veal) carcasses.


ACKNOWLEDGEMENTS

I express my appreciation to Dr. F. C. Parrish, Jr. for serving as my major professor and for assisting and encouraging me during the preparation of this dissertation. I would also like to thank Dr. D. E. Goll, Dr. R. N. Robson, Dr. M. H. Stromer and Mr. John C. Olson for taking an interest in my research program.

A special thank you to B. E. Rust, D. P. Cox and M. Walker for serving on my graduate committee. Appreciation is also extended to Mrs. Sherri Josephson, Dr. Randy Snell, Mr. Doug De Vries, Miss Pat Chen and Mrs. Ruth Smith for their invaluable technical assistance during my research program and also to Mrs. Mary Stimmel for typing this dissertation. I would also like to express my appreciation to my fellow graduate students for their interest and encouragement and especially to William R. Dayton and Ronald E. Allen whose many discussions have immeasurably helped my research planning, execution and interpretation.

Special appreciation is extended to my parents Mr. and Mrs. Johan C. Olson and parents-in-law Mr. and Mrs. F. M. Wooster for their continual encouragement, moral support and financial assistance throughout my graduate career.
Finally and most importantly my deepest appreciation is extended to my wife, Jane, for her patience, encouragement and infallible support and unflattering belief in me during my graduate career.