THE RELATIONSHIP OF ULTRASTRUCTURAL AND BIOCHEMICAL PARAMETERS TO TENDERNESS IN YOUNG BULLS

Dissertation for the Degree of Ph. D.

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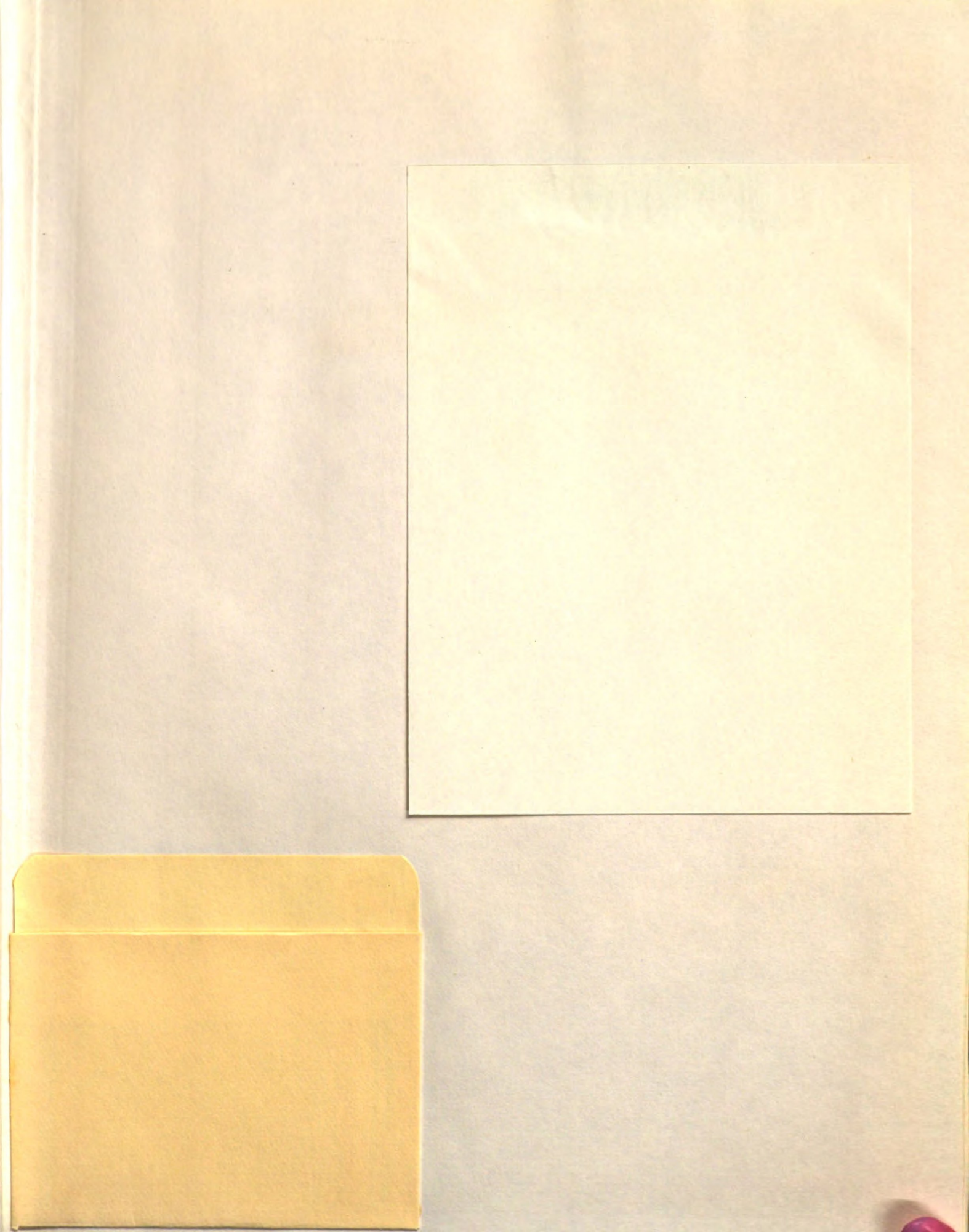

THE RELATIONSHIP OF [illegible]

PARAMETERS TO [illegible]

Two genetic lines of [illegible] selected for tenderness [illegible] were used to study the [illegible] muscle ultrastructure, [illegible] superprecipitation of [illegible] removed from the 12th rib [illegible] were prepared for electron [illegible] frozen in liquid N and [illegible] and taste panel data were [illegible] Sarcomere length was measured [illegible] ITPase and superprecipitation [illegible] toughest samples.

Two groups of fibers, type I [illegible] Z-line width and density and mitochondria [illegible] differ in susceptibility to postmortem Z-line degradation. Type I fibers did not lose Z-line material during postmortem aging, whereas type II fibers consistently lost Z-material in all samples although the amount lost varied between fibers. Some Z-line degradation was apparent at 1 hr. postmortem, however, most of the Z-line degradation occurred between 1 and 48 hr. postmortem. Little additional degradation occurred between 48 and 216 hr. postmortem. Tender and control sample type II fibers were equally susceptible to Z-line degradation during all postmortem aging intervals measured. More fibril fragmentation at the I-Z junction occurred only in the 48 and 216 hr.

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ABSTRACT

THE RELATIONSHIP OF ULTRASTRUCTURAL AND BIOCHEMICAL PARAMETERS TO TENDERNESS IN YOUNG BULLS

by Gary Lee Gann

Two genetic lines of Hereford bulls, consisting of 16 animals, 1 selected for tenderness and the other an unselected tenderness control line, were used to study the effects of postmortem aging on sarcomere length, muscle ultrastructure, protein solubility, ATPase and ITPase activity and superprecipitation of longissimus muscle. Longissimus muscle samples were removed from the 12th rib area 1, 48 and 216 hr. postmortem. Samples were prepared for electron microscopy with the remainder of the sample being frozen in liquid N and powdered in a Waring Blendor. Warner-Bratzler shear and taste panel data were obtained from the 216 hr. postmortem samples. Sarcomere length was measured on the 48 and 216 hr. samples and the ATPase, ITPase and superprecipitation assays were run on the 5 most tender and 5 toughest samples.

Two groups of fibers, type I and type II, classified according to Z-line width and density and mitochondrial location and number, were found to differ in susceptibility to postmortem Z-line degradation. Type I fibers did not lose Z-line material during postmortem aging, whereas type II fibers consistently lost Z-material in all samples although the amount lost varied between fibers. Some Z-line degradation was apparent at 1 hr. postmortem, however, most of the Z-line degradation occurred between 1 and 48 hr. postmortem. Little additional degradation occurred between 48 and 216 hr. postmortem. Tender and control sample type II fibers were equally susceptible to Z-line degradation during all postmortem aging intervals measured. Myofibril fragmentation at the I-Z junction occurred only in the 48 and 216 hr.

postmortem samples. The amount of myofibril fragmentation at 48 hr. postmortem was limited to a few isolated sarcomeres, however, at 216 hr. postmortem, the entire fibers of several samples, were found to be transversely broken. Type I and Type II fibers appeared to be equally susceptible to fragmentation at the I-Z junction. The most tender sample (Warner-Bratzler shear) had more fiber breakage than the toughest sample, although, among all samples, fragmentation and shear score were not highly related.

There were no statistically significant differences between the 48 and 216 hr. postmortem sarcomere lengths nor were there sarcomere length differences between tender and control line samples. The amount of myofibrillar N extracted by KCL at 48 hr. postmortem was significantly ($P < .05$) correlated with 48 hr. sarcomere length, however, no significant correlations were found between 216 hr. KCL or KI extracted myofibrillar N and 216 hr. sarcomere length. Sarcomere length at 216 hr. postmortem was significantly and negatively ($P < .05$) correlated with Warner-Bratzler shear but not with taste panel score.

Sarcoplasmic N increased significantly ($P < .05$) between 1 and 48 hr. postmortem and then decreased to the 1 hr. postmortem level. There were no significant sarcoplasmic N differences between control and tender lines. Shear and taste panel data were not significantly correlated with sarcoplasmic N at any postmortem time interval measured. No significant difference was obtained for 1.1M KI extracted myofibrillar N among the 3 postmortem time periods or between tender and control lines. Myofibrillar N extracted by 1.1M KCL at 216 hr. postmortem was significantly ($P < .05$) greater than that at 1 hr. postmortem, but not from that at 48 hr. postmortem.

No significant differences were obtained between tender and control lines. The KCL extracted myofibrillar N was significantly ($P < .05$) correlated with 1 hr. muscle temperature at all postmortem time intervals measured. There were no significant alterations in NPN as the result of postmortem aging, however, the tender line NPN at 216 hr. postmortem was significantly ($P < .05$) greater than all but the 1 hr. mean of the tender samples. NPN at 216 hr. postmortem was significantly ($P < .05$) negatively correlated with taste panel score, but only approached significance with Warner-Bratzler shear. Stroma N was not significantly different among the 3 postmortem time intervals on control or tender lines determined after extraction of the myofibrillar N by 1.1M KI. However, the 1 hr. postmortem KCL determined stroma N was statistically different ($P < .05$) from the 48 hr. sample but not the 216 hr. postmortem sample. In the present study, it was concluded that protein solubility was not an adequate criterion for categorizing bovine longissimus muscle samples into tough or tender groups.

Ca^{2+}-, Mg^{2+}-, EDTA- and EGTA-modified ATPase activities did not change significantly during the postmortem time intervals measured or between tender and control samples. Ca^{2+}-modified ITPase activity decreased between 1 and 48 hr. postmortem, however, Mg^{2+}-modified ITPase activity changed very little among the 3 postmortem time intervals measured. No significant differences were observed between tender and control line samples at any postmortem time interval measured for the ITPase activities.

The superprecipitation assay (Mg^{2+} + EGTA) of the most tender and toughest samples showed that the 1 hr. postmortem samples had the fastest

rate of turbidity onset. The tender sample low Ca^{2+} (0.05 mM; 100 mM KCL) superprecipitation assay had the fastest rate of turbidity onset as compared to the tough sample, however, in both tender and tough samples the 1 hr. postmortem sample had the fastest rate of turbidity onset.

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By

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INTRODUCTION

As society becomes increasingly more affluent, the consumer not only insists that adequate quantity of foodstuffs be available but also that these foodstuffs be of a consistent and predictable quality. With such a consumer directance the animal scientist has had to become increasingly more sophisticated not only in techniques, but also in innovativeness. Not only has affluence given rise to a discriminating consumer, it has also fueled the arrival of competitive products that are offered and will, in the future, increase both the quality and quantity of alternatives available to the consumer. Economic inflation pressures even the strongest willed consumer to use some of the many substitutes for animal products and, as such, the animal scientist must be continually improving the economic and qualitative position of his products.

In addition to the scruntiny of the consumer, the animal scientist must be cognizant of the restrictions placed on his manipulative procedures by governmental and consumer protective agencies. The questionable status of diethylstilbestrol and sodium nitrite is indicative of the impact that the "potential carcinogen" label can impart to any agent used in the production system. The animal scientist must have foresight and the ability to economically manipulate the animal system within regulatory restraints.

Tenderness has been at the forefront of meat investigations, based on its importance, for many years. The beneficial effects of aging on meat tenderness have been recognized for many years, however, the specific

mechanism by which aging affects tenderness has been elusive. In contemporary marketing methods, the meat packers must efficiently utilize facilities which has prevented the majority of carcasses from receiving adequate storage time to achieve maximum tenderization. Although aging of carcasses has not been a panacea for all tenderness deficiencies, it is sufficient for the animal scientist to search for the components regulating postmortem tenderness changes. Considerable progress has been made on the effects of temperature, pH and histological modifications, however, the animal scientists are still searching for answers to most of the questions.

With these ideas in mind, this project was designed to measure ultrastructural and biochemical parameters of postmortem bovine longissimus muscle. The animals used in this project were young Hereford bulls that had been selected for tenderness or leanness for several generations. Most postmortem studies involve study of biochemical and histological parameters, however, few of these studies have attempted to relate the postmortem observations to tenderness data. This study was designed to not only observe postmortem changes, but to determine if these changes were related to objective and subjective tenderness scores.

REVIEW OF LITERATURE

Tenderness Studies Prior to 1960

For thousands of years the primary concern of man in the handling of meat has been its storage and preservation and only within the last century with the advent of mechanical refrigeration has he been able to preserve meat in the fresh state during all seasons of the year. He has subsequently became concerned with the palatability aspects of fresh meat. With this concern, meat scientists began to investigate the properties of postmortem muscle in an effort to control and manipulate those factors deemed important to the consumer.

Of the several palatability characteristics of meat, tenderness has generally been considered to be the single most important component. With the importance placed on this component, considerable work has been expended on determining the factors which are not only responsible for the inherent tenderness of a muscle, but also in what way these factors promote increases in tenderization during postmortem aging or ripening of meat.

It is not clear when the first ideas concerning tenderization were formulated, however, when histologists first identified the components of a muscle, it became apparent that the connective tissue proteins and the proteins of the muscle fibers would certainly play an important role based solely on their dominance within the system. The early investigators in the field were rather equally divided in belief as to whether the connective tissue proteins (primarily collagen) or the muscle fiber proteins

underwent changes during aging that would account for the tenderization observed.

The first observations that meat hanging for several days improved in tenderness apparently has been lost in antiquity, however, Hoppe-Seyler (1871) made the general observation that dead tissues liquefied without accompanying putrefaction which prompted him to suggest a similarity to digestive fermentation. Whether this observation was the spark that caused many to subscribe to the belief that autolysis was the mechanism of postmortem tenderization is unknown, however, the concept developed a following among many eminent scientists.

Lehman (1907) and associates concluded that the toughness of meat, determined by mechanical measures, was closely related to the connective tissue content. Hoagland, McBryde and Powick (1917) observed that the primary change during postmortem aging was a marked increase in tenderness. This same group concluded that the chemical changes occurring during storage, including the increase in non-protein nitrogen, could be attributed to enzyme activity. Apparently these changes did not manifest themselves microscopically even after 77 days of storage.

Mitchell, Zimmerman and Hamilton (1927) chemically determined the amount of connective tissue in various cuts of meat, but due to low sensitivity of the technique the results were inconclusive. However, they were able to observe relative differences such as that observed between the wholesale shank and the rib of cattle. It was observations such as this that prompted and encouraged some scientists to believe that the

connective tissue proteins were the primary components of tenderness. It was readily ascertained that mere quantity was sufficient to cause differences between anatomical location of muscles and a direct extrapolation to explain the tenderness between the same muscle from two different animals was considered to be a valid inference. Mitchell et al. (1927) didn't make this direct extrapolation, however, they attributed collagen and elastin differences between similar wholesale cuts to condition differences among animals. In a subsequent paper, Mitchell, Hamilton and Haines (1928) correlated collagen and elastin nitrogen in various muscles of the beef carcass with such parameters as age, sex, grade, texture and firmness of steers and heifers fed from 0 to 266 days. A ranking of the various muscles of the carcass was possible utilizing collagen and elastin nitrogen, however, no significant correlations were obtained with age, grade, sex, texture or firmness. While maintaining that connective tissue content was the major factor in meat tenderness, these authors concluded that the parameters used were not a reliable method to determine tenderness of lean meat. The concept of connective tissue quantity dictating tenderness was not universally accepted. Some scientists believed that the aging period was responsible for chemical changes in stroma proteins (connective tissue proteins) that resulted in increased tenderness. Ewell (1940) reported that some German scientists believed that coarse textured (high in connective tissue) cuts of meat which showed a greater relative increase in tenderness than cuts initially more tender supported the concept that postmortem aging altered connective tissue proteins.

Moran and Smith (1929) reported on the postmortem changes in animal tissues and in particular those changes occurring during aging of meat. They remarked that the general consensus among the butcher trade was that hanging meat for a length of time improved the palatability aspects particularly tenderness. In a consideration of the various components of tenderness, the authors reported that muscle fibers, connective tissue and fat affected tenderness. They suggested that much of the tenderness variation between muscles in different anatomical locations could be accounted for by their function. Moran and Smith (1929) reported that unpublished data by Hammond suggested that the quantity of connective tissue might not be the only factor influencing tenderness. The tenderness increase observed upon aging was attributed to changes in both muscle fiber proteins and connective tissue proteins. Moran and Smith (1929) concluded that the lactic acid produced postmortem hydrolyzed the muscle proteins and as aging time proceeded the intrinsic ferments reinforced the hydrolysis by lactic acid. After several days of hanging, the postrigor changes were proposed to be due to softening or swelling of the collagen.

The persistence of the autolysis concept was apparent when Tressler and Murray (1932) reported on the time necessary to effectively age meat. They stated that the tenderizing action observed was brought about by enzymes contained within the tissues themselves.

Warner and Alexander (1932) investigated the palatability changes in lamb legs during varying postmortem storage periods. Utilizing both trained judges and a mechanical shear device, they observed considerable

tenderness increases as the storage period was increased from 0 through 25 days. The most significant observation was that many unaged legs were tender, whereas other fully aged legs were tough. This observation was not clarified or discussed, but the mere fact that aging affected different animals or cuts in a variable manner suggested that the phenomenon probably was influenced by more than one variable.

Ewell (1940) summarized most of the tenderness research work conducted prior to 1940 and observed that a dichotomy of concepts existed between the English at Cambridge and the German group at the Refrigeration Institute. The Cambridge group discounted the autolysis theory, however, they were relatively united behind the belief that during aging collagen was converted to gelatin. The German group subscribed to the concept that the fibers themselves were altered sufficiently to account for the observed ripening. Sadikow and Shoskin (1936) investigated the effect of autolysis on meat proteins. They used several different aging temperatures, i.e., 17, 37 and 55 to 60 C, and toluene as a bacteriostat, and concluded that aging appeared not to be autolytic but apparently something analogous to enzyme stabilization.

Ewell (1940) measured the temperature coefficient (Q10) of the tenderizing process and found that from 0 C to 15.6 C the rate of tenderization increased. He also found that the rate of tenderization increased with temperature elevation, in other words, approximately the same amount of tenderization occurred in 1 to 3 days at elevated temperatures as that which could be obtained in 3 weeks at 1.1 to 2.8 centigrade. Unfortunately, they found that spoilage was a problem at high temperatures. Griswold and

Wharton (1942) utilized similar temperature ranges, however, they coupled high temperatures with ultraviolet light to attenuate microorganism proliferation. Equivocal results were obtained, however, a nonsignificant trend was observed favoring the higher temperatures.

Numerous studies had been made on tenderness up to the early 1940's, however, little emphasis was placed on histological changes possibly due to the fact that Hoagland et al. (1917) reported no changes during aging. Pennington et al. (1917) probably reported one of the earliest studies that involved histological observations on poultry muscle. These authors stated that the muscle cells ruptured which apparently allowed the cell contents to spill out. These cell ruptures followed a time and temperature dependent pattern although it occurred at all temperatures.

Hanson, Stewart and Lowe (1942) reported a comprehensive study on the effect of length of time of storage at 1.7 C on the palatability and histological characteristics of chicken breast and thigh muscles. Each of the muscles studied underwent similar postmortem changes with variance only in the time necessary to achieve the ultimate change. Initially, following rigor, a thinning of cell contents occurred at various points followed by breaks in the muscle fibers. They were not certain that the sarcolemma had been broken, but it appeared that the cell contents had exuded at the breaks in the fibers. Further storage seemed to increase the quantity of breaks and fiber disintegration which they suggested could be due to autolysis. The histological changes were evident in both stored and cooked and stored and uncooked muscles. The primary difference between cooked and uncooked muscle was the time of appearance of the breaks and

disintegrations and the amount of each observed. They surmised that the differences between the cooked and uncooked muscles in the early storage periods could be that the uncooked muscle might be in an incipient stage of breakage which was precipitated as the result of heat treatment. The breast muscle was more tender than the thigh muscle and apparently tenderness was correlated with the quantity of breaks present in the fibers. Additionally, the thigh muscles not only had fewer breaks but needed more time for the development of these breaks. Hanson et al. (1942) observed no particular area of the fiber that was more susceptible to breakage since the breaks were jagged and many times seemed to break obliquely to the longitudinal axis of the fiber. In summary, these authors agreed that postmortem breast and thigh muscles reacted somewhat similarly differing only in time scale and degree of development. Paul, Lowe and McClung (1944) roasted several paired bovine muscles from the round and loin after 0 to 18 days storage at 1.7 centigrade. Histological changes observed during aging varied from poorly differentiated and slightly wavy fibers at death to rigor nodes and increased kinkiness in rigor samples. Many of the kinks disappeared after 4 to 9 days of storage, however, the characteristic effect of aging was the appearance of muscle fiber breaks which became visible during the second day of storage. Longer periods of storage increased the incidence of these breaks. Two types of breaks were observed; one was described as a sharp fracture and the other described as a disintegration of the muscle protoplasm resulting in the loss of both longitudinal and cross striations. Paul et al. (1944) were uncertain as to the reason for the breaks but hypothesized that

autolysis or possibly stresses induced into the system by contraction of the muscle fibers or connective tissues during rigor development. In a subsequent paper, Paul (1963) reiterated the fact that fiber breakage occurred during aging, and in the latter report she said that they frequently appeared at the angles of the Z to Z contraction in passively retracted fibers. Heating appeared to increase the number of breaks in the muscle fibers and apparently the amount of breakage increased with cooking time. Paul (1965) subsequently concluded that rabbit muscle held in cold storage at 5 C reacted similar to bovine muscle except for being more rapid in manifestation of fiber breakage. Although most work has been reported on unfrozen muscle, Hiner, Madsen and Hankins (1945) used several freezing temperatures to determine the effect of low temperatures on shear values. They found that as temperature of freezing decreased shear values also decreased. Histologically, the lower shear values seemed to be associated with fiber splitting and breaking and stretching of the connective tissue associated with muscle fibers and fiber bundles.

Deatherage and Harsham (1947) used loins from U.S.D.A. Good and Commercial grade animals to determine the effect of aging on the palatability aspects of meat. The loins were removed after rigor and put into a cooler at 0.5 to 1.8 C for 7 time periods varying from 2 through 38 days. A summary of the taste panel data revealed that tenderness improved a variable amount from 2 through 31 days and in most cases 2 1/2 weeks was an adequate aging period. They concluded that since tenderness did not follow a linear pattern with aging time it appeared that more than one factor or structure was involved in tenderization.

Ramsbottom and Strandine (1949) used the bovine longissimus muscle to determine what chemical and physical changes could be correlated with tenderness. The sampling times were 2 through 14 hrs. and 1 through 12 days postmortem. The histological observations showed that muscle fibers varied from straight to slightly wavy, during the first few hours or pre-rigor, to the appearance of lumps in rigor muscle which was described as having a washboard appearance. This particular condition persisted for 24 to 72 hours postmortem and was associated with high shear values. Following the cessation of rigor, they described a period during which the muscle began to soften and relax and associated with this condition was a general loss of the waviness or washboard appearance. In some cases, the waviness persisted and failed to subside even after 12 days of storage. Shear values decreased postrigor in association with the loss of waviness and after 8 to 12 days of aging the fibers showed evidence of disintegration which was attributed to enzyme action or autolysis. Microscopically the fibers appeared to break both longitudinally and transversely with the connective tissue, including the endomysium. Accordingly, these individuals associated lower shear values with the distintegration of the muscle fibers and connective tissues.

Bate-Smith (1948), in a classical paper, reviewed all the available information on postmortem aging of muscle. He reported that no one had been able to unequivocably establish, if existent, the one component responsible for the tenderness change observed during aging of meat. Much additional information and considerable sophistication had occurred since Hoagland et al. (1917) published their observations on postmortem aging

of muscle. Bate-Smith (1948) reported that mechanical devices for objectively measuring tenderness of meat, in particular the Volodkewich (1938) machine, had contributed substantially to the knowledge of tenderness. Steiner (1939), as reported by Bate-Smith (1948), used the Volodkewich machine to follow aging induced tenderness changes. After measuring the area under the curve produced by the machine, Steiner (1939) concluded that the connective tissue proteins and muscle fiber proteins both contributed to the resistance when measured across the fiber grain. However, Steiner (1939) could find only connective tissue contribution when measurements were made with the grain. Steiner's work suggested and was the basis for support of the theory that the muscle fibers are the only component that is being modified during aging.

Harrison et al. (1949) using roasts from 4 beef animals, of differing ages which were aged for 1 to 30 days, concluded that the greatest increase in tenderness occurred during the first 10 days of storage. Some animal variation was noted in aged meat and although tenderness increased with storage not all muscles showed tenderness to be directly proportional to time of storage. Histological observations of muscle during the different storage periods revealed progressive changes that were generally associated with the time of storage. These authors noted that as the time of aging progressed beyond 2 days the fibers began to lose the kinks, twists and waves that were prominent in rigor muscle. The most prominent histological observation present in all aged samples was areas of disintegration; one cause being described as increased striation fragility and the other was the loss of longitudinal and cross striations over an

extensive area. The area of disintegration was prominent in all muscles except for the psoas major and young and old animals possessed similar organoleptic and histological characteristics except the older animals required more aging time for expression of these changes. These data generally support the earlier report by Paul et al. (1944).

Strandine, Koonz and Ramsbottom (1949) studied the histological variations in postmortem bovine and chicken muscle and generally found that muscles which contained the most connective tissue had higher shear values and lower organoleptic scores. They also found that muscles containing distinct and prominent muscle bundles received lower organoleptic ratings. Utilizing previous cooking data, Strandine et al. (1949) found that muscle heated quickly to 65.5 C was less tender than other muscles even though the connective tissue had been gelatinized. This indicated to them that other components were involved in meat tenderness.

Husaini et al. (1950a) utilized twenty carcasses of considerable variation in grade, age and sex to determine whether any carcass parameter could be correlated with tenderness scores. They used a 14 day storage period since earlier work suggested that tenderness reached a plateau at this point. Their results implied a close relationship between alkali-insoluble proteins (connective tissue) and tenderness. In an additional study, Husaini, Deatherage and Kunkle (1950b) used shortloins from Hereford, Shorthorn and Holstein cattle aged either 3 or 15 days. Tenderness improvement was noted in all aging periods, however, no relationship was found between 3 day tenderness values and alkali-insoluble protein. A small but insignificant relationship was found between alkali-insoluble protein and 15 day aged samples.

In maintaining the philosophy of the Cambridge group, Callow (1949) discussed the particular merits of the microstructure of muscle in relation to drip and tenderness. He remarked that tenderness was more dependent on the amount of connective tissue present rather than the compactness of the muscle fibers and also that during the aging of meat the lactic acid and phosphates converted the connective tissue to gelatin.

Hiner et al. (1953) in reviewing the literature relevant to meat texture reported that several groups had observed that muscle fibers and muscle bundles are in some way associated with tenderness. In the same article, Hiner et al. (1953) determined the fiber diameter for 9 different muscles from 52 veal and beef cattle varying in age from 10 weeks to 9 years. The carcasses were allowed to age 14 days at 0.6 to 1.7 centigrade. The data indicated that as the diameter of the fiber increased so did shear values.

Beginning in the early 1940's the muscle protein system was being systematically studied biochemically and a better description of the system was becoming available. Wierbicki et al. (1954) used this increased knowledge to investigate the changes in muscle plasma proteins (myofibrillar) during postmortem storage. At the same time, these individuals measured stroma proteins and concluded that since no solubility changes occurred or no changes in hydroxyproline could be documented and since tenderness did increase, it was safe to conclude that connective tissue was not a major component of tenderization. By utilizing a buffer system designed to extract only actin and myosin and not actomyosin, these authors found that tenderness was highly correlated with the nitrogen extracted.

Wierbicki et al. (1954) speculated that the muscle plasma proteins were intimately related to tenderness in a yet undetermined manner. Based on previous observations, they suggested, as had been already reported, that prerigor muscle was tender when cooked (Ramsbottom et al., 1949) and that the formation of actomyosin was associated with toughness. Subsequently they suggested that the dissociation of the actomyosin complex must be of major importance in postmortem tenderization. They proposed that it was necessary for only a small amount of dissociation to occur to effect changes in tenderization. Szent-Gyorgyi (1951) reported some evidence for the involvement of sulfhydryl groups in the combination of the actin and myosin molecules and McCarthy and King (1942) had reported earlier that during postmortem aging the measurable sulfhydryl groups increased. This appeared to corroborate the inference that actomyosin did indeed dissociate during aging. Wierbicki et al. (1954) concluded that postmortem tenderization probably was a combination of proteolysis, actomyosin dissociation and hydration due to a shift of ions. In a subsequent paper, Wierbicki et al. (1956) failed to substantiate their earlier inference that proteolysis and actomyosin dissociation was of major importance in postmortem aging. However, they did conclude that actomyosin formation contributed substantially to initial toughness but it appeared that dissociation of the complex did not occur to any measurable extent during aging. They found an association between the amount of actomyosin isolated and degree of tenderness.

Zender et al. (1958) using aseptic lamb and rabbit muscle stored up to 150 days at 25 C and 15 days at 37 C found support for previous observations that postrigor muscle tenderized as the result of postmortem storage.

Utilizing a unique glycine buffer, they were able to extract more protein from rigor muscle than from prerigor or postrigor muscle. The diminution of protein solubility postrigor was paralleled by a rise in the amount of free amino acids. Microscopically the fibers were found to be easier to separate after 10 to 20 days storage than immediately postrigor, however, the fibers were considerably less extensible than prerigor fibers. Many of the changes reported by Paul et al. (1944) were readily apparent except the aseptic samples changed at a slower rate. Some fiber disruptions were observed between 10 and 20 days, however, it took 40 to 50 days before the fibers disrupted to a large degree. These authors suggested that enzymes were responsible for the disintegrations and most likely these enzymes included the cathepsins. In a subsequent paper, Radouco-Thomas et al. (1959) found that antemortem epinephrine injections had a unique ability to prevent much of the earlier described fiber disintegrations, pH drop and amino acid production during postmortem storage. The authors proposed that epinephrine could be used to improve the distribution of meat since it apparently was an anti-autolytic agent.

Whitaker (1959) in reviewing the literature on chemical changes during meat aging felt that actomyosin dissociation accounted for the resolution of rigor mortis. However, he found no firm support for this occurrence in the literature particularly in light of the conclusions of Wierbicki et al. (1956). This is further supported by Marsh (1954) who found resolution of rigor mortis to be untenable due to the fact that muscle aged for 7 days in an inert atmosphere had the same modulus of elasticity as found in rigor muscle.

Tenderness Studies After 1960

Connective Tissue Proteins

In this literature review, only collagen will be considered, even though the presence of elastin in muscle is well documented. The presence of elastin in muscle is generally considered to be primarily as a component of artery walls. Some work has been reported on this component, but it is generally believed to play an insignificant role in meat tenderness. The quantity and importance of reticulin is unknown and in perusal of the literature this component has been mentioned but little or no work exists that specifically relates this connective tissue protein with tenderness. Generally, when the term connective tissue is used it usually implies all three proteins, but since collagen is the predominate protein in most muscles, this term will be used to implicitly denote collagen in this dissertation.

Before going into recent literature concerning connective tissue proteins and tenderness, it appears appropriate to briefly review some of the molecular information known about collagen. Gross (1961) presented a succinct review on collagen which revealed that the basic collagen molecule is composed of 1000 amino acid residues in each of 3 polypeptide chains. These 3 polypeptide chains are coiled to form a triple helix. The super helix is called tropocollagen which is the basic unit of the collagen fibril. The 3 polypeptide chains of the tropocollagen molecule appear to be held together by interchain H-bonds as the result of the peptide groups of glycine residues (Lehninger, 1970). The tropocollagen

molecule is about 280 nm in length and 1.4 nm in diameter (Lehninger, 1970). X-ray diffraction data indicate that a 70 nm repeat unit occurs and is apparently due to a staggered alignment of the tropocollagen unit with observed density due to the heads of the tropocollagen molecule. The polypeptide chains mentioned earlier have been designated $\alpha 1$ and $\alpha 2$ with the tropocollagen molecule composed of a 2:1 ratio of the α_1 and α_2 chains, respectively (White, Handler and Smith, 1968). White et al. (1968) suggested that even the 2 α_1 chains have subtle differences which allow these to be distinct and as such the tropocollagen molecule is composed of 3 different α chains. However, unequivocal evidence does not exist for the occurrence of 3 different α chains and most individuals still classify these as α_1 and α_2. In denatured preparations of collagen, larger components have been separated and 2 of these components are designated as β and γ which are produced by intramolecular covalent cross-links between the α_1 and α_2 polypeptide chains (Traub and Piez, 1971). Intermolecular crosslinks play an important role in age associated differences in collagen extractability and they also play a role in meat tenderness. The nature of these cross links have intrigued researchers for over a decade. Harding (1965) in a review of the literature on the subject describes two types of cross-links: (1) intermolecular which links one collagen monomer to another and (2) intramolecular which is between individual chains of the triple helix of the tropocollagen molecule. Harding (1965) stated that the increases observed in the shrinkage temperature of collagen is well established as an indicator of the amount of cross-linking by tanning agents.

The intermolecular cross-links have been suggested to be the more important component in stabilizing collagen fibers, and according to Bailey (1969) these are aldimine type bonds (Schiff base). By using collagen reduced with borohydride, which stabilizes intermolecular crosslinks in a reduced form, two of the cross-links have been identified as dehydrohydroxylysinonorleucine and dehydrohydroxylysinohydroxynorleucine and a similar type linkage called Fr. C which has not been characterized (Bailey, 1969; Davis and Bailey, 1971; and Mechanic, Gallop and Tanzer, 1971). The role of these components in tenderness will be considered later in this review.

Connective tissue proteins have played an immense role in the research that has been performed on meat animals over the last century. As alluded to earlier in this review, the predominate emphasis has been placed on simple quantitation of these proteins and subsequent correlation with objective and subjective measures of tenderness. Inconsistent results were obtained in many cases and at best a difference could be obtained between widely divergent muscles such as the psoas major and biceps femoris. In most cases, little difference was found that could be attributed to age differences. One of the earlier suggestions that other than mere quantity of collagen might be involved was that by Wilson, Bray and Phillips (1954). These individuals found a significantly greater amount of collagen in the young bovine as compared to cow or steer samples. They concluded that since veal is more tender than more mature animals qualitative differences probably play a more important role than percentage of connective tissues. In this same light, however, Loyd and Hiner (1960)

made improvements in the hydroxyproline determination for collagen and concluded that collagen content of veal did not differ significantly from other animals.

Lowe and Kastelic (1961) investigated several parameters of beef muscles which included objective and subjective measures of tenderness and connective tissue content on cooked and uncooked muscle tissue. The correlation of shear and taste panel scores to collagen N from cooked samples was nonsignificant. These authors offered no speculations as to why there was no relation between collagen N and shear and taste panel scores other than the effect cooking might play on the system.

Goll, Bray and Hoekstra (1963) studied age associated changes in the content of collagen of bovine animals. By using a modified hydroxyproline technique, these authors failed to find any significant age related differences in collagen content, however, shear data revealed that tenderness decreased with increasing maturity. In a similar experimental design, Goll, Hoekstra and Bray (1964b, c, d) tested for qualitative differences in connective tissue by utilizing collagenase hydrolysis, thermal shrinkage and rate of solubilization at 100 centigrade. The rate of hydrolysis followed an age dependent order except for one group which they speculated to have lipid protection causing a slower hydrolysis. When the same age groupings were utilized for measurement of ninhydrin-positive material and hydroxyproline release along with temperature of thermal shrinkage it was observed that an age dependent relationship again was involved. The rate of solubilization (conversion to gelatin)

occurred most rapidly in younger animals as compared to cows, aged cows and steers in that order. Utilizing the information presented in the three articles, Goll et al. (1964d) concluded that collagen obtained from four different age groups of bovine animals changes in some way with increasing maturation. It was apparent from earlier work that quantitatively collagen did not change with age (Goll et al., 1963; Loyd and Hiner, 1960). With this in mind, Goll et al. (1964d) speculated that either mature collagen contained more cross-links or that the strength of the cross-links increased with age.

Sharp (1963) working with the changes in protein fractions in rabbit and beef muscle stored aseptically at 37 C for up to 6 months observed essentially no changes in solubility of the collagen fraction. McClain et al. (1965) investigated the relationship between alkali-insoluble collagen in the longissimus, semimembranosus and triceps brachii muscles of bovine animals. They found that absolute quantities of alkali-insoluble collagen were not significantly related to shear values in either cooked or uncooked meat. Shear values and collagen were related when the percent alkali-insoluble collagen converted to gelatin was the method of comparison. This supports the contention by Goll et al. (1964d) that the most important aspect of connective tissue content of muscle is not the quantity, but the qualitative changes which occur with maturation.

Hill (1966) determined the solubility of isolated collagen in 1/4 strength Ringer solution upon heating at 77 C and also the hydroxyproline content of the sternomandibularis muscle of cattle, sheep and pigs. He

determined that the percentage of collagen solubilized decreased dramatically with increasing chronological age. He also observed that young calves (8 to 9 weeks) had a higher percent collagen than steers or cows which supports the data reported by Wilson et al. (1954) but is contradictory to that presented by Goll et al. (1963) and Loyd et al. (1960). In summarizing his work, Hill (1966) suggested that the intramuscular collagen undergoes some subtle changes as the animal grows older that prevents more of the collagen from being solubilized during cooking. This causes an increased toughness of the meat and as such less desirability for the consumer.

Carmichael and Lawrie (1967a, b) investigated solubility and electrophoretic changes in bovine longissimus muscle, skin and tendon. Previous observations suggested that in the early stages of collagen fibergenesis certain forms of collagen can be extracted with neutral salt solutions but as maturation progresses collagen extraction requires dilute acids and finally insoluble collagen is present (Carmichael, 1966). Also, according to Piez (1966), cold neutral salt solutions extract largely the newly synthesized collagen and acid extracted collagen contains more β or the cross-linked component than neutral salt extracted collagen. Utilizing this concept, Carmichael and Lawrie (1967a) found that the neutral salt soluble and dilute acid soluble collagen increased rapidly during gestation and declined thereafter up to 1 to 2 years of age. Associated with the fall of the soluble forms, the insoluble form increased significantly after birth and was the predominate form at 1 to 2 years of age. According

to these authors, the solubility characteristics represented a series of increases in the intramolecular and intermolecular cross-linking possibly as a physiological mechanism to handle the increase in bulk of the organism. Coupled with the soluble and insoluble collagen data was the observation that in a 4 year old steer considerably less total collagen was found than in 6 to 12 month steers (Carmichael and Lawrie, 1967a). It was hypothesized that a matter of dilution by myofibrillar and sarcoplasmic proteins possibly could explain this apparent contradiction. Weis and Anesey (1965) reported that the intermolecular bonding between α_1 units appeared to be a function of collagen maturation. In summarizing their work relative to meat tenderness, Lawrie and Carmichael (1967a) suggested that, when connective tissue proteins are utilized for studying tenderness, both total amount and degree of solubility should be examined.

Herring, Cassens and Briskey (1967a) investigated intramuscular collagen solubility as affected by chronological age in bovine animals. The semimembranosus and longissimus muscles were sampled at 5 and 10 days postmortem and analyzed for total collagen, collagen solubility, hydroxyproline and total protein. No age differences in collagen content were obtained in either the longissimus or semimembranosus muscles except for E maturity semimembranosus which was significantly different. Intramuscular collagen solubility showed a significant age effect in both the longissimus and semimembranosus muscles which again supports the contention by Goll et al. (1964d), Carmichael et al. (1967a), Hill (1966) and McClain et al. (1965) that the age related changes in collagen are molecular alterations rather than proliferation of collagen.

In spite of all the information available concerning collagen and tenderness, a certain amount of apathy seemed apparent even when research data have identified the intramolecular and intermolecular cross-link as a factor in maturation. Apparently the age differences were accepted as a real component of tenderness. However, the real problem concerned the observed differences in the same muscle from different but similar aged animals. This certainly has diminished the age associated differences in relationship to tenderness and as such connective tissue proteins have been relegated to a minor role as compared to the myofibrillar proteins.

Utilizing some of these relatively new concepts concerning collagen, Kruggel, Field and Miller (1970) identified and categorized animals into 3 groups based on their Warner-Bratzler shear values; i.e., tender, intermediate and tough. Epimysial connective tissue samples were obtained from uncooked steaks of the same animal and total collagen, acid-soluble collagen, viscosity, ester and aldehyde contents were determined. It was observed that for the most part total collagen was about the same for all animals. By using ultracentrifugation in sucrose density gradients, denatured collagen was separated into its component fractions, α, β, γ or higher cross-linked units. Determination of the percentage of each component revealed that tender samples contained a considerably greater percentage of α-component as compared to the intermediate and tough samples. These authors found that as the intrinsic viscosity of acid-soluble collagen increased, tenderness decreased which they suggested was a reflection of variation in collagen molecule size among the various samples.

It has been suggested that lysine is involved in intramolecular cross-linking (Veis, 1970) and as such the amino acid determination revealed that a significantly ($P < 0.05$) greater quantity of this proposed intramolecular cross-link precursor was present in the tough samples. The authors emphasized that the collagen investigated in this study was epimysial in origin and the relationship between it and intramuscular collagen had not been determined.

Kruggel and Field (1971) subsequently designed an experiment to clarify not only if previous data from epimysial tissue were applicable to intramuscular collagen but what relationship existed between stretched and aged muscle. Using similar techniques as in the earlier work, these individuals found that as the α-chain component decreased the shear values increased. The observed increase in the α-component was related to stretching and aging of the muscle. This observation suggested that stretching, although possibly not an immediate reaction, was responsible for cleavage of intramolecular cross-links. Similarly it appears that in some way the aging process also involves breaking intramolecular cross-links. Subsequent work by the same group, Pfeiffer et al. (1972) verified that initial stretching did not produce immediate changes in the α-component. However, it was suggested that in some way stretched muscle caused molecular structure alteration that allowed in vivo processes to alter the component composition and as such modify resistance to shear.

Shimokomaki, Elsden and Bailey (1972) used borohydride to reduce and stabilize collagen cross-links. With this technique, they found that a

1-year old steer had the highest proportion of reducible cross-links when compared to either fetal samples or samples from animals older than 1 year. This discrepancy was apparently resolved when they found that the aldimine bond in some way becomes stabilized with increasing chronological age (greater than 1 year) and is not reduced by borohydride. Bailey (1972) commented on the importance of labile collagen versus total collagen in cooked meat by stating that rare meat should be affected by total amount of collagen more than labile collagen because it hasn't reached a point of thermal lability or in other words collagen is still in the native state.

Veis (1970) adequately summarized the current information on intermolecular cross-linking by stating that the Schiff base forms that have been isolated are only an intermediate cross-link bond. The lability of this bond is high, however, the knowledge concerning the change from Schiff base to a more stable form is unknown. Until this information is determined, the complete role of connective tissue in meat tenderness will remain unsolved.

In summarizing the overall role of tenderness as affected by connective tissue, it appears that much basic information has been gained. The research work has progressed from a quantitation of collagen to a basic determination of molecular structure. However, one observation on the use of modern ideas of collagen molecular architecture, has been the preponderance of the work being done on age-associated changes of the molecules. This is certainly an area of fruitful research. However, only

a few individuals have used these concepts to help discover why the same muscle from similarly treated animals of the same age can be so divergent in shear values. The normally consumed block beef on the market is obtained from animals of at most a few months difference in age. At the animal science level, this appears to be an area that should elicit considerable investigation. Additionally, the relationship between intramolecular and intermolecular cross-links in relation to tenderness is not clear. Veis (1970) stated that no role has been assigned to intramolecular cross-links. However, Kruggel and Field (1970, 1971) have measured the presence of dimers and trimers of the polypeptide units, yet it is unknown whether all of the components are composed of inter- or intramolecular linkages. Some of these topics need to be clarified to give a clear picture of the tenderness association.

Proteolysis

The origin of the proteolytic concept cannot be found, however, as mentioned previously, Hoppe-Seyler (1871) probably provided an impetus for some scientists to associate it with postmortem tenderization. Probably no early work has been mentioned more often than that of Hoagland et al. (1917) in connection with postmortem aging of meat. These individuals measured the increase in non-coagulable nitrogen during storage and when this was observed to be a considerable amount, they attributed most of it to autolysis. McCarthy et al. (1942) observed that aging of meat whether at conventional cooler temperatures or high temperature, was

associated with increased soluble nitrogen values. Zender et al. (1958) observed considerable increase in free amino acid concentration with decreased solubility of the salt soluble proteins. These authors concluded that the changes reflected the observed changes in tenderness and appeared to be proteolytic in nature. In a subsequent paper, Radouco-Thomas et al. (1959) observed that the release of amino acids as well as other phenomena of postmortem muscle were attenuated or inhibited by the injection of epinephrine. This observation led the authors to propose that epinephrine be used as an anti-autolytic agent which would allow much longer storage of meat. Numerous workers have observed either non-protein nitrogen or amino acid increase with increasing time postmortem (Locker, 1960; Ma, Matlack and Hiner, 1961; Sharp, 1963; Davey and Gilbert, 1966; Suzuki, Nakazato and Fujimaki, 1967; Field and Chang, 1969; Parrish et al., 1969a).

The observed production of non-protein nitrogen (NPN) or amino acids has received considerable attention and in the majority of cases verified to be a normal result of postmortem muscle storage. However, the most seriously studied factor has been the cause of proteolysis and whether or not the production of NPN was directly associated with the observed tenderization. Again, the most prominent suggestion which probably can be associated with this topic is from Hoagland et al. (1917) who suggested that the presence of an endoenzyme or some other endogenous proteolytic substance could degrade native protein. Much attention has been devoted to isolating a component or components which will promote protein degradation in the postmortem muscle storage environment. Balls (1938) isolated

and partially purified a cathepsin from muscle which had a pH optimum of 4.1, however, most of the work has been done using organs such as the spleen which contains greater quantities of the proteolytic enzymes than that found in muscle. Snoke and Neurath (1950) reported the isolation and partial purification of a proteolytic agent from rabbit skeletal muscle. The agent isolated had a pH optimum at 4.0 and had proteolytic acitivity against both crude muscle extracts and denatured hemoglobin. The proteolytic agent was not called a cathepsin, but the authors inferred that it had some similar characteristics to earlier isolated cathepsins. Doty (1950), in reviewing the importance of enzymes to the meat industry, suggested that many of the changes observed in postmortem aging of meat could be attributed to proteolytic enzymes which probably belong to the group called cathepsins. Balls (1960) in reviewing catheptic enzymes of muscle stated that the quantity of this component was very low and optimum activity appears to be at a more acidic level than meat usually approaches during storage. However, he agrees that there are possibilities for and against significant proteolysis occurring in muscle, but no definitive work is available which would suggest that it is not an integral part of postmortem change.

The study of cathepsins isolated from muscle has been complicated considerably not only by the low concentration but also by the fact that this group of enzymes is different from glandular extractions and also there are species to species variations (Landmann, 1963). Sliwinski, Doty and Landmann (1959) purified an enzyme from beef muscle and determined

that the optimum activity occurred at pH 4.4 at 37 C using denatured hemoglobin as a substrate. Koszalka and Miller (1960a, b) purified an enzyme from rat skeletal muscle that had an optimum activity at pH 8.5 to 9.0 on both synthetic and muscle homogenate substrates. Landmann (1963) reported autolytic activity in beef muscle homogenates and he observed that the proteolytic activity had a dual optimum at pH 5.0 and 8.0 to 9.0. Using several different criteria for determining specific catheptic enzyme types, Landmann (1963) concluded that he had isolated cathepsins B and C. Sliwinski et al. (1961) reported that a crude enzyme preparation had been isolated at pH 5.6 and that it contained the majority of all the proteolytic activity in the beef muscle homogenate. These authors noted some similarity between their isolated preparation and cathepsins B and C. After further studies with activators the authors concluded that they had isolated three different enzymes from muscle.

The concept of endogenous muscle proteases has certainly been proposed and their presence has been undoubtedly demonstrated. However, the most important aspect of the concept has not been the presence, but their activity against muscle substrate and relationship to tenderness. Bandock-Yuri and Rose (1961) isolated 2 fractions from chicken breast muscle that exhibited proteolytic activity against synthetic substrates. Even though proteolytic activity was observed, these authors concluded that the activity wasn't sufficient nor did it occur in the right time sequence to account for muscle tenderization in poultry. Davey and Gilbert (1966) used NPN as a measure of proteolysis in stored bovine longissimus muscle. The NPN

values increased somewhat, however, they concluded that tenderization was not paralleled by NPN and generally tenderization was 80% complete before proteolysis could be detected. Additionally, they found that differences in tenderness among several muscles could not be associated with NPN differences. Locker (1960) investigated proteolysis in muscle by N-terminal analysis, free amino acids and NPN analysis. Although a small increase in free amino acids was recorded, the other data were such that led him to conclude that proteolysis probably was of little importance in tenderization. Sharp (1963) investigated the effect of aseptic storage of bovine and rabbit muscle on the production of NPN. Even though considerable quantities of NPN was observed, the author concluded that since no change was observed in the fine structure, NPN was derived from degradation of sarcoplasmic proteins by cathepsins. Bodwell and Pearson (1964a, b) reported on the isolation and characteristics of catheptic enzymes using synthetic and natural substrates. These authors could find little activity of this preparation on actin, myosin or actomyosin but did observe that sarcoplasmic proteins were readily degraded.

Parrish and Bailey (1966, 1967) worked with catheptic enzymes isolated from porcine and bovine muscle that had pH optima at 4.0 and 9.0. All activities tested suggested that the cathepsins isolated were similar to cathepsin from spleen. However, the bovine cathepsins were particulate and from their data they suggested that at least a portion of the activity found in muscle is membrane bound. The intracellular location of the cathepsins has been questioned, however, the presence of organelles (lysosomes) that contain hydrolytic enzymes (deDuve, 1963) has certainly given

credence to speculation that this organelle is the source. The data reported by Parrish and Bailey (1967) support the supposition that the cathepsins are lysosomal bound as determined by the observed hydrolytic latency.

In a series of related papers, Suzuki, Nakazato and Fujimaki (1967), Suzuki and Fujimaki (1968) and Suzuki, Okitani and Fujimaki (1969a, b) studied various parameters of proteolysis in rabbit muscle. The release of NPN seemed to parallel that observed by Wierbicki et al. (1956) and Davey and Gilbert (1966). The former authors also studied the release of peptides during storage and found that this fraction (peptide length index of 10) decreased and the amino acid fraction increased. Whether the amino acids increased at the expense of the peptides was open to speculation. In the subsequent three papers, these Japanese authors isolated and purified a proteolytic component which they classified as cathepsin. This enzyme had a pH optimum at 4.0 and in hydrolyzing the β-chain of insulin it resembled pepsin rather closely. The effect of cathepsin D on several natural substrates revealed that in decreasing activity it hydrolyzed sarcoplasmic > myosin A > actin > myosin B. The sarcoplasmic protein degradation is not surprising since previous work by Bodwell and Pearson (1964) showed no major activity on the salt soluble proteins. Additionally Suzuki et al. (1969b) observed that cathepsin D decreased the Mg^{2+}-enhanced adenosinetriphosphatase (ATPase) activity of muscle at pH 5.0 but not at pH 5.5. The Ca^{2+}-enhanced ATPase activity was not changed irrespective of the pH used.

The concept of the lysosome has elicited considerable interest since the presence of acid pH optima, hydrolytic enzymes seems to fit the mold for a ready reservoir of postmortem autolytic enzymes. However, the concept at first fails since some authors have reported that histochemical enzyme markers suggest that normal muscle exhibits activity only as a part of the vascular system (Smith, 1964), or as the result of dystrophy or atrophy (Pellegrino and Franzini, 1963). However, Bird (1971) reported that by utilizing ultracentrifugation he and his coworkers have been able to define two distinct sources of lysosomal enzymes. By determining relative quantitives of several of the enzymes known to be present in lysosomes, Canonico and Bird (1970) have demonstrated that some of the lysosomal enzymes are present as the result of phagocytic cells but that enzymes are also present in endogenous muscle lysosomes.

However clear the concept of intracellular lysosomes may be, the overriding fact that needs to be clarified is the association of these enzymes with postmortem tenderization. Martins and Whitaker (1968) and Caldwell (1970) suggested that even though difficulty has been encountered in showing catheptic activity towards salt soluble proteins it is possible that a combination of the various cathepsins (A, B, C, D) might be necessary for degradation. Ono (1970) biochemically substantiated the presence of lysosomes in bovine longissimus muscle, however, he did not investigate the capacity of these enzymes to degrade salt soluble protein.

Parrish et al. (1969a) attempted to put the catheptic enzyme-tenderization question in proper perspective. By measuring NPN, free amino groups

and shear values, these authors concluded that postmortem proteolysis did occur, but tenderization did not parallel the proteolytic products. Parrish et al. (1969a) suggested that even if proven that the amino acids come from connective or myofibrillar tissue proteins it would be an assumption that this is related directly to tenderness. These authors proposed that a few highly specific points of myofibrillar protein cleavage, such as those at the I-Z junction, would be more likely to improve tenderness rather than N or C-terminal degradation of actin or myosin.

Eino and Stanley (1973) followed the development of proteolytic activity in psoas major muscles aged at 0 to 5 C up to 10 to 14 days. By utilizing a crude catheptic enzyme preparation and maintaining an optimum pH of 3.8 these individuals found that the activity (cathepsin D) on several natural substrates generally followed the shape of the aging curve. Little additional lysosome release with Triton X could be observed after 8 to 14 days aging which suggested to them that maximal release and activity coincided with tenderization. Even though enzyme assays were conducted in an environment (pH = 3.8) dissimilar to postmortem muscle these authors suggested that the observed maximum release of cathepsins coincident with tenderization should be adequate to implicate these enzymes in tenderization. Additional textural measurements were made which coincided with observations of maximum cathepsin release.

Most investigations have concluded that probably some type of proteolysis is involved in postmortem tenderization, but the proper measurements and conditions have not been found that unequivocably demonstrate this fact.

With this in mind, Goll et al. (1971a, b) attempted to demonstrate that proteolysis was indeed a functioning part of postmortem muscle tenderization. They utilized the concept that the myofibril has certain areas which are sensitive to proteolysis by trypsin. They argued that a mild proteolytic attack on the sensitive myosin and tropomyosin-troponin (TM-TN) complex might be a fruitful indirect approach to determining the role of proteolytic enzymes in postmortem muscle. Their results showed that a mild proteolysis by trypsin effectively mimicked nearly all of the postmortem changes observed in muscle. These changes apparently are prevalent and measurable before the proteolytic activity cleaves myosin into heavy and light meromyosin fractions or destruction of the TM-TN complex. A more in depth review of this work will be made later, however, it appears that work such as this certainly suggests that a mild specific proteolysis probably occurs in postmortem muscle. The most apparent problem that exists is determining what endogenous (if it is such) substance is responsible and how one can properly measure all components of the system.

Although it is not appropriate at this point of this literature review to consider in depth the work reported by Busch et al. (1972b) ,they reported on the isolation of a endogenous component of muscle capable of removing the Z-line from myofibrils. Although complete characterization has not been reported, preliminary results suggest that it is an enzyme which requires activation by greater than 0.1 mM calcium. All other ultrastructural components appear to be unaltered by this enzyme, but until adequate characterization is obtained one can only speculate as to whether this is the elusive proteolytic agent sought after for so many years.

Muscle Protein Solubility

Changes in the myofibrillar proteins during postmortem storage received considerable attention for a number of years. Saxl (1907) observed decreased protein solubility as the result of rigor mortis which he attributed to protein denaturation. Deuticke (1930) found that both postmortem muscle and prerigor muscle that received repeated stimulations yielded about 30% less protein upon extraction with 0.09M potassium phosphate pH 7.2 containing 0.03M potassium iodide (KI). This solubility differential of the stimulated muscle can be associated with early onset of rigor mortis (Helander, 1957).

Any discussion of postmortem muscle protein solubility is intimately involved with rigor mortis. The interpretation of rigor mortis relies solely on the structural aspects of muscle which Huxley (1958) clearly described. The sliding filament model proposes the presence of interdigitating thick and thin filaments. The overlap of these filaments provide not only the striated appearance, but also performs the shortening necessary for force generation. The presence or absence of several ions and specific nucleotide phosphates dictate whether the system is contracting or relaxed. The overall picture of rigor mortis and resolution of rigor will be considered in depth at a later point, however, suffice it to say at this point that in postmortem muscle the absence of the plasticizing effect of Mg adenosine triphosphatase (Mg ATP^{2-}) allows permanent bonds to be formed between actin and myosin. The postmortem contraction and actomyosin formation results in stiffness and is characteristic of the rigor mortis phenomenon.

As mentioned previously, the observation that muscle solubility decreased postmortem prompted the investigation of what caused "resolution of rigor" and the corresponding increases in tenderness. One of the earliest observations in this area was that of Wierbicki et al. (1954) who suggested that initial toughening might be due to formation of actomyosin (rigor) and the observed tenderization could be due to dissociation of actomyosin. Additional results from the same laboratory (Wierbicki et al., 1956) supported the concept that rigor mortis was associated with the formation of actomyosin, however, these authors failed to find support for tenderization to be due to its dissociation. Helander (1957) investigated solubility of contracted and non-contracted muscle, but he was unable to find decreased solubility. However, he assumed that the extracting system, KI which depolymerizes F-actin to G-actin, could be responsible for the apparent discrepancy in extractability.

Zender et al. (1958) in their studies involving aseptic autolysis of muscle found no diminution of the fibril proteins extraction during rigor mortis such as was observed by Wierbicki et al. (1956). However, they used a buffer system consisting of 1 M glycine. Whether or not this buffer can be classified with strong dissociating agents such as KI or act similarly to pyrophosphate in dissociating the actomyosin complex is unknown. These authors did find decreasing extractability of protein from postmortem muscle, but they attributed this to proteolysis since the quantity of free amino acids increased.

Locker (1960) in studying proteolysis in beef noted that the extraction of postmortem muscle with Weber-Edsall solution (W-E) was highly variable

but the trend was for a decline during rigor with an increase following rigor. This increase in many cases equaled or exceeded that extracted from 0 hour samples. Since it is rather well accepted that W-E solution extracts the actin-myosin A (myosin B) complex (Fujimaki et al., 1965b), it would appear that Locker's results support the postulate of Wierbicki et al. (1956) that no actomyosin dissociation occurs postrigor. Whitaker (1959) in a review thought it would be reasonable to assume that the process of tenderization followed a reversal of rigor mortis, however, he concluded that the data do not suggest that this was actually occurring.

Weinberg and Rose (1960) investigated changes in protein extractability during postrigor storage of chicken breast muscle. These authors used several extraction methods based on differential solubility that allowed the quantitation of actomyosin and myosin in prerigor and postrigor muscle. They determined that the increase in extractability postrigor was due to the increase in the actomyosin fraction. These individuals, however, postulated that since the free myosin content decreased postrigor and actomyosin increased that it could have been possible that dissociation of actomyosin occurred but it reassociated during extraction. In concluding their work, Weinberg and Rose (1960) suggested that contrary to the popular autolysis theory it appeared that tenderization was the result of specific cleavage of an actin association responsible for maintenance of the muscle matrix.

Hill (1962) studied the distribution of the various nitrogen fractions in two muscles, longissimus and semitendinosus, among lambs, swine and

cattle. He reasoned that by using two muscles that are considerably divergent in tenderness, information regarding tenderness differences might be determined. The results showed that beef muscle contained a greater quantity of stroma and myofibrillar nitrogen with the latter quantity determined on a stroma nitrogen free basis. Hill (1962) stated that it was assumed that the greater toughness of the semitendinosus versus the longissimus muscle was primarily associated with the stroma content. However, on a stroma free basis, he observed that the semitendinosus had a greater quantity of myofibrillar nitrogen and less sarcoplasmic nitrogen and suggested that in some way an association between these parameters and tenderness existed. Additionally, he suggested that a similar argument could be extended to the observed differences among the species since tenderness ranking appears to coincide with the distribution of myofibrillar and sarcoplasmic nitrogen.

Partmann (1963) in discussing rigor mortis and tenderization concluded that the resolution of rigor mortis is not a simple reversal of those events leading to rigor. However, he observed that if fully aged beef muscle was gently homogenized in isotonic KCl a mixture of fiber fragments was obtained that contracted upon addition of 10^{-2} M ATP. He concluded that this supplied sufficient information to believe that the actomyosin complex has either been dissociated somewhat or became easily dissociated as the result of the aging period. This information and other reported data suggested to him that little change takes place in the fibrillar proteins during aging.

Hegarty, Bratzler and Pearson (1963) investigated the distribution of the various nitrogen fractions in muscle from twenty yearling bulls that had been selected for tenderness. Tenderness was highly correlated with fibrillar solubility when using shear data or taste panel comparisons.

Although studying an abberant muscle situation rather than effects of aging, Sayre and Briskey (1963) found that a combination of pH and temperature could affect the solubility of the myofibrillar proteins. As muscle was exposed to a medium (5.7 to 5.9) or low pH (5.3 to 5.6) in combination with a high temperature (>35 C), myofibrillar protein solubility decreased considerably. This situation is dramatically represented by the condition known as pale, soft exudative pork, however, whether the pH and temperature relationship alters the solubility of bovine muscle is certainly speculative.

Scopes (1964) investigated various temperature and pH combinations and their affect on the solubilities of bovine longissimus muscle proteins. He found a situation paralleling that reported by Sayre and Briskey (1963). Bovine muscle protein extractability dramatically decreased if the muscle preparation was exposed to 37 C temperature for 4 hr. then cooled to 0 Centigrade. Similar samples that were either extracted at 0 · hour or allowed to "age" 20 hr. at 0 C and then extracted showed virtually no changes in extractability. They speculated that the heat denatured sarcoplasmic proteins in some way inhibited high ionic strength KCl from extracting the myofibrillar proteins.

In a somewhat similar study, Goll, Henderson and Kline (1964a) investigated postmortem tenderness and solubility changes in the bovine semitendinosus. Samples were obtained from paired muscles, 1 excised immediately postmortem and the other left on the skeleton. After storage for 1 of 6 time periods, (at 4 C) 0, 6, 12, 24, 72 and 312 hr., the samples were tested for tenderness and protein solubility. It was found that the 2 sample groups, excised and nonexcised, responded differently to the tests performed on them. A negative correlation coefficient ($r = -0.32$) existed between shear and myofibrillar protein solubility of the excised muscle, but a positive correlation ($r = 0.44$) was determined for unexcised muscle. They also observed that the 0 hour excised muscle yielded more protein and was less tender than the unexcised muscle which appears to contradict earlier results (Hegarty et al., 1963). These authors suggested that protein solubility and tenderness were related only casually and that the excised muscles might have an opportunity to cool faster and thus not become denatured during postmortem storage.

Aberle and Merkel (1966) investigated the solubility and electrophoretic behavior of proteins from aged bovine muscle. They found an expected decline in fibrillar protein solubility during rigor mortis, however, contrary to Goll et al. (1964a), they also found a significantly higher solubility at 168 and 336 hr. postmortem. The increase in solubility was reported to be positively correlated with the decrease in Warner-Bratzler shear values at 168 and 336 hours. These authors also found a decrease in sarcoplasmic protein solubility from the semitendinosus muscle postmortem, but no change was observed in the longissimus. A few changes were

also noted in the electrophoretic pattern of the sarcoplasmic proteins as the result of aging. Maier and Fischer (1966) also observed changes in certain bands and increased intensity of others when water soluble proteins were submitted to gel electrophoresis. Fibrillar protein solubility was essentially static during postmortem storage, however, as reported in previous work the latter authors found that the water soluble proteins became less extractable as storage time increased. McIntosh (1967) reported that bovine semitendinosus fibrillar protein including actomyosin became more extractable as aging time increased through 2 weeks. Similar responses were reported for pork and chicken fibrillar proteins except the time scale necessary for chicken was less than that for bovine or porcine muscle.

Cook (1967) studied the effect of certain physical treatments on the solubility of postmortem bovine muscle. He reported that stretching prerigor muscle and allowing rigor to occur, prevented the occurrence of fibrillar solubility changes. This observation led him to suggest that the contractile state played a large part in the solubility of the proteins of the myofibers. The contractile state was only significant for postrigor samples which led him to suggest that the formation of actomyosin was an important aspect of decreased solubility during the rigor state and if actomyosin formation could be inhibited it would improve tenderness attributes of muscle. Buck, Stanley and Commissiong (1970) investigated similar aspects of muscle as that of Cook (1967) and found that stretching significantly increased the extractability of protein from postrigor muscles in all but

one trial. They reported that the increased solubility was positively correlated with tenderness. However, investigation of the properties of the protein extracted revealed that more actomyosin was extracted from the stretched muscle in all trials. These authors were unable to clarify the enigmatic results, however, some evidence for the individual extraction of myosin and actin and subsequent combination of the two in the extracting buffer has been postulated previously by Weinberg and Rose (1960). The data presented by Buck et al. (1970) doesn't suggest that this would account for all the observed discrepancies, however, it is possible that improved resolution of the extracted proteins might clarify this point.

Davey and Gilbert (1968a) investigated the changes in extractability of bovine and rabbit muscle during aging. By using the Hasselbach-Schneider (H-S) buffer which preferentially extracts myosin and the W-E buffer which extracts only actomyosin, these authors determined that fibrillar solubility increased with aging. It was found that less actomyosin was extracted from unaged than aged meat when short extraction periods (10 min.) were used. A time necessary for solubilization and period of aging was described with the aged samples requiring considerably less time. A similar time response was reported when the H-S buffer was used for extraction. The authors concluded that the more extensive and increased ease of extraction of fibrillar proteins was consistent with weakening of the linkages of the proteins and in some way the ultimate pH helped determine the extent of extractability of the proteins. In a subsequent report, Davey and Gilbert (1968b) reported moving boundary electrophoresis data suggesting that actin

was released in increasing amounts into the H-S buffer during aging. They reported that the actin recombined with myosin during dialysis in preparation for electrophoresis and the increased peak area attributed to actomyosin was in essence due to more solubilized actin which had increased during aging. This was consistent with the observation that myosin extraction did not fluctuate as a result of the aging process. From their observations, Davey and Gilbert (1968b) concluded that in some unknown manner the tropomyosin and actin association could be weakened and as a result actin became more extractable with aging time. Some support for dissolution of the Z-line during aging (Davey and Gilbert, 1967b) apparently lends credence to the concept of Davey and Gilbert (1968b). Penny (1968) utilized different extracting buffers and other chemical tests to determine the properties of prerigor and aged rabbit muscle. He found an increasing extraction of proteins during aging when using 1 M KCl. Pyrophosphate extraction yielded similar results, however, by coupling extraction procedures with measurement of Ca^{2+} ATPase activities it was possible to characterize the properties of the increase in extraction. The initial extraction with pyrophosphate yielded all the Ca^{2+} ATPase activity as evidenced by the lack of any further increase with aging. Extraction with KCl yielded less of the Ca^{2+} ATPase activity from prerigor muscle than with the pyrophosphate extraction, however, the KCl Ca^{2+} ATPase activity increased with aging time. Penny (1968) suggested that the differences observed between the two buffered extractants was an indication that more actin and tropomyosin was solubilized as the result of aging. Reduced viscosity data suggested

that no changes occurred in the myosin molecule since a probable component, acto-heavy meromyosin would have revealed results inconsistent with those observed. All of these results allowed Penny (1968) to conclude that a breakdown of bonds had occurred within the myofibrillar component. The probable site of this bond breakage was the complex binding area of thin filament with the Z-line (Davey and Gilbert, 1967b). The weakening of attachments in this area would allow more actin to be solubilized which did occur as was shown by KCl extraction. Results obtained using myofibril suspensions suggested that the component or agent responsible for weakening of the thin filament-Z lattice structure was easily removed by buffered washes of the suspensions.

In two subsequent papers, Penny (1970a, b) sought to more thoroughly investigate and characterize the components being solubilized as the result of aging. In the first paper, Penny (1970a) set out to characterize and quantify the specific proteins extractable with H-S solution and 5 mM tris (pH 8.2) buffer. The composition of the muscle extract was determined to be 47% myosin, 12% tropomyosin and 3% troponin. He stated that actin could not be quantified due to it being complexed with α-actinin, troponin and an insoluble residue. The second paper (Penny, 1970b) verified some of his earlier work that aging was responsible for allowing more myosin, actin, tropomyosin and troponin to be extracted. None of the work firmly suggested that these proteins were degraded during storage. In evaluation of his data and after supplementation with work from other authors, he concluded that α-actinin, as a component of the Z-band, loses its affinity for the thin

filament during aging and as such the thin filament proteins are more readily released upon extraction. Haga et al. (1966) reported that preceding solubilization of actin, a break occurs between the thin filament and the Z-line, Goll et al. (1970) suggested that the strength of the actin-Z-line bonds contributes substantially not only to the extraction of actin but also affects myosin indirectly since actin influences the solubility of myosin.

Sayre (1968) determined the change in extractability of the myofibrillar proteins of chicken pectoralis muscles during 24 hr. of storage. He found that myosin extractability dropped considerably during the first 3 hr. and showed a continuous decline through 24 hours. The loss of myosin extractability was paralleled by an increase in extractability of actomyosin. The constancy of the other measurable protein fractions, NPN, sarcoplasmic and stroma, led Sayre to eliminate the probability that major proteolysis was involved in promoting the changes in extractability. Instead, Sayre (1968) suggested that the combination of actin and myosin during rigor was responsible for the decrease in myosin extractability observed postmortem. The increase in actomyosin extraction that occurred was suggested to be due to either a breakage of the thin filament or in some way a detachment from the Z-line which then allowed the actomyosin to become extracted. These observations support the view of several of the previous studies in that extractability is affected by the actin-myosin combination and by thin filament bonding to the Z-disk.

Valin (1968) used bovine longissimus muscle stored for either 1 or 8 days at 4 C to prepare both actomyosin (W-E) and metin (TM-TN) which was

extracted either directly from muscle or from the isolated actomyosin. Valin (1968) found that more myofibrillar protein was solubilized as postmortem storage time increased and associated with this increase was an increase in extraction of metin from the isolated actomyosin. The importance of the extraction of metin was the lack of extractability after one day of aging and the observation that 8 day aged muscle yielded a considerable quantity. Valin (1968) suggested that an association exists between metin extraction and tenderness since the apparent intimacy of TM-TN with the thin filament and the Z-line coincides with the previous report by Davey and Gilbert (1968b) concerning aging and the disappearance of Z-component.

Chaudhry, Parrish and Goll (1969) investigated the effect of temperature and extracting buffer on the solubility of rabbit and bovine muscle. The sarcoplasmic proteins showed little change in extractability at 2 C, however, these proteins increased in solubility during postmortem storage at 25, 28 and 31 centigrade. At 37 C solubility increased up to 24 hr., however, a decrease of 18 to 25% was observed thereafter. The myofibrillar solubility in 0.5M KCl increased considerably when determined either 48 or 312 hr. postmortem. This increased extraction was apparent in both rabbit and bovine muscle and increased with increasing temperature up to 37 centigrade. Myofibrillar extraction in 1.1M KI generally paralleled KCl extraction up to 25 C, however, KI extractions were not as great as that of KCl. At 28 and 31 C little change was noted and at 37 C a decrease after 6 to 12 hr. was noted. These authors observed that they could not

find any evidence for any of the myofibrillar extracts to contain actomyosin. Chaudhry et al. (1969) could find no association between the solubility of myofibrillar proteins and tenderness. These authors reported a decrease in solubility at 37 C which does not coincide with previous work showing muscle to be more tender at this temperature (Busch, Parrish and Goll, 1967).

The role of the water soluble (sarcoplasmic) proteins in postmortem tenderization has been investigated and in many cases the solubility patterns are similar to those of the myofibrillar proteins, in that a slow decrease occurs postrigor (Goll et al., 1964a; Sayre and Briskey, 1963; Wierbicki et al., 1956). Generally the sarcoplasmic proteins have been shown to change little quantitatively and qualitatively as shown by electrophoresis (Aberle and Merkel, 1966). Fukazawa et al. (1970) found little change in low (< 0.2) and high (> 0.57) ionic strength extracts from pre- and postrigor chicken muscle. However, by extracting the residue of both the low and high ionic strength fractions with 0.03M KCl and H_2O these latter authors found considerable increases occurring during postmortem storage. It was noted that it could be more than fortuitous that this increase is congruent with the loss of Z-line materials. The water soluble extract was fractionated further with NH_4SO_4 and the properties of each of two fractions were tested. They found that considerable differences existed between these fractions from pre- and postrigor muscle on their effect on superprecipitation and gelation of F-actin. The 2 components were identified as α-actinin and native tropomyosin which have been postulated to be associated with the Z-line and thin filament, respectively.

Landes, Dawson and Price (1971) extracted the various protein fractions from treated (sodium pentobarbital) and control turkey breast muscle during pre- and postrigor time periods. They found that fibrillar protein solubility (control) increased rapidly and then leveled off after 3 hr. postmortem. The treated samples revealed a delayed response but increased gradually before leveling off at 12 hr. postmortem. Initially the control birds had the most extractable fibrillar protein, but after aging more protein was extracted from the treated birds.

Wu and Sayre (1971) investigated the effect of aging on myosin from red and white chicken muscle. They found an increase in protein extraction with time postmortem. They also isolated a new component (T) that increased as aging progressed, however, this component was found only in white muscle. Hay, Currie and Wolfe (1972) reported the observation of a peak of unknown composition in sedimentation diagrams of chicken fibrillar protein that corresponded closely to the component T of Wu and Sayre (1971). These authors concluded that myosin becomes more dissociated during long (7 days) periods of aging as compared to that from muscle aged less time. deFremery (1971, 1972) in two closely related papers isolated actin from chicken breast and leg muscle. In the initial paper, he found no change in actin extractability during aging nor could an association between removal of C-terminal amino acids from actin and tenderization be found. In the subsequent paper, some increase in polymerizable actin with aging was reported, however, no correlation with tenderness could be found. Much of the information available concerning aging of meat is contradictory, however,

the breakage or rupture of myofibrils has been relatively well documented by Davey and Gilbert (1967b, 1969). Since considerable evidence exists for the presence of α-actinin in the Z-band (Goll et al., 1969), Penny (1972) regarded the changes of this protein during postmortem aging as of primary importance in myofibrillar disruption. Penny (1972) extracted and quantified α-actinin from 1, 14 and 21 day postmortem bovine longissimus. It was found that the quantity of α-actinin remained the same (1.4%) irrespective of the aging time. When comparing aged to unaged muscle, a small difference was found in the amount of α-actinin that could bind F-actin, however, the author felt that the differences were insufficient to account for all the myofibrillar changes in postmortem muscle.

Hay et al. (1972) and Hay, Currie and Wolfe (1973b) investigated the effect of postmortem aging on the physicochemical changes in chicken actomyosin and fibrils prepared from breast and leg muscle. In the initial paper, Hay et al. (1972) found only minor differences in Ca^{2+} ATPase activities during aging, however, in the latter paper, Hay et al. (1973b) found aging affected electrophoretic patterns. Electrophoresis of sodium dodecylsulfate (SDS) incubated myofibrils quite clearly revealed the disappearance of some and appearance of several new components during a 168 hr. aging period. The authors suggested that the disappearance of a 44,000 Dalton component at 48 hr. in breast muscle could possibly be more than coincidental with the early reported loss of the M-line in electron microscope studies (Hay et al., 1973a). The appearance of a 30,000 Dalton component in both breast and leg muscle was attributed to a possible degradation of myosin.

In summary, only general conclusions can be made concerning protein solubility postmortem and during subsequent aging. It is generally believed that muscle reaches a low point in extractability during rigor and slowly increases during aging. It has been observed that prerigor muscle is tender, however, considerable discrepancy exists in the data as to its protein solubility. Most observations suggest increases in tenderness with aging up to a point, however, not all solubility data reported coincides directly with the increased tenderness. Much of the recent work has focused not only on quantitative changes but also on qualitative changes of postmortem muscles. Biochemical data corresponding to observed histological changes have been promising, but additional sophistication and experimentation will be necessary to validate the observations. A general trend has been seen from the older method of mere quantitation of the various nitrogen fractions postmortem to both quantitative and qualitative evaluation of all protein fractions in an effort to determine if the speculated minor proteolysis actually occurs in muscle (Goll et al., 1971b) and if these changes are associated with tenderization differences.

Water-Holding Capacity

The relationship between water-holding capacity (WHC) and tenderness is well documented (Hamm, 1959, 1960). According to Hamm (1960) there exists a complicated relationship between glycolysis and pH that affects the ultimate hydration of the muscle proteins and therefore the tenderness of meat. Tenderness tends to be at a low point coinciding with the least

hydration of the proteins. Hamm (1959) determined that WHC was more related to the degradation of ATP postmortem than to pH. This relationship was suggested to be involved with the association of ATP with the alkaline earth metals and when ATP is degraded the cations are free to bind to the proteins and cause a tighter structure of less hydration. Hamm (1959) presented a graph of bound water versus pH and it showed a minimum of hydration at approximately pH 5.0 or near the isoelectric point of muscle proteins. The association of WHC with tenderness suggested to several groups that a modification of rigor mortis might prove fruitful to increase tenderness and improve other quality attributes of muscle (Khan and Nakamura, 1971; Weiner and Pearson, 1966; Weiner, Pearson and Schweigert, 1969; deFremery, 1966; Radouco-Thomas et al., 1959). As a general rule, the attenuation of pH fall postmortem has been obtained and as a result increased hydration has been observed. The injection of several of the chemicals used to impede pH fall [1,2-bis-(2-dicarboxymethylaminoethoxy)-ethane (EGTA), ethylenediaminetetracetic acid (EDTA), etc.] have proven to be lethal and the practical application appears to be limited at this time. Another possible limitation could be the probable increased bacterial proliferation if the pH is allowed to remain near neutrality which is the pH optimum for most bacteria (Lechowich, 1971).

Hamm (1960) very adequately summarized the relationship between WHC and tenderness when he said that a correlation is apparent and important only if the difference between samples is relatively great.

Rigor Mortis

Chemical Changes

The literal translation of rigor mortis is "stiffness of death". Much of the early work on rigor mortis involved the association of the observed rigidity with precipitation of the muscle proteins by lactic acid. An early observation by Bernard (1877) suggested that glycogen was the forerunner of the acid production in postmortem muscle and without much difficulty an animal could be depleted of glycogen and alkaline rigor would be established. This appeared to be the first reference that contradicts the necessity for lactic acid for the obtainment of rigor. According to Needham (1971) researchers failed to recognize the implications of Bernard's observation and as such considerable time passed with the lactic acid theory still tenable. This is readily apparent in the report by Moran and Smith (1929) who attributed rigor to coagulation of the muscle proteins. Clarification of the enigmas of rigor mortis appeared to be related to the interpretation and elucidation of the biochemical energy pathways in muscle. The discovery of creatine phosphate (CP) in 1929 was apparently unnoticed in its association with rigor, however, the discovery and demonstration of the ATPase activity of myosin accelerated the work on contraction and rigor (Needham, 1971). Additionally, the discovery (Erdos, 1942) that a close association existed between the rigidity of muscle during rigor and the disappearance of ATP prompted a more thorough investigation of the adenosine triphosphate (ATP) concept (Needham, 1971).

Although earlier workers had reported the association of ATP and rigor, Bate-Smith and Bendall (1947) confirmed and extended this concept. Additionally they determined that animals with low glycogen reserves followed the same pattern except that the pH did not fall to normal levels. Earlier, Bate-Smith (1939) found a rapid change in the modulus of elasticity at pH 6.2. Bate-Smith and Bendall (1947) observed the same occurrence, but they considered that some other mechanism must be involved since a rapid "alkaline rigor" could occur. In a subsequent paper, Bate-Smith and Bendall (1949) reported the effect of animal condition on the onset of rigor, duration of rigor delay, initial pH and ultimate pH. They suggested that the struggle of an animal at death (as verified by muscle relaxants) affected the initial pH, but the glycogen reserves were the only factor that affected the ultimate pH. Bate-Smith (1948) in a classical review on rigor mortis discussed the overall knowledge of rigor mortis which included pH, ATP and CP, however, it was 3 years later before Bendall (1951) reported the precise relationship between CP, ATP and pH. He suggested that no ATP was broken down until approximately 70% of the CP had disappeared. All of this agrees with the observations by Bate-Smith and Bendall (1947, 1949) on the condition of the animal at slaughter. Well rested animals not only have considerable quantities of glycogen reserves, but also the CP reserve is adequate to maintain the ATP supply until it is almost exhausted. Additionally, Bendall (1951) suggested that the shortening observed during rigor, even though differing greatly in time sequence, must be similar in mechanism to physiological contraction.

Marsh (1952a, 1954) extended the rigor mortis and ATP dephosphorylation concept to bovine and whale muscle. Although the investigation of postmortem whale muscle extended and verified earlier observations on other species, it also provided information on previously undescribed states of rigor. Marsh (1952a) reported that fresh whale muscle existed either in a dry and firm, wet and dull or dry, hard and rubbery state. He found an important relationship between pH, ATP dephosphorylation and the physical state of the muscle. If the pH was greater than 6.3, the muscle appeared dry and firm, however, at pH values below 6.1, the muscle was invariably wet and dull. Within the transitional range of pH 6.1 to 6.3 a mixture of wet and dry muscles were observed. In concluding the study on whale muscle, Marsh (1952a) suggested that the transition from the dry to wet states represented the onset of rigor mortis. His observations on beef muscle verified the earlier observations on rabbit muscle (Bate-Smith and Bendall, 1947, 1949). Certain variations in temperature effects were observed between rabbit and bovine muscle but the dependence of rigor onset on ATP dephosphorylation unequivocably demonstrated that ATP content dictated the time course of rigor mortis. Marsh (1954) also reported the verification of the report by Bendall (1951) that shortening during rigor was essentially a slow and irreversible physiological contraction. He suggested that the resolution of rigor was in no way a reversal of the inextensibility obtained during rigor since the modulus of elasticity did not change after 7 days of storage at 7 C in a nitrogen atmosphere.

Since the shortening aspect of rigor mortis has been suggested to be essentially the same as contraction except for the time sequence (Bendall, 1951), the accumulation of information concerning contraction certainly should give an insight to the mechanism of rigor shortening. Marsh (1952b) discovered the method of how muscle maintained the relaxed state without splitting ATP. The presence of a relaxing factor in the supernatant and additional experimental evidence allowed him to speculate that the relaxing property was due to the binding of Ca^{2+} ions. Porter and Palade (1957) described a three-dimensional membrane and tubular structure surrounding muscle which has been implicated in conduction of nerve impulses resulting in Ca^{2+} release (Huxley, 1965). Subsequent work has verified and extended this concept and showed that the relaxing factor is particulate in nature and is composed of components of the sarcoplasmic reticulum (SR) (Marsh, 1966). The clarification of the role of the SR in contraction and the suggestion that rigor shortening is essentially the same as contraction (Bendall, 1951) has led to the development of the theory that the increase in free sarcoplasm Ca^{2+} causes rigor shortening (Schmidt, Cassens and Briskey, 1970). Goll et al. (1971b) proposed that the loss of Ca^{2+} accumulating ability was the direct and immediate cause of rigor onset. They also proposed that limited proteolysis of the SR might be the direct cause of the loss of Ca^{2+} accumulating ability and the subsequent onset of rigor mortis. Needham (1971) succinctly summarized the sequence in the development of rigor mortis. The loss of circulation prevents

oxidative phosphorylation which necessitates the replenishment of ATP by PC and glycolysis. The usage of glycogen stores for anaerobic glycolysis causes the production of lactic acid which accumulates during the delay period. The pH drop is dependent on the quantity of glycogen reserves with exhausted animals showing little or no pH fall. Rapid ATP hydrolysis ensues when a critical pH is reached and the actomyosin ATPase appears to be activated when the Ca^{2+} pump fails to maintain the concentration gradient. The actomyosin ATPase initiates hydrolysis if ATP is available and fiber contraction may occur. The muscle fibers become inextensible when ATP is unavailable for plasticizing myosin and actin. Certain modifications of the sequence of events are seen if elevated temperatures are used or if insulin and muscle relaxants alter the normal situation.

Structural Changes

Before the introduction of such sophisticated research tools as the electron microscope, it had been difficult to explain physical measurements taken on prerigor and rigor muscle (Bendall, 1960). The increased knowledge of the muscle proteins and the knowledge that actin and myosin will combine when ATP is absent led to a clarification of the contractile mechanism (Szent-Gyorgyi, 1944). The explanation of the mechanism of shortening by the sliding of thin and thick filaments past each other contributed substantially to the interpretation of contraction and rigor (Huxley, 1958). Earlier structural models were unable to account for

certain observations made on muscle, however, the Hanson and Huxley model of filament sliding fulfilled nearly all the requirements necessary to explain rigor mortis changes (Bendall, 1960). Earlier proposals that suggested similarity between physiological contraction and rigor shortening seemed to fit nicely into the sliding filament hypothesis. The isolation and characterization of several new proteins of the myofibril (Bailey, 1948; Ebashi and Ebashi, 1964; Ebashi and Kodama, 1966) has more definitively defined the contractile process whether it be physiological or postmortem. The previously mentioned relaxing factor described by Marsh (1952b) and the regulatory proteins discovered by Ebashi and coworkers (1964, 1966) in addition to the biochemical explanation of rigor mortis by a host of workers (Bate-Smith, 1939; Bate-Smith and Bendall, 1947, 1949; Bendall, 1951) has allowed considerable insight into mechanisms which govern muscle activity.

Although this description of rigor mortis is at best superficial, it does report the empirical observations necessary to account for most of the changes responsible for its development. The interpretation of postrigor changes which are responsible for the observed tenderness changes are no less difficult than that encountered in explaining the onset of rigor mortis. A detailed account of these postrigor changes implicated as tenderness factors follows under the title of "resolution of rigor mortis."

Postmortem Shortening of Myofibrils

Factors Affecting Shortening

Rigor Shortening. The observation that muscle shortens as it passes into rigor has long been known, but one of the earliest observations on rigor shortening in meat producing animals was reported by Bate-Smith (1939). He reported that rigor shortening appeared to be an unusual situation rather than a normal occurrence. He suggested that shortening appeared to be related to a rapid onset of rigor. Bate-Smith and Bendall (1947) concluded that shortening occurred only when the stiffening of rigor was associated with a pH higher than 6.2 and is not a normal concomitancy of rigor. Bate-Smith and Bendall (1949) investigated the time course of rigor and reported a temperature dependent relationship for rigor shortening. Bendall (1951) suggested that rigor shortening, although involving a different time sequence, was similar to physiological contraction in that the contractile elements were involved in both cases. Marsh (1954) reported results that supported the observation by Bendall (1951) that rigor shortening was similar to physiological contraction. In addition, he also suggested that this shortening was a slow and irreversible contraction.

All of the early work reported by Bate-Smith (1939), Bendall (1951) and co-workers defined all aspects of rigor mortis including shortening based on isotonic measurements. Jungk et al. (1967), Goll (1968) and Busch et al. (1972a) developed an isometer that measured tension produced

by postmortem muscle and this apparatus definitively showed that muscle shortened during rigor onset. The overall implication of this type of measurement will be discussed in the section concerning rigor resolution, however, at this point it is sufficient to note that shortening, irrespective of amount, plays an important role in the overall concept of rigor mortis.

Temperature Effects. Although the discovery of cold shortening has been attributed to Locker and Hagyard (1963), the foundation for this work has a much longer history. Although Lowe and Stewart (1946) did not associate their observed toughness of prerigor excised chicken breast muscle with myofibril shortening, it was one of the earliest observations of this phenomena. Bate-Smith and Bendall (1949) and Bendall (1951) reported that muscle stored at 17 C shortened considerably less than muscle stored at 37 centigrade. Thus the early work implied a linear association between storage temperature and muscle shortening.

Locker and Hagyard (1963) investigated the effects of temperature on shortening using a range of temperatures between 0 and 37 centigrade. They observed that maximum shortening occurred at 0 C and a minimum was reached between 14 to 19 centigrade. Shortening increased between 19 and 37 C although the extent of shortening at 37 C was not as great as that observed at 0 centigrade. The authors commented on the complexity of this enigma by reporting that rabbit muscle did not cold shorten. Jungk et al. (1967) and Henderson, Goll and Stromer (1970) extended the cold shortening phenomena by clearly demonstrating that cold shortening was a temperature and

species dependent phenomenon. They reported the similar minimum and maximum temperature for bovine muscle as reported by Locker and Hagyard (1963). Henderson et al. (1970) reported that rabbit muscle shortened, however, the maximum occurred at 37 C and shortening was minimal at 0 to 2 centigrade. Rabbit muscle shortened slightly between 2 to 16 C, remained constant between 16 to 25 C and then shortened dramatically above 25 C and reached a maximum at 37 centigrade. Henderson et al. (1970) also reported that the shortening of porcine muscle was temperature dependent. Temperature dependent shortening of porcine muscle was reported to be intermediate compared to that of bovine and rabbit muscle but more nearly approximating rabbit muscle. Porcine muscle shortened more at 2 C than 16 or 25 C, however, the maximum occurred at 37 centigrade. Busch et al. (1972a) verified and extended all the previous reports on temperature dependent muscle shortening.

Prerigor Muscle Excision. Locker and Hagyard (1963) reported that the stimulus of excision caused a small quantity of the contraction ultimately obtained. The overall implications of prerigor excision is its effect on tenderness which will be discussed subsequently.

Effect of Shortening on Tenderness. As mentioned previously, Lowe and Stewart (1946) observed that prerigor excised breast muscle from chicken was considerably tougher than muscle that was left attached to the skeleton. Although these authors did not suggest that shortening was responsible for the tenderness differences, it was one of the earlier reports that described a tenderness relationship between excised and non-

excised samples. Ramsbottom and Strandine (1949) removed the longissimus from beef carcasses before chilling and reported that these samples were considerably less tender than paired non-excised muscles even after 12 days of storage. The authors attributed the tenderness differences to the change in the chemical and physical state of the muscle possibly due to the stimulation of muscle and nerve cells by cutting.

The classical papers in the literature concerning shortening and tenderness are those of Locker (1960) and Locker and Hagyard (1963). In the initial paper, Locker suggested that the muscles of the ox go into rigor in various states of contraction. Locker (1960) suggested that the restraint a muscle has imposed on it as a result of hanging the carcass determines the final contractile condition of each muscle. The removal of a muscle either completely or by severing one attachment allows shortening to occur, and more importantly, the shortened muscle is tougher than a less contracted muscle. In the subsequent paper, Locker and Hagyard (1963) expanded the original observation to include temperature dependent shortening. The latter observation becomes important since in situ muscles exposed to low temperatures may shorten and as a result be tough. Goll et al. (1964a) verified the tenderness differences between excised and non-excised muscles and concluded that aging improved the tenderness of excised muscles but they were still less tender than controls after 312 hours.

In a series of papers, Herring et al. (1965a, b, 1967b) investigated the influence of sarcomere length on the organoleptic qualities of bovine muscle. They found that prerigor excised muscle was considerably tougher

than prerigor excised but stretched-restrained muscle. This phenomenon existed for both psoas major and semitendinosus muscles which were considerably divergent in initial sarcomere length and original shear scores. These authors concluded that it was more important to prevent shortening than to stretch the muscle. Additionally, in light of previous observations on fiber diameter (Hiner et al., 1953; Tuma et al., 1962), Herring et al. (1965b) found a positive relationship between shear force and fiber diameter. However, the relationship became complicated since they were unable to ascertain the contribution of shortening to fiber diameter versus that due to inherent differences in diameter of the fibers.

Gothard et al. (1966) measured sarcomere lengths of semimembranosus and longissimus muscles at slaughter through 7 days of aging. They found considerable shortening in both muscles, and the results suggested a strong association between final contractile state and tenderness. They also reported the tendency for sarcomeres to lengthen once maximum contraction was obtained. These authors concluded that final contractile state played a role in overall tenderness although it apparently was not the major contributing factor.

Cook and Langsworth (1966a, b) reported that several pre- and postmortem treatments affected both the shortening and shear values of ovine muscle. They found cold shortening to occur maximally at 0 C, a minimum occurred at 5 C followed by an insignificant rise at 20 C and another maximum at 40 C that approached the overall maximum that appeared at 0 centigrade. The shear values did not correspond to the amount of shortening in all cases. Maximum shear values were obtained from the 0 C samples,

however, the minimum value was obtained from the 40 C samples which were shortened to approximately the same degree as those at 0 centigrade. Marsh and Leet (1966) reported results that conflicted somewhat with Cook and Langsworth (1966a, b). Marsh and Leet (1966) reported shortening to highly influence shear values, however, certain limits were defined that govern the amount of shear resistance of a sample. Up to 20% shortening did not appear to affect shear resistance, however, shear values increased from a low at 20% shortening to a maximum at 40 percent. Between 40 to 60% shortening, shear values began to decrease and at 60% the amount of shear resistance approached the minimum obtained at 20 percent. Although all measurements were obtained from excised muscles, Marsh and Leet (1966) suggested that certain muscles in the carcass such as the longissimus can shorten in situ due to lack of bony attachments on both ends of the fibers. Indeed, Marsh, Woodhams and Leet (1968) found that ovine longissimus in situ increased significantly in toughness if exposed to low temperatures within 16 hr. of slaughter. These authors suggested that the toughness was probably due to the shortening of the muscle fibers as previously shown to occur in excised muscle strips (Marsh and Leet, 1966). Parrish et al. (1969b) reported work on Choice beef carcasses that contradicted the observations of Marsh et al. (1968) on lamb carcasses. Several different temperature combinations were used to allow the phenomenon of cold shortening to manifest itself if indeed it was a problem of in situ muscles. These authors found that shear values were essentially the same for all time-temperature treatments. Thus this work suggested that

2 or 15 C temperatures did not influence the tenderness of bovine muscle that remained attached to the carcass. Parrish et al. (1969b) suggested that the rate of cooling could have been attenuated enough by the larger muscle mass and fat covering of the beef carcass which prevented fiber shortening.

Davey, Kuttel and Gilbert (1967a) investigated the relationship between cold shortening and meat aging. They found a similar association between the percentage of shortening and tenderness as reported by Marsh and Leet (1966). In addition, they reported that aging (shear decrease) was dramatically affected by the extent of shortening. Below 20% shortening, shear values were lowest and the effects of aging were maximized, however, beyond 20% shortening, the effects of aging decreased and they were minimal at 40% shortening. They surmised that at 40% shortening, or peak toughness, the sarcomere had decreased in length to the point that no I band was visible, i.e., the sarcomere was the same length as the A band (1.5 μm).

Jungk et al. (1967), utilizing isometric tension measurements, suggested that the increase and decrease in tension postmortem, probably corresponds to similar phases of decreasing and increasing tenderness. However, Busch et al. (1967) reported that isometric tension measurements are not necessarily a valid method for determination of shear values. They concluded similarly to previous reports (Gothard et al., 1966; Marsh and Leet, 1966) that shortening contributes to muscle tenderness, but probably is not the main contributor.

Buck et al. (1970) determined the force necessary to shear rabbit muscle that was excised and allowed to shorten and on a similar sample that was stretched and both stored at 1 to 2 C for 24 hours. The muscle that was allowed to shorten required more force to shear than the stretched sample in all cases. Bouton et al. (1973b)reported similar results using bovine muscle. The contractile state influenced the shear values significantly, and in addition these authors found an interaction between pH and sarcomere length.

Most of the evidence overwhelmingly suggests that the contractile state of a muscle influences the eating quality of the final cooked product. Voyle (1969) recognized the amount of literature that supports the phenomena of toughening and shortening; however, he considered the situation to be considerably more complex than a mere shortening of the sarcomere. Additionally, he suggested that the compression of the myosin filaments against the Z-discs might be the most important aspect of muscle shortening.

Weidemann, Kaess and Carruthers (1967) reported that prerigor unrestrained (cooked without restraint) bovine semitendinosus muscle was always tender, however, prerigor muscle that was restrained during cooking was always tough. Muscle stored unrestrained at 0 C remained tough even during 1 to 5 days storage. Stretched muscle underwent tenderization during one day of storage and remained tender throughout storage. Klose, Luyet and Menz (1970) utilized prerigor restrained and contracted chicken muscle to determine if shear values were related to the contractile condition. They reported that the restrained samples had shear values

approximately twice as large as the contracted muscle samples. The authors concluded that the difference between samples could be obviated if the shear values were reported on a per filament basis.

Hegarty and Allen (1972) stretched rigor turkey leg muscle and compared the shear values to a paired group of folded muscles on the opposite leg. Unexpectedly, the authors reported a significantly lower shear value for the folded muscles versus the stretched muscles even though before cooking the stretched muscles had a significantly greater sarcomere length. The authors could not explain these discrepancies of their data in light of those in the literature.

Considering all the reports involving the effect of cold shortening on tenderness, Schmidt and Gilbert (1970) and Parrish et al. (1973) stated that they could possibly circumvent this problem. Schmidt and Gilbert (1970) reported that muscles excised from prerigor beef carcasses could be maintained in a tender state if stored for 24 hr. at 15 centigrade. An additional 24 hr. of storage produced a considerable aging effect in several of the excised muscles. Parrish et al. (1973) examined both prerigor excised muscle stored at 2 and 16 C and similar samples that were left in situ at the same temperature. They found that one day of aging at 16 C vastly improved the tenderness of bovine longissimus muscle. These authors suggested that 2 C chilling of muscles allowed to remain on the carcass apparently was not a deterrent to tenderness possibly due to the slowed rate of cooling as effected by muscle mass and fat cover. Smith, Arango and Carpenter (1971) reported similar results in that bovine

longissimus muscle increased significantly in tenderness by holding carcasses at 16 C for 16 hr. postmortem and then placed in a 2 C cooler. McCrae et al. (1971) found similar relationships when they reported that a 16 hr. delay at 18 C before freezing lamb carcasses considerably improved tenderness of several muscles.

The original work on cold shortening (Locker and Hagyard, 1963) certainly pointed out the hazard involved with exposing excised muscles to near freezing temperatures. Subsequent work has verified this early observation, however, others have failed to find a one to one association between shortening and tenderness. Some researchers have suggested that cold shortening probably doesn't affect those muscles normally restrained on the carcass. Marsh (1972) suggested that shortening in situ must be considered on an individual muscle basis since carcass restraint will minimize shortening in some but will not prevent it in others.

Restraints on Shortening. Marsh and Thompson (1958) observed that muscle which was restrained during rigor did not shorten once the restraint was removed. Locker (1960) suggested that the final contraction state of a muscle depended on the restraint put on it in the hung carcass and it could be modified by excision or partial detachment.

Herring et al. (1965a) investigated muscle shortening and alteration of the normal carcass suspension method. The right sides of fraternal twins were hung normally by the achilles tendon and the left sides were placed horizontally, bone down, on a flat surface. The limbs of the left side were tied perpendicular to the long axis which approximated the

standing position. The vertically suspended carcasses had sarcomere lengths ranging from 1.8 μm to 3.6 μm with the horizontal carcasses varying from 2.0 μm to 2.7 μm. They suggested that the long sarcomeres of a few muscles from the vertically suspended sides in particular the psoas major could be attributed to stretching. Due to the anatomy of the carcass, other muscles were not under tension and could shorten appreciably. Horizontal suspension prevented several muscles from shortening that were free to do so in vertically suspended sides and conversely several muscles had tension removed and could shorten. In view of earlier work (Locker, 1960) these authors found that shortened muscles had larger fiber diameters and higher shear values. It was suggested that practical application might be possible since horizontal placement improves the tenderness of several important muscles. Indeed, Eisenhut et al. (1965) reported that horizontal placement markedly changed fiber angles of the longissimus muscle relative to the spinous and transverse processes and the sarcomere length.

Hostetler et al. (1970) compared 5 muscles from vertically suspended (leg suspension) carcasses and carcasses suspended from the ischium (hip suspension). One muscle, triceps brachii (TB) (long head), from the thoracic limb, two from the lumbar region, longissimus (LD) and psoas major (PM), and two from the pelvic limb, semimembranosus (SM) and semitendinosus (ST) were studied. The SM, ST and LD had significantly greater sarcomere lengths due to the hip suspension method and the SM and LD had lower shear scores and higher taste panel scores. The ST shear and taste panel scores were not significantly different from leg suspended carcasses.

The PM had shorter sarcomeres and higher but nonsignificant shear values. The TB was not significantly different for sarcomere length, taste panel or shear scores. Hostetler et al. (1972) extended these observations to include modifications of the hip suspension (hip tied) and new methods, neck tied and horizontal. The observations revealed that the hip free method as used previously was the most beneficial in improving tenderness of the major muscles and in particular the muscles of the loin and round. Bouton et al. (1973a) used some variation of the techniques reported by Hostetler et al. (1972), however, they also concluded that the aitch bone suspension (hip free) yielded shear values at 2 to 3 days postmortem that were comparable to muscles aged 2 to 3 weeks in the carcass form suspended by the achilles tendon. Similar conclusions were made by Quarrier et al. (1972) and Bouton and Harris (1972) concerning ovine muscles.

Again with the phenomena of shortening and sarcomere length as with connective tissue proteins and all other tenderness associated phenomena, there appears to be no unanimity among the researchers in the field as to the contribution of these factors to tenderness. Although conclusive evidence has been reported by numerous reseachers concerning sarcomere length and tenderness, Hostetler et al. (1972) reported that sarcomere length could account for only 12% of the variation in tenderness among animals. So it appears that shortening and sarcomere length appear along with the other variables in the complex equation involving tenderness and not as the only variable.

Resolution of Rigor Mortis

Concept of Rigor Resolution

The usage of "rigor resolution" has been loosely implied to describe the postrigor changes in muscle which apparently influences the eating quality of meat. Prior to the work of Bendall (1951) and Bate-Smith (1939) at Cambridge and Marsh (1954) in New Zealand, it was assumed that tenderness increases were in some way related to a reversal of the events causing the stiffness of muscle. The observations by Moran and Smith (1929) attributed the onset of rigor mortis to protein coagulation and the subsequent "resolution of rigor mortis" to the change of the proteins back to a soluble form. However, Bate-Smith (1939) and Bendall (1951) began using the measurement of extensibility to define phases of rigor mortis. This type of observation allowed them to conclude that little or no change or "resolution of rigor" occurred as could be measured isotonically. Accordingly, the elucidation of many of the secrets of the contractile process has contributed to the apparent demise of the concept in its literal interpretation. The argument for nonexistence of resolution has firm roots both biochemically and physiologically. The concept of proteolysis doesn't stem necessarily from the philosophy of rigor resolution, however, the maintenance and proliferation of this concept is based considerably on the feeling that "rigor resolution" does not occur in the form of a reversal of rigor onset. Even though the majority of reported data do not support the possibility of proteolysis occurring

(Locker, 1960), as measured by conventional methods, the concept has been attractive to help explain the increased tenderness apparent in aged muscle. Increased extensibility during postrigor storage has been ruled out by the observations of Bate-Smith (1939), Bendall (1951) and Marsh (1954) who could not find any evidence of changes during storage. Many other reports support the theory that inextensibility is maintained during aging and that proteolysis of the myofibrillar proteins or alterations in stroma proteins as measured by changes in content or presence of degraded products apparently cannot be found, at least with the present level of instrumental sophistication.

Goll and co-workers (1968) have added a new technique to postmortem muscle measurements that apparently does not contradict the originally defined philosophy of rigor resolution. Jungk et al. (1967) developed an isometer in an effort to describe rigor and postrigor changes in terms of isometric tension. The observations were made using both rabbit and bovine skeletal muscle. Temperature played a role in the time of onset and tension developed, however, in bovine muscle isometric tension developed at all temperatures and reached a peak which subsequently declined to levels much lower than the maximum levels reached. Goll (1968) redefined "resolution of rigor mortis" in terms of isometric tension which did not refute earlier insistence that rigor resolution was non-existent. He divided rigor mortis into a shortening or contraction aspect and loss of extensibility. The extensibility aspect was divided into a macroscopic and molecular phase to facilitate the extension of the concept of "resolution of rigor mortis". The macroscopic aspect of extensibility is manifested as

inextensibility as measured and reported by Bate-Smith and Bendall (1949). The molecular extensibility occurs at the sarcomere level when myosin and actin combine and are unable to freely slide past each other as is apparent in physiologically contracted muscle. Goll (1968) attributed macroscopic inextensibility to be the result of numerous sarcomeres having lost molecular extensibility. The stiffness or rigidity aspect of rigor mortis, although not necessarily a prerequisite for rigor, was a result of the shortening or contraction phase mentioned previously. All the aspects of rigor were directly influenced by the ATP loss during rigor development.

The presence of tension development in postmortem muscle in itself is not surprising since some shortening has been observed by Bendall (1951) and by various other individuals. The point of most interest is the observed inability of a muscle to maintain the isometric tension obtained at the height of rigor mortis (Jungk et al., 1967). Goll (1968) suggested that the inability of muscle to maintain isometric tension corresponded to "resolution of rigor mortis." Additionally, he attributed isometric tension loss to inability of the muscle to maintain the contracted or shortened state. The concept of rigor resolution appears to be a viable topic when considered from the perspective presented by Goll (1968). The validation of the concept rests on determining the causes and effects of rigor resolution which will be presented by discussing the various instruments and criteria that are utilized to visualize postmortem muscle changes.

Rigor Resolution and Tenderness

The overall concept and definition of "rigor resolution" is dependent totally on the methodology used for measurement. As alluded to previously, Bendall (1951) and others have decried usage of the term "rigor resolution" since it implicitly requires that rigor onset must be a reversible reaction. Marsh (1952b) had shown previously that muscle stored in an inert atmosphere does not become freely extensible as seen for prerigor muscle.

The concept of rigor resolution has been reported earlier in this review only as a definition by Goll (1968). The overall implication is not its existence since by definition it has been shown to exist (Goll, 1968), but what affect it has on muscle structure and tenderness. Since the dimensions of rigor resolution are microscopic, measurements of this change must, at best, be indirect and to be reported later, alteration of ATPase activities, supercontraction, sulfhydryl content and histological evidence are used to describe these changes.

As with its inception, by far the majority of the work on rigor resolution has emanated from the laboratories of Goll (1968) and co-workers. The association of tenderness and rigor resolution has been an inference from this group and their concepts will be the basis for the review of this topic.

Goll (1968) and Goll et al. (1971b) have proposed two main causes that might explain the inability of postmortem muscle to maintain isometric tension. Criteria used to characterize the loss of isometric tension are a modification of the actin-myosin interaction measured as a change in the

nucleoside triphosphatase (NTPase) activity, in vitro contractile activity (superprecipitation) modification of actomyosin, lengthening of rigor-shortened sarcomeres and modification of dissociability of actomyosin by ATP and secondly, the loss of the integrity of the Z-disk resulting in fragmentation.

Goll (1971b) based much of the causes of rigor resolution on the information gained from observing the characteristics of trypsin treated muscle. Not only will trypsin initiate rigor onset, it also produces similar changes in NTPase activities as seen during postmortem storage and it induces the normally seen reduction in time for turbidity response. Additionally, trypsin lengthens rigor shortened sarcomeres similar to that described for postrigor muscle (Gothard et al., 1966)

Trypsin also selectively degrades the Z-line of muscle which has been implicated by several researchers as a primary characteristic of aged muscle. Although earlier workers (Davey and Gilbert, 1969; Davey and Dickson, 1970) have reported fiber breakage at the I-Z junction more frequently than mere loss of fibrillar structure, the effects of trypsin on this particular loss of Z-disk continuity has been left unexplained.

The interrelationship of the above phenomena and tenderness merely shows that during the aging or postrigor period many of these conditions accompany the simultaneous increase in tenderness. Based on previous observations that sarcomere length and tenderness are associated (Marsh, 1972) and if the modification of the actin-myosin interaction does result in a slippage or lengthening of the sarcomere (Goll, 1968) the association

with tenderness is more than mere conjecture. The weakening and degradation of the Z-disk appears to have strong theoretical grounds for diminishing shear values, however, explicit data supporting this concept have not been reported.

In summary, the character of postmortem muscle has been measured innumerable ways and many of these characteristics appear to have an intimate association with tenderness. However, the contribution of each component to tenderness still is unresolved and if a "most important component" does exist it has not been elucidated with respect to the myofibrillar proteins during postmortem aging.

Morphological Changes

Z-line Structure

Most of the early workers studying the histological changes associated with postmortem storage reported that some degree of fiber breakage occurred and apparently in the region of the I-Z junction (Hanson et al., 1942; Paul et al., 1944). The Z-line has been recognized as a distinctive component of the sarcomere for many years, however, its structure and composition has been and still is somewhat unclear.

Knappeis and Carlsen (1962) suggested that the actin (I) filaments terminated on each side of the Z-line as rod-like projections. The thin filaments as such do not extend through the Z-line, but one I filament is positioned between two I filaments from the adjacent sarcomere. This pattern in effect imparts a zig-zag appearance to the Z-band.

Other structures have been suggested to account for the morphology of the Z-band, however, they do not explain the ultrastructural properties and biochemical data. Kelly (1966) investigating desmosomes and related structures found that intracellular filaments which attach at the desmosome sites actually loop past the area of attachment and return into the cell interior. Kelly (1967) proposed two models based upon electron microscopic observations of skeletal muscle Z-disks. In the first model, the actin double helix separates into its two component strands adjacent to the Z-line and each of the separated strands enters into the Z-band and loops over an actin filament from the adjacent sarcomere. After looping over, the actin filament returns to its original sarcomere and becomes incorporated into a double helix of an adjacent actin filament. Certain inconsistencies with known biochemical data exist for this model, however, Kelly (1967) in describing the second model suggested that the filament looping through the Z-band could be the tropomyosin molecule which would follow the same pattern as suggested for the actin filament. Rowe (1971) expanded Kellys (1967) observations to include a model that would satisfy certain inconsistencies with reported lattice dimensions. Rowe (1973) included the looping filament model to explain Z-disk dimension differences observed for the red, white and intermediate fibers with a modification of the looping filament model.

Certainly the looping filament model doesn't account for all the known observations either biochemically or ultrastructurally and the report that α-actinin resides in the Z-line (Goll et al., 1969) raises the question as

to its role in the looping filament model. Whatever the discrepancies, the looping filament model fulfills many of the ultrastructural observations on skeletal muscle and as additional information concerning the Z-disk becomes available the model of the actual Z-line ultrastructure will become more definitive.

In an earlier section of this review, Hanson et al. (1942), Paul et al. (1944), Ramsbottom and Strandine (1949) and others reported that postmortem aging was accompanied by fiber splitting and transverse breakage. Sharp (1963) found that storage at 5 C for 19 days produced fibers that broke transversely and eventually produced short sections of fibrils. Paul (1963) described the effect of heating on fiber breaks as possibly occurring at the angles of the Z-Z contractions and these breaks apparently increase in number with heating. This implies an inherent weakness at this point in the myofibril which is expressed upon heating. Cook and Wright (1966) came to similar conclusions when they suggested that the Z-line may be the first structural component degraded by heat treatment. In a subsequent paper, Paul (1965) observed fiber breakage beginning in the I band which she suggested was a greater ability for the actin filament to break as compared to the thick filament. Gothard et al. (1966) attempted to section samples that were aged 6 to 7 days and generally the fibers fragmented very easily. The fibers were reported to always shatter at the level of the I band. This was interpreted as a possible degradation of the protein actin as the result of postmortem aging.

Fukazawa and Yasui (1967) and Takahashi, Fukazawa and Yasui (1967) studied the change in the zig-zag configuration of the Z-line and fragmentation

of myofibrils of chicken pectoral muscle during postmortem storage. They determined that myofibrils isolated immediately postmortem were in the form of intact myofibrils, however, as aging progressed the isolated myofibrils were obtained in progressively smaller fragments. Accordingly, they attributed the fragmentation to either a Z-line loss or a disruption of the bands between tropomyosin and actin. Fukazawa et al. (1969) recognized 2 types of Z-line distruction when 24 hr. stored chicken pectoral muscle samples were blendorized. First, the Z-line was degraded or disappeared completely and secondly, the sarcomeres broke at the Z-line and I-filament junction. Fukazawa et al. (1970) reported that protein extractability increased at the same time that the changes occurred in the Z-line.

Davey and Gilbert (1967b) found that bovine sternomadibularis muscle removed from the carcass underwent 2 primary changes during aging. First, they observed a complete loss of the Z-line and a lengthening of the A-band at the expense of the I-zone, and secondly, they suggested that the disintegration of the Z-line allowed the actin filaments to collapse onto the thick or myosin filaments. These changes parallel closely the objective and subjective increases in tenderness which occur during aging. In a subsequent paper, Davey and Gilbert (1969) found that aged myofibrils disrupted both laterally and transversely (Z-line dissolution) during brief homogenization as compared to the refractory unaged myofibrils. These authors also reported that the disappearance of Z-lines and loss of lateral association was completely inhibited by EDTA. Davey and Dickson (1970) extended the previous observations to include stretched and cold shortened

muscle. Ultrastructural observations of these preparations revealed fiber breakage when aged muscle was placed under light extension loads. The breaks were indicative of a weakness in the myofibril structures. Unaged meat only responded after much greater extension loads and generally by a withdrawal of thin filaments from beteen the thick filaments. The shortened fibers broke in the shortened I-zone, however, the slightly stretched myofibrils broke near the Z-disk. The authors concluded that the weakening of the fiber at the level of the I-filament-Z-disk junction contributed more to meat aging than the loss of the lateral linkages. Weidemann et al. (1967) concurred when they stated that the degree of tenderness was related to the degree of disruption of the myofilaments.

Sayre (1970) investigated the relationship between fragmentation and tenderness in chicken pectoralis major muscle. He observed that the myofibrils always broke in the I-band region and the A-band never broke which supports the early observations of Fukazawa, Hashimoto and Tonomura (1963). Sayre (1970) found that the Z-line didn't disappear during 24 hr. storage, but rather fragments of it were attached to the I-band and generally no I-filaments were found to protrude from the fragmented Z-line. This contradicts observations by Davey and Gilbert (1967b) that the Z-line completely disappears in 4-day aged bovine muscle. Sayre (1970) observed that fragmentation takes place at the same time as tenderization, however, he did not find evidence that would conclusively link fragmentation with tenderness.

Henderson et al. (1970) compared ultrastructural changes in bovine, porcine and rabbit muscle stored at 2, 16, 25 and 37 C for 4, 8 or 24 hr.

postmortem with at death muscle in each of the species. Z-line degradation occurred much sooner postmortem and to a greater extent when the incubation temperature was 25 or 37 C as compared to lower temperatures. Bovine muscle was reported to be considerably more resistant than rabbit or porcine muscle, however, many of the same changes were evident in bovine muscle if incubation time was extended. The M-lines of rabbit and porcine muscle were frequently lost at higher temperatures, however, after aging up to 24 hr. bovine M-lines were not degraded. Some fragmentation of myofibrils was observed to occur mainly at the level of the Z-line, however, no particular significance was attributed to this phenomenon (Henderson et al., 1970). Bovine muscle myofibrils exhibited little change during postmortem storage at 2 or 16 centigrade. It was suggested that Z-line degradation might be the result of disruption of the bond between the Z-line and the thin filament rather than a disruption of the Z-line itself. Parrish et al. (1973) concurred with the observation that Z-line fragmentation occurs primarily at or near the Z-line and that fragmentation seems to result in greater tenderness. Moller, Westergaard and Wismer-Pedersen (1973) reported that measurements of fragmentation occurring after homogenization of raw muscle tissue was an important measure of tenderness in heated bull longissimus muscle.

Several observations in the literature suggest the presence of endogenous proteolytic enzymes or other agents that control the degradation of the Z-line in postmortem muscle. Davey and Gilbert (1969), as reported earlier, were able to inhibit Z-line degradation with EDTA and Fukazawa et al. (1969) found the extraction of sarcoplasmic proteins from muscle to influence the

degree of fragmentation during storage. Using these observations and others concerning cathepsins and the observation that trypsin can remove the Z-line (Goll et al., 1971a) Busch et al. (1972b) isolated a sarcoplasmic factor that removed Z-lines exclusively from rabbit muscle. These authors reported that the sarcoplasmic factor was protein in nature and required Ca^{2+} for activation. Incubation of muscle samples with a Ca^{2+} chelator (EGTA) prevented Z-line removal. The isolation of this factor fulfills the search for a cathepsin or other enzyme that will effect degradation of Z-lines, however, greater than known physiological levels of Ca^{2+} are required for optimum activation. The clarification of the role of this component in postmortem muscle and its relationship to tenderness must be awaited, however, the implications are of major importance.

The alteration of skeletal muscle Z-lines has been demonstrated quite clearly, however, the existence of several fiber types differing not only morphologically (Gauthier, 1970) but also biochemically and physiologically (Brooke, 1970) suggests possible differing effects of postmortem storage. Few data have been reported supporting this concept, however, Goll et al. (1970) reported that the Z-line of red muscle fibers might be less labile than those of white muscle. The ultrastructure of chicken breast muscle has been reported previously, however, Hay et al. (1973a) reported that chicken leg muscle Z-lines were virtually resistant to degradation even after 168 hr. of storage, whereas, at the same time period, breast muscle Z-lines were virtually absent. The authors suggested that Z-line degradation might not totally reflect changes in tenderness in light of the red muscle Z-lines

not being degraded during postmortem storage. Dutson et al. (1974) reported data on normal and low quality porcine muscle that supports the observation that white muscle fiber Z-lines are more labile than red muscle fiber Z-lines.

ATPase Activity

Of the studies concerned with research on muscle, few can rival the discovery that myosin has the capability to hydrolyze the terminal phosphate from ATP. This remarkable discovery was first reported by Engelhardt and Lyubimova (1939). This report was subsequently verified by Needham (1942) and Bailey (1942). Bailey (1942) suggested that the enzyme was activated by Ca^{2+} and Mn^{2+} and that no inconsistencies were found to rule out the fact that the enzyme and myosin were one and the same entity. Some complications exist on interpretation of early work concerning activators of the enzyme since no information was available concerning the presence of other proteins in the system and much was done unknowingly on either myosin A or myosin B and sometimes both (Needham, 1971). Suffice it to say at this point that highly purified myosin A is activated by Ca^{2+} and inhibited by Mg^{2+} and myosin B is activated by Mg^{+2} and traces of Ca^{2+} (Gergely, 1970; Seidel, 1969a).

Early observations suggested that tenderization could proceed by dissociation into separate actin and myosin components, however, the extensibility concept virtually refutes this speculation (Bendall, 1960). Fujimaki et al. (1965a) utilized the previously mentioned properties of actin,

myosin and actomyosin to ascertain postmortem changes in the muscle proteins. By utilizing the activities of Ca^{2+} and Mg^{2+} on the ATPase properties of rabbit fibrillar proteins, they determined that postrigor muscle had 1.2 to 1.3 times the Mg^{2+}-activated ATPase activity of prerigor muscle. Aged muscle (7 days) Mg^{2+} ATPase activity decreased from postrigor values, but never below prerigor levels. The Ca^{2+} activated ATPase activity increased during rigor, however, the aged muscle activity decreased below that observed for prerigor muscle. They also found that sensitivity to dissociation increased as postmortem time increased. Other physicochemical measurements supported their suggestion that the contractile proteins and in particular the actin-myosin interaction was undergoing some modification during the transition from prerigor to aged condition. Okitani, Takagi and Fujimaki (1967) reported that the interaction between actin and myosin was weakened by storage at low temperatures, low pH or high ionic strength conditions. They speculated that myosin B became denatured either by spontaneous aggregation or irreversible dissociation into myosin A and actin. Scharpf, Marion and Forsythe (1966) reported some evidence utilizing gradient ultracentrifugation and Mg^{2+} and Ca^{2+}-modified ATPase activity that they considered as some evidence for dissociation of myosin B into its components actin and myosin.

In a report that bears some importance to the measurement of ATPase activity is that of Hayashi and Tonomura (1966) who found that ATPase activity was related to the sarcomere length of the muscle fibers. A peak of activity was noted at 2.57 μm with a fairly sharp drop of activity on each side of the maximum.

In a series of reports, Goll and Robson (1967), Robson, Goll and Main (1967), Chaudhry et al. (1969) and Arakawa, Goll and Temple (1970a, b) investigated the ATPase and ITPase activities of bovine and rabbit myofibrils and myosin B. The initial work involved activities of sucrose prepared myofibrils incubated at 2 and 16 C from 0 to 312 hr. postmortem. The Ca^{2+} and Mg^{2+} ATPase activities showed a variable response, however, at 24 hr. both values were 20 to 50% higher than at death values. Little change was observed in the EDTA-modified ATPase activity, but the EGTA-modified response increased slightly at 24 hours. All except the Mg^{2+}-modified enzyme remained higher than the 0 hr. samples. The ITPase activities were less responsive with the Mg^{2+}-modified enzyme showing some increase by 24 hr., but the Ca^{2+}-modified enzyme showed no response during postmortem storage. Some temperature and ionic strength interactions were involved, but at > 0.5 ionic strength the actomyosin complex appeared to be dissociated and only the myosin enzyme could be assayed. In a subsequent paper, Robson et al. (1967) utilized myosin B preparations to determine ATPase activities. Little difference could be found between at death preparation and samples stored at 2 or 16 C. It was hypothesized that possibly the lack of α-actinin might be responsible for the results. Chaudhry et al. (1969) reported a complete lack of Ca^{2+} and Mg^{2+} ATPase activity, but the authors suggested exhaustive dialysis or KI extraction as possible reasons rather than postmortem changes. Although the role of the minor protein components will be considered in depth later, Arakawa et al. (1970a) reported that α-actinin and the TM-TN complex are not the primary causative agents for the variable NTPase activities.

Penny (1967, 1968) investigated the solubility and ATPase activities of aged rabbit muscle and model systems to determine the mechanism of the reported ATPase fluctuations. He concluded in the initial report that loss of ATPase activity was primarily due to denaturation of fibrillar protein. The loss of the Ca^{2+}-activated enzyme occurred earlier since myosin not bound to actin is denatured easier than the Mg^{2+}-activated enzyme associated with the more resistant actomyosin. Penny (1967) also found a parallel between loss of extractability and ATPase activity and considered that uncoiling of the myosin molecule might be responsible. In the subsequent paper, he found no effects of aging on Ca^{2+}-activated ATPase activity, but the Mg^{2+}-activated enzyme showed a definite decline after 4 days at 15 to 18 and 4 centigrade. He reported that denaturation of myosin would be inconsistent with the Ca^{2+}-activated stability and concluded that the myosin-actin linkage must be weakened in some way.

Herring et al. (1969a, c) studied the physiochemical properties of natural actomyosin from tough and tender bovine longissimus aged 0 to 10 days. The highest ATPase values (1.1 to 1.2 times the 0 hour sample) were obtained from the 12 and 24 hr. aged samples. The Mg^{2+}- and Ca^{2+}-modified activities of both tough and tender samples responded similarly and as such little relationship between tenderness and ATPase activity was reported.

Yang, Okitani, Fujimaki (1970) and Okitani and Fujimaki (1970a, b) investigated the postmortem changes in rabbit myofibril and actomyosin ATPase activity. In the initial report, Yang et al. (1970) observed that the Mg^{2+}-activated ATPase activity increased

with increased aging time. At the same time, they found that aging increased the dependence of this activity on ionic strength. Due to some similarities between isolated actomyosin and 8 day aged myofibrils the authors suggested that Mg^{2+} ATPase activities appeared to be related to the structural continuity of the myofibril. The aging of muscle was reported to produce similar Mg^{2+}-activated ATPase activities as seen in high ionic strength extractions of muscle. Since aging has been reported to alter myofibril structure, the authors suggested an association between myofibril structural alterations and ATPase activities. In the second paper, Okitani and Fujimaki (1970a) reported that the loss in Ca^{2+}-enhanced ATPase activity when stored in 0.6 M KCl can be attributed to inactivation of myosin A ATPase activity whereas Mg^{2+}-enhanced ATPase (low ionic strength) activity loss was due to a loss of the activating ability of F-actin. In the subsequent paper, Okitani and Fujimaki (1970b) expanded on the loss of activating influence of myosin A and F-actin. They found that if the ATPase activity of myosin A was lost, it still retained the ability to combine with F-actin. However, if F-actin lost ATPase activating ability it also lost the ability to combine with myosin A. These same authors also concluded that the actin-myosin interaction became insensitive to dissociation by ATP during storage.

Earlier reports on ATPase activities of bovine and rabbit actomyosin showed an increase in both Ca^{2+}- and Mg^{2+}-modified ATPase activity during rigor mortis (Fujimaki et al., 1965a, b; Goll and Robson, 1967; Robson et al., 1967). Hay et al. (1972) and Jones (1972) reported that chicken muscle actomyosin ATPase activities responded somewhat differently than that reported for rabbit and bovine muscle. Hay et al. (1972) reported values for

chicken breast and leg muscle actomyosin and observed that Ca^{2+}-activated ATPase did not change from 0 to 168 hr. of storage except for chicken breast muscle actomyosin during rigor. The Mg^{2+}-modified ATPase activities increased during rigor and after 168 hr. the values were lower than rigor but not as low as 0 hr. samples. The Mg^{2+}-modified values paralleled those reported by Goll et al. (1971b) for bovine myofibrils, but the Ca^{2+} mediated values are considerably different than those reported by these same authors. Hay et al. (1972) suggested that the results might reflect the actin content of the actomyosin preparation. Jones (1972) used chicken breast actomyosin and reported values similar to Hay et al. (1972), however, he suggested that rather than containing less actin, the rigor actomyosin possibly contained more actin that was enzymatically inactive than that of 0 hr. samples. Jones (1972) additionally suggested that stored chicken actomyosin underwent some unknown modification of the actin-myosin interaction.

Most of the data reported to date involves changes in ATPase activities of postmortem muscle as related to the reported phenomena of "resolution of rigor mortis", however, few data relate these activities to actual sensory and objective evaluations of tenderness. Parrish et al. (1973) utilized Choice grade steers in an effort to interrelate most of the known measurements of postmortem muscle changes with both taste panel and Warner-Bratzler shear values. The carcasses utilized were stored at either 2 or 16 C and samples were obtained at 4 postmortem time periods varying from 0 hr. to 7 days. The Mg^{2+}, Ca^{2+} and EGTA-modified ATPase activities increased

with postmortem storage at 2 and 16 C with the 16 C values generally greater but not significantly greater. The association between observed ATPase values and tenderness was not discussed, however, the authors utilized these measurements in an effort to clarify the changes occurring in postmortem muscle. The relationship between the histological observations and tenderness will be included in a later section of this review.

In summary, the ATPase data reported are not consistent, however, the procedures used and techniques utilized were almost as varied as the results. The concept of muscle fiber type and ATPase activity was not included in this discussion. The differences in biochemical, physiological and histological structure has been well documented (Dubowitz, 1970; Gauthier, 1970); the overall implication of these differences was considered earlier. Generally, it can be concluded that the ATPase activities vary during postmortem storage which must reflect rigor and postrigor changes in the state of the muscle proteins. Even though the reported values vary with laboratories and experiments, it is important to use this type of technique in an effort to determine the molecular mechanism of the myofibrillar changes.

Regulatory Proteins

Superprecipitation

The phenomenon of superprecipitation of actomyosin was first reported by Szent-Gyorgyi (1944). Under *in vitro* situations of low ionic strength

which resembles that in living muscle, the interaction of actin and myosin forms a fine suspension which does not settle readily (Szent-Gyorgyi, 1947). In the presence of Ca^{2+}, Mg^{2+} and ATP, the fine suspension of the combined actin and myosin contracts with the comcomitant splitting of ATP (Briskey and Fukazawa, 1971). Szent-Gyorgyi (1947) described the transformation of the loose flocculi of the actomyosin in the gel state to that of a granular precipitate of reduced volume upon the addition of ATP. The use of the word superprecipitation was destined to separate it from low KCl precipitation and was classified as an in vitro model of contraction (Briskey and Fukazawa, 1971). The response of an in vitro model to ATP in the presence of Mg^{2+} and absence of Ca^{2+} has been called clearing which is analogous to in vivo relaxation (Spicer, 1952).

Since superprecipitation has been described as an in vitro model of muscle contraction, it has been used to describe various parameters of the contractile process. Difficulty in quantitatively measuring superprecipitation existed at first, but Ebashi (1961) gained considerable success by using turbidity changes as an index of superprecipitation. The scattering of incident light by actomyosin after ATP induced syneresis has been attributed to an increase in refractive index of the actomyosin gel which appears to more than compensate for the decreased volume reducing scattering (Endo, 1964).

Considerable work has been reported on the mechanism of superprecipitation (Weber and Winicur, 1961; Watanabe and Yasui, 1965; Yasui and Watanabe, 1965; Tada and Tonomura, 1966; Matsunaga and Noda, 1966; and Sekine and Yamaguchi, 1966), however, superprecipitation has also been used to determine

the effects of aging on the postmortem muscle system. Although some questions concerning the exact comparisons of superprecipitation to *in situ* contraction have been issued, the system has been used to characterize the changes in the postmortem muscle system as compared to at death muscle. This section of the review will be primarily concerned with the work reporting changes in the postmortem system that might clarify the mechanism of tenderization.

The recent discovery of the presence of several new proteins in the myofibril has certainly complicated measurement of postmortem protein alterations. The regulatory proteins account for 25% or less of the total myofibrillar protein, however, they exert profound influence on the total system. This regulatory influence coupled with superprecipitation has given the meat scientist a tool to gain insight into the molecular alterations.

Ebashi and Ebashi (1964) reported the isolation and characterization of a protein from muscle which they called α-actinin. The protein was isolated from native tropomyosin preparations and was found to markedly influence the superprecipitation of synthetic actomyosin. The authors reported that α-actinin accelerated the turbidity response of an actomyosin preparation. From additional research by Maruyama and Ebashi (1970), they determined that α-actinin was composed of a 6S and 10S component with the 6S component being the active part of the system.

It has been recognized for several years that myofibrillar ATPase activity increased after a short time period of storage postmortem (Fujimaki *et al.*, 1965a). Herring *et al.* (1969b) measured the turbidity changes in

postmortem bovine muscle to determine if differences between tender and tough muscles could be ascertained. Aged muscle (12 to 24 hr.) had an increased rate of turbidity rise when using natural actomyosin in 100 mM KCl. The authors suggested that 12 to 24 hr. postmortem muscle had a stronger actin-myosin interaction than prerigor samples. They also observed that when the assay was conducted in 100 mM KCl, 5 days aging was adequate to observe a decrease in rate of superprecipitation of tender muscles, however, tough muscles had to be aged 10 days to elicit a comparable change. The rate of turbidity response for tender and tough muscle in 50 mM KCl showed a more rapid response for tough muscle, however, in 100 mM KCl this relationship was reversed. The authors suggested that a possible interaction between ionic strength and α-actinin enhanced superprecipitation. Additionally, they surmised that a possible difference in either α-actinin or another regulatory protein existed between tough and tender muscle.

The earlier mentioned discovery of α-actinin and the previously described regulatory protein troponin by Ebashi and Ebashi (1964) led Arakawa et al. (1970a, b) to speculate that postmortem modification of either of these proteins could be responsible for the observed changes. They considered that proteolysis of troponin would in effect derepress the inhibitory activity that this protein exhibited towards the interaction of actin and myosin. α-actinin has been isolated from the Z-line (Goll et al., 1967) and they suggested that Z-line disintegration might release this protein to induce an increase in Mg^{2+}-modified ATPase activity (Arakawa et al., 1970c). By utilizing rabbit muscle stored at several temperatures and pH the authors prepared α-actinin and troponin and measured the response these

2 proteins had on superprecipitation. The authors concluded that even though postmortem storage appeared to decrease the time necessary to observe the turbidity response, all of the difference could not be attributed to α-actinin and troponin modification. Their results showed that α-actinin slowly lost its capability to accelerate turbidity and ATPase activity. This was certainly contrary to the results which would be necessary to account for the increased Mg^{2+}-modified ATPase activity and decreased time for turbidity to occur. The (TM-TN) system also appeared to have considerable resistance to proteolysis and appeared to not be involved in modifying the activities of postmortem muscle. Some change was noted in both systems, however, most could be attributed to abnormal pH and temperatures not generally encountered in normal postmortem muscle environment.

The use of superprecipitation as an indicator of molecular alterations has been demonstrated quite adequately and in particular has been a very essential tool to measure the modifications of some of the minor components of the myofibril.

Jones (1972) observed much the same modifications of the time course of superprecipitation in chicken muscle as that observed by Arakawa et al. (1970a) for rabbit muscle and suggested that some protein in the regulatory group was being modified with postmortem storage. Removal of low ionic strength proteins was accomplished by exhaustive dialysis and centrifugation, however, the results suggested that regulatory protein modification was not primarily responsible for the observed changes. Fukazawa et al. (1970) obtained somewhat different results from chicken pectoralis muscle which they suggested was the result of an increased release of α-actinin occurring in

conjunction with Z-line dissolution. These authors isolated a fraction (Fr.2) which originally contained superprecipitation depressing activity on trypsin-treated myosin B. The activity of Fr.2 decreased with postmortem storage and Fukazawa et al. (1970) concluded that it was troponin. Another fraction (Fr.1) was considered to be α-actinin, specifically the 10S or inactive component and not the active 6S component.

Other Indicators of Regulatory Changes

Penny (1970a, b) extracted the proteins from bovine longissimus muscle after aging for 8 and 15 days at 4 centigrade. He found no evidence that would suggest that any protein fraction had been degraded or disappeared during aging. However, his data suggested that the actin-α-actinin complex had been altered in some unknown manner during storage. In a subsequent paper, Penny (1972) prepared α-actinin from 7, 14 and 21 day aged bovine muscle. No quantitative differences were obtained as the result of aging, however, a small and insignificant effect on α-actinin binding properties was obtained. Penny (1972) concluded similarly to that of Arakawa et al. (1970a, b) that some small changes were occurring in properties of α-actinin, however, he felt that these changes were not primarily responsible for observed postmortem alterations.

Hay et al. (1973b) investigated the effects of aging chicken breast and leg muscle on the SDS disc gel electrophoresis patterns. The appearance and disappearance of several bands, particularly in the breast muscle, was considered to be rather consistent with ultrastructural observations on similar muscles (Hay et al., 1973a). Although several band changes were

discussed, the authors considered the disappearance of a 44,000 Dalton component and the appearance of a 30,000 Dalton component during aging to be the most important of the changes. It was suggested that the 44,000 Dalton component could possibly be the result of the disappearance of the M-line as reported earlier (Hay et al., 1973a). The leg muscle did not show a similar loss, however, the 0 hr. gels did not contain a band corresponding to 44,000 Daltons. The authors also reported the appearance of a 30,000 Dalton component which was apparent at 48 hr. in breast muscle, but did not appear until 168 hr. of aging in the leg muscle. Hay et al. (1973b) interpreted this to be either a degradation product of myosin or possible one from troponin-B although they considered the latter to be unlikely. Other aspects of the investigation support the contention that α-actinin and actin are not being degraded. These latter authors felt that since the myosin band was broad and relatively diffuse it probably masked subtle alterations if they occurred in this fraction.

The physiological and biochemical properties of slow (red) and fast (white) muscle have been known for a number of years (Dubowitz, 1970). Interspecies differences in ATPase activity and superprecipitation have been reported (Barany et al., 1967) along with subunit variations between red and white myosin (Locker and Hagyard, 1968). Suzuki et al. (1973) reported little apparent difference in the biochemical properties of α-actinin prepared from red and white portions of the semitendinosus muscle. The biochemical properties are divergent enough to possibly impart some importance to some of the parameters measured in muscles, however, the use

of mixed fiber muscles precludes accurate biochemical determination of the contributions of each type to the observations.

Sulflydryl Groups

The ATPase activity of myosin preparations is a universally known phenomenon. However, the molecular architecture of the active site has not been characterized as clearly, but it is known that myosin contains two sulfhydryl groups (SH) that affect the ATPase activity differently (Blum, 1962a). Blum (1962b) reported that the two SH groups can be blocked with N-ethylmaleimide(NEM), however, the amount of blocking action is dependent on the reaction time. One SH group can be blocked that activates Ca^{2+}-modified ATPase activity without inhibiting superprecipitation or the effect of relaxing factor grana. Increased reaction time will effectively block both ATPase activity and superprecipitation. Yamaguchi and Sekine (1966) called the SH group responsible for activating the Ca^{2+}-activated ATPase S1 and the group that inhibits ATPase activity S2. They also determined that the Ca^{2+}-activated ATPase SH group is present as one group per myosin subunit. Seidel (1969b) selectively blocked the slower reacting S2 group and determined that by blocking only S2 does not inhibit Ca^{2+} ATPase activity. He concluded that both groups lead to a conformational change in the regulatory site and it necessitates blockage of both groups to lose superprecipitation and ATPase activity. Daniel and Hartshorne (1972) reported that the SH groups that are responsible for Ca^{2+} sensitivity of natural actomyosin apparently are located on the heavy subunits of myosin

(subfragment 1). Other additional data suggested to them that the Ca^{2+} sensitivity of myofibrils may be affected more by the myosin molecule than earlier suspected. This could change the complexity of the relationship of the TM-TN complex to ATPase activity.

The measurement of postmortem changes in SH content in relation to tenderness has been rather limited. One of the earlier attempts was conducted by McCarthy and King (1942) in conjunction with work on the "Tenderay" process of aging beef. They reported that the number of titratable SH groups increased during aging at both normal cooler temperatures and at elevated "Tenderay" process temperatures. No definite conclusions were made except for the observation that the increase in tenderness was parallel by the increase in titratable SH groups.

Much of the work associating the role of SH groups with tenderization has been done with poultry muscle. Chajuss and Spencer (1962a, b) reported work that suggested that SH groups may play a role in the onset and "resolution of rigor mortis". They hypothesized that the formation, cleavage or reorientation of disulfide bonds may be intimately associated with the rigor state in muscle. The relaxation of the rigor state or "resolution of rigor" by an exchange reaction involving disulfide-sulfhydryl groups was a suggested mechanism. Gawronski, Spencer and Pubols (1967) concurred with this hypothesis when they observed a modification of rigor and tenderization with NEM modified muscle preparations. NEM was reported to ultimately increase the shear resistance of muscle, however, they concluded that no firm role for SH groups could be stated until the nature of NEM alterations are fully understood. Caldwell and Lineweaver (1969) investigated similar parameters

with chicken muscle, however, they were unable to substantiate the role of the SH group as a rigor initiator or tenderization mechanism. Wu and Sayre (1971) concurred with the observation of Caldwell and Lineweaver (1969) that aged chicken muscle did not differ from fresh muscle in its SH content. Hay et al. (1972) measured the SH groups in chicken red and white muscle by several methods and except in one instance all were similar to that reported by Wu and Sayre (1971). In the one exception the quantity of SH groups exposed by 20 mM KCl in breast muscle was increased during rigor mortis. These authors suggested a possible association between this observation and the reduction of Ca^{2+} ATPase activity during the same time period.

Stranberg et al. (1973) investigated the effect of various SH protecting reagents and postmortem storage on ATPase and superprecipitation activities of rabbit muscle myosin B. A variety of results were obtained particularly as modified by the ionic strength of the assay medium. They reported that quantitative differences in SH groups were apparent only during storage at elevated temperatures (3 days, 25 C). Based on quantitation of SH groups, the authors concluded that the postmortem change in ATPase activity and superprecipitation could not solely result from SH group alteration. Iodoacetamide (IAA) and NEM modified myosin B reacted similarly to postmortem myosin B preparations. Stranberg et al. (1973) observed that 50% blockage of total SH groups of at death myosin B accelerated the rate of turbidity response and that of the Mg^{2+} + Ca^{2+} and Mg^{2+} + EGTA-modified ATPase activities. This is similar to previous reports (Goll, 1968) that postmortem storage modifies ATPase activity and superprecipitation. Some protection

against increased ATPase activity could be conferred to myosin B by dialyzing it against 2-mercaptoethanol (MCE). However, at low ionic strength (0.052) dialysis against MCE did not prevent an increase in Mg^{2+} + EGTA ATPase activity. The authors were cautious in interpreting the results, however, they concluded that modification of SH groups should be credited with partial responsibility for superprecipitation and ATPase changes in postmortem myosin B. However, Strandberg et al. (1973) reported that the increase in Mg^{2+} + EGTA ATPase activity was indicative of loss of Ca^{2+} sensitivity which suggested that proteolysis might play a role in postmortem muscle. Previous work by Arakawa et al. (1970a) showed that α-actinin and the TM-TN complex was not changed in sufficient magnitude to account for observed postmortem changes. With Ca^{2+} sensitivity attributed to the TM-TN complex, the source responsible for the increased Mg^{2+} EGTA activity is presently unknown.

Proteolytic Probes

The size of the myosin molecule has contributed to the difficulty of determining molecular weight, α-helix content and dimension.(Needham, 1971). Earlier attempts had been made to disrupt secondary structure linkages by urea or other agents, however, the use of trypsin to cleave the molecule into smaller fragments has clarified many aspects of myosin structure (Gergely, 1950; Perry, 1950). Subsequent work by Szent-Gyorgyi (1953) helped clarify earlier observations when he obtained two sub-units from trypsin proteolysis and named them light meromyosin (LMM) and heavy meromyosin (HMM). The important results of this and previous work showed that LMM maintained

much of the solubility characteristics of the parent molecule, whereas HMM was water soluble but possessed most of the ATPase activity.

The structure of myosin known to date has been determined from the use of proteolytic tools such as trypsin, papain, chymotrypsin and others together with X-ray diffraction data. The present day evidence suggests that the myosin molecule is composed of two major polypeptide chains running the length of the molecule (Lowey et al., 1969). The myosin molecule can be split into smaller fragments by proteolytic enzymes as reported earlier by Szent-Gyorgyi (1953). These smaller fragments are called LMM and HMM, however, the HMM appears to be able to be subdivided into two additional fractions called heavy meromyosin subfragment 1 (HMMS-1) and heavy meromyosin subfragment a (HMMS-2) (Lowey et al., 1969). The junction between HMM and LMM, as previously mentioned, is susceptible to trypsin hydrolysis, however, with the use of insoluble complexes of papain (Nihei and Kay, 1968; Lowey et al., 1969) it was determined that the globular head containing the ATPase activity is connected to LMM by a highly helical fraction, HMMS-2 (Huxley, 1969).

Although the description of the myosin molecule has been considerably superficial, the clarification of molecular structure has allowed the pursuit of postmortem perspectives that allow an insight into the changes occurring during the transition of muscle to meat.

Proteolysis has been covered in a previous section of this review, however, most measures of proteolysis that have been used are of the type that measure degraded products such as peptides or amino acids. Suzuki et al. (1969a, b) and Okitani et al. (1972) utilized cathepsin D as a proteolytic

agent to determine postmortem changes in rabbit muscle. Suzuki et al. (1969b) reported that cathepsin D does not degrade native tropomyosin in myosin B which indicates a specificity difference between it and other commonly used proteolytic agents. Cathepsin D lowered Mg^{2+}-enhanced ATPase activity when the assay was run at pH 5.0, but when pH 5.5 or what has been considered to be approximately the ultimate pH of meat was used, no lowering of the ATPase activity was observed. Cathepsin D showed no effect on Ca^{2+}-activated ATPase irrespective of pH which suggests that no activity occurs against myosin A. Suzuki et al. (1969a) reported similar specificities for pepsin and cathepsin D when using the oxidized B-chain of insulin as substrate. Pepsin was reported to significantly decrease the Mg^{2+}-activated ATPase activity of myosin B which is in contrast with the effect of cathepsin D. Okitani et al. (1972) observed the same ATPase activities under conditions reflecting normal postmortem situations. They concluded that cathepsin D does not play a major role in postmortem degradation of muscle proteins of the myofibril.

Yang et al. (1972) incubated actomyosin and myofibrils from rabbit skeletal muscle with trypsin and measured the alteration of the ATPase activities. They found that the Mg^{2+}-enhanced ATPase activity underwent an alteration that was dependent on ionic strength and time of aging. Actomyosin and myofibril preparations underwent qualitatively similar modifications with the changes of the former being considerably larger than the latter. These authors concluded that aging of myofibrils results in some alteration of the structural components with the Z-line and tropomyosin the most probable sites of change.

Goll et al. (1971a) reported data that support the concept that a brief incubation of myofibrillar protein with trypsin modifies the actin-myosin interaction before the cleavage into HMM and LMM fractions occurs. These authors listed three specific modifications that support this concept. First, 30 min. of tryptic digestion causes an 8-fold increase in the Ca^{2+} modified inosine triphosphatase (ITPase) activity of actomyosin without causing a similar response in myosin preparations. In addition to the ITPase activity trypsin causes a 10 to 30% increase in the Mg^{2+} + Ca^{2+}-modified ATPase activities during the first 1 to 2 min. but 60 min. of digestion caused a drop in activity to only 20% of the original value. Secondly, trypsin caused an increase in the rate of turbidity response of actomyosin suspensions initially, but after 60 min. the rate of response was much less than control samples. The final line of evidence presented was the observation that supercontracted myofibrils (ATP contracted to 50% of initial length) incubated for 4 min. with trypsin lengthen to 70% of resting length. This lengthening was accompanied by cleavage of myosin into HMM and LMM, but the authors concluded that lengthening was primarily due to the sliding of thick and thin filaments rather than by cleavage of the myosin molecule into HMM and LMM. This latter observation supports the earlier data reported by Stromer, Goll and Roth (1967) that trypsin lengthened rigor shortened myofibrils.

The culmination of all work on muscle utilizing proteolytic probes must be either as a method to investigate and relate to in vivo molecular structure or as a tool to help determine causes of postmortem changes. The former method has been demonstrated quite adequately by Gergely (1950), Perry (1950)

and Lowey et al. (1969), however, only a few researchers utilized proteolytic enzymes to study postmortem changes. Goll et al. (1971b) reported the striking similarities between normal postmortem muscle parameters and those produced by limited tryptic proteolysis. Greaser et al. (1969) attributed rigor mortis onset to the loss of Ca^{2+} accumulating ability of the SR. Goll et al. (1971a) concurred with this observation, however, information gained from limited tryptic incubation suggests that proteolysis of the SR causes the loss of Ca^{2+} accumulating ability of these membranes. The resolution of rigor mortis apparently is more complex in origin than rigor onset, but many of the known parameters of aged muscle can be duplicated by mild tryptic proteolysis (Goll et al., 1971b). Trypsin incubation mimics the changes in the Mg^{2+} and Ca^{2+}-activated ATPase activities that have been reported earlier (Arakawa et al., 1970a). The Mg^{2+}-activated ATPase was elevated in postmortem muscle (24 to 72 hr.) and also in trypsin treated (2 min.) at death myofibrils, however, longer incubations (4 min.) caused the decline in Mg^{2+} ATPase activity which paralleled the activity in 312 hr. postmortem muscle. The Ca^{2+}-activated ATPase activity increased in a similar trial and remained elevated in both cases even after 312 hr. of storage.

A second line of evidence reported by these same authors (Goll et al., 1971b) is the increased rate of turbidity response in both normal postmortem muscle and trypsin treated muscle. Some inconsistencies in this comparison were reported between 8-day aged muscle and greater than 5 min. trypsin treatment. The authors concluded that proteolysis may be only a partial explanation for postmortem changes.

Two final lines of evidence presented by Goll et al. (1971b) to support the similarities between postmortem changes and trypsin incubation are the lengthening of shortened myofibrils and Z-disk alterations. The former observation was reported in a earlier paper (Goll et al., 1971a) concerning the effects of trypsin on mimicking postmortem changes. Normal rigor shortened muscle lengthens during storage much the same as trypsin treated muscle. The latter observation was considered in detail earlier, however, these authors again cite the similarities between normal untreated aged muscle and trypsin treated at death myofibrils. The Z-disk in both cases was reported to undergo a gradual disintegration and eventual loss of continuity.

Goll et al. (1971b) concluded that it appears that very limited proteolysis plays an important role in determining not only the onset of rigor but also the controversial "resolution of rigor mortis." They also reported that other factors probably contribute to the observed changes and cautioned that changes in postmortem muscle are probably affected by other alterations in the muscle system.

EXPERIMENTAL METHODS

Experimental Animals

Sixteen Hereford bulls, 14 to 16 months of age, were used in this study. The bulls were obtained during the last year of a 12 year study that was designed to genetically select for tenderness. One-half of the bulls were unselected for tenderness, but selected for leanness. The latter line served as the tenderness control line. Semen was collected from each bull prior to slaughter and frozen. The semen from the 2 most tender bulls was used to inseminate the females in the original herd designated as the tender group; likewise, semen from the 2 leannest bulls was used to inseminate the females in the lean group to obtain the next years calf crop. The 2 most tender and leanest bulls were identified by a tenderness or leanness index as follows: tenderness index = 10 + 1.4 X taste panel score - shear value; index of leanness = weight of the round, rump and loin divided by carcass weight and the percentage obtained was adjusted to a carcass weight of 500 pounds by linear regression.

Sample Preparation

Approximately 1 hr. after exsanguination, a 300 to 400 g sample of the longissimus muscle was removed adjacent to the 12th rib. The sample was trimmed free of epimysium and a pH determination was made on the muscle surface using a Corning model 12 pH meter.

A 1 hr. postmortem temperature was obtained by inserting a thermometer in the longissimus muscle anterior to the point where the sample for electron microscopy and protein fractionation was removed. Several small bundles of fibers were removed from different locations within the muscle sample for electron microscopy preparation. The remainder of the sample was cut into smaller portions and frozen in 2-methoxybutane cooled with dry ice. The frozen samples were placed in Whirl-Pak bags (Nasco, Ft. Atkinson, Wisconsin) and stored at -29 C until used.

Electron Microscopy

Sampling. The prerigor muscle samples were removed from the right longissimus muscle approximately adjacent to the 12th rib at 1 hr. postmortem. Muscle samples were removed after 48 and 216 hr. of postmortem storage from the same area of the left longissimus. An attempt was made to prevent shortening of the prerigor samples, however, no mechanical restraining device was used. The samples were trimmed to approximately 2 mm in cross sectional area and 1 cm in length and placed in glutaraldehyde fixative.

Fixation and Embedding. The glutaraldehyde fixation was a modified procedure of Karlson and Schultz (1965) described by Sjostrand (1967). The samples were fixed for 2 hr. in a 1.25% glutaraldehyde solution pH 7.4 in sodium phosphate (PO_4) buffer as described in Appendix I. The fixative and PO_4 buffer also contained NaCl to provide an approximately in situ osmotic condition to help prevent osmotic damage.

After the samples were fixed for 2 hr., the fixative was removed and the samples were washed 3 times for 20 min. each using only the PO_4 buffer containing NaCl (Appendix II).

After rinsing, the samples were transferred to a 1% osmium tetroxide solution made up in veronal acetate buffer pH 7.4. The tonicity of the veronal acetate buffer was adjusted to that of blood (300 milliosmolar) by adding NaCl, KCl and $CaCl_2$ (Appendix III). The procedure for preparing the 1% osmium tetroxide solution was reported by Sjostrand (1967). The samples were postfixed in osmium tetroxide with gentle agitation for 1 hour.

The samples were dehydrated with a graded series of ethanol, 25, 50, 75 and 95% for 10 min. each. The samples were then allowed two 15 min. changes in absolute (100%) ethanol for final dehydration. The samples were placed into propylene oxide for two 30 min. changes and then into a 1:1 (v/v) mixture of Epon 812 and propylene oxide for 12 hr. in a dessicator.

After the 12 hr. period in the propylene oxide : epon mixture, the samples were removed and trimmed to approximately 1.0 mm x 0.5 millimeter. These trimmed samples were then transferred to 00 size gelatin capsules containing 100% epon (Appendix IV). Three replicates were embedded for each sample. The capsules were placed in a dessicator under slight vacuum for 12 hours. After the 12 hr. settling period, the samples were oriented for proper longitudinal sectioning and placed in a 60 C oven and allowed to polymerize for 48 hours. The polymerized epon blocks were removed from the oven and stored in a dessicator until used (Appendix V).

Section Preparation and Staining. The epon embedded tissue blocks were hand trimmed, using razor blades, into the shape of a truncated pyramid with no dimension greater than 0.5 millimeters. The trimmed tissue block was then sectioned on a LKB 4801A ultramicrotome using either glass or diamond knives. Sixty to 100 nm (silver to gold) sections were picked up on 300-mesh uncoated copper grids or 75 to 200 mesh formvar (0.25%) coated copper grids. Three to 5 grids were collected for each replicate which gave a total of 9 to 15 grids per muscle sample.

The copper grids containing the tissue sections were stained by floating on a saturated solution of aqueous uranyl acetate (Sjostrand, 1967) for 30 min. or for 5 min. on a 3% alcoholic (ethanol : methanol, 3:1; Appendix VI, VII) phosphotungstic acid (PTA) solution. After the required staining period, the grids were rinsed thoroughly with a jet of glass distilled water for the uranyl acetate stained grids or a ethanol : methanol (3:1) solution for PTA stained grids (Appendix VI, VII). In the case of the uranyl acetate stained grids, a second staining was accomplished by floating the grids on a lead citrate stain, proposed by Reynolds (1963), for 5 min. or a modification of this method for 10 sec. (personal communication, Richard Ruffing)(Appendix VI). After staining, a jet of 0.02N NaOH followed by glass distilled water was used for rinsing and then the grids were allowed to dry before use. The PTA stained sections were not doubly stained and were ready for use after drying.

Specimen Observation and Photography. Specimen containing grids previously stained were placed in a Philips EM-300 electron microscope and

observed at an accelerating voltage of 60 kilovolts. At least 9 grids were observed for each sample and representative photographs were taken using Kodak 8.25 cm x 10.16 cm sheet film. The film was developed for 4 min. in Kodak D-19 developer, washed for 1 1/2 min. in running water, fixed for 8 to 10 min. in Kodak Fixer, washed in running water for 1 min., rinsed in Kodak Hypo-Cleaning Agent and washed for 10 min. in running water. The washed negatives were dipped in Kodak Photo-Flo solution and dried for 45 min. with warmed air. All processing from the latent image to the final negative was performed on an Arkay nitrogen burst machine.

The 8.25 cm x 10.16 cm negatives were placed in a Durst S-45-EM point light source enlarger and Ilford Ilfoprint rapid stabilization process paper exposed. The Ilfoprint paper was developed in an Ilford model 1501 rapid stabilization processor using Ilford activator and stabilizer chemicals. Selected prints were fixed in Kodak fixer, washed in Orbit bath, flattened with Pakosol, washed in running water and dried on a ferrotype dryer.

Sarcomere Measurement

Sample Preparation. Only the postrigor aged samples (48 hr. and 216 hr.) were utilized for sarcomere measurements. Approximately 3 g of powdered muscle were weighed into a stainless steel homogenization cup and approximately 35 ml. of a 0.25 M sucrose solution added. The powdered muscle was then homogenized at high speed with a Virtis "23" macro-homogenizer for 1 minute. A drop of the homogenized sample was placed on a 75 x 25 mm

microscope slide and a 22 x 22 mm cover slip was placed over the sample and tapped lightly to remove entrapped air bubbles and to help prevent the formation of more than one layer of myofibrils. The slide preparation was viewed using a Zeiss WL research microscope with a 100X phase contrast oil immersion objective. A filar micrometer was used to count 25 separate 10 sarcomere fields for each muscle sample. The filar micrometer was calibrated with a stage micrometer having 0.1 mm and 0.01 mm divisions. Representative areas were photographed using either Kodak Panatomic-X or Plus-X 35 mm black and white film.

Protein Extraction

Prior to extraction, the frozen muscle samples were placed in a Waring Blendor which had been previously cooled in a -29 C freezer and tempered with liquid nitrogen. The samples were powdered in a -29 C freezer as described by Borchert and Briskey (1965). Chipped dry ice and frozen muscle were placed in the blendor jar and powdered by a 30 to 45 sec. burst of the blendor and then sifted with the coarse material replaced in the blendor and the procedure repeated. All material was placed into a pan and thoroughly mixed to insure that the coarse connective tissue was equally distributed. The powdered muscle samples were placed in Whirl-Pak bags and allowed to remain unsealed at -29 C for 12 hr. for sublimation of the dry ice. After 12 hr., the bags were sealed and stored at -29 C until used.

Sarcoplasmic Protein. The powdered muscle was extracted using a modification of the method of Helander (1957) and Borton (1969). A 2 g sample was weighed into a 250 ml wide mouth polypropylene bottle equipped with a screw cap. Fifty ml of pre-cooled (3 C) 0.015M PO_4 buffer pH 7.4 (Appendix VIII) were added to the 2 g of powdered muscle. A stirring bar was added to the bottles and they were placed on a magnetic stirrer and gently stirred at 3 C for 20 minutes. The mixture was centrifuged at 3500Xg for 25 min. in a Sorvall RC2-B automatic refrigerated centrifuge. The supernatant was then passed through 6 layers of cheese cloth and collected in a 100 ml graduated cylinder. The residue was resuspended in 50 ml of 0.015M PO_4 buffer, extracted, centrifuged and filtered as previously described. The 2 supernatants were combined, the volume recorded and designated sarcoplasmic protein (SP).

Myofibrillar Protein. The residue from the sarcoplasmic fraction was suspended by adding either 50 ml of 1.1M KCl or 1.1M KI in 0.1M PO_4 buffer pH 7.4 (Appendix VIII). The mixture was gently stirred for 1 hr. on a magnetic stirrer and then centrifuged at 3500Xg for 25 minutes. The supernatant was collected as described previously and the residue reextracted following the same procedure as described above. The 2 supernatants were combined and designated total myofibrillar protein fraction (MP).

Non-Protein Nitrogen. Fifteen ml of the sarcoplasmic protein fraction were placed in a centrifuge tube and 5 ml of a 10% TCA solution were added (Appendix VIII). The mixture was allowed to stand for 4 hr. and then centrifuged at 3,000Xg for 25 minutes. The supernatant was designated as the non-protein nitrogen (NPN) fraction.

Total Nitrogen. Total nitrogen (TN) was determined on 0.5 g of powdered muscle by the micro-Kjeldahl method as described below for the SP, MP and NPN fractions. Fifteen ml of the SP, MP and NPN fractions were placed in separate micro-Kjeldahl flasks and 3 glass beads, 1 g solid Na_2SO_4, 1 ml 10% $CuSO_4$ solution and 7 ml of concentrated H_2SO_4 were added to each flask. The mixture was digested for approximately 45 min., cooled and 15 ml water added. Approximately 10 ml of a 40% (w/v) solution of NaOH were added and the nitrogen distilled and trapped in a 2% (w/v) boric acid solution containing brome cresol green (Appendix IX). The boric acid solution was titrated using a standardized H_2SO_4 solution. The results were reported as total nitrogen in the muscle sample and/or in each protein fraction.

Stroma Nitrogen. The sum of the SP, MP and NPN nitrogen was subtracted from the TP nitrogen to determine the stroma nitrogen fraction.

ATPase and ITPase Activity

The ATPase activity and superprecipitation assays were run on only 10 muscle samples, 5 from the tender group and 5 from the control (lean) group. The 5 bulls from the tender group with the lowest shear and highest taste panel scores and the 5 bulls from the control (lean) group with the highest shear and lowest taste panel scores were used.

Preparation of Myofibrils. The method of Stromer, Goll and Roth (1967) (Appendix X) was followed with some modification. Five g of powdered

muscle were weighed into a centrifuge bottle as the myofibril source rather than ground muscle, otherwise the procedure of Stromer et al. (1967) was followed explicitly.

Ca^{2+}-Activated ATPase Activity. One ml of myofibrils containing 0.2 to 0.8 mg protein per ml was placed in a test tube to which the following were added: 1 ml 0.2M tris-acetate pH 7.0, 1 ml 0.35M KCl, 5 ml H_2O and 1 ml 0.01M Ca^{2+} (Appendix XI). The assay was activated by adding 1 ml of 0.01M ATP (Sigma Chemical Company, St. Louis). The assay volume totaled 10 ml with a final concentration of 0.02M tris-acetate, 0.04M KCl (includes 0.005M KCl in myofibril preparation), 0.001M Ca^{2+} and 0.001M ATP. Thirty sec. after the addition of the ATP a 1 ml aliquot was removed and added to 1 ml of 15% TCA solution. The remaining solution was incubated for 15 min. at 25 centigrade. Another 1 ml aliquot was removed and treated the same as the 30 sec. sample. Both the 30 sec. and 15 min. aliquots were run in duplicate. The ATPase assay was a modification of the procedure of Goll and Robson (1967) and Briskey and Fukazawa (1971).

Mg^{2+}-Activated ATPase Activity. This assay was the same as that described above for Ca^{2+} ATPase except 1 ml of 0.01M Mg^{2+} rather than Ca^{2+} was used as the activator.

EGTA + Mg^{2+}-Activated ATPase Activity. The EGTA mediated ATPase activity reaction mixture was the same as described previously for Ca^{2+}-activated ATPase except EGTA was added to the reaction mixture to a final concentration of 0.0002M which included 0.01M Mg^{2+}. The EGTA was added

in solid form to the reaction vessel to provide the proper reaction conditions.

EDTA-Activated ATPase Activity (high ionic strength). This assay was modified by adding 2 ml of 2.42M KCl and 1 ml of 0.01M EDTA and only 4 ml H_2O in contrast to 5 ml H_2O in the Ca^{2+} ATPase assay described above. All other procedures were the same as described previously for the Ca^{2+} mediated ATPase assay.

Mg^{2+} and Ca^{2+}--Activated ITPase Activity. The ITPase activities were identical to the ATPase assay except ITP (0.01M, Sigma Chemical Company, St. Louis) was substituted for ATP and the Mg^{2+} and Ca^{2+} activators were added in the same proportions as previously described.

Phosphate Determination. Inorganic phosphate was determined by a modification of the Fiske and Subbarow (1925) procedure as described by Leloir and Cardini (1957)(Appendix XII). The values were reported as micrograms phosphate (μg) per milligram (mg) of protein per minute.

Myofibril Protein Determination. The biuret reaction was used to determine the protein for standardization (Gornall, Bardawill and David, 1949)(Appendix XIII).

Superprecipitation

Natural Actomyosin Preparation. Myosin B (natural actomyosin) was extracted by following a modification of the procedure of Arakawa et al. (1970a). Five g of the powdered muscle were suspended in 30 ml of W-E solution (Appendix XIV). This suspension was stirred magnetically at 2 C for 16 to 24 hours. Ten ml of the extracted solution were removed after 16 to 24 hr. and centrifuged at 15,000Xg for 20 minutes. The supernatant containing myosin B and SP was diluted with distilled water to 0.15M KCl and centrifuged at 15,000Xg for 20 minutes. The precipitate (myosin B) was dissolved in 1.0M KCl and then diluted with distilled water to a final concentration of 0.5M KCl. The precipitation and dissolution cycle was repeated twice and the final precipitate was adjusted to 0.5M KCl and dissolved by gentle magnetic stirring overnight and then clarified by centrifugation at 15,000 Xg for 20 minutes.

Superprecipitation Assay. All readings were obtained from a Beckman DU spectrophotometer equipped with a Gilford attachment and a Sargent SR recorder. All solutions except myosin B and ATP were kept at 27 C prior to the assay determinations. Myosin B was stored at 3 C and ATP was stored at -20 C prior to their use.

The superprecipitation assay followed the procedure outlined by Arakawa et al. (1970a, c) and Briskey and Fukazawa (1971). Myosin B concentration was determined by a modification of the biuret procedure of Gornall et al. (1949).

Mg^{2+} + EGTA Superprecipitation. The total reaction volume of each assay was 3 ml in a Beckman cuvette. The final concentration of the reagents, added in order, were as follows: myosin B, 0.4 mg/ml; tris-acetate, .01M pH 7.0; KCl, 0.1M; $MgCl_2$, 0.001M; EGTA, 0.001M; and ATP, 0.001M. The solution was mixed after the addition of each reagent except for ATP which was added to the cuvette after it was placed in the spectrophotometer. Prior to the addition of ATP, a reading was made and then the ATP was carefully added to the center of the cuvette and the reaction plotted on the recorder.

Low Ca^{2+} Superprecipitation. The procedure for this assay was identical to the previous assay except that $CaCl_2$ was added to a final concentration of 0.00005M and EGTA was deleted from the assay mixture.

Statistical Analyses

Data were analyzed on the CDC 6500 Computer at Michigan State University. Product moment correlations and a one way analysis of variance was first performed on the data and then the data were treated as a split plot design with treatments as main effects and time as the subeffect. Duncan's New Multiple Range Test (Steel and Torrie, 1960) was applied when analysis of variance data were significant in order to detect the significantly different means. The more important correlation coefficients are listed in Appendix XVI through XIX.

RESULTS AND DISCUSSION

Electron Microscopy

1 Hr. Postmortem. The samples obtained approximately 1 hr. postmortem were designated as controls to which the postrigor aged samples, 48 hr. and 216 hr., respectively, could be compared. The control samples (1 hr.) will be compared to literature observations whenever possible since these samples were not obtained immediately postexsanguination.

Mitochondria. Figure 1 is a group of myofibrils (X17,500) with an intermyofibrillar row of mitochondria. Several of the mitochondria, as indicated by the single arrows, are swollen and considerable cristal damage can be seen. Other mitochondria, as indicated by double arrows, appear to be approximately normal in size and the cristae are in a more normal and organized condition.

Figure 2 shows a higher magnification (X37,800) electron micrograph with several subsarcolemmal mitochondria which are oriented in various planes. Several mitochondrial areas, as indicated by arrows, are disrupted and the cristae have been degraded. The intact cristae tend to be somewhat denser and in a slightly different configuration than the cristae shown in figure 1.

Figure 3 represents another myofiber area showing mitochondria sectioned in longitudinal and transverse planes. Some swelling can be seen in many of the mitochondria and areas of disrupted cristae, indicated by arrows, can be seen.

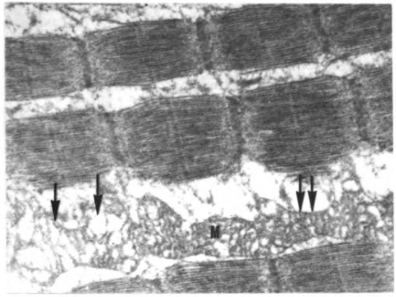

Figure 1. 1 hr. postmortem bovine longissimus muscle fiber. M = intermyofibrillar row of mitochondria. Swollen mitochondria shown by single arrows; double arrows show less swollen mitochondria. (X 17,500)

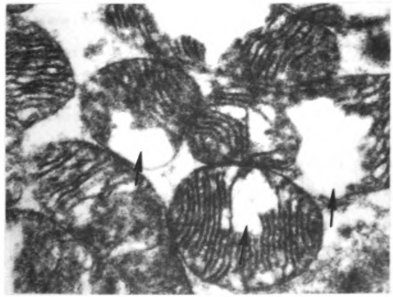

Figure 2. 1 hr. postmortem bovine longissimus muscle subsarcolemmal mitochondria. Arrows indicate areas of cristal degradation. (X 37,800)

Little attention has been given to the morphology of postmortem bovine muscle mitochondria. Considerable research has been reported on other species relative to the determination of fiber type by mitochondrial number and cristae density. Gauthier (1970) reported the division of rat diaphragm muscle fibers into three classifications, partially by the quantity and location of mitochondria. Extrapolating her observation for rat muscle to include bovine muscle is difficult, however, mitochondrial disruption is obvious in the 1 hr. sample when compared to the mitochondria in fibers reported for rat muscle.

Dutson et al. (1974) investigated postmortem changes in normal and low quality porcine longissimus muscle mitochondrial ultrastructure. They found no obvious changes in mitochondrial continuity in either normal or low quality animals at 15 min. postmortem. The swelling and disruption of the mitochondrial membranes in the 1 hr. samples suggest that an alteration in the cell environment has occurred between exsanguination and sampling. It is difficult to ascertain whether the changes in the mitochondria are the result of sample excision, fixative osmolarity, or postmortem changes inherent to the animals tissue.

Z-line. In an effort to more effectively discuss changes in Z-line morphology, the fibers in this study have been arbitrarily classified as type I and type II based on Z-line density and width and number and location of mitochondria. This classification is based entirely on subjective evaluation of the ultrastructure of the Z-line and mitochondrial population. Dubowitz (1970) classified fibers as type I and type II by using reciprocal

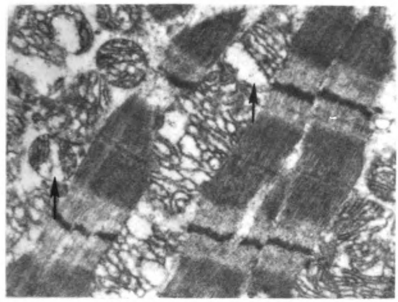

Figure 3. 1 hr. postmortem bovine longissimus muscle fiber. Arrows indicate areas of mitochondria cristae degradation (X 17,500)

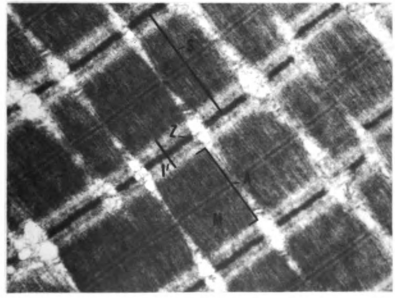

Figure 4. 1 hr. postmortem bovine longissimus muscle type I fiber. A = A-band; M = M-line; I = I-band; Z = Z-line; S = sarcomere (X 17,500)

histochemical stains. However, the type I and type II fibers in the present study are similar to the type I and type II fibers of Dubowitz (1970) only if the ultrastructural observations and histochemical stains identify the same fiber characteristics.

Figure 4 shows a group of myofibrils having thick and dense Z-lines characteristic of type I fibers. The Z-lines have a dense fibrillar configuration that is readily evident and a sharp division between the I-band and Z-line is apparent. The Z-lines are continuous across the myofibril which imparts an unaltered appearance to their entire structure.

Figure 5 is another representative type I fiber which is similar in most respects to the fiber in figure 4. The Z-lines are very distinct, wide and dense, which is characteristic of type I fibers and a number of intermyofibrillar mitochondria (indicated by arrows) are also very apparent.

Figure 6 shows several myofibrils which are representative of the type II fibers. The Z-lines in these myofibrils appear to be moderately wide and dense, but are clearly more diffuse and distinctly less fibrillar than type I Z-lines. The demarcation between the I-band and Z-band is not as obvious as that observed for type I fibers.

Figures 7 and 8 shows 2 additional examples of type II fibers differing only slightly from that seen in figure 6. The myofibrils in figure 7 have Z-lines that are more diffuse and in certain areas, as indicated by arrows, only limited structure can be seen. The Z-lines of the myofiber in figure 8 are thinner, but less diffuse and slightly more distinct than those in figure 7.

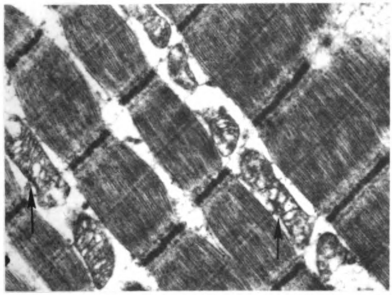

Figure 5. 1 hr. postmortem bovine longissimus muscle type I fiber. Arrows indicate several intermyofibrillar mitochondria (X 17,500)

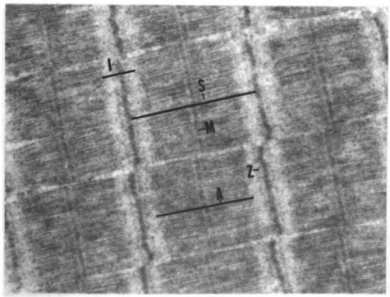

Figure 6. 1 hr. postmortem bovine longissimus muscle type II fiber. A = A-band; M = M-line; I = I-band; Z = Z-line; S = sarcomere (X 17,500)

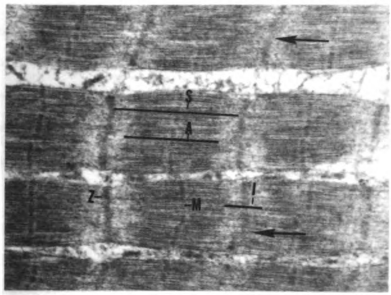

Figure 7. Type II fiber in 1 hr. bovine longissimus muscle. A = A-band; M = M-line; I = I-band; Z = Z-line; S = sarcomere. Arrows indicate areas of loss of Z-line structure.

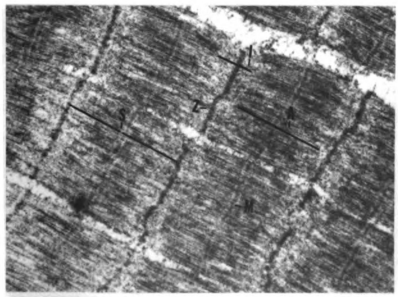

Figure 8. Type II fiber in 1 hr. bovine longissimus muscle. A = A-band; M = M-line; I = I-band; Z = Z-line; S = sarcomere (X 17,500)

Electron micrographs of early postmortem muscle fibers in the literature show distinct Z-lines which frequently illustrate a prominent fibrillar configuration. A similar condition can be seen in type I fiber samples at 1 hr. postmortem in the present study, however, the type II fibers appear to be altered slightly in fibrillar configuration. Gauthier (1970) reported that 3 types of fibers were present in rat diaphragm muscle. The Z-lines in all 3 fiber types of rat muscle are distinct and fibrillar and do not tend to be diffuse as was seen in several of the type II fibers in the present study. Dutson et al. (1974) reported ultrastructure data that showed no change in Z-line ultrastructure of red and white fibers of porcine longissimus muscle at 15 min. postmortem in either normal or low quality carcasses. Goll (1968) and Henderson et al. (1970) reported no discernible changes in at death samples of bovine, porcine and rabbit muscle. The samples in the present study appear to have fibers that are divided into 2 relatively distinct populations having divergent Z-line morphology and stability at 1 hr. postmortem.

Contractile State. The contractile condition of the fibers at 1 hr. postmortem varied from little or no contraction to maximal contraction. Figure 9 represents a group of myofibrils with a clear and distinct I-band, however, the H-zone is indistinct suggesting that some contraction has occurred in this fiber. The fiber in figure 10 has a very small I-band and the close proximity of the A-band to the Z-line indicates that maximal contraction has occurred. Figure 11 is a fiber area that appears to be relaxed or slightly stretched as indicated by a wide I-band and a distinct H-zone.

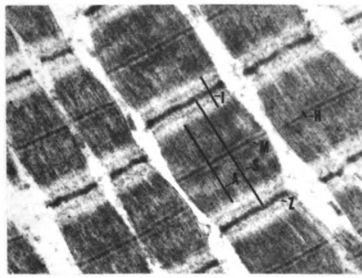

Figure 9. 1 hr. postmortem bovine longissimus muscle type II fiber illustrating partially relaxed muscle sarcomeres. A = A-band, M= M-line; I = I-band; Z = Z-line; H = H-zone; S = sarcomere (X 17,500)

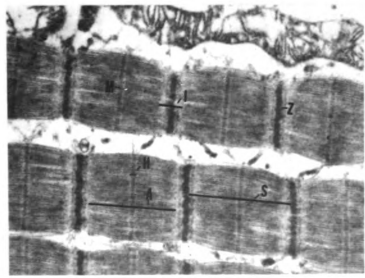

Figure 10. 1 hr. postmortem bovine longissimus muscle type I fiber illustrating contracted muscle sarcomeres. A = A-band; M = M-line; I = I-band; Z = Z-line; H = H-zone; S = sarcomere (X 17,500)

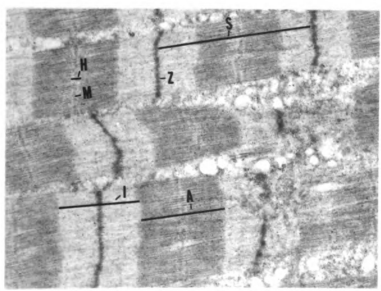

Figure 11. Type II fiber, 1 hr. postmortem, illustrating relaxed condition. A = A-band, M = M-line; I = I-band; Z = Z-line; H = H-zone; S = sarcomere (X 17,500)

The contractile condition of the 1 hr. samples indicates that many of the fibers were not adequately restrained before fixation. Since no mechanical device was used to restrain the samples, the appearance of contracted fibers was expected and not easily prevented. The contracted condition did not seem to alter the ultrastuctural morphology of the fibers.

M-Line. The M-line was readily apparent in all preparations as a dark line bisecting the center of the A-band as shown in figure 4. No distinct differences in M-line morphology were found between fiber classifications, treatments or contractile condition of the fibers.

Glycogen. Generally, at death samples have an abundant supply of 15 to 30 nm diameter glycogen granules (Lehninger, 1970) in the intermyofibrillar cytoplasm and in the cytoplasm in other locations within the muscle fiber. These granules were conspicuously absent in the 1 hr. postmortem samples. The dearth of glycogen in the 1 hr. samples can be interpreted in several different ways. Since all animals were bulls, the possibility exists that initial glycogen reserves were low, and by the time the samples were taken, glycolysis had proceeded far enough to deplete all or most of the reserves. Sample removal may have contributed to the glycogen loss since contraction was rather violent during excision. The literature of at death muscle samples reported by Goll (1968), Henderson et al. (1970) and Dutson et al. (1974) have a variable but obvious quantity of glycogen in the cytoplasm. No firm conclusion can be drawn concerning the lack of glycogen in the 1 hr. samples from the data obtained in this study.

Treatment Effects. The 1 hr. postmortem samples tend to show no discernible effects due to treatment. The group of animals selected for tenderness appeared to show an equal amount of mitochondrial disruption, Z-line alterations and glycogen depletion as that found in the group not selected for tenderness. Likewise, contractile state did not appear to differ between tenderness groups.

48 Hr. Samples

Mitochondria. Considerable mitochondrial morphological variation was present in the postrigor (48 hr.) samples, however, there was more of a tendency for all of these organelles to be disrupted as compared to the 1 hr. samples. Figure 12 represents a myofiber area showing several mitochondria in various states of disintegration. The intermyofibrillar mitochondria, as indicated by M2, appeared to retain more of the characteristic morphological features than the mitochondria encircling the I-band-Z-line area, which are indicated by M1. The mitochondria, indicated by M, in figure 13, are more disrupted than those in figure 12. The outer membrane in several mitochondria appears to be broken and the cristae in most of the mitochondria are severely disrupted. Cassens et al. (1963) reported mitochondrial disruption in normal porcine longissimus at 24 hr. postmortem. Dutson et al. (1974) reported that some mitochondrial disruption or loss of cristal density could be found in all 24 hr. samples, but was particularly obvious in the white fibers (type II). Although not investigated thoroughly, it appeared that the mitochondria in type I fibers are less labile than mitochondria in type II fibers in the present study.

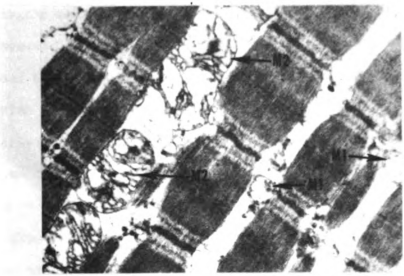

Figure 12. Type I fiber, 48 hr. postmortem. M 1 = mitochondria which encircle the myofibril; M 2 = intermyofibrillar mitochondria. (X 17,500)

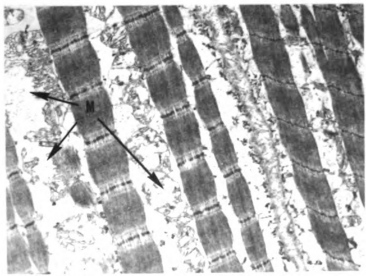

Figure 13. Two type II fibers, 48 hr. postmortem. M = disrupted intermyofibrillar mitochondria (X 7,840)

Z-Line. Z-line line ultrastructure in some postrigor (48 hr.) fibers has undergone considerable modification when compared to 1 hr. samples. Again, however, 2 relatively distinct fiber groups are apparent as distinguished by Z-line morphology and mitochondrial density. Type I fibers, having wide and dense Z-lines, as indicated by figures 14, 15 and 16, appear essentially unaltered and are similar in ultrastructure to prerigor type I fibers. The only discernible alteration in type I fibers appeared to be a less distinguishable fibrillar nature of the Z-line. Type II fibers, although somewhat more variable in appearance than type I fibers, are easily distinguishable from type I fibers by the appearance of breaks in the Z-line evident at 48 hr. postmortem. Figure 17 is a group of myofibrils having Z-lines approaching the thickness and density of type I fibers, however, as indicated by arrows, a number of breaks can be seen in the Z-line. Figure 18 is a higher magnification of a Z-line from the same fiber shown in figure 17. The arrows indicate 2 areas of loss of continuity in Z-line structure. Figure 19 is an electron micrograph of a portion of 2 fibers exhibiting differing amounts of Z-line breakage. Fiber A is similar to that in figure 17 in that only minor Z-line breakage had occurred, (shown by arrows), whereas fiber B had more evidence of Z-line breakage, as indicated by arrows.

Figure 20 shows another group of myofibrils representing the type II fiber group. The major difference between this fiber and previous examples is the plethora of Z-line disintegrations. Several areas, as indicated by arrows, are almost devoid of distinguishable Z-line material. Figure

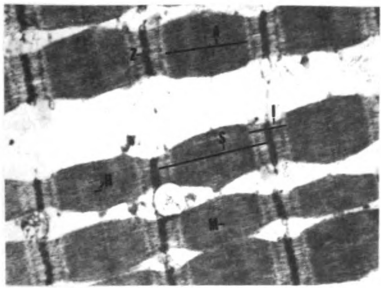

Figure 14. Type I fiber from 48 hr. postmortem bovine longissimus muscle. A = A-band; I = I-band; H = H-zone; M = M-line; Z = Z-line; S = sarcomere (X 17,500)

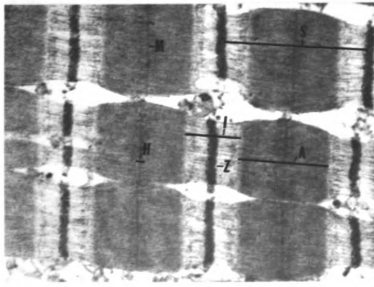

Figure 15. Type I fiber from 48 hr. postmortem bovine longissimus muscle. A = A-band; I = I-band; H = H-zone; M = M-line; Z = Z-line; S = sarcomere (X 17,500)

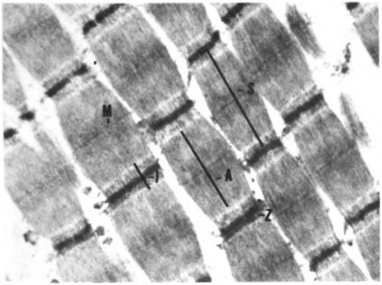

Figure 16. Type I fiber from 48 hr. postmortem bovine longissimus muscle. A = A-band; I = I-band; M = M-line; Z = Z-line; S = sarcomere (17,500)

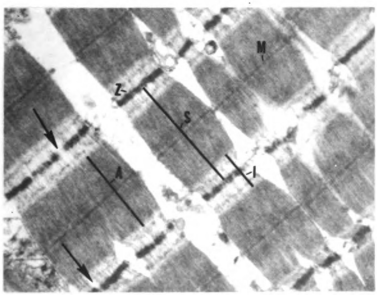

Figure 17. Type II fiber, 48 hr. postmortem, showing Z-line degradation. Arrows indicate breaks in Z-line structure. A = A-band; I = I-band; M = M-line; Z = Z-line; S = sarcomere (X 17,500)

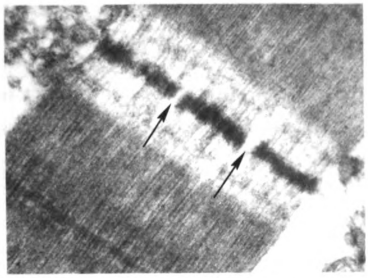

Figure 18. Higher magnification of Z-line area from figure 17. Arrows indicate breaks in the Z-line (X 37,800)

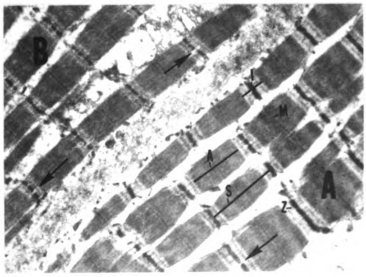

Figure 19. Two type II fibers, A and B. Arrows indicate breaks in the Z-line. A = Z-band; I = I-band; M = M-line; Z = Z-line; S = sarcomere (X 9520)

21 shows a fiber exhibiting a similar condition to that seen in figure 20, however, the remaining Z-line material appears to be slightly less dense and considerably more diffuse.

Figures 22 and 23 differ only slightly from previous examples of type II fibers, except for the proportion of Z-line disintegration. The double arrows in both figures indicate areas of longitudinal splitting. In figure 23, the single arrows indicate Z-lines that are almost totally devoid of typical Z-line material. Even without obvious Z-line material, several sites can be seen in this figure where filamentous material appears to be continuous through the Z-line area.

I-Z Breakage. The ultrastructural change common to both types of fibers is the disruption of the connection between the thin filaments and the Z-line. The type I fibers exhibit distinct I-Z breakage since in many cases the Z-line can be found intact on one or the other half of the I-bands. In figure 24, the arrows indicate an area of an I-Z junctional breakage which is unilateral and the Z-line material remains attached to I-band filaments only in one-half I-band. The I-band-Z-line junction has almost completely separated with little I-filament remaining attached to the Z-line. Figure 25 exhibits a similar but more massive case of I-Z breakage, however, as indicated by the arrows, some connection still exists between the Z-line and thin filaments. It appears that the thin filaments have been stretched rather than totally severed from the Z-line.

Figure 26 is a low magnification electron micrograph of a portion of 2 fibers. The arrows indicate several areas of apparent I-Z junctional

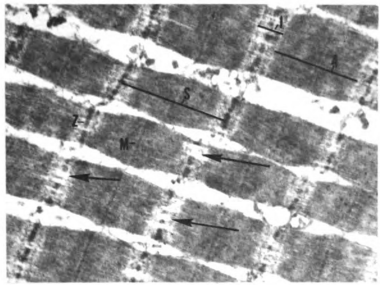

Figure 20. 48 hr. postmortem type II fiber. Arrows indicate areas of Z-line degradation. A = A-band; I = I-band; M = M-line; Z = Z-line; S = sarcomere (X 17,500)

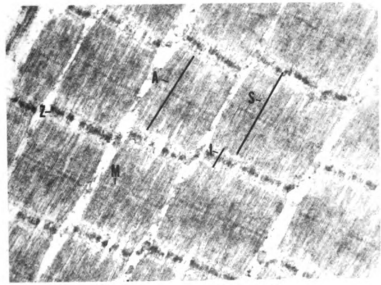

Figure 21. 48 hr. postmortem type II fiber. A = A-band; I = I-band; M = M-line; Z = Z-line; S = sarcomere (X 17,500)

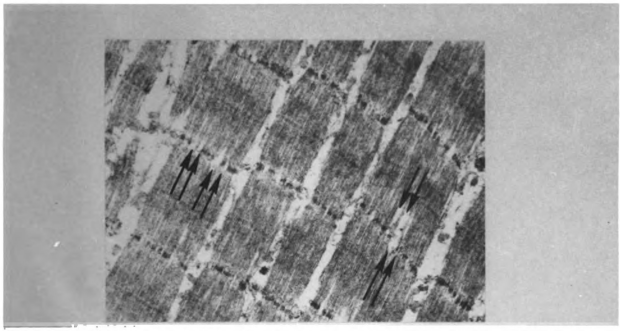

Figure 22. Type II fiber in 48 hr. postmortem bovine longissimus muscle. Double arrows indicate areas of longitudinal splitting (X 17,500)

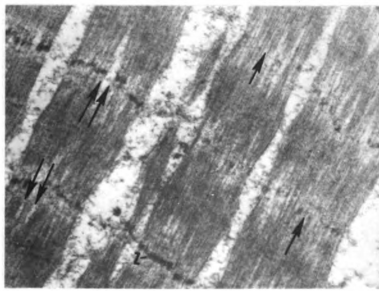

Figure 23. Type II fiber in 48 hr. postmortem bovine longissimus muscle. Single arrows show Z-lines that are practically devoid of typical dense Z-line material, double arrows indicate areas of longitudinal splitting. Z = Z-line (X 17,500)

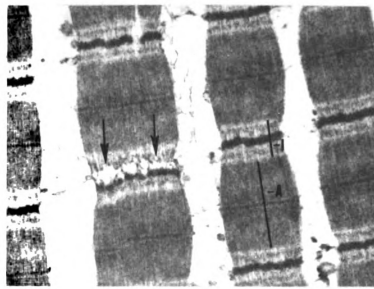

Figure 24. Type I fiber in 48 hr. postmortem bovine longissimus muscle. Arrows indicate a break at the junction of the I-band and Z-line. A = A-band; I = I-band (X 17,500)

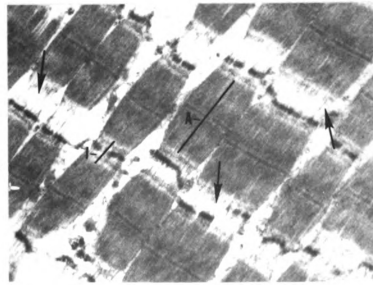

Figure 25. 48 hr. postmortem bovine longissimus muscle type I fiber. Arrows indicate filamentous material connecting Z-line and I-band. A = A-band; I = I-band (X 17,500)

breakage, which are not easily discerned at this level of magnification. Figure 27 is a higher magnification of these areas which clearly shows that the breakage has occurred at the I-Z junction. The Z-lines are typical of type I fibers as indicated by their width, density and unaltered ultrastructural appearance. Generally, as in previous examples, the Z-line remains on one side of the original I-Z-I band connection and in most examples the breakage is not total as illustrated by a residual connection between I-band and Z-lines. This observation is clearly depicted in figure 27 (arrows), however, this is a general rule applicable to the majority of postrigor type I fibers and occasionally a I-Z breakage can be seen in which the I filaments and Z-line material have completely separated from each other.

Figure 28 shows a type I fiber exhibiting an atypical Z-line breakage or splitting. The single arrow indicates the Z-line that appears to be undergoing symmetrical Z-line division rather than an I-Z junction breakage. This is an uncommon condition and it is difficult to ascertain whether it results from postmortem storage or is an abberation inherent to the in vivo fiber. The double arrows indicate an area of breakage at the I-Z junction, however, the Z material adheres to both halves of the I-band filaments rather than one as shown in figure 27.

All previous examples represented type I fibers exhibiting I-Z junctional splitting, however, type II fibers exhibit a similar condition. Figure 29 shows a fiber with considerable Z-line breakage and I-Z junctional splitting. In several areas, indicated by single arrows, breaks have

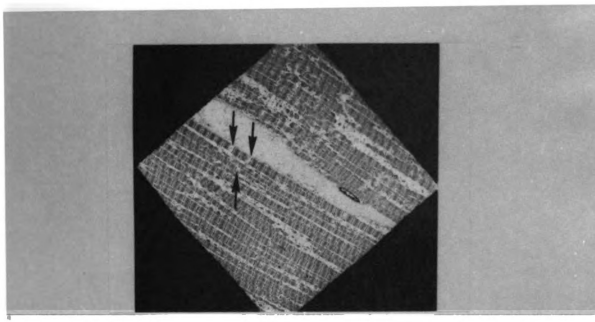

Figure 26. **Low magnification electron micrograph of a portion of 2 fibers in 48 hr. postmortem bovine longissimus muscle. Arrows indicate areas of I-Z junction breakage (X 1,120)**

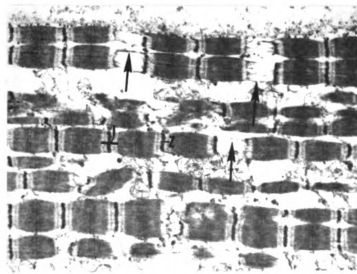

Figure 27. Higher magnification of figure 26 showing I-Z junction breakage. Arrows indicate filamentous connection between the I-band and Z-line. I = I-band; Z = Z-line (X 7,840)

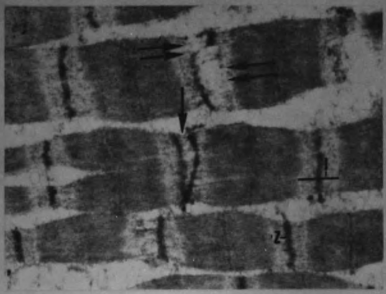

Figure 28. Type I fiber in 48 hr. postmortem bovine longissimus muscle. Single arrow shows symmetrical Z-line splitting, double arrows show an area of breakage at the I-Z junction. I = I-band; Z = Z-line (17,500)

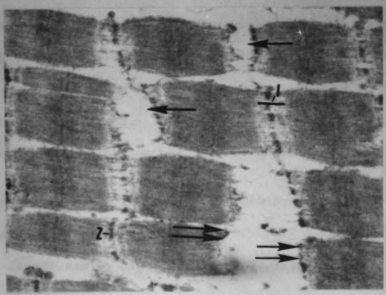

Figure 29. Type II fiber in 48 hr. postmortem bovine longissimus muscle. Single arrows indicate breaks that occur at the junction of the I-band and Z-line, double arrows show Z-line material on both portions of I-band. I = I-band; Z = Z-line (X 17,500)

occurred at the junction of the thin filaments and Z-line. The double arrows indicate an area of breakage that has allowed Z-material to partially remain on both sides of the divided I-band as compared to the type I fiber I-Z breakage which predominately leaves Z material attached to only one of the halves of the I-bands.

Figure 30 shows a low magnification electron micrograph of 2 fiber portions. In fiber A, arrows indicate several areas of I-Z breakages in contiguous myofibrils. The I-Z breakage in this fiber tends to follow a definite pattern, whereas previous examples appeared to indicate a more random distribution of breaks in that only rarely did 2 I-Z junction breaks occur in contiguous sarcomeres. Figure 31 shows a higher magnification of the area represented in the square in figure 30. The higher magnification electron micrograph shows that the breakage occurs at the junction of the thin filament and the Z-line. The double arrows indicate Z-line breakage both at an I-Z junction and at another Z-line where I-Z splitting has not occurred. The single arrows indicate portions of Z-material that has remained on both halves of the I-band.

M-Line. The M-line in most preparations seemed to be unaltered, however, occasionally, as seen in certain myofibrils of figures 22 and 23, the M-line is not easily discerned. Generally, the M-line can be seen, in an apparent unaltered state, in all samples.

Contractile State. The postrigor fibers appeared to have undergone little perceptible alteration in sarcomere length when compared to prerigor

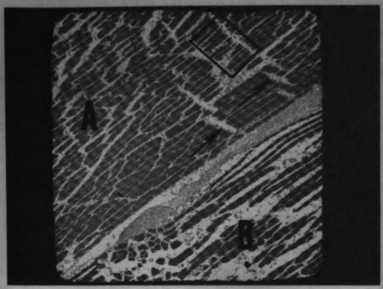

Figure 30. Low magnification electron micrograph of a portion of 2 fibers, A and B. The arrows indicate myofibril breakage (X 1,400)

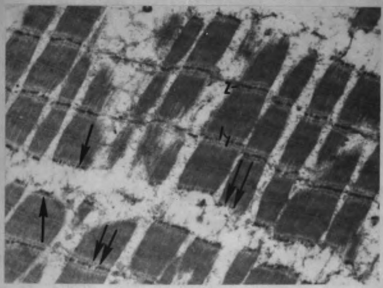

Figure 31. Higher magnification electron micrograph of the area within the square in figure 30. Single arrows show Z-line material remaining on both halves of the I-band, the double arrows indicate Z-line degradation. I = I-band; Z = Z-line (X 9,520)

fibers. Fibers with distinct and wide I-bands (figure 14 and 15) were frequently encountered, however, many fibers (figures 21, 22 and 23) with narrow, almost indistinguishable I-bands, could be found in most samples.

Glycogen. No granules having the dimensions or morphology commensurate with that reported for glycogen could be found in any of the postrigor samples.

These results indicate an apparent dichotomy in Z-line condition of postrigor samples. Davey and Gilbert (1969) reported that the Z-line condition in bovine sternomandibularis muscle was dependent somewhat on the animal from which the myofibrils were isolated. Some preparations contained Z-lines that were unaffected by storage, whereas others apparently suffered total Z-line loss. No direct comparison can be made between this study and that reported by Davey and Gilbert (1969) since they used a homogenization procedure for myofibril isolation. This procedure might contribute to the leaching of partially degraded Z-lines (Goll, 1968) and as such either the Z-lines are completely removed or are unaffected. Dutson et al. (1974) reported that Z-lines of red fibers (type I) from both normal and low quality porcine longissimus muscles were virtually unaltered by 24 hr. of postmortem storage. However, white fibers from the same animals underwent substantial Z-line alterations after 24 hr. of storage. Henderson et al. (1970) found no changes in bovine semitendinosus myofibrils after 24 hr. at 2 or 16 C, however, considerable disruption could be found in electron micrographs after 24 hr. when the samples were stored at 25 or 37 centigrade. Hay et al. (1973a) found little evidence of morphological

alterations in chicken leg muscle during postmortem storage, however, considerable alteration in Z-line structure was found in chicken breast muscle as early as 48 hr. postmortem. Fukazawa et al. (1969) found that Z-lines in myofibrils prepared from chicken pectoral muscle were either degraded, completely removed, or were broken at the junction of the Z-line and I-filaments.

The degradation of the Z-line, as considered previously, and I-Z breakage seem to be independent events. The occurrence of the latter phenomenon appears to be a function of the linkage between the Z-line and I-filament and not related to Z-line degradation. Fukazawa et al. (1969) considered that a breakdown of structures at the junction of the I-band and Z-line was a prerequisite for myofibril fragmentation during homogenization. Davey and Dickson (1970) observed that aged bovine muscle broke at the I-Z junction when tension was placed on muscle strips. These authors found no indication of breakage in unstretched samples prior to application of tension to the muscle strips. In unaged samples the strips lengthened predominately by withdrawal of thin filaments from the A-band, although considerable tension was necessary to force this stretching.

As reported by Hay et al. (1973a) and Dutson et al. (1974) the condition of the Z-line in postmortem fibers is dependent on the fiber type. Hay et al. (1973a) reported that the chicken leg muscle red fibers maintained Z-line continuity even after several days of postmortem aging. The type I fibers in the present study reacted similar to chicken leg muscle fibers (Hay et al., 1973a) and porcine longissimus muscle red fibers

(Dutson et al., 1974). On the other hand, the type II fibers lose Z-line structure similar to that of chicken breast muscle (Hay et al., 1973a) and porcine longissimus muscle white fibers (Dutson et al., 1974). Implications of this phenomenon with postmortem aging and tenderness will be considered later.

Little evidence could be found that would suggest any consistent M-line alterations occurring as the result of 48 hr. postmortem storage. An occasional fiber, as mentioned earlier, could be found that contained altered M-lines, however, these appeared to be the exception and not the general situation.

The glycogen content and contractile state were apparently unchanged after 48 hr. of storage. The glycogen reserves were considered earlier and the contractile state will be considered later in association with sarcomere length changes and tenderness.

216 Hr. Samples

Mitochondria. The morphology of mitochondria after 216 hr. of postmortem storage varies from fiber to fiber. Figure 32 shows portions of 2 fibers with the arrows indicating 2 mitochondria in 1 fiber. The mitochondrion indicated by the single arrow is relatively dense and the cristae have been altered very little. The mitochondrion indicated by the double arrow has an area devoid of cristae indicative of severe degradation. Figure 33 shows portions of another 2 fibers with the arrows indicating several intermyofibrillar mitochondria. These mitochondria appear swollen

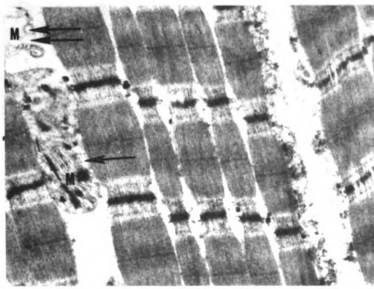

Figure 32. **Mitochondria in 216 hr. postmortem bovine longissimus muscle. M = mitochondria, single arrow indicates a mitochondrion with recognizable cristae, double arrows show a portion of a degraded mitochondrion (X 17,500)**

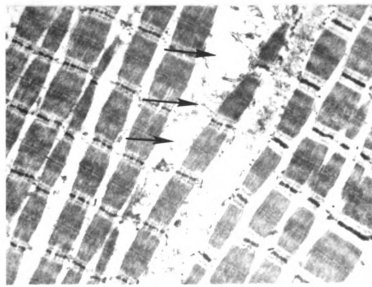

Figure 33. Two fiber portions of 216 hr. postmortem bovine longissimus muscle. Arrows indicate disrupted intermyofibrillar mitochondria (X 9,520)

and the cristae are either completely missing or degraded sufficiently to make organelle identification difficult. Figure 34 shows several myofibrils with a few mitochondria that are intermediate to those in the previous 2 examples. The organelle is easily identified, but the cristae show considerable disruption.

Few data can be found in the literature concerning postmortem mitochondrial morphology. The variable condition of the mitochondria suggests a fiber type-degradation relationship, however, this was not investigated and can not be substantiated without further investigation.

Z-Line. The condition of the Z-lines in the aged samples (216 hr.) resembles that seen for postrigor samples (48 hr.) in many respects. The type I fibers in the aged samples are almost indistinguishable from the type I fibers at 48 hr. postmortem. Figure 35 shows a group of myofibrils representing a type I fiber. The Z-lines are virtually unaltered and still maintain the thickness and density observed for the same fiber types in 1 hr. and 48 hr. samples. Figure 36 is a higher magnification of several myofibrils having very thick and dense Z-lines that have maintained much of the fibrillar structure seen in the 2 previous time periods. Figure 37 is another representative sample of type I fibers. Although the Z-lines in this fiber are not as dense as those seen in figure 36, they are almost completely unaltered from that at 1 hr. postmortem. The fibrillar nature of the Z-lines is discernible, however, this fiber has a tendency to be more diffuse than type I fibers in prerigor and early postrigor (48 hr.) samples.

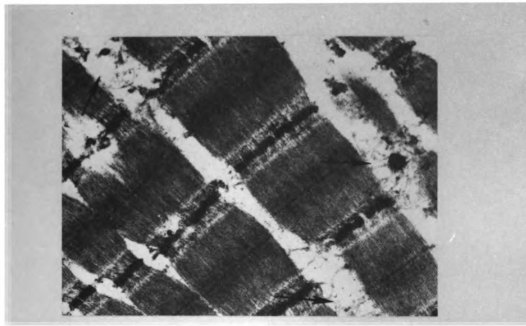

Figure 34. 216 hr. postmortem bovine longissimus muscle fiber. The arrows indicate several mitochondria in various states of degradation (X 17,500)

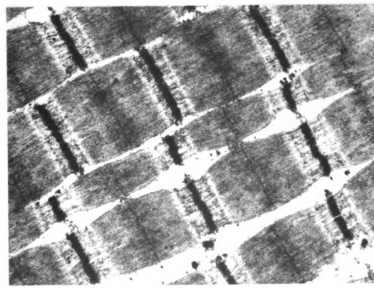

Figure 35. 216 hr. postmortem bovine longissimus muscle type I fiber showing essentially no change (X 17,500)

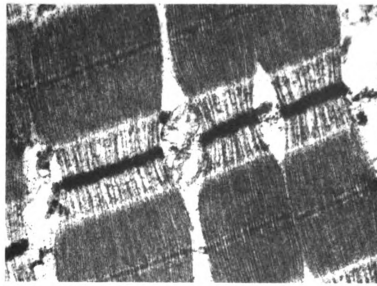

Figure 36. Higher magnification of a portion of a 216 hr. postmortem bovine longissimus muscle type I fiber showing essentially no change. (X 28,000)

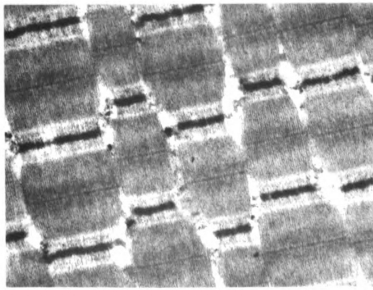

Figure 37. Type I 216 hr. postmortem bovine longissimus muscle fiber showing essentially no change (X 17,500)

Figures 38 and 39 show representative myofibrils of type II fibers in the 216 hr. samples. The myofibrils in figure 38 have relatively broad and dense Z-lines, however, as indicated by the arrows, several breaks have occurred in the Z-line. Figure 39 shows a group of myofibrils that are similar to those in figure 38. The large arrows indicate breaks in the Z-line which are associated with what appears to be longitudinal splitting (small arrows) of a portion of the myofibril especially in the I-band.

Figures 40 and 41 show 2 additional representative electron micrographs of type II fibers, however, these myofibrils show considerably more Z-line degradation (large arrows) than the previous micrographs of type II fibers. The small arrows indicate areas of longitudinal splitting which appear to be primarily associated with areas of Z-line breakage. Figure 42 shows a portion of 2 fibers, A and B, that represent the variation in Z-line breakage found in aged (216 hr.) type II fibers. Fiber A has relatively broad and dense Z-lines with only minor breakage, however, fiber B has narrower and less dense Z-lines and numerous areas of Z-line degradation.

I-Z Breakage. The breakage occurring at the junction of the thin filament with the Z-line follows a similar pattern to that seen in the 48 hr. samples. The principal difference between 48 hr. and 216 hr. samples is the greater abundance of I-Z splitting which was found in most fibers of the 216 hr. samples. Figure 43 shows a type I fiber exhibiting several areas of I-Z breakage as indicated by the arrows. The small arrows indicate areas that are breaking on both sides of the Z-line. This type of

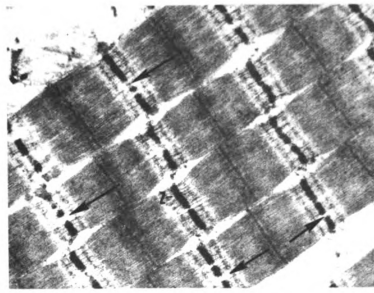

Figure 38. 216 hr. postmortem bovine longissimus muscle type II fiber. Arrows indicate breaks in the Z-line. Z = Z-line (X 17,500)

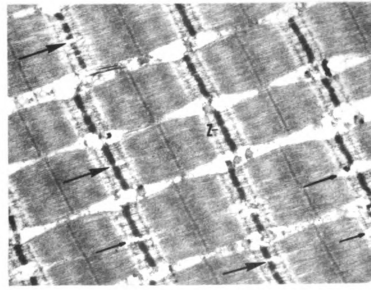

Figure 39. Type II fiber in 216 hr. postmortem bovine longissimus muscle. Large arrows show breaks in the Z-line, small arrows indicate longitudinal splitting. Z = Z-line (X 17,500)

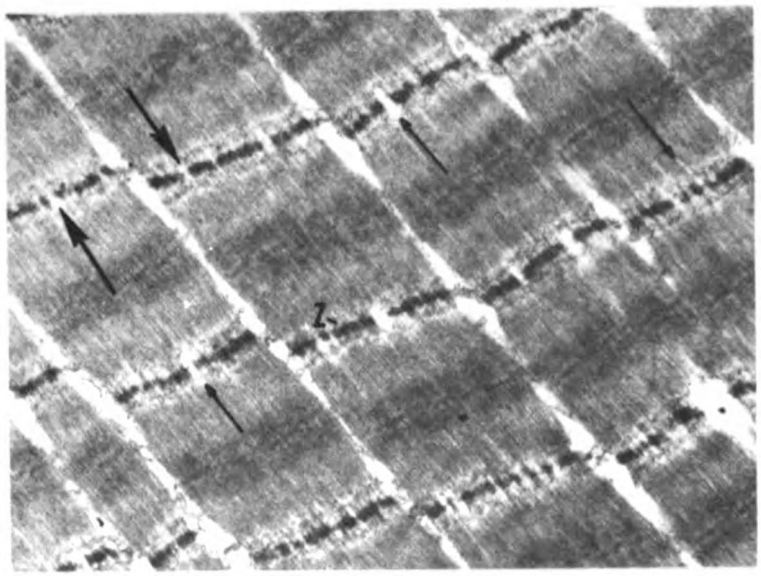

Figure 40. 216 hr. postmortem bovine longissimus muscle type II fiber. Large arrows indicate Z-line degradation, small arrows show areas of longitudinal splitting. Z = Z-line (X 17,500)

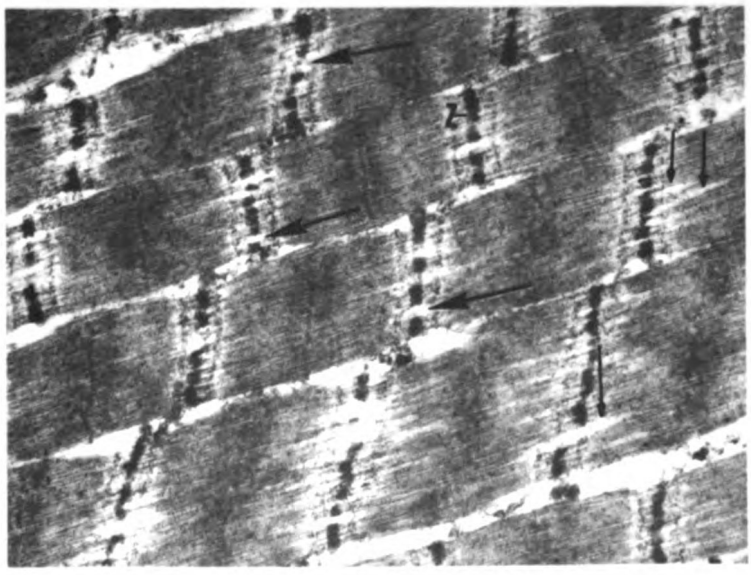

Figure 41. Type II fiber in 216 hr. postmortem bovine longissimus muscle. Large arrows show Z-line degradation, small arrows indicate longitudinal splitting. Z = Z-line (X 17,500)

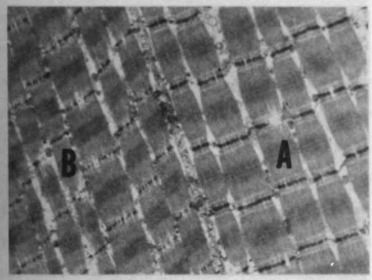

Figure 42. Two type II fibers, A and B, in 216 hr. postmortem bovine longissimus muscle (X 11,480)

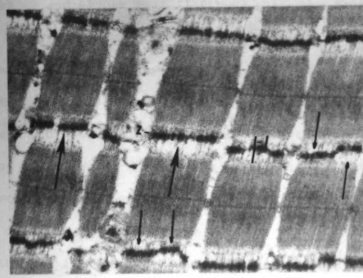

Figure 43. Type I 216 hr. postmortem bovine longissimus muscle fiber. Large arrows indicate I-Z breakage occurring on 1 side of the Z-line, small arrows indicate I-Z breakage occurring on both sides of Z-line. I = I-band; Z = Z-line (X 17,500)

breakage generally is not seen in type I fibers, however, this particular fiber represents an exceptional case and was not observed to occur in most type I fibers. Figures 44 and 45 show additional representatives of type I fibers, however, in these myofibrils the breakage has progressed to the point that they have been completely severed at the I-Z junction. The single arrows in figure 44 indicate 2 areas that appeared to have pulled away from the Z-lines but have not been completely severed. The double arrows in the same figure indicate areas that have broken in the I band region rather than at the I-Z junction. The single arrows in figure 45 indicate several areas that have broken predominately at the level of the I-Z junction.

Figures 46 and 47 are type II fibers that have broken at the I-Z junction. The single arrows in figure 46 indicate areas of breakage at the I-Z junction and which have retained the majority of the Z-line on the visible myofibril fragment. The double arrows indicate areas of Z-line breakage and longitudinal splitting. Figure 47 is another type II fiber having an area of I-Z breakage, but it has not progressed to the point of total I-Z severance as shown for the myofibrils in figure 46. The arrows indicate areas of Z-line breakage within Z-lines which are adjacent to the I-Z junction breakage. Figure 48 shows a portion of a fiber similar to that in figure 46. The single arrows indicate areas of Z-line breakage and the double arrows indicate an area of myofibrils that are obliquely sectioned.

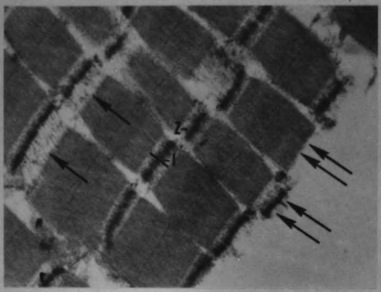

Figure 44. 216 hr. postmortem bovine longissimus muscle type I fiber. Single arrows indicate incomplete I-Z breakage, double arrows indicate apparent breakage in the I-band area. I = I-band; Z = Z-line (X 17,500)

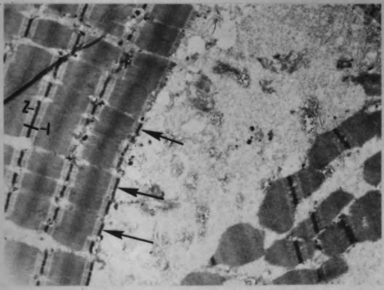

Figure 45. 216 hr. postmortem bovine longissimus muscle type I fiber. The single arrows indicate areas that have broken at the level of the I-Z junction. I = I-band; Z = Z-line (X 7,840)

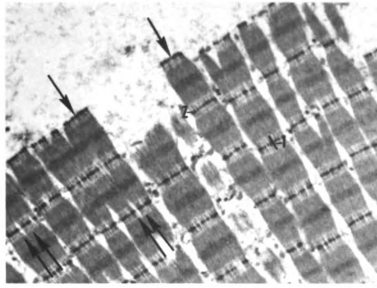

Figure 46. 216 hr. postmortem bovine longissimus muscle type II fiber. The single arrows indicate I-Z breakage and the double arrows show Z-line degradation and longitudinal splitting. I = I-band; Z = Z-line (X 9,520)

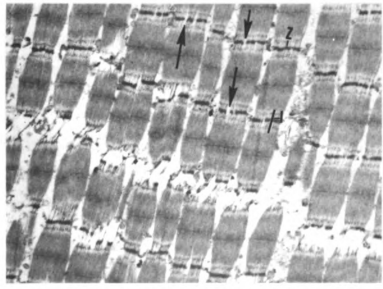

Figure 47. Type II fiber in 216 hr. postmortem bovine longissimus muscle. The single arrows indicate areas of Z-line breakage adjacent to the I-Z junction breakage. I = I-band; Z = Z-line (X 9,520)

Fiber Breakage. Several of the 48 hr. postmortem samples had numerous areas of I-Z breakage, however, most of the breakages involved only random Z-lines and only rarely were areas found that suggested incipient transverse fiber division. Figure 49 shows a low magnification electron micrograph of portions of three fibers. As indicated by the arrows, fiber A has several areas of partial transverse breaks. One of the areas of fiber breakage (double arrows) shows greater myofibril separation near the transverse center of the fiber and the break decreases in width as it extends in one direction towards the sarcolemma. Figure 50 shows another fiber exhibiting oblique breakage that extends across the entire fiber. The arrows indicate a small area of complete I-Z breakage that has occurred near the sarcolemma.

Figures 51 and 52 show low magnification electron micrographs of several fibers that have complete transverse fiber breakage. In figure 51, fiber C has not only broken completely but it has also been pulled apart leaving a large gap between the 2 broken halves of the fiber. Figure 52 shows a different fiber, which broke similarly to that in figure 51 and likewise has a gap between the two broken fiber fragments. Figure 53 shows a higher magnification of a fiber similar to the 2 previous fibers. The double arrows indicate areas that have obviously broken at the level of the Z-line, whereas the single arrows indicate the adjacent portions of these myofibrils which apparently had moved sufficiently from the longitudinal orientation during breakage and consequently they were sectioned obliquely.

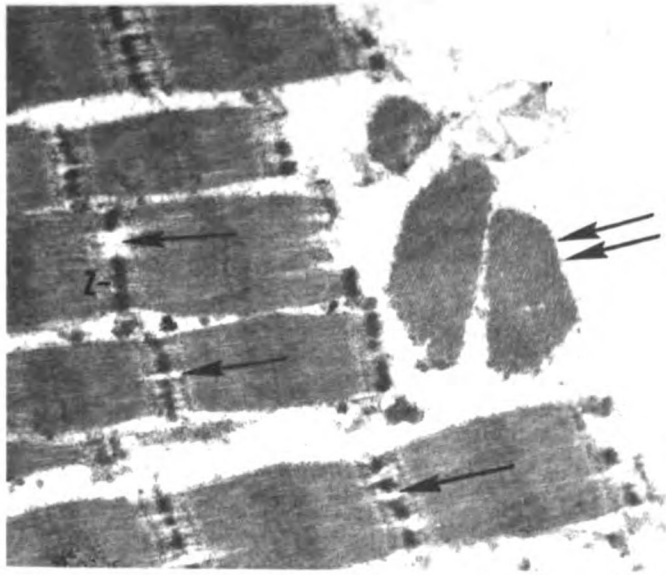

Figure 48. 216 hr. postmortem bovine longissimus muscle type II fiber. The single arrows indicate Z-line breakage and the double arrows show an area of obliquely sectioned myofibrils. Z = Z-line (X 17,500)

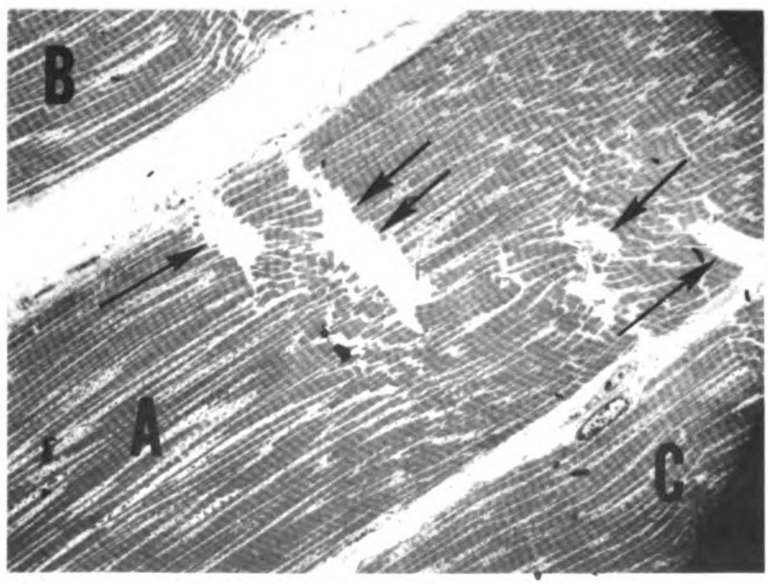

Figure 49. Portions of 3 fibers, A, B and C, from 216 hr. postmortem bovine longissimus muscle. The arrows indicate areas of partial fiber breakage and the double arrows indicate an area extending across 1/2 of the fiber (X 780)

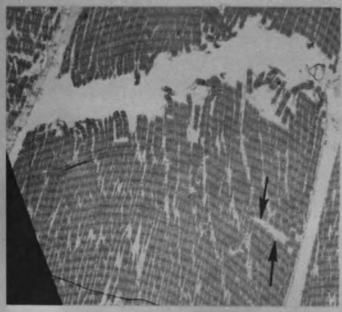

Figure 50. Low magnification electron micrograph of an area of oblique fiber breakage in 216 hr. postmortem bovine longissimus muscle. Arrows indicate a small area of I-Z breakage (X 1,400)

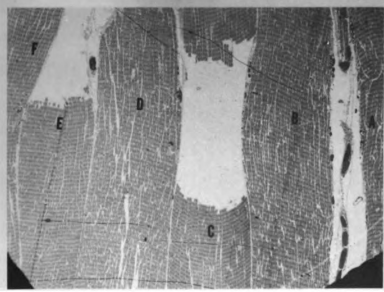

Figure 51. Low magnification electron micrograph of a portion of 6 fibers, A, B, C, D, E and F, of 216 hr. postmortem bovine longissimus muscle. Fiber C has broken transversely and retracted (X 1,400)

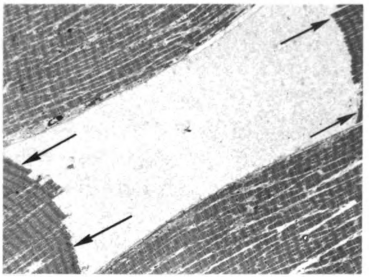

Figure 52. **Portion of a broken fiber from 216 hr. postmortem bovine longissimus muscle. The arrows indicate the 2 broken halves of the fiber (X 1,400)**

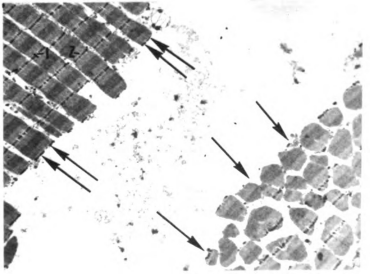

Figure 53. Higher magnification electron micrograph of a transversely divided 216 hr. postmortem bovine longissimus muscle fiber. The single arrows show obliquely sectioned portions of myofibrils and the double arrows indicate I-Z breakage. I = I-band, Z = Z-line (X 5,460)

M-Line. The M-line did not appear nearly as discrete and prominent as in earlier postmortem time periods, however, it was present in all fibers at 216 hours.

Contractile State. The actual sarcomere lengths of all the samples will be presented later, however, the ultrastructural observations suggest that little sarcomere lengthening has occurred after 216 hr. of postmortem aging.

Glycogen. As was the case at 1 hr. and 48 hr. postmortem, no glycogen granules could be found in any sample at 216 hours. The absence of glycogen is consistent with other published data in postrigor muscle. The absence of these particles in the 1 hr. samples has been discussed previously.

The structure of the Z-line changed very little between 48 and 216 hr. postmortem. The type I fiber Z-lines became slightly more diffuse, however, the alteration was very subtle. The type II fibers were similar in most respects to the 48 hr. samples, however, the Z-line degradation tended to be more constant from fiber to fiber although some fiber variation still existed. As mentioned previously, Davey and Gilbert (1969) found 2 fiber populations, based on the presence or absence of Z-lines, in myofibrils of muscle aged for 20 days. The predominate myofibril population was devoid of Z-lines, while these authors found that other samples contained Z-lines. Fukazawa and Yasui (1967) reported that homogenized chicken muscle myofibrils lost all evidence of Z-line material after 24

hr. of postmortem storage. In contrast to these observations, no sample in the present study could be found that was devoid of Z-lines, although many had lost as much as one-half of the Z-line material. This apparent observation can be partially explained by different methods of sample preparation since Davey and Gilbert (1969) and Fukazawa and Yasui (1967) used myofibril preparations that possibly allowed leaching of material from the labile Z-lines. Henderson et al. (1970) reported a total loss of Z-lines from rabbit muscle after storage at 37 C for 24 hr., however, lower storage temperatures prevented the complete loss of Z-lines. The latter authors observed that bovine muscle Z-lines were not removed from myofibrils by homogenization until 168 hr. postmortem. These results are confusing, however, an apparent fiber type-species relationship seems to exist in postmortem muscle Z-line lability.

The amount of I-Z breakage became much more prominent in muscle at 216 hr. than was observed at 48 hr. postmortem. At 216 hr., there appeared to be a tendency for considerable numbers of I-Z breakages to be present in those muscles where they were observed at all since some fibers showed no I-Z breakages. This contrasts somewhat to the 48 hr. samples where only isolated and seemingly random I-Z breakages occurred. The most obvious difference among the three time periods is the appearance of total transverse fiber splitting as the result of massive breaks at the I-Z junction in the 216 hr. samples. Hanson et al. (1942), Paul et al. (1944) and Ramsbottom and Strandine (1949) reported the presence of fiber breaks in postmortem muscle. Paul et al. (1944) found these breaks occurred as early as 24 hr. in bovine muscle and increased with postmortem storage time.

Ultrastructure and Tenderness. Most of the ultrastructural observations on muscle have been concerned with postmortem structural alterations and not the direct association between ultrastructure and tenderness. The association between these alterations and tenderness has primarily been by inference and not by objective or direct measurement. Goll (1968) suggested that since Z-line degradation and postmortem tenderization followed a similar time sequence that a cause and effect relationship could exist. Davey and Gilbert (1967b) found that bovine sternomandibularis muscle aged for 3 days at 15 C suffered a complete loss of Z-lines. These authors suggested that the loss of normally refractory Z-lines was closely associated with the effects of aging. Weidemann et al. (1967) found that the degree of disruption of myofilaments was a good indication of tenderness. Davey and Gilbert (1969) suggested a possible relationship between loss of myofibrillar lateral linkages and tenderness. Davey and Dickson (1970) substantiated this observation but concluded that other changes probably were more important to overall tenderness variation. Hay et al. (1973a) reported that chicken breast myofibrils underwent substantial alterations, particularly at the Z-line, whereas leg myofibrils underwent little perceptible morphological change up to 168 hr. postmortem. Since the leg muscle, adductor longus, is considered to be a predominately red muscle and the breast muscle, pectoralis superficialis, predominately white the authors attributed the postmortem muscle changes to a possible difference in lability between red and white fibers. The red and white fibers presented by Hay et al. (1973a) corresponds closely to the type I

and type II fibers, respectively, in the present study both morphologically and the type of postmortem alterations. If in fact postmortem aging and ultrastructural alterations have a cause and effect relationship, then the observation that Z-line degradation is a primary facet of tenderization must be evaluated in a different perspective. This is not to suggest that Z-line degradation does not impart fragility to muscle fibers, but the presence of a population of fibers that apparently do not undergo perceptible Z-line alteration suggests that another, at least equally important, alteration exists which could account for tenderization in these fibers.

The relationship between Z-line degradation and tenderness in the present study is difficult to assess. The fibers classified as type II were extremely variable in susceptibility to Z-line degradation although all fibers within this group were affected to some degree. The amount of degradation seen for any one sample was dependent on the presence of degradation in the particular section of the fiber that was sampled and particularly dependent on the fiber types present in each sample. No fibers were seen in the 48 hr. and 216 hr. samples that were classified as type II fibers that were free of Z-line degradation. If Z-line degradation imparts fragility to the fiber system, then the type I fibers apparently do not become fragile or tenderize by the same mechanism as that observed for the type II fibers.

In addition to the degradation and disappearance of the Z-line in 24 hr. postmortem chicken pectoral muscle, Fukazawa et al. (1969) found that the junction of the I-Z bands was weakened and broke during homogenization.

Davey and Dickson (1970) and Goll et al. (1970) reported that the weakening of the junction of the I-filaments with the Z-line could account, at least partially, for the loss of tensile strength and rigor resolution. Davey and Dickson (1970) studied the breakdown at the I-Z junction and suggested that the weakness at this point was primarily responsible for the effects of meat aging. Generally, the morphological expression of I-Z weakness is breakage and separation at the junction of these myofibrillar components. However, these same authors found that the inherent weakness imparted by postmortem aging, at the I-Z junction could be present without ultrastructural expression.

If, in fact, the observations by Davey and Dickson (1970) are true, then the interpretation of the ultrastructural data in the present study becomes very tenuous and difficult to relate to tenderness. The ultrastructure of 1 hr. and 48 hr. bovine longissimus muscle was variable and no association between morphological changes and tenderness groups could be found. However, at 216 hr. a subtle association was found between the amount of fiber breakage (maximal I-Z splitting) and tenderness. This association showed that the most tender (shear) sample contained more fiber breakage than the toughest sample. Early work by several groups (Hanson et al., 1942; Paul et al., 1944; and Ramsbottom and Strandine, 1949) reported a relationship between fiber breakage and shear values. Hanson et al. (1942) reported that chicken breast muscle had more and an earlier occurrence of fiber breakage than thigh muscles and generally longer storage periods resulted in more breakage. This latter group also

found that breast muscle became tender sooner than thigh muscle when scored by a taste panel. The most tender muscle in the study, the pectoralis secundis, was found to microscopically exhibit postmortem changes earlier than the other muscles studied. Moller et al. (1973) reported that myofibril fragmentation was positively associated with tenderness. This myofibril fragmentation was induced by homogenization and is difficult to compare with unhomogenized muscle samples. The amount of fiber breakage and shear value in the present study did not appear to be related when the differences in shear value were less than that observed for the most tender and toughest sample. However, the observation by Davey and Dickson (1970) that the weakness at the I-Z junction did not necessarily express itself ultrastructurally leads one to question the classification of samples as tough if fiber breaks or I-Z breaks are not apparent.

Thus, it appears that a muscle sample was tender if a large percentage of the fibers broke during storage. However, the lack of fiber breakage, in the present study, appears to be ineffective as a criteria for classifying a sample as tender or tough. A combination of biochemical and ultrastructural data may be necessary to effectively evaluate shear and taste panel differences between samples, particularly if these differences are small. A combination of several biochemical parameters and ultrastructural data and their interrelationship with tenderness will be considered later.

Sarcomere Length

Sarcomere lengths were measured only on the 48 and 216 hr. longissimus muscle samples. The sarcomere length means of the tender and control groups at 48 and 216 hr. postmortem time periods are presented in table 1.

Table 1. MEAN SARCOMERE LENGTH OF THE LONGISSIMUS MUSCLE OF EACH LINE AND AGING PERIOD[a]

Postmortem time	Tenderness line		
	Tender	Control	Overall
48 hr.	2.12 ± 0.02	2.13 ± 0.03	2.14 ± 0.02
216 hr.	2.10 ± 0.02	2.09 ± 0.03	2.10 ± 0.03

[a]Mean sarcomere length is expressed in μm ± S.E.

Sarcomere length of the 48 and 216 hr. samples were similar, however, sarcomere lengths of the 216 hr. samples were slightly although nonsignificantly ($P > .05$) shorter than those at 48 hours. The treatment means (control and tender groups) are essentially the same within each aging time period with the 48 hr. samples having slightly longer sarcomeres than the 216 hr. samples. These observations are not in agreement with those of Gothard et al. (1966) who found considerable lengthening of bovine longissimus and semimembranosus sarcomeres during postmortem aging. Parrish et al. (1973) reported no change in bovine longissimus muscle sarcomeres after 7 days aging, however, bovine semitendinosus muscle sarcomeres increased in length during each postmortem aging interval examined. The lack of a difference between the sarcomere lengths of the control and tender

groups was surprising in light of the effect of sarcomere length on tenderness (Locker, 1960). However, Marsh (1972) reported that shortening of 20 percent or less had little effect on tenderness. If the approximate resting sarcomere length of bovine muscle is considered to be 2.4 μm (Bendall, 1971), then the sarcomeres in the present study have contracted less than the 20 percent reported to be necessary to have an effect on shear values.

Some correlation coefficients between sarcomere length and muscle properties are presented in table 2. Sarcomere length of the 48 hr. samples was significantly ($P < .01$) and negatively correlated ($r = -.78$) with the amount of KCL extracted myofibrillar N and also with the KCL extracted myofibrillar N to sarcoplasmic N ratio (MF-N:SP-N) ($r = -.71$, $P < .05$). The 216 hr. KCL and KI extracted myofibrillar N were significantly negatively correlated ($r = -.62$ and $r = -.66$, respectively) with sarcomere length at 48 hr. but nonsignificantly correlated with sarcomere length at 216 hr. postmortem. Cook (1967) reported that postrigor (48 hr.) bovine sternomandibularis myofibrillar protein extraction increased with an increase in sarcomere length. In the present study, sarcomere length of 48 hr. aged samples was significantly correlated ($r = 0.74$, $P < .05$) with 1 hr. postmortem temperature.

The sarcomere lengths at 216 hr. were significantly correlated ($r = -.64$, $P < .05$) with shear values, but not with taste panel scores ($r = 0.22$). The shear value correlation concurs with previous observations for the relationship with sarcomere length (Locker, 1960), however, the taste panel and sarcomere length correlation disagrees with that reported by Herring et al. (1965a).

Table 2. SIMPLE CORRELATION COEFFICIENTS BETWEEN SARCOMERE LENGTH AND BIOCHEMICAL AND TENDERNESS DATA.

	KCL2[a]	KCL3[b]	KI2[c]	KI3[d]	MF-N: SP-N[e]	Warner-Bratzler shear	Taste panel	TMP[f]
Sarcomere length (48 hr.)	-.78**	-.63*	-.04	-.66*	-.71*	-.33	-.01	0.74*
Sarcomere length (216 hr.)	-.44	-.21	-.15	-.05	-.28	-.64*	0.22	0.44

[a]KCL2 = KCL extracted N at 48 hr.
[b]KCL3 = KCL extracted N at 216 hr.
[c]KI2 = KI extracted N at 48 hr.
[d]KI3 = KI extracted N at 216 hr.
[e]48 hr. KCL extracted myofibrillar N to sarcoplasmic N ratio
[f]Temperature of Longissimus at 1 hr. postexsanguination
*P < .05
**P < .01

Protein Fractionation

Sarcoplasmic Proteins. The means of low ionic strength protein N (sarcoplasmic) extracted from muscle after 1, 48, and 216 hr. of postmortem storage are presented in table 3. The 48 hr. (mg N/g of tissue) means are significantly ($P < .05$) greater than the 1 hr. or 216 hr. means. This observation is in contrast with the values reported by Goll et al. (1964a) who found sarcoplasmic protein extraction to be highest immediately after slaughter. Aberle and Merkel (1966) reported that sarcoplasmic N extracted from bovine longissimus muscle did not change significantly during postmortem aging, however, some minor modifications were apparent in their electrophoretic patterns. In the present study, no sarcoplasmic N differences were obtained between the control and tender groups. Additionally, no significant correlation coefficients were observed between sarcoplasmic N and either taste panel or shear data.

Myofibrillar Proteins. The mean fibrillar N extracted with KI decreased slightly but not significantly from 1 to 48 hr. but then showed a small but nonsignificant increase from 48 to 216 hr. (table 3). Myofibrillar N extracted with KCl increased significantly ($P < .05$) from 1 hr. through 216 hr. postmortem. While the KCl extracted myofibrillar N increased between 48 and 216 hr., the increase was not significant. In contrast to the present study, Goll et al. (1964a) reported that bovine muscle protein extracted with KI was highest at 0 hr. when compared to longer postmortem storage times (6, 12, 24, 72 and 312 hr.). Aberle and Merkel (1966) found myofibrillar N (KI extraction) decreased from 0 to 24 hr. postmortem, however,

Table 3. MEAN PROTEIN FRACTION N OF THE LONGISSIMUS MUSCLE OF EACH LINE AND AGING PERIOD[a,b,c]

Protein fraction	Line	Postmortem period 1 hr.	48 hr.	216 hr.	$\bar{x}$
SP-N[d]	Control	6.38	6.87	6.28	6.51 ± 0.08[o]
	Tender	6.37	6.88	6.40	6.55 ± 0.08[o]
	$\bar{x}$	6.38 ± 0.55[m]	6.88 ± 0.55[n]	6.34 ± 0.55[m]	
MF-N (KI)[e]	Control	17.80	17.31	17.74	17.62 ± 0.42[o]
	Tender	17.71	17.28	17.23	17.40 ± 0.42[o]
	$\bar{x}$	17.76 ± 0.42[m]	17.30 ± 0.42[m]	17.48 ± 0.42[m]	
MF-N (KCL)[f]	Control	6.70	7.95	8.18	7.61 ± 0.05[o]
	Tender	6.82	7.69	8.39	7.61 ± 0.05[o]
	$\bar{x}$	6.76 ± 0.23[m]	7.82 ± 0.23[n]	8.29 ± 0.23[n]	
NPN[g]	Control	4.89	4.85	4.78	4.84 ± 0.25[o]
	Tender	4.91	4.89	5.10	4.97 ± 0.25[o]
	$\bar{x}$	4.90 ± 0.07[m]	4.87 ± 0.07[m]	4.94 ± 0.07[m]	
ST-N (KI)[h]	Control	5.94	4.96	5.89	5.60 ± 1.19[o]
	Tender	5.02	4.29	5.60	4.97 ± 1.19[o]
	$\bar{x}$	5.48 ± 0.29[m]	4.63 ± 0.29[m]	5.74 ± 0.29[m]	
ST-N (KCL)[i]	Control	16.94	14.46	15.45	15.62 ± 1.72[o]
	Tender	15.90	13.89	14.43	14.74 ± 1.72[o]
	$\bar{x}$	16.42 ± 0.24[n]	14.17 ± 0.24[m]	14.94 ± 0.24[m,n]	
TP-N[j]	Control	34.92	33.86	34.65	34.48 ± 1.15[o]
	Tender	34.00	33.34	34.32	33.89 ± 1.15[o]
	$\bar{x}$	34.46 ± 0.92[m]	33.60 ± 0.92[m]	34.49 ± 0.92[m]	

Table 3. (Continued)

Protein fraction	Line	Postmortem period 1 hr.	48 hr.	216 hr.	$\bar{x}$
MF-N:SP-N (KI)[k]	Control	2.79	2.52	2.83	2.71 ± 0.09[o]
	Tender	2.79	2.51	2.71	2.67 ± 0.09[o]
	$\bar{x}$	2.79 ± 0.28[n]	2.52 ± 0.28[m]	2.77 ± 0.28[n]	
MF-N:SP-N (KCL)[l]	Control	1.05	1.16	1.30	1.17 ± 0.00[o]
	Tender	1.07	1.12	1.32	1.17 ± 0.00[o]
	$\bar{x}$	1.06 ± 0.23[m]	1.14 ± 0.23[m]	1.31 ± 0.23[n]	

[a]Means are expressed as mg N/g of tissue ± S.E.
[b]Means on a line with the same superscript are not significantly different ($P > .05$)
[c]Means in a column with the same superscript are not significantly different ($P > .05$)
[d]Sarcoplasmic N
[e]Myofibrillar N extracted by KI
[f]Myofibrillar N extracted by KCL
[g]Non-protein N
[h]Stroma N from KI extraction
[i]Stroma N from KCL extraction
[j]Total N
[k]Myofibrillar N:sarcoplasmic N ratio, KI extraction
[l]Myofibrillar N:sarcoplasmic N ratio, KCL extraction

myofibrillar N increased significantly between 24 and 168 hours. Chaudhry et al. (1969) reported that 0.5M KCl extracted myofibrillar protein from bovine semitendinosus muscle increased between 16 to 24 hr. and 312 hr. of storage. Chaudhry et al. (1969) also reported that 1.1M KI extracted myofibrillar protein increased in a similar manner as that for KCl extracted protein except that the KI extraction had a smaller increase during postmortem aging. The changes in myofibrillar N between 48 and 216 hr., in the present study, are consistent with those reported by Chaudhry et al. (1969) with KI and KCL extraction. Penny (1968) reported an increase in extractability of N (KCL) from rabbit longissimus muscle during aging at 15 to 18 C and 4 C, however, muscle stored at the lower temperature yielded less myosin than muscle stored at room temperature. Davey and Gilbert (1968a) reported that postmortem aging decreased the time necessary for extracting 75% of bovine longissimus muscle protein. No differences were found in the extractability of myofibrillar N between control and tender groups in the present study which is in agreement with Goll et al. (1964a) but disagrees with the results of Hegarty et al. (1963). Only myofibrillar N extracted by KI at 48 hr. postmortem was significantly correlated ($P < .05$) (table 4) with taste panel score, however, neither of the other 2 KI myofibrillar N nor KCL myofibrillar N fractions was significantly correlated with taste panel or shear values. The KCL extracted myofibrillar N at all time periods was highly negatively correlated ($P < .05$) with 1 hr. temperature (table 4). An association between temperature and protein solubility has been reported (Scopes, 1964; Sayre and Briskey, 1963), however, in most

Table 4. SOME SIMPLE CORRELATION COEFFICIENTS BETWEEN PROTEIN FRACTION N AND PALATABILITY, MUSCLE pH AND TEMPERATURE

		Correlation Coefficients		
		Postmortem period		
Variable I	Variable II	1 hr.	48 hr.	216 hr.
KI[a]	Taste panel[b]	-.15	-.64*	0.04
KCL[c]	Temperature[d]	-.68*	-.78**	-.72*
KI	pH[e]	0.11	0.05	0.50
KCL	pH	-.38	0.11	-.31
KIS[f]	Taste panel	-.17	0.67*	0.06
NPN[g]	Taste panel	-.72*	-.29	0.10
NPN	Shear[h]	0.31	-.22	-.61
Total N[i]	Temperature	-.26	-.40	-.79**
KIMF-N:SP-N[j]	Taste panel	-.60	-.88**	-.29

[a]KI extracted myofibrillar N
[b]216 hr. sensory evaluation
[c]KCL extracted myofibrillar N
[d]1 hr. postexanguination muscle measurement
[e]1 hr. postexanguination muscle measurement
[f]Stroma N determined as difference from KI myofibrillar N extraction
[g]Non-protein N
[h]216 hr. Warner-Bratzler shear value
[i]Total sample N
[j]KI extracted myofibrillar N:sarcoplasmic N
*P < .05
**P < .01

cases a combination of high temperature and low pH was reported to be responsible for decreased solubility of the fibrillar proteins. However, no significant correlation was found between 1 hr. pH and fibrillar N solubility in the present study.

NPN. The mean TCA soluble N did not change significantly during postmortem storage (table 3). The tender line 216 hr. NPN mean (5.10) was significantly different ($P < .05$) from all other line and aging period means except for the 1 hr. tender line mean. These results disagree with

those of Sharp (1963) and Aberle and Merkel (1966) who found NPN to increase significantly during postmortem storage of bovine muscle. Moran and Smith (1929) and others assumed that the increase in tenderness of aged muscle was due to proteolysis and used TCA soluble N as a measure of proteolysis. However, Sharp (1963) and Parrish et al. (1969a) concluded that the increase in NPN was primarily due to changes in the sarcoplasmic proteins and not myofibrillar degradation. Results from the present study concerning CA^{2+} ITPase activity will be presented later in this thesis, but these data support the concept that no major myofibrillar degradation had occurred and the increased NPN in the tender line was probably due to sarcoplasmic protein degradation. NPN at 1 hr. was significantly and negatively correlated ($P < .05$) with taste panel score and 216 hr. NPN approached significance with Warner-Bratzler shear (table 4). The latter observation suggests that proteolytic degradation in muscle was associated with lower shear values.

Stroma. The stroma N obtained after extracting the myofibrillar N of longissimus muscle with KI, in the present study, varied slightly during postmortem storage from 1 hr. to 216 hr. (table 3). There was a tendency for the 48 hr. samples to have less stroma, however, these differences were not statistically significant. In all aging periods, more stroma N was extracted from the control group than from the tender group and these group differences approached significance ($P < .10$). The stroma N obtained after extracting myofibrillar N with KCL decreased significantly ($P < .05$) between 1 and 48 hr. postmortem. However, the stroma N (KCL) between 1 and 216 hr.

means were not statistically different. Penny (1967) investigated the extraction of rabbit myofibrillar proteins by KCL and concluded that KCL does not extract all of the actomyosin from the myofibrillar structure. Penny (1968) also reported that pyrophosphate containing solutions extracted more of the myosin than KCL which are similar to the KI extracted muscle N values in the present study. The only significant correlation coefficient between stroma N and tenderness data was that between 48 hr. KI stroma N and taste panel score ($r = 0.67$, $P < .05$), however, the coefficient at 216 hr. was not significantly correlated with either shear or taste panel data (table 4). These data suggest that quantitatively stroma N values were not highly related to tenderness and the significant differences in stroma N between aging periods following myofibrillar extraction with KCL probably resulted from the incomplete myofibrillar extraction and the corresponding increase in stroma N which was determined by difference.

Total N. The amount of total N did not vary significantly among the 3 aging periods in the present study (table 3). There was a small but nonsignificant decrease in total N at 48 hr. when compared to 1 and 216 hr. mean values. No significant differences were observed between the control and tender groups, however, the control group contained slightly more total N at each time period than the tender group. Few data have been reported on the effect of postmortem aging on total N in muscle, perhaps due to the assumption that total protein (Kjeldahl) in muscle is static, whereas the various fractions can be altered by postmortem muscle conditions. Cook (1967) reported a variable but nonsignificant total N content between postrigor (48 hr.) shortened muscle and postrigor (48 hr.) stretched muscle.

There were no significant correlations between total N and objective and subjective tenderness scores. The muscle temperature 1 hr. postmortem was highly ($P < .05$) correlated with total protein N extracted at 216 hr. This latter observation is in agreement with Scopes (1964) who found that allowing rigor mortis to occur at 0 C did not affect myofibrillar solubility, but higher temperatures (37 C) decreased solubility by one-half. A combination of high temperature and low pH has been reported to affect myofibrillar protein solubility (Sayre and Briskey, 1963), however, no correlation was found between 1 hr. pH and solubility in the present study (table 4).

Myofibrillar N to Sarcoplasmic N Ratio (MF-N:SP-N). The myofibrillar and sarcoplasmic N values were considered earlier as separate fractions. The 48 hr. KI MF-N:SP-N ratio was significantly ($P < .05$) different from the 1 and 216 hr. mean values (table 3). This difference was primarily due to the increased extractability of the sarcoplasmic N at 48 hr. and not due to increased myofibrillar N extraction. The KCL MF-N:SP-N ratio increased significantly ($P < .05$) between 1 hr. and 216 hr., however, the 1 and 48 hr. means were not significantly different (table 3). In contrast to the KIMF-N:SP-N ratio, increase in the KCL MF-N:SP-N ratio was due primarily to the increased quantity of myofibrillar N extracted at each time period. No significant differences were obtained between the control and tender groups for either KCL or KIMF-N:SP-N ratios. The 48 hr. KIMF-N:SP-N ratio was significantly and negatively correlated ($r = -.88$, $P < .05$) with taste panel score (table 4), however, the ratio was

nonsignificantly correlated with shear value. None of the other objective and subjective tenderness scores was significantly correlated with either KCL or KIMF-N:SP-N ratios at the other aging periods.

The recognition that postmortem NPN content has been reported to increase, led to the suggestion that postmortem tenderization was the result of myofiber degradation (Hoagland et al., 1917). However, recent research suggests that little proteolysis occurs in postmortem muscle and the proteolysis detected has generally been attributed to breakdown of sarcoplasmic proteins (Locker, 1960; Sharp, 1963; Parrish et al., 1969a). These observations are consistent with those in the present study. The increase in myofibrillar solubility during postmortem storage has been associated with increased tenderness (Aberle and Merkel, 1966; Davey and Gilbert, 1968a), however, the factors responsible for the increased solubility have not been characterized. The quantity of connective tissue in muscle is known to influence tenderness, however, recent work suggests that molecular architecture of collagen, between similar muscles, contributes more to muscle tenderness (Pfeiffer et al., 1972; Bailey, 1972) than connective tissue quantity. The nonsignificant change in KI extracted stroma N in the present study supports the contention that quantity of connective tissue is less important than molecular structure when comparing the same muscles among animals.

Protein Solubility and Ultrastructure. The association between KI extracted myofibrillar N and ultrastructural observations at 1, 48 and 216 hr. is apparently small since KI extracted N did not vary significantly

among the 3 postmortem time periods (table 3). The KCL extracted myofibrillar N increased significantly from 1 to 48 hr. and further increased, although nonsignificantly, between 48 and 216 hr. postmortem. Most of the Z-line degradation occurred between 1 and 48 hr. postmortem and while further degradation occurred between 48 and 216 hr. postmortem, the changes were less extensive than that prior to 48 hours. Davey and Gilbert (1968a) suggested that increased solubility of myofibrillar proteins during aging was consistent with weakening of protein linkages with insoluble cell components, or possibly the destruction of the Z-line and SR contributed to the increased solubility during aging. There were no detectable differences in myofibrillar N extracted between control and tender groups at any time period measured in the present study. This observation suggests that tenderness differences cannot be assessed by myofibrillar quantitation or that the samples in the present study were not sufficiently divergent in tenderness to be detected by protein solubility differences.

Adenosine Triphosphatase Activity

Ca^{2+}-Modified ATPase. The 1 mM Ca^{2+}-modified ATPase (40 mM KCL) activities changed little between 1 hr. and 216 hr. (table 5). There were no significant differences among the 3 time periods (1, 48 and 216 hr.) or between control and tender longissimus muscle samples. These results are in contrast to those of Goll and Robson (1967) and Parrish et al. (1973) who reported that Ca^{2+}-modified ATPase activity of bovine muscle myofibrils increased 20 to 50% (312 hr.) and 2-fold (7 days), respectively, when

Table 5. MEAN ADENOSINE TRIPHOSPHATASE ACTIVITIES BY LINE AND AGING PERIOD[a]

Activator	Tenderness line	Postmortem period 1 hr.	48 hr.	216 hr.	$\bar{x}$
1 mM CA^{2+}	Control	0.11	0.12	0.13	0.12 ± 0.02[c]
	Tender	0.12	0.13	0.14	0.13 ± 0.02[c]
	$\bar{x}$	0.12 ± 0.02[b]	0.12 ± 0.02[b]	0.13 ± 0.02[b]	
1 mM Mg^{2+}	Control	0.12	0.11	0.12	0.11 ± 0.01[c]
	Tender	0.12	0.11	0.13	0.12 ± 0.01[c]
	$\bar{x}$	0.12 ± 0.01[b]	0.11 ± 0.01[b]	0.12 ± 0.01[b]	
1 mM EDTA	Control	0.09	0.09	0.09	0.09 ± 0.01[c]
(High Ionic Strength)	Tender	0.08	0.10	0.10	0.09 ± 0.01[c]
	$\bar{x}$	0.08 ± 0.01[b]	0.09 ± 0.01[b]	0.10 ± 0.01[b]	
0.2 mM EGTA - 1 mM Mg^{2+}	Control	0.08	0.07	0.07	0.07 ± 0.01[c]
	Tender	0.08	0.07	0.08	0.07 ± 0.01[c]
	$\bar{x}$	0.08 ± 0.01[b]	0.07 ± 0.01[b]	0.08 ± 0.01[b]	

[a]Means expressed as μg Pi/min/mg protein ± S.E.
[b]Means on a line with the same superscript are not significantly different ($P > .05$)
[c]Means in a column with the same superscript are not significantly different ($P > .05$)

samples were stored at 2 centigrade. Hay et al. (1972, 1973a) reported little change in CA^{2+}-modified ATPase activity of chicken leg and breast natural actomyosin and myofibrils, respectively, when stored at 2 C for 168 hours. Penny (1968) found little change in the Ca^{2+}-modified ATPase activity during aging with rabbit myofibrils. Jones (1972) reported that chicken pectoralis major actomyosin (stored at 25 C) Ca^{2+}-modified ATPase activity decreased during postmortem storage. Since ATPase of myosin is activated by K^{+} or Ca^{2+} (Seidel,1969a) any change in this activity should be a measure of the condition of the myosin molecule or the actin-myosin interaction. The data in the present study suggest that little change has occurred in myosin, as the result of postmortem aging, which can be associated with tenderness measurements. This conclusion is supported by the fact that there were no significant correlations between Ca^{2+}-modified ATPase activity and objective and subjective tenderness measurements. Ca^{2+}-modified ATPase (216 hr.) activity was significantly correlated ($r = 0.63$, $P < .05$) with NPN (216 hr.; table 6), however, the 1 and 48 hr. Ca^{2+}-modified enzyme activity was not significantly correlated with NPN at the respective time periods.

Mg^{2+}-Modified ATPase. The 1 mM Mg^{2+}-modified ATPase (40 mM KCL) activity (table 5) was similar in magnitude and variation to that reported for the Ca^{2+}-modified enzyme in the present study. No significant differences were found among the 3 time periods or between the control and tender groups. Goll and Robson (1967), Hay et al. (1972), Jones (1972) and Parrish et al. (1973) reported an increase in the Mg^{2+}-modified ATPase activities of bovine,

Table 6. SIMPLE CORRELATION COEFFICIENTS FOR ATPase, ITPase AND NPN

Variable 1	Variable 2					
			1 hr.			
NPN1[a]	CAP[b]	MAP[c]	EDAP[d]	EGAP[e]	CIP[f]	MIP[g]
	0.41	-.06	0.04	-.23	0.14	0.36
			48 hr.			
NPN2[h]	0.54	-.02	0.66*	0.18	0.21	0.66*
			216 hr.			
NPN3[i]	0.63*	0.59	0.65*	0.68*	0.00	0.49

[a]Non-protein N, 1 hr. postmortem
[b]Ca^{2+}-modified ATPase activity
[c]Mg^{2+}-modified ATPase activity
[d]EDTA-modified ATPase activity
[e]EGTA-modified ATPase activity
[f]Ca^{2+}-modified ITPase activity
[g]Mg^{2+}-modified ITPase activity
[h]Non-protein N, 48 hr. postmortem
[i]Non-protein N, 216 hr. postmortem
*$P < .05$

chicken (leg and breast), chicken and bovine muscle, respectively. However, Penny (1968) reported a decrease in Mg^{2+}-modified ATPase activity for rabbit myofibrils during postmortem storage, whereas Hay et al. (1973a) reported that chicken breast and leg muscle myofibril activity changed little during storage. Mg^{2+} activates only actomyosin ATPase (Penny, 1968) and a change in the activity of this enzyme has been used to indicate an alteration in the actin-myosin interaction (Goll et al., 1970). Yang et al. (1970) reported that homogenization of myofibrils resulted in an increase in the

Mg^{2+}-modified ATPase activity (low ionic strength) of rabbit muscle. These same authors reported that the extent of myofibril breakdown could be monitored by measuring actomyosin ATPase activity in either homogenized, rigor or postrigor muscle. The observations, as indicated by no change in Mg^{2+}-modified ATPase activity, in the present study suggest that no change had occurred in the actin-myosin interaction during postmortem aging. Furthermore, the ultrastructural modifications in the present study do not coincide with the myofibril alterations reported by Yang et al. (1970) since no change in Mg^{2+}-modified ATPase activities were apparent. There were no significant correlations between Mg^{2+}-modified ATPase activity and either shear values or taste panel scores. However, the correlation between Mg^{2+}-modified ATPase activity and NPN at 216 hr. was approaching significance ($r = 0.59$, table 6).

EDTA-Modified ATPase. The 1 mM EDTA-(high ionic strength, 500 mM KCL) modified ATPase activity changed nonsignificantly between 1 and 216 hr. postmortem (table 5). These results are similar to those reported by Goll and Robson (1967) for low ionic strength activation, however, these same authors reported an increase in high ionic strength activation followed by a decrease to 0 hr. values after 312 hr. of storage. Goll and Robson (1967) suggested that high ionic strength activation in combination with 5 mM ATP dissociated the actin-myosin interaction and EDTA-modified enzyme activity exclusively measured the myosin enzyme. The results in the present study do not support the observations of Goll and Robson (1967), however, only 1 mM ATP was used in the assay and this might have been insufficient to

dissociate the actomyosin complex. The EDTA-modified ATPase activity was not significantly correlated with taste panel score or shear values. However, 48 and 216 hr. EDTA-modified ATPase was significantly ($P < .05$) correlated with 48 and 216 hr. NPN ($r = 0.66$ and 0.65, respectively).

EGTA + Mg^{2+}-Modified ATPase. The 0.2 mM EGTA + 1 mM Mg^{2+}-modified ATPase activity did not change significantly among the 3 time periods or between the control and tender groups (table 5). The ATPase activity measured with Mg^{2+} in the presence of a Ca^{2+} chelator (EGTA) has been reported to be a measure of the condition of the TM-TN system in postmortem muscle (Goll and Robson, 1967; Arakawa et al., 1970a). Goll et al. (1970) concluded that of the many changes observed in postmortem muscles, proteolysis of the TM-TN complex apparently did not contribute substantially to postmortem changes in the myofibrillar proteins. There were no significant correlations between EGTA-modified ATPase activity and either shear or taste panel results in the present study. The 216 hr. NPN values were significantly correlated ($r = 0.68$, $P < 0.05$) with 216 hr. EGTA-modified ATPase activity (table 6), however, the importance of this observation is difficult to evaluate.

Inosine Triphosphatase Activity

Ca^{2+}-Modified ITPase. The 1 mM Ca^{2+}-modified ITPase (40 mM KCL) activity decreased significantly ($P < .05$) between 1 and 48 hr. (table 7), however, the enzyme activity showed no further change through 216 hours. There were no significant differences between the control and tender groups

Table 7. MEAN INOSINE TRIPHOSPHATASE ACTIVITIES BY LINE AND AGING PERIOD[a]

Activator	Tenderness line	Postmortem period 1 hr.	48 hr.	216 hr.	$\bar{x}$
1 mM Ca^{2+}	Control	0.047	0.034	0.033	0.038 ± 0.00[d]
	Tender	0.050	0.035	0.034	0.040 ± 0.00[d]
	$\bar{x}$	0.049 ± 0.02[c]	0.034 ± 0.02[b]	0.034 ± 0.02[b]	
1 mM Mg^{2+}	Control	0.088	0.080	0.082	0.089 ± 0.01[d]
	Tender	0.089	0.084	0.095	0.083 ± 0.01[d]
	$\bar{x}$	0.088 ± 0.01[b]	0.082 ± 0.01[b]	0.089 ± 0.01[b]	

[a]Means expressed as μg Pi/min/mg protein ± S.E.
[b,c]Means on a line with the same superscript are not significantly different ($P > .05$).
[d]Means in a column with the same superscript are not significantly different ($P > .05$).

Ca^{2+}-modified ITPase activity. Goll and Robson (1967) reported that the Ca^{2+}-modified ITPase activity of bovine muscle myofibrils did not change with postmortem storage. However, Goll et al. (1971a) reported a marked increase in the 1 mM Ca^{2+}-modified ITPase (100 mM KCL) activity when reconstituted actomyosin was incubated with trypsin. These authors concluded that trypsin modified the actin-myosin interaction exclusively since trypsin did not alter the Ca^{2+}-modified ITPase of myosin alone. Goll et al. (1971b) reported that trypsin modified muscle mimics most of the changes that occur during postmortem storage and suggested that proteolysis might be involved in postmortem muscle alterations. The Ca^{2+}-modified ITPase data do not support the occurrence of any significant proteolysis in the present study. Although Goll et al. (1971a) used 100 mM KCL to accentuate the ITPase activities, lower ionic strength preparations showed similar but smaller changes. The ITPase activities in the present study were ran at less than 100 mM KCL (40 mM KCL), however, any increased activity should have been apparent if significant proteolysis of myofibrillar proteins had occurred. In fact, a decrease in Ca^{2+}-modified ITPase activity of greater than 30% occurred between 1 and 48 hours. No significant correlations were found between Ca^{2+}-modified ITPase activities and either shear or taste panel data. In contrast to the earlier observations that Ca^{2+}, Mg^{2+}, EDTA and EGTA-modified ATPase activities were either significantly correlated or approaching significant correlations with 216 hr. NPN values, Ca^{2+} ITPase activities were nonsignificantly correlated with 216 hr. NPN values (table 6). This observation is further support for the lack

of any significant myofibrillar proteolysis having been observed in the present study.

Mg^{2+}-Modified ITPase Activity. The 1 mM Mg^{2+}-modified ITPase activities are shown in table 7. These values did not change during postmortem storage and no significant differences were observed between the control and tender groups. This is in contrast with Goll and Robson (1967) who reported an increase in Mg^{2+}-modified ITPase activity of bovine myofibrils after 24 hr. storage at 2 centigrade. Goll et al. (1971a) reported a 2-fold increase in the 1 mM Mg^{2+} + 0.05 mM Ca^{2+}-modified ITPase activity following incubation with trypsin and when assayed in 10 mM KCL. However, when the same system was assayed in 100 mM KCL the activity decreased with increasing time of postmortem storage. The results in the present study suggest that the conditions of the assay were sufficiently different from those of Goll et al. (1971a) and they either were not measuring proteolytic activity or possibly the proteolysis which may have occurred was insufficient in magnitude to be measured. There were no significant correlations between Mg^{2+}-modified ITPase activity and either taste panel scores or shear values. Mg^{2+}-modified ITPase activity (48 hr.) was significantly correlated ($r = 0.66$, $P < .05$) with 48 hr. NPN values, however, the importance of this observation is not readily apparent.

The measurements of ATPase and ITPase activities have been well documented as a tool to assess alterations in the actin-myosin interaction of postmortem muscle (Goll and Robson, 1967; Goll, 1968; Hay et al., 1972, 1973a; Penny, 1968). Most researchers report variable but positive increase

in ATPase activity as the result of postmortem storage and Ca^{2+}-modified ITPase activity appears to be sensitive to trypsin induced proteolysis. Goll et al. (1971b) reported striking similarities between trypsin treated muscle and postmortem muscle. The data in the present study do not show an increase in ATPase and ITPase activities as the result of postmortem storage and in fact the Ca^{2+} ITPase activity decreased. These observations are difficult to interpret in light of the literature on postmortem muscle and trypsin treated muscle; however, the diversity of the muscle preparations and assay conditions in the previous studies compared to those in the present study may have contributed somewhat to the differences obtained.

Natural actomyosin, reconstituted actomyosin and myofibrils have been used in ATPase and ITPase assays, however, the preparative procedure and final product differs for each system. The results of any such assay must therefore be interpreted only on the basis of the specific system used since dissolution in high ionic strength salt solutions and subsequent low ionic strength precipitation may alter the preparation from the normal in situ condition. The myofibril preparation has been used as the most representative of the in situ myofibrillar proteins (Goll and Robson, 1967). Ionic strength and type of activator also affect the results and these variables must be evaluated along with the type of myofibrillar preparation used.

ATPase, ITPase and Ultrastructure. Most of the reported changes in ATPase and ITPase activities represent subtle alterations in molecular structure of myofibrillar proteins which cannot be seen at the present

resolution capability of transmission electron microscopy. As reported in previous sections of this dissertation, considerable modification of myofibril structure had occurred as the result of postmortem storage. No enzyme assay in the present study appeared to be highly associated with the Z-line degradation observed. Goll et al. (1971a, b) reported that trypsin selectively removed Z-lines from muscle preparations. This appears to be in partial agreement with the present study, however, not all Z-lines in this study were degraded as the result of postmortem storage. Additionally, trypsin has been reported to "relax" or lengthen rigor shortened sarcomeres (Goll et al., 1971a, b), however, sarcomere length was essentially unchanged between 48 and 216 hr. postmortem in the present study. Thus, it appears difficult to biochemically assess postmortem ultrastructural changes observed in muscle in the present study.

Superprecipitation

Mg^{2+} + EGTA Superprecipitation. The time curves for turbidity development in the presence of 1 mM Mg^{2+} and 0.1 mM EGTA, of the lowest (tender) and highest (control) tenderness indexing (see experimental methods) bulls are presented in figure 54. At 1 hr. postmortem, the most tender sample required the least, and at 9 day postmortem this same sample required the most time for turbidity development. The 48 hr. postmortem control sample became turbid faster than the 9 day control sample, whereas it required more time than the 1 hr. and 9 day control and 1 hr. and 48 hr. tender line samples. These results disagree with those of Goll et al. (1970) and

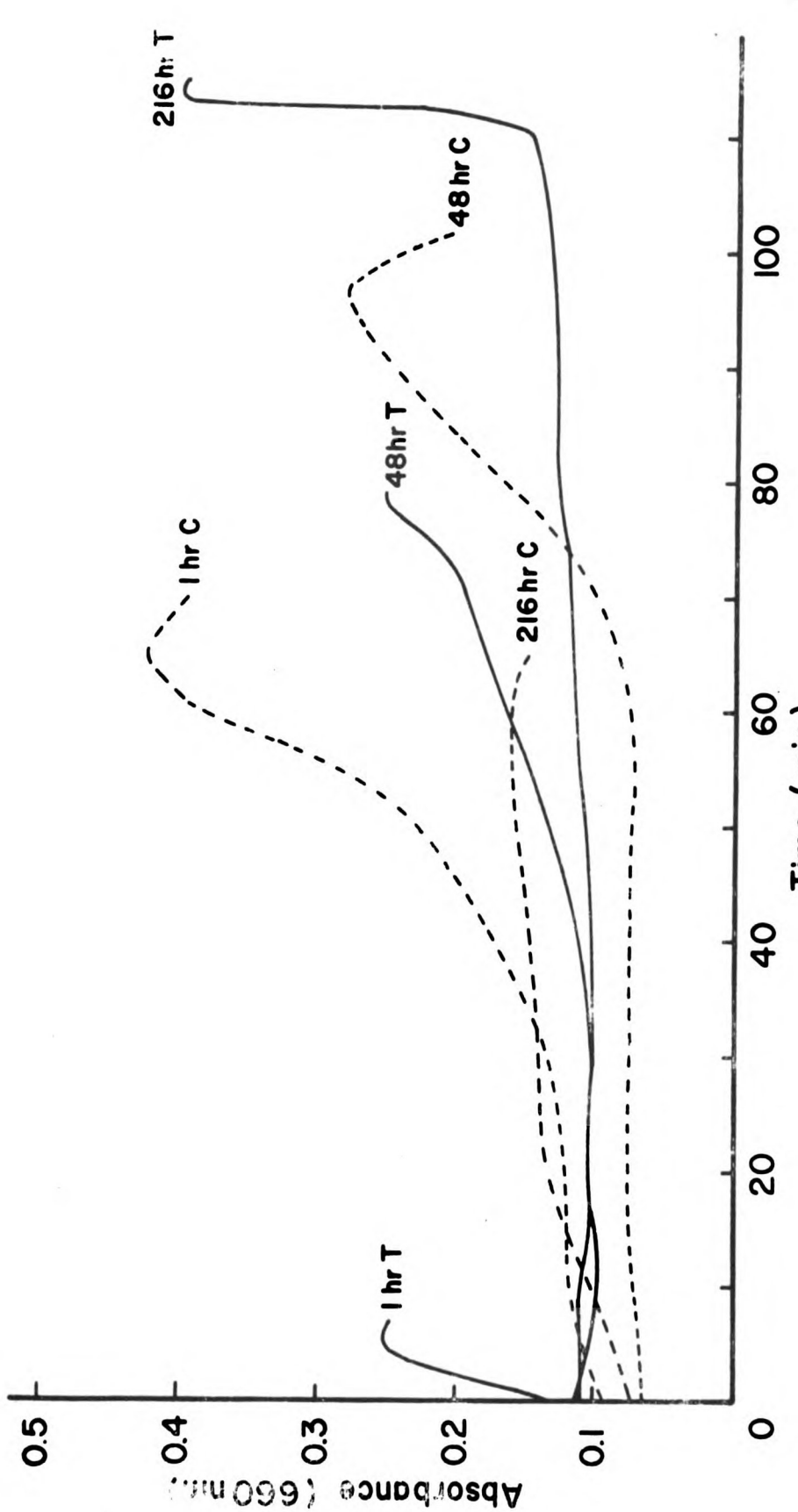

Figure 54. 1 mM Mg^{2+} + 0.1 mM EGTA superprecipitation assay of most tender and toughest samples. T = tender sample; C = control sample

Arakawa et al. (1970a) who found that bovine natural actomyosin prepared from aged muscle required less time for turbidity development than unaged muscle. Since superprecipitation in the presence of a Ca^{2+} chelator (EGTA) is a measure of Ca^{2+} sensitivity (Goll et al., 1970), the more rapid onset of turbidity by aged muscle suggests the loss of the contractile repressing ability of the TM-TN system during postmortem aging (Arakawa et al., 1970a). Goll et al. (1971b) concluded that the postmortem increase in the rate of turbidity development was not the result of selective destruction of troponin or tropomyosin, but an alteration of the actin-myosin complex.

Low Ca^{2+} Superprecipitation. Curves of turbidity development in the presence of added Ca^{2+} (0.05 mM), in the same 2 muscle samples as described for Mg^{2+} + EGTA superprecipitation are presented in figure 55. The tender line sample became turbid at a much faster rate than the control line. The 1 hr. postmortem samples (tender and control) had the fastest rate of turbidity development which disagrees with results reported by Arakawa et al. (1970a) and Goll et al. (1971b) who found that postmortem aging decreased the time necessary for turbidity development. Herring, Cassens and Briskey (1969b) reported that tough muscle (based on Warner-Bratzler shear) required a longer time period for development of turbidity in 100 mM KCL (no added Ca^{2+}) than tender muscle. The low Ca^{2+} (0.05 mM) superprecipitation assay in the present study generally supports the observations of Herring et al. (1969b) for tender and tough muscles, however, it is difficult to interpret the more rapid turbidity development of the 1 hr. samples compared to the slower rate of 48 and 216 hr. samples.

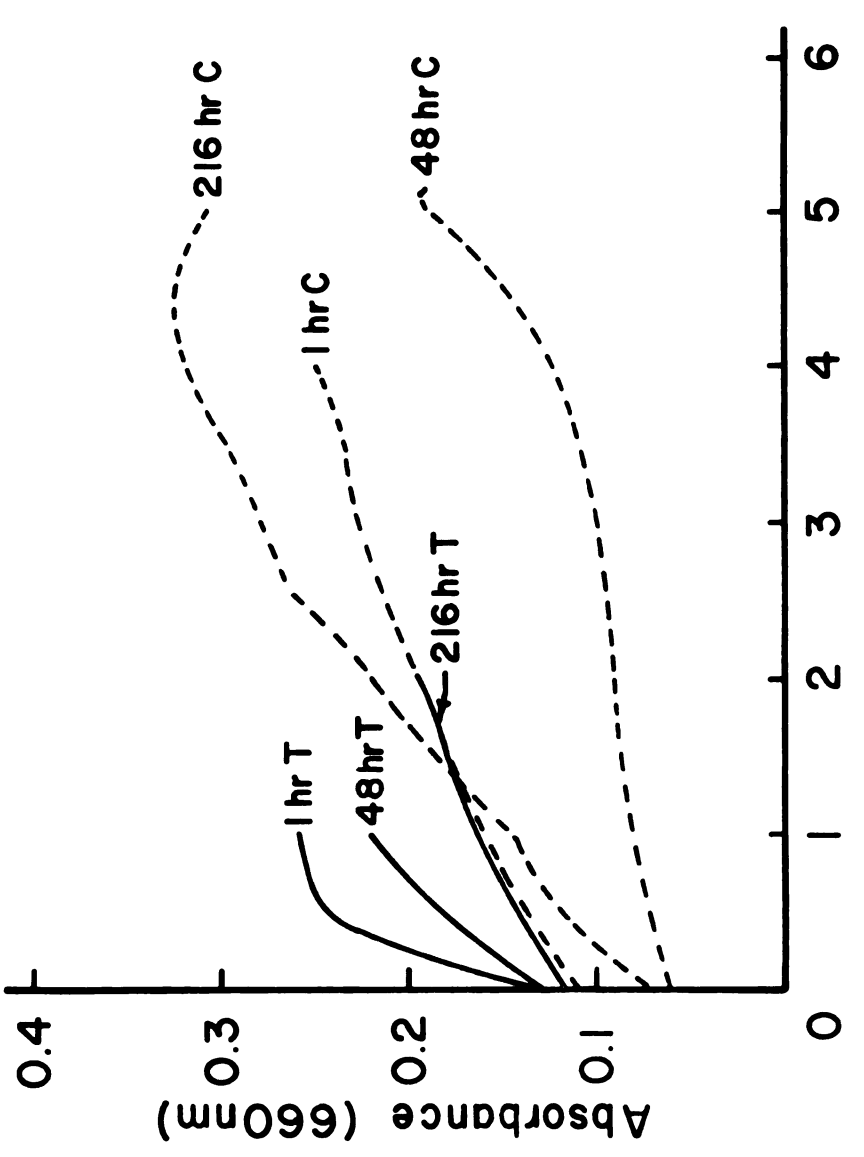

Figure 55. Low Ca^{2+} (0.05 mM) superprecipitation assay of most tender and toughest samples. T = tender sample; C = control sample

The Mg^{2+} + EGTA modified superprecipitation assay is difficult to interpret since it should be a measure of the TM-TN and actin-myosin complexes which have been reported to be altered during postmortem storage (Arakawa et al., 1970a), however, Briskey, Seraydarian and Mommaerts (1967) reported that turbidity measurements are difficult to accurately interpret. The low Ca^{2+} (0.05 mM) + Mg^{2+} superprecipitation assay in the present study generally agrees with the superprecipitation assay results for tender and tough muscle as reported by Herring et al. (1969b), but the more rapid onset of the 1 hr. samples, in the present study, makes accurate interpretation of these results difficult.

SUMMARY

Sixteen Hereford bulls which were the progeny of 2 genetic lines, 1 selected for tenderness and the other unselected (control) were slaughtered and the carcasses stored at 2 C for 216 hr. to determine the effects of aging on sarcomere length, ultrastructure, protein solubility, ATPase, ITPase and superprecipitation of the longissimus muscle. Samples were removed from the 12th rib area of the longissimus muscle after 1, 48 and 216 hr. of postmortem aging. Warner-Bratzler shear and taste panel data were obtained from the 216 hr. postmortem samples. Sarcomere length was measured only on the 48 hr. and 216 hr. samples and the ATPase, ITPase and superprecipitation assays were run on the 5 most tender and 5 toughest samples.

Z-line degradation was observed in some fibers as early as 1 hr. postmortem, however, most of the Z-line degradation occurred between 1 and 48 hr. postmortem. Little additional Z-line degradation occurred between 48 and 216 hr. postmortem. Two groups of fibers, type I and type II, differing in susceptibility to Z-line degradation, occurred in all samples of both tender and control lines. Type I fibers lost little Z-line material during postmortem aging, whereas the type II fiber group showed Z-line degradation which ranged from a slight to moderate amount. Myofibril fragmentation (I-Z junction) did not occur in the 1 hr. postmortem samples but appeared in some samples by 48 hr. postmortem. At 216 hr. postmortem, myofibril fragmentation was evident in most samples and fiber breakage was observed in several samples. Both type I and type II fibers appeared

equally susceptible to fragmentation at the I-Z junction. There were no apparent differences in Z-line degradation or myofibril fragmentation between tender and control samples, however, at 216 hr. the most tender sample (Warner-Bratzler shear) had more fiber breakage than the toughest sample.

Sarcomeres were slightly shorter at 216 hr. as compared to the 48 hr. samples, however, the differences were not statistically significant. There was no sarcomere length difference between control and tender lines at 48 and 216 hr. postmortem. Forty-eight hr. sarcomere length was significantly ($P < .01$) correlated with KCL extracted myofibrillar N, however, no significant correlations were found between 216 hr. sarcomere length and 216 hr. KCL or KI extracted myofibrillar N. Sarcomere length at 216 hr. was significantly ($P < .05$) and negatively correlated with Warner-Bratzler shear but not with taste panel score.

Sarcoplasmic N extracted at 48 hr. was significantly ($P < .05$) greater than that from either the 1 or 216 hr. postmortem samples. There were no differences between tender and control lines in amount of sarcoplasmic N extracted at any postmortem time interval. No significant correlations were obtained between sarcoplasmic N and shear or taste panel data. Less myofibrillar N was extracted by 1.1M KI at 48 hr. postmortem as compared to that at 1 or 216 hr; however, these differences were not significant. No significance differences in myofibrillar N were obtained between tender and control samples at any postmortem time period. Myofibrillar N extracted by KCL at 216 hr. postmortem was significantly ($P < .05$) greater than that at 1 but not 48 hr. postmortem. The KCL extracted myofibrillar N was

significantly correlated ($P < .05$) with 1 hr. muscle temperature at all postmortem aging intervals. NPN did not change significantly during postmortem storage, however, the tender 216 hr. sample mean was significantly ($P < .05$) greater than all but the 1 hr. tender sample mean. NPN at 216 hr. approached significance with Warner-Bratzler shear, but was significantly ($P < .05$) and negatively correlated with taste panel score. There were no significant differences among postmortem aging time intervals measured or control or tender lines for stroma N determined after removal of myofibrillar N by 1.1M KI. Stroma N determined after myofibrillar N extraction by 1.1M KCL was significantly greater at 1 hr. postmortem as compared to 48 hr., however, 48 hr. stroma N was not statistically different from that at 216 hours. Total N did not vary significantly during postmortem aging or between the control and tender lines. It may be concluded from these data that protein solubility was not a good criterion for classifying bovine longissimus muscle samples as tough or tender.

The Ca^{2+}-, Mg^{2+}-, EDTA- and EGTA-modified ATPase activities did not vary significantly during postmortem aging periods or between control and tender lines. Ca^{2+}-modified ITPase activity decreased between 1 and 48 hr. postmortem and remained constant between 48 and 216 hours. Mg^{2+}-modified ITPase activity changed very little during postmortem aging. No significant differences were observed between tender and control lines at any time period for the ITPase activities.

The Mg^{2+} + EGTA superprecipitation assay of the most tender and toughest samples showed that 1 hr. postmortem samples were fastest in onset of

turbidity. The tender sample low Ca^{2+} superprecipitation assay required less time for onset of turbidity than the tough line sample.

The ATPase, ITPase and superprecipitation assays, in this study, do not support those reported by others that postmortem aging alters the actin-myosin interaction or regulatory protein complex.

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APPENDIX

Appendix I. Schedule for Preparation of 1.25% Glutaraldehyde Fixative

Compound	Amount	Molarity	Final molarity
$NaH_2PO_4 . H_2O$	1.8 g	0.0127	0.006
$Na_2HPO_4 . 7\ H_2O$	23.25 g	0.085	0.042
NaCl	5.0 g	0.084	0.043
50% Biological grade glutaraldehyde	50.0 ml	0.244	0.123
H_2O	975 ml		

Dilute 1:1 with glass distilled water to obtain 1.25% glutaraldehyde and approximately 415 milliosmolar

Appendix II. Schedule for Preparation of Washing Buffer

Compound	Amount	Molarity
NaH_2PO_4	1.8 g	0.014
Na_2HPO_4	23.25 g	0.094
NaCl	5.0 g	0.092
H_2O	925 ml	

Appendix III. Osmium Tetroxide Fixation (1%)

A. Stock Solution A

Sodium acetate	9.714 g
Veronal-sodium	14.714 g
Distilled water to make	500 ml

B. Stock Solution B

Sodium chloride	40.25 g
Potassium chloride	2.10 g
Calcium chloride	0.90 g
Distilled water to make	500 ml

The solutions are mixed according to the following scheme:

Solution A	10 ml
Solution B	3.4 ml
0.1N HCl	about 11 ml

Distilled H_2O is added to make 50 ml and pH adjusted to pH 7.2-7.4 with 0.1N HCl. To this mixture, 0.5 g osmium tetroxide is added and stored in a brown stoppered glass bottle in the refrigerator.

Appendix IV. Epon Embedding Media*

Mixture A	Mixture B
94 g DDSA[1,2]	98 g NMA[3,4]
80 g Epon 812	100 g Epon 812

Use separate 8 oz bottles for mixture A and B. Use a jet of freon into each bottle, cap and mix thoroughly. Seal bottles with Parafilm and place in refrigerator; normally usable for 6 months if kept refrigerated.

Schedule for mixing A and B

Add 7 g of mixture A, 3 g of mixture B and 0.14 g of DMP-30[5]. Thoroughly mix for at least 5 min. and vacuum slightly if considerable amounts of air becomes incorporated during mixing.

[1]Dodecenyl Succinic Anhydride

[2]Based on a weight per epoxy equivalent of 159-160

[3]Nadic methyl anhydride

[4]Based on a weight per epoxy equivalent of 159-160

[5]Dimethyl amino methyl phenol

*All mixing instructions based on schedule reported by Ladd Research Industries (Burlington, Vermont) for embedding in Epon 812.

Appendix V. Procedure for Embedding Muscle Fibers

1) Fix in glutaraldehyde (1.25%) solution for 2 hrs.

2) Rinse in washing buffer - 3 rinses, 20 min. each

3) Fix in osmium tetroxide solution for 1 hr.

4) Dehydrate in 25, 50, 75 and 95% ethanol - 10 min. in each

5) Dehydrate in absolute ethanol 2 times for 15 min. each

6) 2 periods, 30 min. each, in propylene oxide (100%)

7) Add propylene oxide : epon (1:1) overnight in dessicator

8) Place samples in "00" gelatin capsules containing epon for 12 hrs. in dessicator (under light vacuum)

9) Place capsules in oven at 60 C for 48 hrs.

10) Remove from oven and store in a dessicator until used

Appendix VI. Stain Preparation Procedure

A. Lead citrate stain - modified Reynolds method (Richard Ruffing)

1 g lead citrate-Pb_3 $(C_6H_5O_7)_2$ in 50 ml glass distilled water

Shake, and let sit; repeat several times, but do not exceed 5 min.

Add approximately 0.5 ml 10N $NaOH_1$ invert and then add 1 drop of 10N NaOH, invert and repeat until solution becomes clear.

Pour off 45 cc and throw remainder away

Solution usable for 2 weeks or until solution turns cloudy

B. Reynolds lead citrate stain

Lead nitrate-Pb $(NO_3)_2$	1.33 g
Sodium citrate - Na_3 $(C_6H_5O_7).2$ H_2O	1.76 g
H_2O (freshly boiled distilled H_2O and cooled)	30 ml

Shake intermittently and vigorously for 30 min., a white precipitate will form. Add 8 ml of 1N NaOH dilute to 50 ml with boiled distilled H_2O and mix by inversion until the precipitate is dissolved. The pH should be approximately 12.

C. Phosphotungstic acid stain

Phosphotungstic acid	0.3 g
Ethanol:methanol (3:1)	10 ml

Mix by inversion and use immediately, the mixture appears to be good almost indefinitely, however, it cannot be used on coated grids due to the low pH. Seems to disrupt the formvar film.

Appendix VI (continued)

D. Saturated solution of uranyl acetate

Uranyl acetate	8 g
Glass distilled H_2O	100 ml

Allow to sit 1-2 days; a portion of the saturated solution can be filtered before use or the amount to be used can be removed by pipette without disturbing the precipitate.

Appendix VII. Staining Procedure for Thin Sections

A. Uranyl Acetate

1. Place a parafilm or dental wax sheet on workbench
2. Place drops of uranyl acetate on the sheet and cover
3. Place grid, section down, on drop
4. Cover and stain for 30 min. or longer
5. Remove and rinse thoroughly with a jet of distilled water
6. Dry with the edge of a filter paper sheet

B. Modified Reynolds citrate stain procedure

1. Put drops of stain on parafilm or wax sheet
2. Put several pellets of solid NaOH around edge of cover
3. Stain one grid at a time on a drop and remove after 5 sec.
4. Rinse with a jet of distilled H_2O followed by a jet of 0.02N NaOH
5. Remove excess liquid with an edge of filter paper
6. Allow to dry before use

C. Reynolds lead citrate stain

1. All procedures are the same as the modified procedure except the grids are allowed to stain for 5 min.

Appendix VIII. Reagent Preparation for Protein Fractionation

1. 0.015M PO_4 buffer pH 7.4

   0.326 g KH_2PO_4 (Potassium phosphate monobasic)

   2.18 g K_2HPO_4 (Potassium phosphate dibasic)

   Place into 1 liter volumetric and add distilled, deionized H_2O to make 1 liter.

2. 0.1M PO_4 buffer pH 7.4

   2.180 g KH_2PO_4

   14.631 g K_2HPO_4

   Place into 1 liter volumetric and add distilled, deionized H_2O to make 1 liter.

3. 1.1M KCl (Potassium chloride)

   KCl 82.0 g

   Add to 1 liter volumetric, add 0.1M PO_4 buffer and bring into solution. Bring to 1 liter with PO_4 buffer.

4. 1.1M KI (Potassium iodide)

   KI 182 g

   Place into 1 liter volumetric, add 0.1M PO_4 buffer and bring into solution and then add PO_4 to make 1 liter.

5. 10% Trichloroacetic acid (TCA)

   TCA 10 g

   Add sufficient water to make volume 100 ml.

Appendix IX. Reagents for Kjeldahl Nitrogen Determination

1. Concentrated H_2SO_4
2. 40% NaOH (10N)
3. 0.1% Brom cresol green
4. Sodium sulfate (Na_2SO_4)
5. 2% Boric acid
6. Standard H_2SO_4 solution
7. 10% $CuSO_4$

Appendix X. Procedure for Myofibril Preparation

Solutions:

1. 0.001M EDTA, 0.25 sucrose, 0.05M tris, pH 7.6
2. 0.001M EDTA, 0.05M tris, pH 7.6
3. 0.15M KCl
4. 0.001M EDTA
5. Deionized glass distilled H_2O

Procedure:

1. Weigh 5 g powdered muscle in a centrifuge bottle.
2. Add 25 ml of solution 1 from above and stir at low speed on magnetic stirrer for 1 hr.
3. Centrifuge at 2500Xg for 10 min.
4. Discard supernate and resuspend the residue in solution 2 and stir for 30 min.
5. Pass through a wire screen to remove connective tissue.
6. Centrifuge at 2500Xg for 10 min.
7. Discard supernate and resuspend in 25 ml of solution 3 and stir gently for 15 min.
8. Centrifuge and resuspend for 15 min each, in order: 25 ml 0.001M EDTA, pH 7.6; deionized glass distilled water; 0.15M KCl.
9. Resuspend a second time in 0.15 KCl and store at 2 C.
10. Run biuret assay.

Appendix XI. ATPase and ITPase Assay Procedure

Solutions: Concentration in 10 ml

a. Protein (myofibrils) 0.2-0.8 mg/ml

b. 0.02M tris-acetate pH 7.0

c. KCl (including KCl in protein preparation (0.100-0.025M) in this case 50 mM KCl)

d. Deionized glass distilled H_2O

e. Mg_2Cl-0.001M

f. Ca_2Cl-0.001M

g. EDTA-0.001M

h. ATP-0.001M

i. ITP-0.001M

j. EGTA-0.0002M

k. KCl including KCl in protein (0.5M)-for high ionic strength EDTA assay

All stock solutions are 10X final concentration values

Incubate all solutions to 25 C except myofibrils, ATP and ITP

Procedure:

1. Suspend 1 ml myofibrils in reaction mixture which includes 1 ml 0.02M tris-acetate pH 7.0, 1 ml 0.35 KCl, 5 ml H_2O.
2. Add 1 ml of activator, Mg^{2+} (0.01M), Ca^{2+}/0.01M), 1 ml Mg^{2+} (0.01M) + 0.7 mg EGTA or 2 ml 2.42M KCl + 1 ml 0.01M EDTA deleting 1 ml H_2O for high ionic strength activation.
3. Add 1 ml of ATP or ITP (0.001M in each case).
4. After 30 secs, remove 1 ml aliquot and add to 1 ml 10% cold TCA to stop reaction.
5. Incubate remaining solution at 25 C for 15 min.

Appendix XI (continued)

6. Remove another 1 ml aliquot and stop reaction with TCA.

7. Do both the 30 sec and 15 min assay in duplicate for each sample.

8. Centrifuge each tube to remove protein and run phosphate determination on the samples.

Appendix XII. Phosphate Determination

Standard Solution - 0.3509 anhydrous KH_2PO_4/liter (80 μg/ml)

Molybdate Solution - 2.5% ammonium molybdate in 5N H_2SO_4

Sodium-meta-bisulfite ($NaHSO_3$) - 3% (w/v) solution

Elon (p-methylaminophenol) - 1.0 g in 100 ml $NaHSO_3$

Standard Curve: 2 μg/ml of KH_2PO_4

4 μg/ml of KH_2PO_4

8 μg/ml of KH_2PO_4

12 μg/ml of KH_2PO_4

16 μg/ml of KH_2PO_4

Phosphate Determination Procedure

1. Add 1 ml of TCA to standard
2. Centrifuge standard and add 1.5 ml of H_2O to standard and to sample
3. Add 0.5 ml molybdate solution
4. Add 1 ml Elon
5. Incubate for 15 min at room temperature
6. Read at 660 nm

Appendix XIII. Biuret Reagent

1. Cupric sulfate ($CuSO_4 \cdot 5\ H_2O$) k,5 g

2. Sodium potassium tartrate ($NaKC_4H_4O_6 \cdot 4\ H_2O$) 6.09

Place in 1 liter flask, add 500 ml H_2O

3. NaOH-10% solution (carbonate free)

Add 300 ml of 10% NaOH to previous 1 liter flask, make to volume with H_2O

Keeps indefinitely - discard if black or reddish precipitate appears.

Biuret Standard

1. Bovine serum albumin - 10 mg/ml

Standard curve - 1 mg/ml

3 mg/ml

5 mg/ml

7 mg/ml

10 mg/ml

Biuret Assay Procedure

1. Dilute 1 ml of myofibrils to 2 ml with 0.15M KCl

2. Add 8 ml biuret reagent to either 2 ml of sample or 2 ml of standard, mix and incubate at room temperature for 30 min.

3. Read at 540 nm on spectrophotometer.

Appendix XIV. Myosin B (Natural Actomyosin) Preparation

Solutions:

Weber-Edsall Solution

0.6M KCl

0.03 $KHCO_3$

0.01 K_2CO_3

1.0M KCl

Procedure:

1. Suspend 5 g of powdered muscle in 30 ml W-E solution.
2. Suspension allowed to extract for 16-24 hrs at 2 C.
3. 10 ml of the extracted solution removed and centrifuged at 15,000 Xg for 20 min.
4. Supernatant diluted to 0.15M KCl with distilled H_2O.
5. Precipitate collected by centrifugation at 15,000Xg for 20 min.
6. Precipitate dissolved in 1.0M KCl
7. Volume adjusted to a final concentration of 0.5M KCl
8. Precipitation, dissolution cycle repeated twice.
9. After final precipitation the myosin B was adjusted to 0.5M KCl and dissolved by gentle magnetic stirring overnight.
10. Dissolved myosin B clarified by centrifugation at 15,000Xg for 20 min.

Appendix XV. Definition of Variable Names

Name	Definition
WSP1, 2, 3	1, 48 and 216 hr. water soluble N
KI1, 2, 3	1, 48 and 216 hr. 1.1M KI extracted N
KCL1, 2, 3	1, 48 and 216 hr. 1.1M KCL extracted N
NPN1, 2, 3	1, 48 and 216 hr. non-protein N value
TP1, 2, 3	1, 48 and 216 hr. total N value
KIS1, 2, 3	1, 48 and 216 hr. KI stroma N values
KCLS1, 2, 3	1, 48 and 216 hr. KCL stroma N values
KIR1, 2, 3	1, 48 and 216 hr. KI:WS N ratios
KCLR1, 2, 3	1, 48 and 216 hr. KCL:WS N ratios
CAP1, 2, 3	1, 48 and 216 hr. Ca^{2+}-modified ATPase value
MAP1, 2, 3	1, 48 and 216 hr. Mg^{2+}-modified ATPase value
EDAP1, 2, 3	1, 48 and 216 hr. EDTA-modified ATPase value
EGAP1, 2, 3	1, 48 and 216 hr. EGTA-modified ATPase value
CIP1, 2, 3	1, 48 and 216 hr. Ca^{2+}-modified ITPase value
MIP1, 2, 3	1, 48 and 216 hr. Mg^{2+}-modified ITPase value
TMP	1 hr. longissimus muscle temperature
SH	216 hr. postmortem Warner-Bratzler shear value
TP	216 hr. postmortem taste panel value
pH	1 hr. longissimus muscle surface pH
Sar 1, 2	48 and 216 hr. sarcomere length means

Appendix XVI. Simple Correlation Coefficients Between Shear and Sensory, Biochemical and Histological Parameters.

Variable I	Variable II	r	Variable I	Variable II	r
Shear	WSP 1	-.27	Shear	MAP 2	-.30
Shear	KI 1	0.16	Shear	MAP 3	-.21
Shear	KCL 1	0.01	Shear	EDAP 1	-.15
Shear	NPN 1	0.31	Shear	EDAP 2	-.41
Shear	WSP 2	-.26	Shear	EDAP 3	-.35
Shear	KI 1	0.09	Shear	EGAP 1	-.26
Shear	KCL 2	0.15	Shear	EGAP 2	-.03
Shear	NPN 2	-.22	Shear	EGAP 3	-.47
Shear	WSP 3	-.21	Shear	CIP 1	0.04
Shear	KI 3	0.15	Shear	CIP 2	-.14
Shear	KCL 3	-.02	Shear	CIP 3	-.32
Shear	NPN 3	-.61	Shear	MIP 1	-.11
Shear	KIS 1	-.10	Shear	MIP 2	-.12
Shear	KCLS 1	-.13	Shear	MIP 3	0.44
Shear	TP 1	-.16	Shear	Temp	-.43
Shear	KIS 2	-.09	Shear	pH	-.05
Shear	KCLS 2	0.06	Shear	Sar 1	-.33
Shear	TP 2	0.01	Shear	Sar 2	-.64
Shear	KIS 3	0.20	Shear	KIR 1	0.31
Shear	KCLS 3	0.38	Shear	KCLR 1	0.17
Shear	TP 3	0.05	Shear	KIR 2	0.39
Shear	CAP 1	-.39	Shear	KCLR 2	0.33
Shear	CAP 2	-.36	Shear	KIR 3	0.32
Shear	CAP 3	-.36	Shear	KCLR 3	0.17
Shear	MAP 1	-.08	Shear	Taste panel	-.52

Appendix XVII. Simple Correlation Coefficients Between Taste Panel and Shear, Biochemical and Histological Parameters

Variable I	Variable II	r	Variable I	Variable II	r
Taste panel	WSP 1	0.56	Taste panel	MAP 2	-.13
Taste panel	KI 1	-.15	Taste panel	MAP 3	-.07
Taste panel	KCL 1	0.13	Taste panel	EDAP 1	-.33
Taste panel	NPN 1	-.72	Taste panel	EDAP 2	-.49
Taste panel	WSP 2	0.06	Taste panel	EDAP 3	-.05
Taste panel	KI 2	-.63	Taste panel	EGTA 1	0.21
Taste panel	KCL 2	0.27	Taste panel	EGTA 2	-.34
Taste panel	NPN 2	-.29	Taste panel	EGTA 3	0.20
Taste panel	WSP 3	0.26	Taste panel	CIP 1	0.30
Taste panel	KI 3	0.04	Taste panel	CIP 2	0.14
Taste panel	KCL 3	0.42	Taste panel	CIP 3	0.25
Taste panel	NPN 3	0.10	Taste panel	MIP 1	0.04
Taste panel	KIS 1	-.17	Taste panel	MIP 2	-.37
Taste panel	KCLS 1	-.27	Taste panel	MIP 3	0.02
Taste panel	TP 1	-.14	Taste panel	Temp	0.07
Taste panel	KIS 2	0.67	Taste panel	pH	-.02
Taste panel	KCLS 2	-.14	Taste panel	SH	-.52
Taste panel	TP 2	0.19	Taste panel	Sar 1	-.01
Taste panel	KIS 3	0.06	Taste panel	Sar 2	0.22
Taste panel	KCLS 3	-.24	Taste panel	KIR 1	-.60
Taste panel	TP 3	0.28	Taste panel	KCLR 1	-.19
Taste panel	CAP 1	0.04	Taste panel	KIR 2	-.88
Taste panel	CAP 2	-.30	Taste panel	KCLR 2	0.19
Taste panel	CAP 3	0.02	Taste panel	KIR 3	-.29
Taste panel	MAP 1	0.32	Taste panel	KCLR 3	0.15

Appendix XVIII. Simple Correlation Coefficients

Variable I	Variable II	r	Variable I	Variable II	r
Sarcomere 2	WS 1	-.31	Sarcomere 2	MAP 3	-.12
Sarcomere 2	KI 1	-.32	Sarcomere 2	EDAP 1	0.39
Sarcomere 2	KCL 1	-.29	Sarcomere 2	EDAP 2	0.53
Sarcomere 2	NPN 1	0.03	Sarcomere 2	EDAP 3	-.33
Sarcomere 2	WS 2	-.02	Sarcomere 2	EGAP 1	0.05
Sarcomere 2	KI 2	-.04	Sarcomere 2	EGAP 2	0.03
Sarcomere 2	KCL 2	-.78	Sarcomere 2	EGAP 3	-.45
Sarcomere 2	NPN 2	0.81	Sarcomere 2	CIP 1	-.23
Sarcomere 2	WS 3	-.45	Sarcomere 2	CIP 2	0.05
Sarcomere 2	KI 3	-.66	Sarcomere 2	CIP 3	0.43
Sarcomere 2	KCL 3	-.62	Sarcomere 2	MIP 1	0.26
Sarcomere 2	NPN 3	-.00	Sarcomere 2	MIP 2	0.39
Sarcomere 2	KIS 1	0.17	Sarcomere 2	MIP 3	-.17
Sarcomere 2	KCLS 1	0.31	Sarcomere 2	TMP	0.74
Sarcomere 2	TP 1	0.07	Sarcomere 2	pH	0.29
Sarcomere 2	KIS 2	0.04	Sarcomere 2	SH	-.33
Sarcomere 2	KCLS 2	0.48	Sarcomere 2	TP	-.01
Sarcomere 2	TP 2	-.21	Sarcomere 2	Sar 3	0.28
Sarcomere 2	KIS 3	0.27	Sarcomere 2	KIR 1	0.18
Sarcomere 2	KCLS 3	0.19	Sarcomere 2	KCLR 1	-.14
Sarcomere 2	TP 3	-.57	Sarcomere 2	KIR 2	-.02
Sarcomere 2	CAP 1	0.34	Sarcomere 2	KCLR 2	-.71
Sarcomere 2	CAP 2	0.35	Sarcomere 2	KIR 3	0.08
Sarcomere 2	CAP 3	-.42	Sarcomere 2	KCLR 3	-.20
Sarcomere 2	MAP 1	-.03			
Sarcomere 2	MAP 2	-.05			

Appendix XIX. Simple Correlation Coefficients

Variable I	Variable II	r	Variable I	Variable II	r
Sarcomere 3	WS 1	-.27	Sarcomere 3	MAP 2	0.35
Sarcomere 3	KI 1	-.11	Sarcomere 3	MAP 3	0.01
Sarcomere 3	KCL 1	-.46	Sarcomere 3	EDAP 1	0.28
Sarcomere 3	NPN 1	0.04	Sarcomere 3	EDAP 2	0.36
Sarcomere 3	WS 2	-.18	Sarcomere 3	EDAP 3	0.50
Sarcomere 3	KI 2	-.15	Sarcomere 3	EGAP 1	0.36
Sarcomere 3	KCL 2	-.44	Sarcomere 3	EGAP 2	-.30
Sarcomere 3	NPN 2	-.08	Sarcomere 3	EGAP 3	0.54
Sarcomere 3	WS 3	-.19	Sarcomere 3	CIP 1	-.38
Sarcomere 3	KI 3	-.05	Sarcomere 3	CIP 2	-.10
Sarcomere 3	KCL 3	-.21	Sarcomere 3	CIP 3	-.36
Sarcomere 3	NPN 3	0.48	Sarcomere 3	MIP 1	-.29
Sarcomere 3	KIS 1	0.00	Sarcomere 3	MIP 2	-.12
Sarcomere 3	KCLS 1	0.27	Sarcomere 3	MIP 3	0.29
Sarcomere 3	TP 1	-.04	Sarcomere 3	TMP	0.44
Sarcomere 3	KIS 2	0.17	Sarcomere 3	SH	-.64
Sarcomere 3	KCLS 2	0.30	Sarcomere 3	TP	0.22
Sarcomere 3	TP 2	-.14	Sarcomere 3	KIR 1	0.25
Sarcomere 3	KIS 3	-.20	Sarcomere 3	KCLR 1	-.31
Sarcomere 3	KCLS 3	-.09	Sarcomere 3	KIR 2	0.02
Sarcomere 3	TP 3	-.28	Sarcomere 3	KCLR 2	-.28
Sarcomere 3	CAP 1	0.29	Sarcomere 3	KIR 3	0.19
Sarcomere 3	CAP 2	-.02	Sarcomere 3	KCLR 3	-.01
Sarcomere 3	CAP 3	0.01	Sarcomere 3	pH	0.01
Sarcomere 3	MAP 1	-.03			

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