A METHOD FOR MEASURING SHEAR FORCE FOR AN INDIVIDUAL MUSCLE FIBER

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Meat is a complex food made primarily of muscle, connective tissue, and fatty tissues. A single muscle is surrounded by a layer of connective tissue from which septa develop at regular intervals. These connective septa hold the muscle fibers, the basic muscle unit, into bundles.

The shear force of muscle tissue has been extensively investigated, but rarely at the fiber level. Presently the Warner-Bratzler instrument is the most widely used physical method of measuring the shear force of muscle. However, this method is concerned with cooked pieces of meat, one-inch in diameter. The Warner-Bratzler instrument tells little about the influence of the individual muscle tissue components on tenderness or texture. Therefore, a need clearly exists for a method that would record the shear force of the individual muscle fiber.

Some work on the physical properties of the muscle fiber has been reported (Stanley et. al., 1971), however, most studies have been concerned with the tensile strength of the fiber, its elasticity or breaking strength. There has been no investigation into the role of shear force of individual muscle fibers in determining quality attributes of meat. The purpose of this study was to evaluate a method whereby the shear force of the individual muscle fiber can be measured.

CHAPTER II

REVIEW OF LITERATURE

Physical Characteristics of Muscle

Muscle Structure

There are three types of muscle tissue found in vertebrates: (1) skeletal muscle which is cross striated, (2) smooth or involuntary muscle characterized as having no striation, and (3) cardiac muscle with branched striated fibers. In dealing with meat, one is usually concerned with muscle of the skeleton. Consequently, the scope of this research will involve only the skeletal muscle fiber.

A whole intact muscle is surrounded by a layer of connective tissue called the epimysium. This connective tissue layer contains septa which pass into the interior of the muscle to form a second connective tissue layer called the perimysium. The perimysium surrounds a fascisuli (bundles of muscle fibers). There are also extensions from the perimysium which extend deeper into the interior of the muscle to surround the individual muscle fibers. These extensions form a third layer of connective tissue called the endomysium. Although one may distinguish these three layers of tissue, Briskey (1967) has shown that the structures are continuous around and within the muscle. Immediately beneath the endomysium is a layer of membranous tissue called the sarcolemma. The sarcolemma in the muscle fiber is analogous to the cell membrane in

other cells. Lorincz and Biro (1963) observed thin filaments of collagenous connective tissue within and between the sarcolemma and the endomysium. These thin connective tissue strands were found to be reticular fibers.

The structural unit of muscle is the fiber. These range in diameter from 10-100 microns and extend a considerable distance up and down the muscle. A fiber is a cylindrical, elongated, nonbranching structure. Fibers, when held together, make up the muscle which ends in tendinous bone connections. A muscle fiber contains elongated myofibrils which are bathed in the sarcoplasm (analogous to the cytoplasm in other cells). Each fiber is multinucleated and the nuclei are generally located beneath the sarcolemma but may be found around or between the myofibrils. An average muscle fiber of 50 microns in diameter will contain about 2000 myofibrils (Bendall 1966). Each fibril is approximately one micron in diameter and is considered to be the region of muscle contraction. The light and dark appearance of a skeletal muscle fiber, as seen under the microscope, is due to striations on the myofibrils. The striations are oriented regularly among all of the myofibrils of an individual muscle fiber. With the aid of a high power light microscope, it has been observed that the striation pattern is an alteration of light isotropic I-bands and dark anisotropic A-bands (Bendall 1966). The light I-bands are made of the protein actin, and the dark A-bands are made of the protein myosin. When the vertebrate muscle fiber is relaxed, the length of a single A-band of a myofibril is approximately 1.5 microns and the length of a single I-band is about 0.8 microns (Huxley 1957). In the center of the A-band section is a light area called the H-zone (Huxley and Hanson 1960). Within the center of the H-zone is a densely

stained region running vertically down the center, called the M-line. The protein composition of the M-line is at this time undetermined. In the center of the light I-band is a dark region called the Z-line. The distance between two Z-lines is known as a sarcomere, the functional unit of contraction.

When the myofibril is examined under the electron microscope, two types of filaments can be observed, one about twice as thick as the other. The thicker filaments are about 100 Å in diameter and about 1.5 microns long. The thinner filaments are approximately 50 Å in diameter and about 2 microns long. Each filament is seen arrayed with other filaments of the same kind, and the two arrays overlap for part of their length. This overlapping is responsible for cross banding on the myofibrils. The dense A-band consists of overlapping thick and thin filaments and the lighter I-band reflects the thin filaments alone. The H-zone consists only of thick filaments. Halfway along their length, the thin filaments pass through a narrow area of dense material, designated as the Z-line by Huxley (1957).

Surrounding the myofibrils in the muscle fiber is a mass of protoplasm called the sarcoplasm. Within the sarcoplasm are the muscle cell organelles, including the lysosomes, mitochondria, golgi apparatus, lipid droplets, and the sarcoplasmic reticulum which is analogous to the endoplasmic reticulum in other cells. In addition, the nuclei are found within the sarcoplasm around or between the myofibrils. The major function of the sarcoplasm seems to be that it serves as the nutrient source for the myofibrils.

Muscle Proteins

About 20 percent of the weight of the muscle fiber is represented by proteins. This 20 percent is made of three general classes of protein: (1) myofibrillar, the fraction which includes the contractile proteins, actin and myosin (Perry 1965), (2) stroma, the fraction which includes the connective tissue proteins, collagen and elastin, and (3) sarcoplasmic, those containing the proteins that are soluble in low salt concentrations, such as myoglobin, hemoglobin, cytochrome C, and various enzymes.

Myofibrillar Proteins

Myosin is found to constitute 50-55 percent of the myofibrillar protein in skeletal muscle. It is an alpha helix type protein with a molecular weight of about 420,000 (Lowey and Cohen 1962). Myosin has been fragmented into two fractions (meromyosins), a heavy and a light function, which together make up the myosin molecule. Heavy meromyosin has a molecular weight of about 300,000 and light meromyosin of about 120,000.

The myosin molecule possesses enzymatic activity which will catalyze the hydrolysis of ATP; Szent-Györgi (1953) found that this ATP-ase activity was associated with the heavy meromyosin. Szent-Györgi (1958) also determined that practically all of the ATP present in muscle fibers is bound to myosin, one mole of ATP being bound per 18,000 grams of myosin.

Myosin seems to be present in the muscle fiber as the magnesium myosinate; it binds monovalent and divalent ions although it does not distinguish between sodium and potassium ions. Szent-Györgi (1958) re-

ported that the ATP is bound to the magnesium of the myosinate molecule since the ultraviolet absorption spectrum of the myosin-ATP complex is different from that obtained by summation of the absorption of myosin and ATP separately.

Myosin is the myofibrillar protein which makes up the heavy anisotropic A-bands. Szent-Györgi (1953) reported that the heavy meromyosin may be the projection that attaches itself to the actin filament during contraction. It seems certain that the heavy meromyosin is the portion of the myosin molecule that binds with actin to form actomyosin during contraction.

Actin constitutes 20-25 percent of the protein in the myofibrillar class of skeletal muscle proteins. Actin binds four equivalents of calcium per molecule, and apparently exists in the muscle fiber as calcium actinate. In the absence of salts, actin exists in the globular form, with a molecular weight of approximately 70,000. In the presence of ATP, actin forms dimers, and in the presence of ATP and 0.1 M potassium chloride, it forms polymers of long strands. Actin, therefore, can exist in two forms: (1) G-actin, the globular form, and (2) F-actin, the fibrillar form.

Huxley (1963) reported that actin appears to be asymmetrical and to be made of two coils of globular units wound in a double helix with crossover points 360 degrees apart. Huxley (1963) also stated that when actin and myosin combine, this arrangement is repeated. The actin has a period of 400 $\mathring{A}$ according to X-ray diffraction and electron microscope studies. The role of this transformation of actin between the globular and fibrillar protein forms in muscle contraction has not been adequately explained.

When actin and myosin are mixed in the presence of salts, they interact to form actomyosin. The mechanism of this interaction is not completely understood, although it is clear that actin and myosin have been found to combine in stoiciometric ratios. The role of ATP in this reaction between actin and myosin is also rather ambiguous; ATP is necessary for the contraction of the sarcomere and the formation of actomyosin. It serves as a contracting agent when bound to the myosin in the presence of magnesium. However, the disassociation of actin and myosin is maintained only as long as ATP is present, and the linkage between actin and myosin will relax if the ATP is bound to the myosin, but not split by its ATP-ase activity. The formation of actomyosin in the absence of ATP results in rigor mortis, the irreversible contraction of the muscle fibers (Briskey 1967).

Tropomyosin has a molecular weight of approximately 50,000 and it closely resembles light meromyosin in several respects including amino acid composition and iso-electric point. The role of tropomyosin in the contraction is not clear, however, Huxley (1957) reported it to be associated with G-actin, and the Z-line. Tropomyosin is a helical structure although it may also appear as a single strand in the Z-line.

Stroma Proteins and Sarcoplasmic Proteins

Since the scope of this study is limited primarily to the contraction of muscle, an attempt will be made to briefly describe the relationship between these protein classes, and not provide a detailed discussion of their role in other muscle functions. Stroma proteins are not considered to be directly involved in the contractile process. Their function is connection and maintenance of the integrity of the muscle struc-

tures. Sarcoplasmic proteins are related to contraction only in the sense that they supply necessary enzymes and energy source materials for the contractile process; examples would include creatine phosphokinase as a necessary enzyme and creatine phosphate as an energy source.

Muscle Contraction

Contractility has reached its highest development in the skeletal muscle fiber; the muscle fiber being adapted specifically to this function. The skeletal muscle fiber is made up of contractile myofibrils which contain the basic unit of contraction, the sarcomere. The sarcomere consists of thick and thin filaments that make up the A-bands and the I-bands. Contraction takes place as the electrical potential around the fiber is changed and these filaments slide together. A model showing this action was developed by H. E. Huxley (1957) and is known as the sliding filament theory of muscle contraction.

The first step in the contraction of muscle is the nerve impulse that stimulates the muscle fiber and propagates the action potential, changing the electrical potential around the fiber. As the nerve impulse travels through the myoneural junction, the action potential causes a change in permeability of the sarcolemma. This change in sarcolemma permeability leads to the establishement of an electrical potential change that travels the length of the sarcolemma, and to the Z-bands by way of the transverse tubules. At this point, calcium ions, in the presence of ATP and magnesium, activate ATP-ase, an enzyme which catalyze the hydrolysis of ATP. The high energy phosphate bond, that is released in the hydrolysis of ATP, provides the energy necessary for contraction.

During muscle contraction, the A-band remains constant, but the

length of the I-band may vary depending upon the degree of contraction (Bendall, 1966). The length of the H-zone fluxuates with the length of the I-bands, but during complete contraction, the H-zone completely disappears. It has been shown that when a muscle fiber contracts, the two sets of filaments slide past each other (Huxley and Hanson, 1959). When a muscle fiber is contracted to its fullest extent, the filaments meet at their ends, and new bands often appear on electron micrographs. Huxley (1957) explained these bands as a result of the shortening process, not the cause of contraction. Finally, during the contractile process, actin and myosin chemically react in the presence of ATP to form actomyosin. Weber (1959) has shown evidence for a relaxing factor which in the presence of ATP can dissociate the actomyosin complex. This relaxing factor seems to sequester calcium ions from the sarcoplasmic reticulum in excitation-contraction coupling (Weber et. al., 1964; Marsh, 1960). With the removal of calcium from the system, the ATP-ase is inactivated and the fiber is relaxed.

Rigor Mortis

Rigor mortis is the irreversible contraction of muscle due to the depletion of ATP. Although physiological contraction and rigor mortis utilize the same basic mechanism of contraction, Bendall (1960) showed that contraction due to rigor mortis only involved a fraction of the muscle fibers, distinguishing it from simple muscle contraction.

In order to appreciate the significance of rigor mortis and its effect on meat, certain physical and chemical aspects of rigor mortis must be considered. Rigor mortis manifests itself in meat as a loss of extensibility, accompanied by a lowered pH and a loss in the water-

holding capacity of the muscle. The loss of extensibility, or stiffening of the muscle, is due to the formation of a complex between actin and myosin, actomyosin. Lawrie (1966) pointed out that this loss of extensibility occurs in two stages: (1) the delay period; in this stage stiffening proceeds slowly, and (2) the fast phase; here the loss of extensibility proceeds quite rapidly. After the fast phase, the extensibility of the muscle remains constant, but at a low level.

Bendall (1960) classified three general patterns describing the onset of rigor mortis: (1) acid rigor; characterized in immobilized animals by a long delay period and a short fast phase, and in struggling animals by a drastic curtailment of the delay period. (At body temperature, stiffening is accompanied by a shortening of the muscle.) (2) alkaline rigor; characterized by a rapid onset of stiffening and by a marked shortening even at relatively low temperatures, and (3) intermediate type; characterized in starved animals by a curtailment of the delay period, but not of the rapid phase; there is some shortening.

The environmental temperature of the animal post-mortem influences the effect of rigor mortis, as is manifested in a loss of extensibility and in the shortening of the muscle. Lawrie (1966) stated that muscle shortening is minimal in the temperature range of 14-19 degrees C. Locker and Hagyard (1963) reported that below this 14 degree C. level, a cold shortening was observed, and Marsh (1962) observed a great increase in shortening, accompanied with a marked decrease in tenderness with post mortem temperatures over 43 degree C.

The chemical significance of rigor mortis was demonstrated when Erdös (1943) found that the development of rigor mortis was associated with the depletion of ATP from the muscle. The reason for this depletion

of ATP has been examined at length by Bate-Smith and Bendall (1947, 1949) and Bendall (1951, 1960). As the oxygen supply in the muscle is decreased after exsanguination of the animal, the metabolism of the muscle shifts from the highly efficient aerobic tricarboxylic acid cycle to the inefficient method of anaerobic glycolysis, which results in the decreased synthesis of ATP. Marsh (1954) reported that all glycolytic processes in beef muscle are completed thirty six hours post-mortem. DeFremery and Pool (1960) observed that the fast phase of extensibility loss in chicken muscle did not begin until the level of ATP was down to 30 percent of its initial concentration. A necessary level of ATP can be briefly maintained by the synthesis of ATP from ADP and creatine phosphate, however, Briskey (1959) showed that the creatine phosphate in muscle is broken down enzymatically soon after death. Briskey (1959) also found accumulations of lactic acid, the end-product of anaerobic glycolysis, in muscle shortly after death, accounting for the drop in muscle pH as rigor mortis develops. This drop in pH also contributes to the decrease in the water-holding capacity of the muscle.

The time period required for complete shortening of muscle due to rigor mortis varies between animals within and between species. Smith et. al. (1969) observed that shortening due to rigor mortis was complete within three hours in chicken muscle, and within five hours in turkey muscle. T-I Ma et. al. (1971) reported a complete loss of extensibility in the pectoralis muscle of turkey within a time range of twenty-five minutes and six and one-half hours post-mortem, indicating widespread variation between animals of the same species. In porcine muscle, shortening due to rigor mortis is completed within five hours post-mortem (Sayre and Briskey, 1963). Marsh (1952) reported that whale muscle main-

tained in vivo levels of ATP and muscle pH for as long as twenty-four hours post-mortem, indicating variation between species in time required for the development of rigor mortis.

Ramsbottom and Strandine (1949) demonstrated that pre-rigor meat is quite tender, but becomes progressively less tender with the development of rigor mortis. Although the exact cause of the decrease in tenderness is not known, it is believed to be associated with the loss of extensibility and the shortening of the muscle. This problem in meat tenderness has been extensively investigated, and there are several methods available to reduce the toughening effect of rigor mortis on meat. The most common, and perhaps the most successful means presently in use, is the process of aging. It has been repeatedly demonstrated that meat increases in tenderness when aged (Paul et. al. 1952, Whitaker, 1959, Weinberg et. al., 1960, Aberle et. al., 1966, and Davey et. al., 1968). The reason for this increase in tenderness with aging has been shown to be twofold: (1) the autolysis of muscle and connective tissue by proteolytic enzymes secreted from the lysosomes of the muscle fiber soon after the death of the animal, and (2) the microbial proteolysis of myofibrillar and connective tissue proteins. The combined effect of these processes results in decreased stiffening of the muscle, and in the amount of connective tissue within the muscle, both contributing to the increased muscle tenderness.

A second method in widespread use is to place the muscles under tension to prevent the shortening effect of rigor (Herring et. al. 1965a, 1965b, Gillis and Henrickson, 1968). A third, less common method involves the injection of a calcium chelator to block the actin-myosin interaction (Weiner and Pearson, 1966). Finally, a method which has

recently come into large scale commercial use, involves the ante-mortem injection of proteolytic enzymes into the vascular system of animals to artifically speed up the natural effects of aging, the enzymatic breakdown of myofibrillar and connective tissue proteins, increasing the tenderness of the muscle.

Muscle Excision--Hot and Cold Boning

As pointed out earlier, the decline in tenderness associated with rigor mortis is decreased if the muscles are placed under tension to prevent shortening during the development of rigor mortis. Herring et. al. (1965b) reported that carcass position has a pronounced effect on the contractile state of bovine muscle. Locker (1960) demonstrated that the vertical suspension of the beef carcass places certain muscles in a stretched state, while other muscles are in a shortened state. Herring (1965a) observed that bovine muscles excised pre-rigor were more tender when tension was applied during rigor mortis.

Reddy (1962), studying the effect of pre-rigor excision on three bovine muscles, reported that the amount of fiber distortion, as measured in terms of percent kinkiness, was found to be greater for pre-rigor excised Longissimus dorsi muscle than post-rigor excised muscle. However, the same author observed the opposite effect for the Gluteus medius muscle, supporting the results reported by Locker (1960), that the vertical suspension of the bovine carcass results in different strains among the various muscles within the carcass.

Gillis and Henrickson (1968), studying the effect of induced tension on pre-rigor excised muscle, reported that muscle fiber diameter decreased as tension increased up to 1000 grams. The same authors also

observed that muscle fiber distortion decreased as the tension on the muscle increased, and that an increase in muscle fiber distortion resulted in an increased shear force. Reddy (1962) also investigating the effect of pre-rigor excision on bovine muscle, reported that fiber diameter and shear force were not significantly affected in Longissimus dorsi muscle by pre-rigor excision. However, the same author did observe significant increases in muscle fiber diameter and shear force in the Semitendinosis muscle, supporting subsequent work by Gillis and Henrickson (1968).

Lowe and Stewart (1946) observed that breast muscle in chicken was generally less tender when excised soon after death, before the onset of rigor. The same authors reported that the faster the muscle was excised post-mortem, the less tender the product, and that when the muscle was excised after the onset of rigor mortis, no significant decrease in tenderness was observed. These results were confirmed and enlightened by T-I Ma et. al. (1971) who reported that the less ATP present in muscle, the smaller the effect of muscle excision on tenderness.

Ramsbottom and Strandine (1949) demonstrated that pre-rigor excised bovine muscle was less tender than beef chilled on the carcass, and that muscle was more tender two hours post-mortem than after six hours of aging. The same authors also observed that beef muscle excised two hours post-mortem was less tender than beef which had been allowed to age for twelve days. Goll et. al. (1964) partially confirmed this finding when they reported that muscles held restrained on the carcass were least tender immediately post-mortem, but gradually increased in tenderness with aging.

Evaluation of the Physical Properties of Meat

Tenderness of meat is perhaps the most important quality factor determining consumer acceptance. For this reason there have been numerous attempts to develop methods for the assessment of tenderness. The term tenderness implies subjective evaluation and must ultimately be measured organleptically. However, texture, used here to connote mechanical properties, can be measured objectively. The physical properties of meat are measured to provide indices of texture and ultimately of tenderness. The physical property most widely used as an objective measure of texture is shear force. The first mechanical measure of meat texture involved a determination of shear force (Lehmann, 1907), and the most popular device used by present day investigators to measure meat texture, the Warner-Bratzler shear instrument, also involves a determination of shear force.

Until recently, every mechanical measure of texture utilized samples of meat tissue, usually cooked cores of meat one-inch in diameter. Presently, the most widely used objective method for the evaluation of meat texture involves various means by which force is applied via a blunt edge and the force required to shear a sample is recorded. Szczesniak and Torgeson (1965) pointed out that there are several variables inherent in this procedure that are difficult or impossible to control, including orientation of muscle fibers, sample temperature, speed of shearing, and blade dullness. The same authors also reported that these methods only measure the maximum shear force, not the slope of the shear force curve which they suggested to be more meaningful. Sharrah et. al. (1965) pointed out that it is not clear whether these determinations of maximum shear force measure the same characteristics

of meat as do sensory panels. Finally, Pool and Klose (1969) observed that meat samples subjected to shearing stress are distorted to the point that part of the applied shear force is altered to a tensile stress of the stretching fibers.

Perhaps the most innovative development in the measurement of the physical properties of meat is the method whereby tensile strength of muscle fiber bundles is determined with the aid of the Instron Universal Testing Machine. Stanley et. al. (1971), working with rabbit Longissimus dorsi muscle, reported that the Instron Universal testing machine was able to successfully measure tensile strength of muscle as well as other related physical properties. In the same study, working with bovine Psoas major and shank muscles, these authors also demonstrated that the Instron could distinguish between these two muscles in terms of texture as measured by tensile strength.

It is clear that a method for the objective assessment of meat texture is necessary, however, as Stanley et. al. (1971) pointed out, it is likely that more than one physical property of meat will have to be measured in order to provide a good estimate of meat texture.

CHAPTER III

MATERIALS AND METHODS

The Microsensitive Shear Instrument

A microsensitive shear instrument was utilized in this study to determine the shear force of individual muscle fibers, Figure 1. This shear instrument was developed and described by Henrickson et. al. (1967) as a research tool for measuring shear force, a physical property of the muscle fiber. The instrument consists of a shear gauge, equipped with a torque dial which is easily read from the top, Figure 2. The shear gauge is strung with a wire 1/100 inch in diameter which supports a blunt edged knife that passes through the fiber to make a shear (Figure 3). The top end of the wire is connected to the torque dial so when the shearing blade passes through the fiber the required torque is recorded. The fiber is held, but not tightly clamped, between a plexiglass and an aluminum plate. A shallow V-cut was made into the aluminum plate, and the fiber placed into this groove, Figure 4. The two plates are then attached by a clamp to an adjustable specimen holder which orients the fiber in a vertical position. The holder is placed under a physiological saline solution while the shear is being made. The torque required to shear the fiber is converted to force per unit area by the following formula: Force in grams per micron^2 is equal to $\frac{\text{torque x } 0.7759469}{\text{fiber diameter}^2}$ (Henrickson, et. al., 1967).

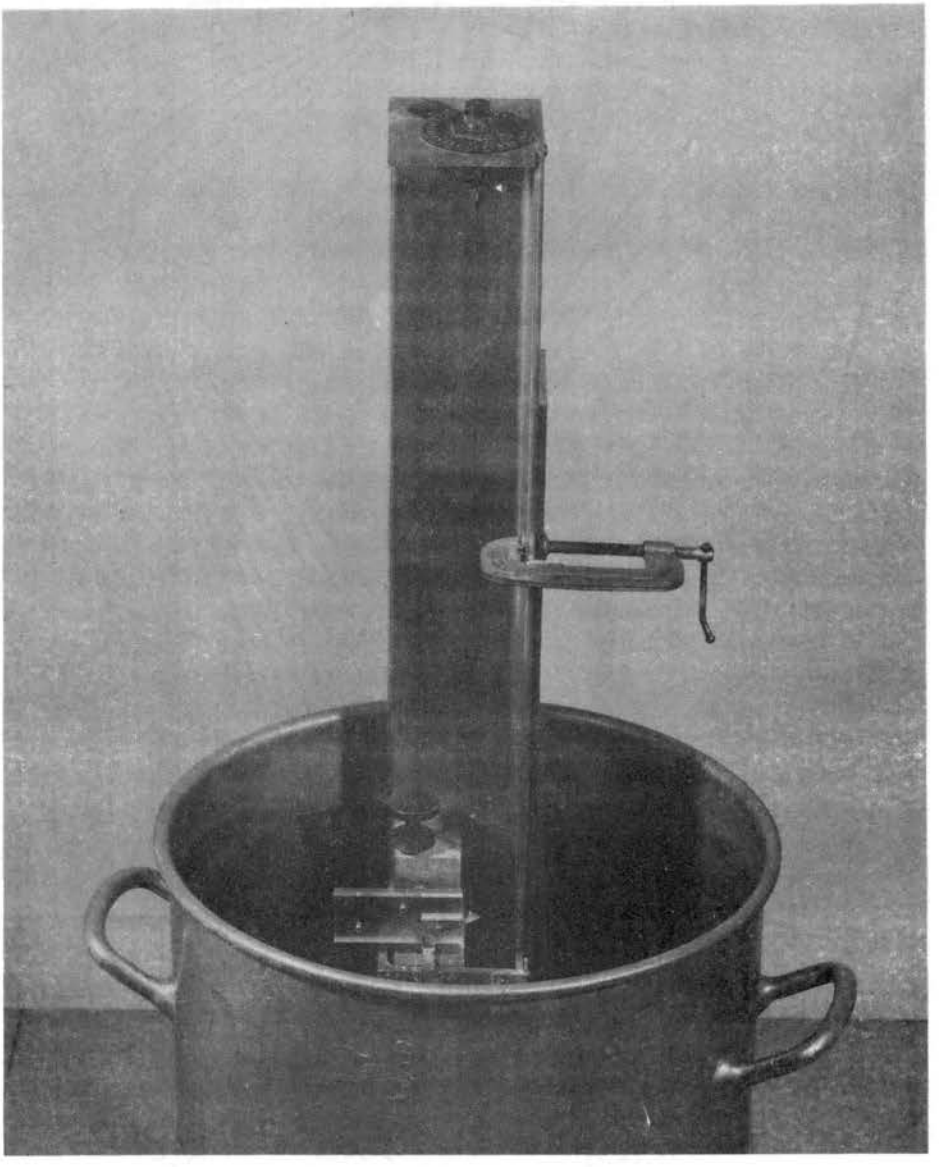

Figure 1. Microsensitive Shear Instrument Fully Assembled

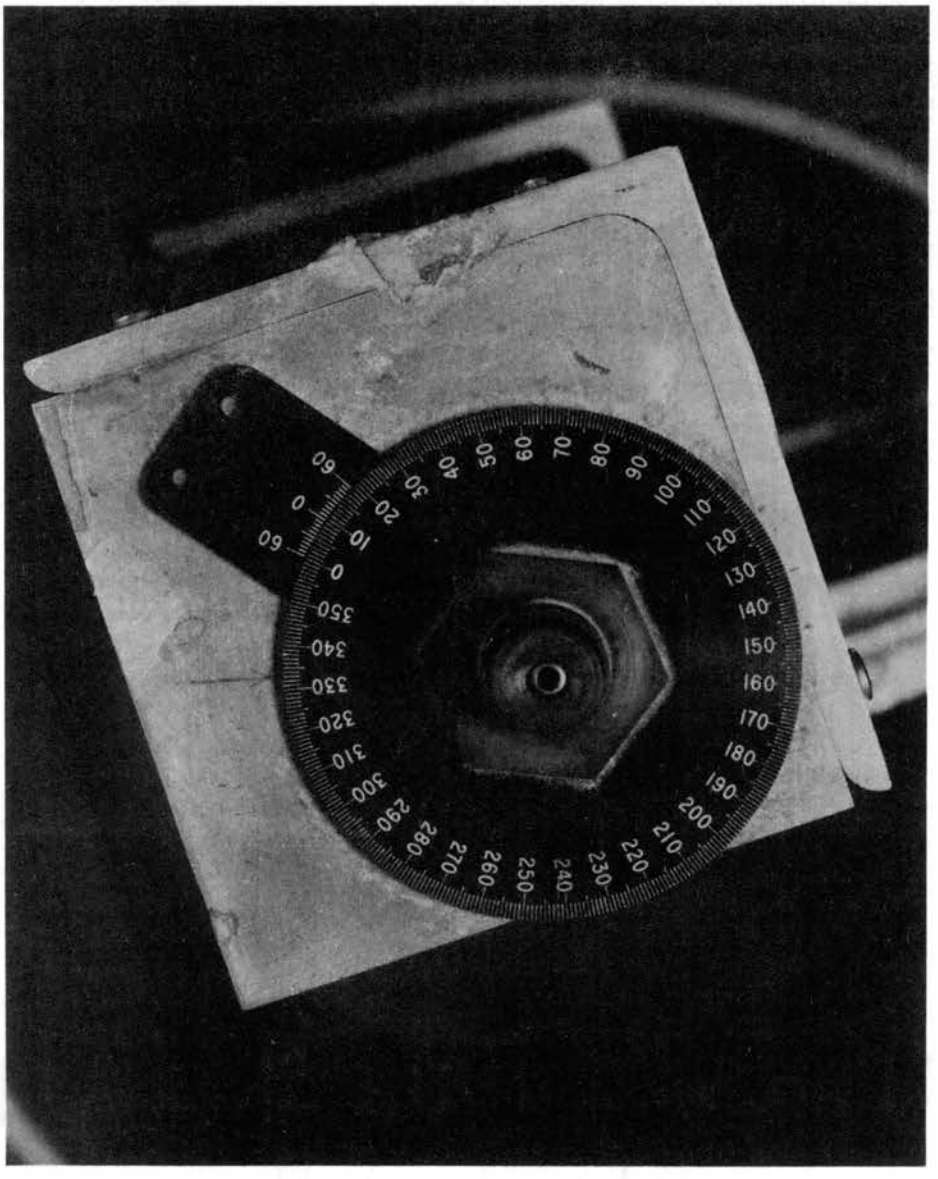

Figure 2. Torque Dial of the Microsensitive Shear Instrument

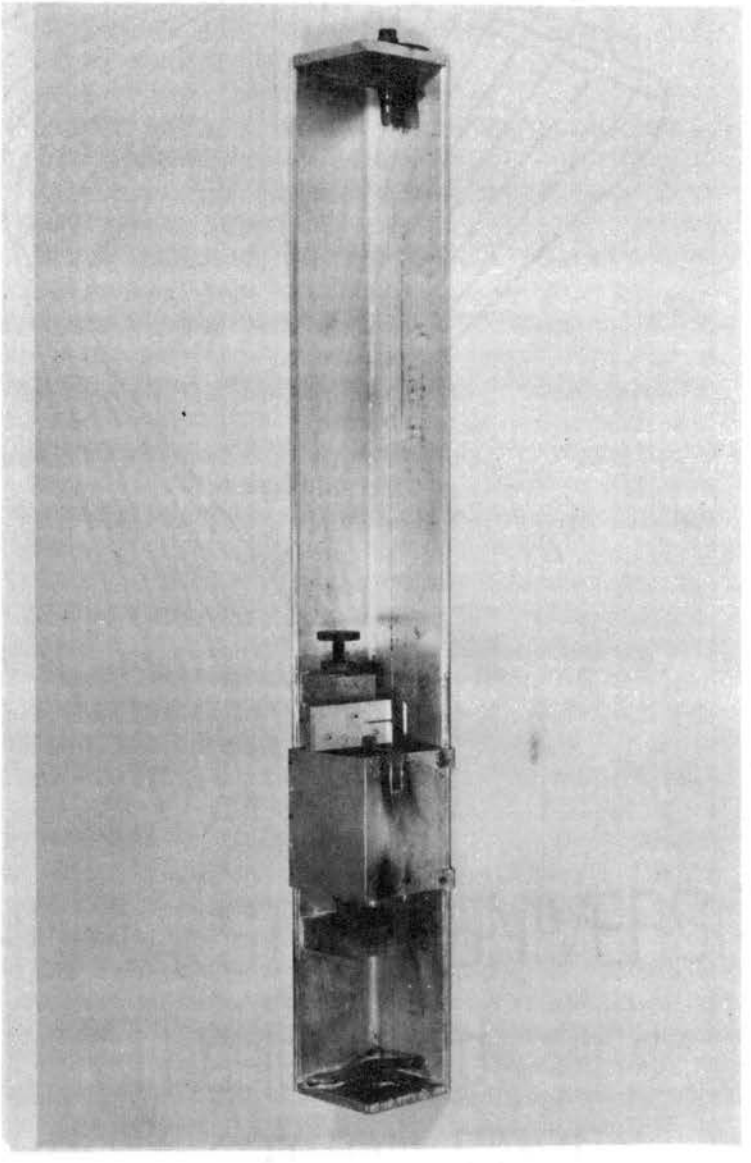

Figure 3. Assembled Shearing Mechanism and Wire Leading to Torque Dial

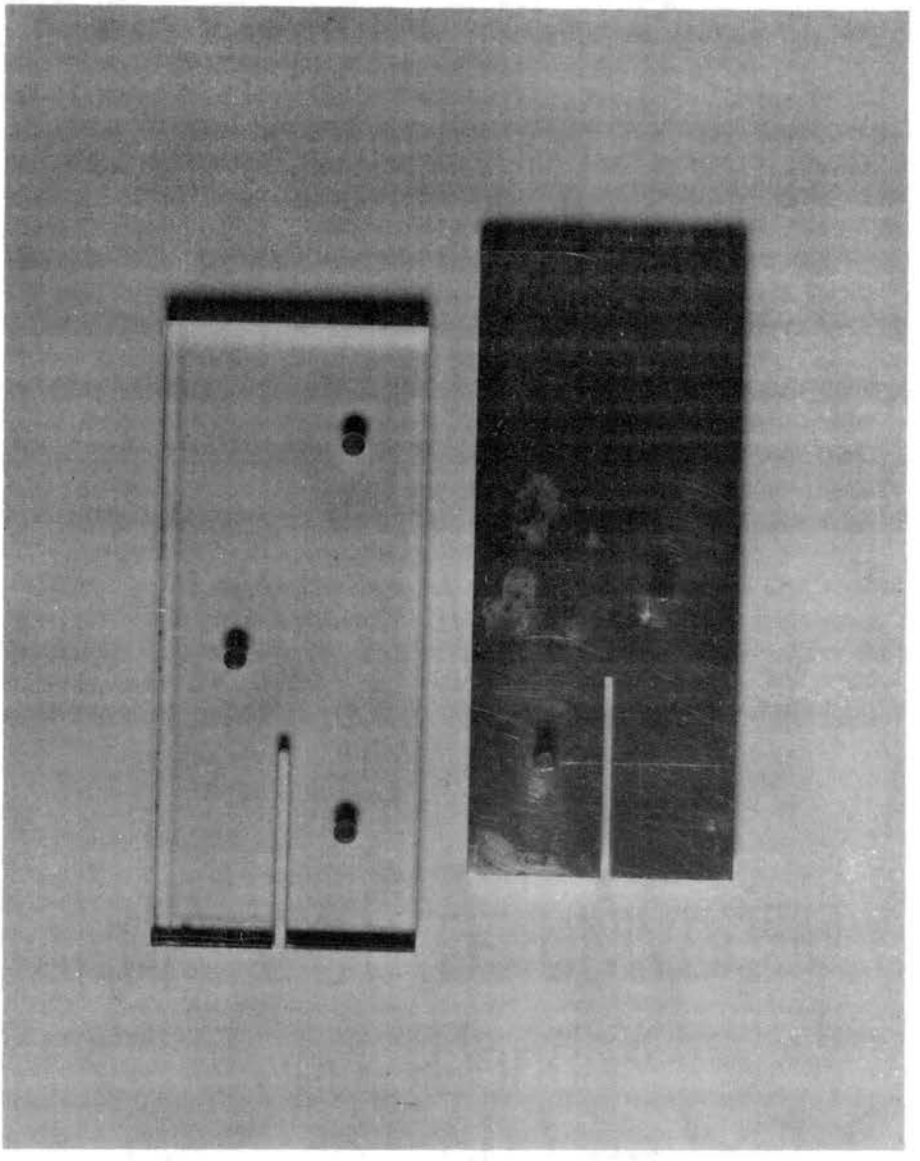

Figure 4. Aluminum and Plexiglass Holders

Uniformity Trial

In order to determine the reliability and precision of the microsensitive shear instrument, a uniformity trial was initiated. One Hereford steer was slaughtered under Federal inspection, split into sides and graded (choice). The carcass was held at 2 degrees C. for a period of 48 hours. Then the Sartorius muscle was excised and a section of the muscle removed and placed in a 10% formalin solution. When the muscle section was thoroughly fixed, a group of fasiculi was removed, separated, and its fibers harvested using a modified Waring blender. Three hundred fibers were randomly selected from the composite group of fibers and determinations were made on each fiber for diameter, degree of rigor mortis, and shear force. The results were analyzed statistically for reliability and measurement precision.

Two Hour Holding Period

Upon completion of the uniformity trial, the main objective of the study was a determination of the ability of the microsensitive shear instrument to measure a difference between muscles with respect to average shear force for 100 individual muscle fibers. This research was carried out in conjunction with another experiment investigating the effects of hot boning of the bovine carcass (Kastner 1972).

Six Hereford steers were utilized in this test of the shear instrument. These animals were of the same approximate weight and market grade. The animals were each rendered unconscious with a Cash Percussion Stunner, raised off of the floor by both hind legs and bled in the traditional manner. Skinning and eviseration were accomplished within 45 minutes after death. The carcasses were split, Federally inspected, and

the hot weights were recorded. The sides were randomly assigned to one of two treatments, (1) removing the muscles after a 2 hour restraining period, after which the muscles were allowed to contract freely ("hot boning"), or (2) removing the muscles after restrainment on the carcass at 2 degrees C. for a period of 48 hours ("cold boning"). The effect of these treatments was to allow the muscles excised hot to freely contract, while the muscles that were excised after a 48 hour restraining period would not go into as complete a state of contraction as the "hot boned" group.

Muscle Excision - "Hot Boning"

The side designated for the "hot boning" treatment was suspended from the rail during muscle excision, along with the side designated for the "cold boning" treatment. The temperature in the cutting room was held at 24 degrees until all muscles were removed from the hot boned side (minus chuck, shank, and brisket). The sample involved in this study, the Sartorius muscle, was placed in a loose Cry-O-Vac bag, and chilled at a temperature of approximately 4 degrees C. Carcass dissection of this muscle was completed within 2 hours post-mortem.

Muscle Excision - "Cold Boning"

After a forty-six hour cooling period with all muscles restrained on the carcass, the side designated for "cold boning" was broken down in the same manner as the hot boned side. The Sartorius muscle was excised and placed in a loose Cry-O-Vac bag. Carcass dissection of all muscles was accomplished approximately 48 hours post-mortem.

Formalin Fixation

Longitudinal sections approximately two inches long and one-quarter inch wide were removed from the Sartorius muscles from both sides of the carcass. These sections were fixed in 10 percent formalin solution, buffered with sodium acetate. Fixation was accomplished by placing the muscle sections in labeled bottles and covering the sections with the buffered formalin solution. After twenty-four hours, the original formalin solution was removed and replaced with a fresh buffered 10 percent solution. All muscle sections were thoroughly fixed before any fiber shear determinations were attempted.

Harvesting Muscle Fibers

After the Sartorius muscle sections from each of the six carcasses were thoroughly fixed, samples were chosen at random for harvesting of individual muscle fibers for fiber diameter, degree rigor, and shear force determinations. Fasiculi were teased from the formalin fixed sections with a needle and placed in approximately 100 ml. of buffered 10 percent formalin solution in a Waring blender equipped with a reverse blade, and harvested after low speed mixing. The blender was turned on for a period of one minute, and the result was a suspension of individual muscle fibers. This suspension was placed in a four ounce jar held at 34 degrees F. until all 100 fibers could be measured.

Determination of Fiber Diameter

At the time of measurement, the fiber suspension in the jar was thoroughly shaken and a small portion was poured into a two-inch diameter petri dish. The petri dish was placed on an American Optical microscope

equipped with an ocular micrometer, and the fibers were allowed to settle to the bottom of the dish. The only fibers measured were those which appeared steady in the microscopic field and were at least the length of the field. One hundred muscle fibers from each sample were measured for diameter at their widest point as seen in the magnified field.

Determination of Degree Rigor

Degree rigor or percent kinkiness was assessed at the same time fiber diameter was measured. After a determination of fiber diameter was made on an individual muscle fiber, a subjective score for kinkiness was assigned on a scale from 1-7, depending upon the amount of kinkiness and the condition of the fiber. A weighted score for the 100 total fibers was then calculated and converted to percent kinkiness for each Sartorius muscle by the procedure outlined by Cagle et. al. (1970).

Shear Force Determination for Individual Muscle Fibers

After the individual muscle fibers were measured for fiber diameter and assessed for kinkiness, each fiber was carefully removed from the petri dish, with a tweezer, and placed on a steel and plexiglass holding mechanism in preparation for shearing (Figure 4). After the fiber was tightly secured between the two components of the holding mechanism, the fiber was placed on the shearing platform of the microsensitive shear instrument. The actual shearing of the fiber was accomplished under a physiological saline solution. This shearing procedure consisted of the slow and steady turning of the blade until it was visibly in contact with the fiber. This reading was used as the starting point. The blade

was steadily turned by the operator until it passed through the fiber. At this point the operator recorded the torque required to shear the fiber by the subtraction of the reading on the torque dial at the point where the fiber was sheared, from the point where the blade first came into contact with the fiber. This procedure was repeated for 100 muscle fibers from each Sartorius muscle. The torque required to shear each fiber was converted to force by the computation formula outlined by Henrickson et. al. (1967), and these force determinations for the 100 muscle fibers were averaged to yield a mean force assignment for each muscle.

Statistical Analysis

The Analysis of Variance in conjunction with the F-test was used to analyze differences in fiber diameter, percent rigor, and shear force between the hot and cold excised muscles. In addition, simple means were calculated for fiber diameter, percent rigor, and shear force for both the uniformity trial and the hot and cold excised muscles.

Five Hour Holding Period

As determined by Kastner (1972), muscle tenderness presented the greatest problem with the hot processed muscle. For this reason, the investigators decided to repeat the above procedure, utilizing a second group of six animals, with one major change; the holding period was extended from two hours to five hours. In this experiment, the hot boned side was held five hours at 16 degrees C. prior to muscle excision. This change in holding time was used in an attempt to reduce Warner-Bratzler shear differences between hot and cold processed muscles. The

advantage of holding muscles at 16 degrees C. has been cited by several authors (Locker and Hagyard, 1963; Forrest et. al., 1969, and Smith et. al., 1969).

The muscle fiber experiment, using the Sartorius muscle, was accomplished in the same manner with these six carcasses held at 16 degrees C. for five hours as with the first six held for two hours. All fiber diameter, percent rigor, and shear force measurements were made in the same manner previously outlined.

Eight Hour Holding Period

In order to further decrease differences between hot and cold processed muscles, Kastner (1972) made a second change in the holding period of the muscles. In the third experiment, the muscles were held at 16 degrees C. for a period of eight hours. As in the two previous experiments, the animals were of similar grade and weight and genetic background, and a total of six animals were utilized. The muscle fiber investigation was accomplished in exactly the same manner as in the two previous experiments. Again all muscle fiber diameter, percent rigor, and shear force measurements were made by the same procedure as outlined for the two hour holding period. This third holding period resulted in the fiber study to be made of three experiments, each consisting of six animals. Two Sartorius muscles were taken from each carcass and held at two, five, and eight hour holding periods. One hundred muscle fibers were sheared from each Sartorius muscle, resulting in a total of 3,600 muscle fiber shears.

CHAPTER IV

RESULTS AND DISCUSSION

Uniformity Trial

Although a more extensive uniformity study should be initiated to examine the precision and reliability of the microsensitive shear instrument, the results of this study demonstrated that it is feasible to measure the shear force of individual muscle fibers with a reasonable degree of precision.

The mean shear force for the 299 muscle fibers measured in the preliminary study was 2.30×10^{-2} gm./u^2, with a standard deviation of 1.05 (Table I). The shear force for individual muscle fibers ranged from $0.62 - 8.20 \times 10^{-2}$ gm./u^2. Both the standard deviation and the range indicate a large amount of variation among fibers in terms of shear force, however, the standard error of estimate for the measurement of shear force for these 299 fibers was 0.06, indicating that the microsensitive shear instrument measured shear force with a reasonable degree of precision. A large part of the variation in shear force among muscle fibers can be attributed to differences in fiber diameter and degree rigor.

Fiber size varied within a given muscle. When the data was grouped according to fiber diameter one may note that as diameter increased, the shear force, expressed in terms of force per unit area (gm./u^2), decreased (Table II, Figure 5). The only explanation that the author

TABLE I

UNIFORMITY TRIAL: MEAN, STANDARD DEVIATION AND RANGE FOR FIBER DIAMETER, DEGREE RIGOR AND SHEAR FORCE

Variable		N	Mean	Standard Deviation	Range
Diameter	u	299	54.35	10.64	30.00 - 90.00
Degree Rigor	1-7[a]	299	3.08	0.62	2.00 - 5.00
Shear Force	$x\ 10^{-2}$ gm./u^2	299	2.30	1.05	0.62 - 8.20

[a]Degree rigor was expressed by using a photograph standard ranging in value from 1-7. The lower a value assigned for degree rigor, the less damaged and kinked was the fiber.

TABLE II

UNIFORMITY TRIAL: EFFECT OF DIAMETER ON SHEAR FORCE

Diameter u	N	Shear Force $\times 10^{-2}$ gm./u^2	Standard Deviation
30	3	3.25	0.82
40	54	3.20	1.36
50	110	2.46	0.96
60	83	1.95	0.61
70	42	1.57	0.44
80	5	1.04	0.32
90	2	1.20	0.09

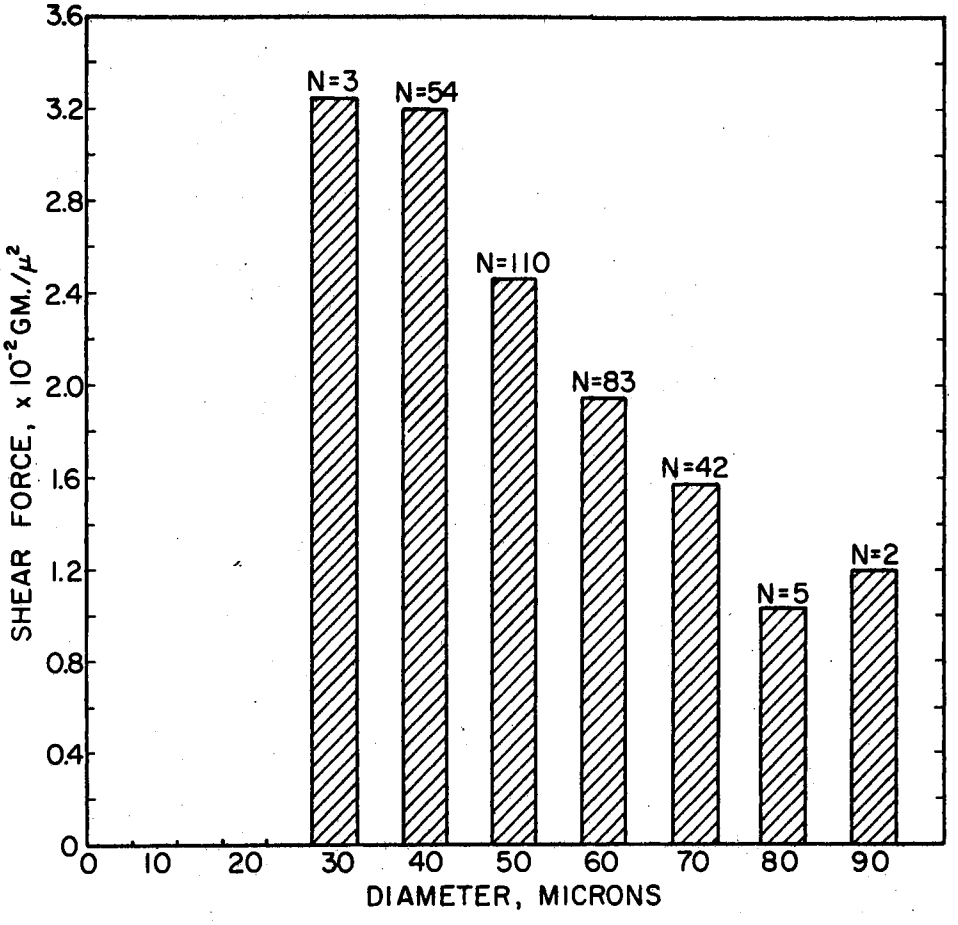

Figure 5. Uniformity Trial: Effect of Fiber Diameter on Shear Force

can give for this phenomenon is that when the fiber is sheared, a large force is required to pass through the endomysium and sarcolemma surrounding the fiber, but the blade passes with relative ease through the interior of the fiber. This would result in a decrease in force per unit area for a fiber with a large diameter when shear force was expressed in terms of force per unit area. When shear force was expressed in units of direct force, the relationship was reversed. Fiber kinkiness or degree rigor also influenced the shear force. As the degree rigor of the fibers increased, there was a trend for the shear force, measured in force per unit area, to decrease (Table III). This relationship held true even after correcting for the effect of fiber diameter. The reason for this unexpected relationship has not been determined, and warranted further investigation.

Effect of Hot and Cold Processing - Fiber Diameter

A difference in fiber diameter between the hot and cold excised muscles for the two hour holding period was statistically significant ($p < 0.01$). Table IV and Figure 6 illustrate that this difference in fiber diameter decreased in the five hour and eight hour holding periods; the difference was statistically significant only for the two hour holding period. Figure 6 clearly shows that an increase in the post-mortem restraining period for the carcasses, held five and eight hours before muscle excision, corrected for the increase in fiber diameter apparent in the muscle held for two hours.

The reason for the increase in fiber diameter in the muscles that were processed hot and held at 24 degrees for two hours before muscle excision is closely related to the development of rigor mortis. These

TABLE III

UNIFORMITY TRIAL: EFFECT OF RIGOR ON SHEAR FORCE

Degree Rigor (1-7)	N	Shear Force $\times 10^{-2}$ gm./u^2	Standard Deviation
2	45	2.68	1.05
3	185	2.31	1.06
4	68	2.03	0.97
5	1	2.37	0.00

TABLE IV

EFFECT OF PROCESSING METHOD AND HOLDING TIME ON FIBER DIAMETER, DEGREE RIGOR, AND SHEAR FORCE

Holding Time	2 Hour			5 Hour			8 Hour		
	Fiber Diameter u	Degree Rigor %	Shear Force x 10^{-2} gm./u^2	Fiber Diameter u	Degree Rigor %	Shear Force x 10^{-2} gm./u^2	Fiber Diameter u	Degree Rigor %	Shear Force x 10^{-2} gm./u^2
Processing Method									
HOT	59.9	61	2.90	44.6	35	1.95	51.9	36	1.71
COLD	47.6	28	2.10	46.6	31	1.83	51.6	34	1.83

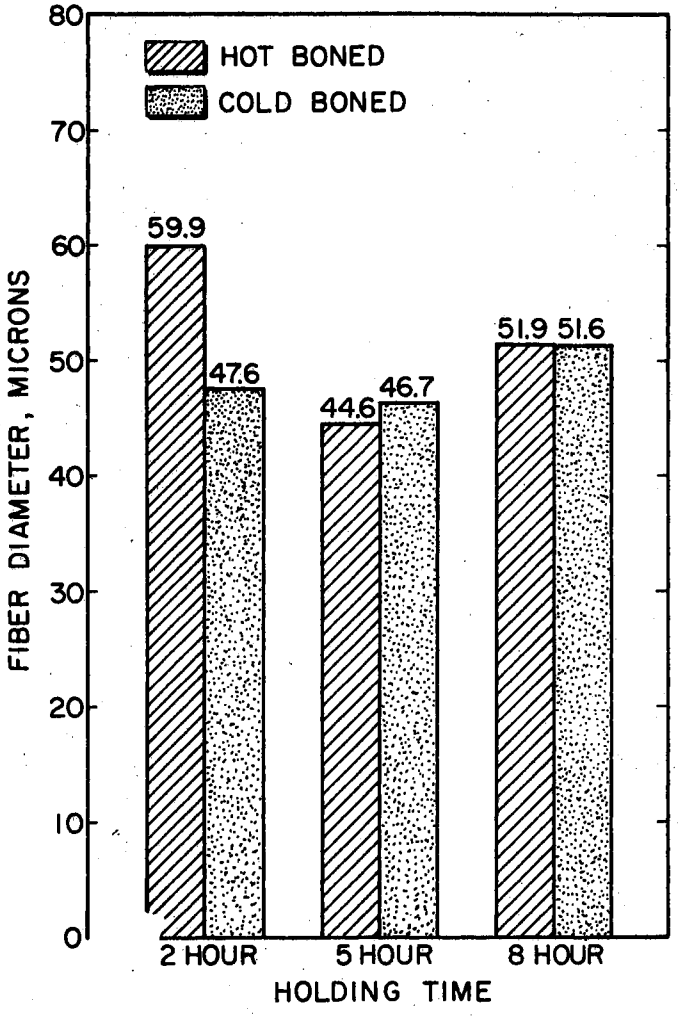

Figure 6. Effect of Holding Time and Processing Method on Fiber Diameter

muscles were removed prior to the onset of rigor, and thus were allowed to contract freely when rigor did develop. There was no tension placed on these muscles due to restrainment on the carcass during the development of rigor, and the fibers therefore were allowed to contract to the fullest extent, resulting in an increase in fiber diameter.

The results of this study indicate that a carcass restrainment of the Sartorius muscle for a five hour period post-mortem before excision is adequate to prevent increases in fiber diameter due to contraction of the fibers in rigor mortis. The eight hour holding period also accomplished the same results, but with no significant additional benefit. These results apply only to the Sartorius muscle, and only to the holding conditions outlined in the chapter on materials and methods.

Degree Rigor

The differences in degree rigor between the hot and cold processed muscles in the two hour holding period were statistically significant ($p < .01$). The differences in degree rigor between the hot and cold processed Sartorius muscles in the five and eight hour holding periods were not statistically significant. Again it is illustrated (Table IV and Figure 7) that the five hour holding period was adequate in preventing the unrestrained development of rigor mortis observed in the hot processed muscles held two hours post-mortem before muscle excision.

As was the case for the fiber diameter, the reason for the development of the severe rigor in the hot processed muscles held two hours at 24 degrees C., was simply that the muscles were removed before the onset of rigor and when rigor mortis developed, it was allowed to proceed unrestricted. In the case of the muscles held five and eight hours post-

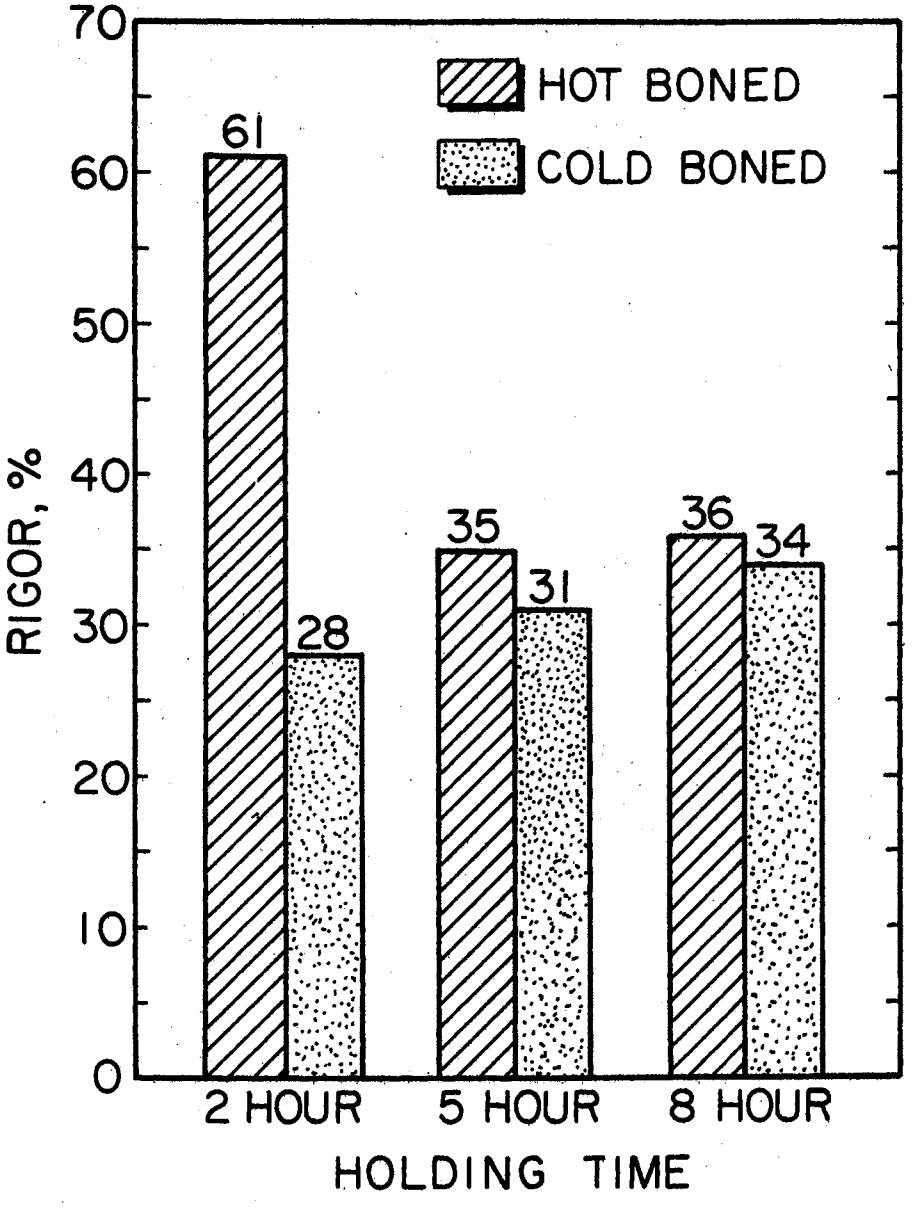

Figure 7. Effect of Holding Time and Processing Method on Percent Rigor

mortem before muscle excision, the restrainment of the muscles on the carcass during the onset and development of rigor mortis prevented the unrestricted shortening of the muscle.

Shear Force

The difference in shear force between the hot and cold processed muscles was statistically significant only in the two hour holding period ($p < .01$). In the five and eight hour holding periods, the shear force was comparable between the hot and cold processed muscles (Table IV and Figure 8). In the five hour holding period, the shear force was slightly greater for the hot processed muscles, but in the eight hour holding period, shear force was actually less for the hot processed muscles. Within the two hour holding period, there was a rather great difference in shear force for the two processing methods, the hot processed muscles requiring 2.90×10^{-2} gm./u^2 while the cold processed muscle required 2.10×10^{-2} gm./u^2 (Figure 8).

The reason for the increase in the shear force required for the hot processed muscle, held for two hours at 24 degrees C. before muscle excision, must ultimately be related back to the development of rigor. These results indicate that increased rigor increased the shear force for individual muscle fibers. These results do not support the results of the uniformity trial which demonstrated an opposite relationship. Although the fiber diameter increased with the development of rigor, the force required to shear the fibers decreased, even when force was expressed as force per unit area. This indicated that the increase in rigor exceeded the effect of fiber diameter in the determination of shear force for the muscle fibers.

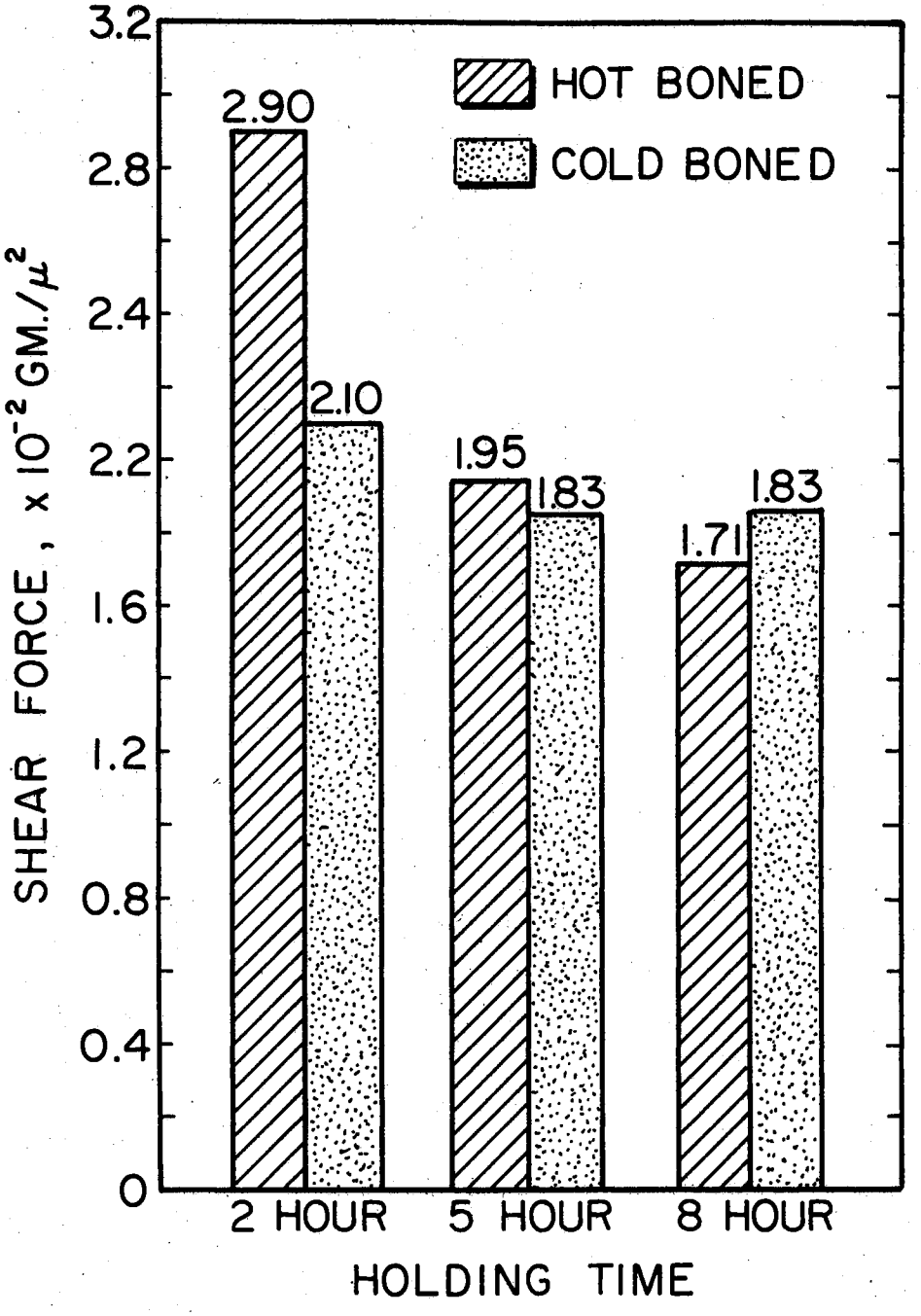

Figure 8. Effect of Holding Time and Processing Method on Shear Force

It is not clear why these data do not coincide with the results of the uniformity trial. The only explanation that the author can give is that the degree rigor does not have a toughening effect on the fibers until it reaches the severe stages. In the uniformity trial, the fibers were obtained from Sartorius muscle processed in the conventional manner, and the degree rigor assignments were all in the low to intermediate range. However, the rigor assignments made on the muscle processed hot and held at 24 degrees C. two hours post-mortem before muscle excision were in the intermediate to high range. This indicates that the effect of the degree rigor on the shear force of the fibers is greatest in the high range of rigor development, and the effect is low or minimal in the low to intermediate range.

Some Sources of Variation Associated With Shear Force of One Muscle Fiber With Possible Methods to Adjust for Each

When shearing a muscle fiber, the blade must pass through the endomysium, reticulum fibers, sarcolemma, sarcoplasm, any intracellular structures which may lie in the path of the blade, (mitochondria, nucleus, golgi apparatus, etc.), sarcoplasmic reticulum, myofibrils, and again through the opposite side of the sarcolemma, reticular fibers, and endomysium. The accumulative properties of these micro structures, with their alteration by processing, may be of significant importance to the force required to shear a single muscle fiber. The sarcoplasm, and other intracellular structures would offer little resistance to the shearing blade, therefore, in the opinion of the author, most of the resistance will be influenced by the following.

Endomysium

The endomysium is loose areolar connective tissue, composed of collagen fibers and small amounts of elastic fibers. The amount of endomysium left attached to the fiber after formalin fixation and fiber separation can cause shear force variation among fibers of the same diameter and degree of rigor-mortis. One way to determine the amount of endomysium present on the external surface is to treat the fibers in Mallory's triple connective tissue stain or some related connective tissue stain before making the diameter measurement. An estimate of the amount of endomysium remaining on the fiber could then be measured. Fibers with varying amounts of endomysium present could be sheared, thus determining the effect of endomysium on shear force, if all other factors were equal.

Possible ways of removing any endomysium present on the fiber is to (1) dissolve the endomysium with a weak acid, or enzyme (collagenase) or (2) cook (by boiling in water) small pieces of muscle before fixation, thus hydrolyzing any collagen present to gelatin. In this way, the variation due to the amount of endomysium would be reduced.

Reticular Fibers

Reticular fibers, first observed by Lorinz and Biro (1963) are thin filaments of collagen, like connective tissue which connect the endomysium and the sarcolemma. These filaments could possibly be removed with the endomysium and sarcolemma by dissolving these structures with a weak acid or enzyme.

Sarcolemma

The sarcolemma is a delicate tubular membranous sheath which encloses the entire muscle fiber. Robertson (1957) reported that the sarcolemma is a homogenous structure approximately 100 Å thick, adjacent to a surface membrane complex. He described the complex as consisting of two components: an inner zone 200-300 Å thick, and an outer more dense and granular zone 100-300 Å thick. Beyond these zones is a network of small connective tissue fibrils (identification based on their periodicity 100 Å) and even smaller fibers (periodicity 100 Å) which would be unresolved under the light microscope.

The sarcolemma does affect shear force of the muscle fiber. How much variation in shear force exists between muscle fibers of the same diameter is not known. The amount of variation due to the sarcolemma possibly could be determined by: (1) staining the sarcolemma so it may be seen under the microscope, (2) dissolving muscle substance with trypains, leaving sarcolemma intact, and (3) shearing sarcolemmas from fibers of the same diameter. This may not be feasible due to the thickness of the sarcolemma.

Sarcoplasmic Reticulum

The sarcoplasmic reticulum is a continuous system of tubules that extend throughout the sarcoplasm and form a closely meshed canalicular network around each fibril not easily seen with the light microscope. The sarcoplasmic reticulum would surely affect shear force to some degree. The amount of sarcoplasmic reticulum in a single muscle fiber would be a function of the number and size of fibrils present. The author cannot visualize a way of determining shear force variation due to

the sarcoplasmic reticulum alone, because of its size. Variation in shear force due to fibrils would also include variation due to the sarcoplasmic reticulum.

Myofibrils

Myofibrils are long thin structures approximately 2-3 micron in diameter, arranged parallel to one another, and running the entire length of the muscle fiber. They are the contractile structure of the fiber, and are composed principally of the fibrillar proteins, myosin, actin, and tropomyosin.

The greatest part of the shear force value of a muscle fiber would likely be due to the fibrils, since the muscle fiber is composed chiefly of this structure. The greater the muscle fiber diameter, the greater the number of fibrils or the greater the size of each individual fibril. Variation in shear force among muscle fibers containing the same number of fibrils, then, must be due to the degree of rigor-mortis (degree of contraction) of the fibril at the time of shearing, considering all other factors are equal. Thus, muscle fibers with the same diameter and the same degree of rigor-mortis should have the same shear force.

To have an accurate comparison among muscle fibers of different diameter, the degree of rigor-mortis of each muscle fiber must be the same. Degree of rigor-mortis can be determined by the amount of kinkiness of the individual muscle fiber scored on a 1-7 basis.

Fiber Diameter

As was pointed out earlier, diameter of the muscle fiber affects shear force due to (1) greater number of myofibrils, thus (2) greater

amounts of sarcoplasmic reticulum, and (3) a different size sarcolemma, when all other factors are the same. The muscle fiber varies in diameter along its length, so the point where the fiber is measured and where it is sheared (if different) will cause variation in shear force. This variation may be avoided by marking the point of measurement with a biological ink or dye. The point of measurement could then be placed at the cutting point by use of the dissecting microscope.

Shear Machine

One source of variation associated with shear force may be due to the hand turning of the blade mechanism. Force cannot be applied to the blade evenly and constantly when the operator pauses in turning the blade mechanism or turns it at different speeds. Some type of mechanical force, which is constant, should be used in the turning of the blade mechanism. The shearing blade should fit securely in the fiber holder opening and also the opening of the fiber holder and the shearing blade should be smaller to insure that the point measured on the fiber is the point sheared.

CHAPTER V

SUMMARY AND CONCLUSIONS

The microsensitive shear instrument was evaluated in a uniformity trial using 299 muscle fibers from conventionally processed Sartorius muscle in terms of its ability to measure shear force for individual muscle fibers. The influence of fiber diameter and degree rigor on this shear measurement was also evaluated. In addition, the ability of the shear instrument to distinguish between muscles of different degrees of tenderness by assessing shear force for individual muscle fibers was ascertained using Sartorius muscles from 18 bovine carcasses. The Sartorius muscles from 18 sides of beef were excised hot at 2, 5, and 8 hours post-mortem. Those from the opposite sides were excised after the carcass had chilled at 32 degrees F. for 48 hours. One hundred fibers from each muscle were appraised for shear force, fiber diameter, and degree rigor mortis. Thus, 1800 formalin fixed fibers from hot excised muscles were compared to 1800 fibers harvested after the muscle had chilled.

The results of the uniformity trial demonstrated that the microsensitive shear instrument was capable of measuring the shear force of individual muscle fibers. The mean force for the 299 fibers was 2.30 $\times 10^{-2}$ gm./u^2, with a standard deviation of 1.05. The coefficient of variation for the shear force of the 299 fibers was 45.8 percent, indicating that there is a large amount of variation associated with the measurement. It was interesting to note that the effect of fiber di-

ameter and degree rigor on shear force was not the effect that was expected. As fiber diameter increased, the force per unit area required to shear the fiber decreased, and there was also a trend for force per unit area to decrease as degree rigor increased. These parameters suggested that the shear force measured per unit area may not be a good estimate of meat texture.

The results of the fiber diameter, degree rigor, and shear force determination on the hot and cold excised Sartorius muscles from the two hour holding period supported the expectations of the author. Fiber diameter, degree rigor, and shear force were all significantly different ($p < 0.01$) for the muscles excised hot. However, for the five hour holding period, none of the variables were significantly different, as was the case for the eight hour holding period. These results support the general findings of Kastner (1972) who completed a study using the same three conditioning periods. He reported that the Warner-Bratzler shear value for the Longissimus dorsi muscle was significantly different for the hot processed muscles. The cold processed muscles were more tender at the two hour conditioning period than those excised hot, however, the five and eight hour holding periods at least in part reduced the tenderness differences. The results of shear force measurements of individual fibers closely follow these results.

The data reported in this study indicate that it is feasible to measure the shear force of individual muscle fibers with a reasonable degree of precision. It also confirmed the ability of the microsensitive shear instrument to distinguish between Sartorius muscles of varying states contraction through the measurement of shear force of muscle fibers.

Additional research should be initiated to improve the precision and

the reliability of the microsensitive shear instrument through improvement of instrument design and the technique in shearing muscle fibers.

LITERATURE CITED

Aberle, E. D. and R. A. Merkel. 1966. Solubility and electrophoretic behavior of some proteins of post-mortem aged bovine muscle. J. Food Sci. 31:151-156.

Bate-Smith, E. C. and J. R. Bendall. 1947. Rigor mortis and adenosine-triphosphate. J. Physiol. 106:177-185.

Bate-Smith, E. C. and J. R. Bendall. 1949. Factors determining the time course of rigor mortis. J. Physiol. 110:47-65.

Bendall, J. R. 1951. The shortening of rabbit muscles during rigor mortis: its relation to the breakdown of adenosine triphosphate and creatine phosphate and to muscular contraction. J. Physiol. 114:71.

Bendall, J. R. 1960. Post-mortem changes in muscle. The Structure and Function of Muscle III. Edited by G. H. Bourne. Academic Press, New York, pp. 227-272.

Bendall, J. R. 1966. As reported by E. J. Briskey. Proceedings of the Meat Industry Research Conference, pp. 3-4.

Briskey, E. J. 1959. Changes occurring during rigor mortis and subsequent ripening of muscle tissue. Proc. 12th Recip. Meat Conf., p. 108.

Briskey, E. J. 1967. Myofibrillar proteins of skeletal muscle. Proceedings of the Meat Industry Research Conference, pp. 3-5.

Cagle, E. D. and R. L. Henrickson. 1970. Influence of slicing warm porcine muscle on fiber diameter, kinkiness, and shear force. J. Food Sci. 35:270.

Davey, C. L. and K. V. Silbert. 1968. Studies in meat tenderness: IV Changes in the extractability of myofibrillar proteins during aging. J. Food Sci. 33:2-7.

DeFremery, D. and M. F. Pool. 1960. Biochemistry of chicken muscle as related to rigor mortis and tenderization. Food Res. 25:73.

Erdös, T. 1943. Die wirking der polyphenoloxdase in tierkoper. Stud. Inst. Med. Chem. Univ. Szeged. 3:51. As reported by R. A. Lawrie. Meat Science. 1966. Pergamon Press Ltd., New York, p. 80.

Forrest, J. C., C. G. Haugh, and C. E. Allen. 1969. The myotron: An instrument for the study of physical properties of muscle under controlled conditions. Paper No. 69-876 ASAE; Chicago, Ill.

Gillis, W. A. and R. L. Henrickson. 1968. The influence of tension on pre-rigor excised bovine muscle. J. Food Sci. 34:375-377.

Goll, D. W., D. W. Henderson, and E. A. Kline. 1964. Post-mortem changes in physical and chemical properties of bovine muscle. J. Food Sci. 29:590-595.

Henrickson, R. L., F. C. Todd, and J. J. Dunn. 1967. Mechanical properties of beef muscle fibers. Unpublished. Oklahoma State Univ. Agricultural Experiment Station, Stillwater, Oklahoma.

Herring, H. K., R. G. Cassens, and E. J. Briskey. 1965a. Sarcomere length of free and restrained bovine muscles at low temperatures as related to tenderness. J. Sci. Food Agr. 16:379.

Herring, H. K., R. G. Cassens, and E. J. Briskey. 1965b. Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. J. Food Sci. 30:1049.

Huxley, H. E. 1957. The double array of filaments in cross striated muscle. J. Biophys. and Biochem. Cytol. 3:631.

Huxley, H. E. 1963. Electron microscope studies of natural and synthetic protein filaments from striated muscle. J. Molec. Biol. 7:281-308.

Huxley, H. E., and J. Hanson. 1959. The structural basis of the contraction mechanism in striated muscle. Ann. N. Y., Acad. Sci. 81:403-408.

Huxley, H. E., and J. Hanson. 1960. The molecular basis of contraction in cross-striated muscle. Structure and Function of Muscle I. Bourne. Academic Press; New York, pp. 183-225.

Kastner, C. L. 1972. Influence of "hot" boning on bovine muscle. Ph.D. Thesis, Oklahoma State Univ., pp. 25-73.

Lawrie, R. A. 1966. Meat Science. Pergamon Press Ltd., New York, pp. 47, 80-83, 130.

Lehmann, K. B. 1907. Studies of the causes of toughness in meats. Arch. Hyg. 63:134.

Locker, R. H. 1960. Degree of muscular contraction as a factor in tenderness in beef. Food Res. 2:304-307.

Locker, R. H. and C. J. Hagyard. 1963. A cold shortening effect in beef muscle. J. Sci. Food Agr. 11:787.

Lorincz, F. and G, Biro. 1963. Acta Morph. Budapest 12:15. As reported by Lawrie. 1966. Meat Science. Pergamon Press Ltd., New York, p. 47.

Lowe, B. A. and G. F. Stewart. 1946. The cutting of breast muscle of poultry soon after killing and its effect on tenderness after subsequent storage and cooking. Adv. in Food Res. 1:232.

Lowey, S. and C. Cohen. 1962. Studies on the structure of myosin. J. Mol. Biol. 4:293.

Marsh, B. B. 1952. Observations on rigor mortis in whale muscle. Biochem. Biophys. Acta. 9:217-132.

Marsh, B. B. 1954. Rigor Mortis in beef. J. Sci. Food Agr. 5:70.

Marsh, B. B. 1960. Identification of the muscle relaxing factor. Biochem. Biophys. Res. Comm. 3:233-238.

Marsh, B. B. 1962. Fourth Meat Ind. Res. Conf. Hamilton, Meat Ind. Res. Inst. N.Z. Inc,, Pub. No. 55:32. As reported by Lawrie. 1966. Meat Science. Pergamon Press Ltd.; New York, p. 130.

Paul, P., L. J. Bratzler, E. D. Farwell, and K. Knight. 1952. Studies on tenderness of meat: I. rate and heat penetration. Food Res. 17:504.

Perry, S. V. 1965. Muscle. Edited by W. M. Paul, E. E. Daniel, C. M. Kay, and G. Moncklon. Pergamon Press, N.Y., p. 29, 582. As reported by E. J. Briskey. Proceedings of the Meat Industry Research Conference. March 1957, p. 1.

Pool, M. F. and A. A. Klose. 1969. The relation of force to sample dimensions in objective measurement of tenderness of poultry meat. J. Food Sci. 34:524.

Ramsbottom, J. M. and E. J. Strandine. 1949. Initial physical and chemical changes in beef as related to tenderness. J. Animal Sci. 8:398-409.

Reddy, S. G. 1962. The influence of pre- and post-rigor excision of some bovine muscles. M.S. Thesis, Oklahoma State Univesrity, Stillwater, Oklahoma.

Robertson, J. D. 1957. The cell membrane concept. J. Physiol. 140:58.

Sayre, R. N. and E. J. Briskey. 1963. Protein solubility as influences by physiological conditions in the muscle. J. Food Sci. 28:472-477.

Sharrah, N., M. S. Kunze, and R. M. Pangborn. 1965. Beef tenderness: comparison of sensory methods with the Warner-Bratzler and L. E. E. Kramer shear presses. Food Technol. 19:238.

Smith, M. C., Jr., M. D. Judge, and W. J. Stadelman. 1969. A "cold shortening" effect in avian muscle. J. Food Sci. 34:42.

Stanley, D. W., G. P. Pearson, and V. E. Coxworth. 1971. Evaluation of certain physical properties of meat using a universal testing machine, J. Food Sci. 36:256-260.

Szczesniak, A. S. and K. W. Torgeson. 1965. Methods of meat texture measurement viewed from the background of factors affecting tenderness. Adv. Food Res. 14:33.

Szent-Györgi, A. G. 1953. Meromyosins, the subunits of myosin. Arch. Biochem. Biophys. 42:305.

Szent-Györgi, A. G. 1958. Muscle Research. Science. 128:699-702.

T-I Ma, R., P. B. Addis, and E. Allen. 1971. Response to electrical stimulation and post-mortem changes in turkey pectoralis major muscle. J. Food Sci. 36:125-129.

Weber, A., R. Herz, and I. Reiss. 1964. The regulation of myofibrillar activity by calcium. Proc. Roy. Soc. (London) B160:489-501.

Weber, H. H. 1959. The relaxation of the contracted actomyosin system. Ann. N. Y., Acad. Sci. 81:409-421.

Weinberg, B. and R. Dyson. 1960. Changes in protein extractability during post-mortem tenderization of chicken breast muscle. Food Technol. 14:376-379.

Weiner, P. D. and A. M. Pearson. 1966. Inhibition of rigor mortis by ethyleneamine tetraacetic acid. Proc. Soc. Exptl. Biol. Med. 123:185.

Whitaker, J. R. 1959. Chemical changes with aging of meat with emphasis on the proteins. Advan. Food Res. 9:1.

A P P E N D I X

TABLE V

UNIFORMITY TRIAL: STATISTICAL ANALYSIS

Variable	N	Mean	Standard Dev.	Variance	Sum	Corrected S.S.	C.V. %
Animal	299	3.50	1.71	2.93	1050.00	875.00	48.88
Observation	299	25.50	14.46	208.95	7650.00	62475.00	56.69
Diameter	299	54.27	10.72	114.85	16280.00	34338.67	19.75
Rigor	299	3.08	0.62	0.39	922.00	114.91	20.14
Torque	299	82.65	31.08	966.59	24710.00	288042.71	37.62
Force	299	2.29	1.06	1.12	687.77	336.08	46.25

TABLE VI

TWO HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE DIAMETER

Source	DF	Sum of Squares	Mean Square	F
Animal	5	32027.75	6405.55	
Treatment (Hot or Cold)	1	45756.75	45756.75	320.19**
Treatment x Animal	5	11895.75	2379.15	
Residual	1188	169769.00	142.90	
Corrected Total	1199	259449.25	216.39	

*$p < 0.05$
**$p < 0.01$

TABLE VII

TWO HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE RIGOR

Source	DF	Sum of Squares	Mean Square	F
Animal	5	178.49	35.70	
Treatment (Hot or Cold)	1	1148.56	1148.56	583.21**
Treatment x Animal	5	9.85	1.97	
Residual	1188	834.42	0.70	
Corrected Total	1199	2171.32	1.81	

*$p < 0.05$
**$p < 0.01$

TABLE VIII

TWO HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE SHEAR FORCE

Source	DF	Sum of Squares	Mean Square	F
Animal	5	178.49	35.70	
Treatment (Hot or Cold)	1	1148.56	1148.56	583.21**
Treatment x Animal	5	9.85	1.97	
Residual	1188	834.42	0.70	
Corrected Total	1199	2171.32	1.81	

*$p < 0.05$
**$p < 0.01$

TABLE IX

FIVE HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE DIAMETER

Source	DF	Sum of Squares	Mean Square	F
Animal	5	5596.42	1119.28	
Treatment (Hot or Cold)	1	138.75	138.75	1.26
Treatment x Animal	5	3137.75	627.55	
Residual	1188	130985.00	110.26	
Corrected Total	1199	139857.92	116.65	

*$p < 0.05$ **$p < 0.01$

TABLE X

FIVE HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE RIGOR

Source	DF	Sum of Squares	Mean Square	F
Animal	5	115.33	23.07	
Treatment (Hot or Cold)	1	18.01	18.01	2.42
Treatment x Animal	5	37.11	7.42	
Residual	1188	332.21	0.28	
Corrected Total	1199	502.66	0.42	

*$p < 0.05$
**$p < 0.01$

TABLE XI

FIVE HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE SHEAR FORCE

Source	DF	Sum of Squares	Mean Square	F
Animal	5	79.00	15.80	
Treatment (Hot or Cold)	1	4.45	4.45	0.19
Treatment x Animal	5	119.47	23.89	
Residual	1188	962.89	0.81	
Corrected Total	1199	1165.80	0.97	

*$p < 0.05$
**$p < 0.01$

TABLE XII

EIGHT HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE DIAMETER

Source	DF	Sum of Squares	Mean Square	F
Animal	5	12385.42	2477.08	
Treatment (Hot or Cold)	1	18.75	18.75	0.14
Treatment x Animal	5	7588.75	1517.75	
Residual	1188	159667.00	134.40	
Corrected Total	1199	179659.92	149.84	

*$p < 0.05$
**$p < 0.01$

TABLE XIII

EIGHT HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE RIGOR

Source	DF	Sum of Squares	Mean Square	F
Animal	5	63.72	12.75	
Treatment (Hot or Cold)	1	4.94	4.94	1.08
Treatment x Animal	5	22.82	4.57	
Residual	1188	598.61	0.50	
Corrected Total	1199	690.10	0.58	

*$p < 0.05$ **$p < 0.01$

TABLE XIV

EIGHT HOUR HOLDING PERIOD: ANALYSIS OF VARIANCE FOR VARIABLE SHEAR FORCE

Source	DF	Sum of Squares	Mean Square	F
Animal	5	76.84	15.37	
Treatment (Hot or Cold)	1	4.08	4.08	0.49
Treatment x Animal	5	42.08	8.42	
Residual	1188	741.42	0.62	
Corrected Total	1199	864.41	0.72	

$^{*}p < 0.05$

$^{**}p < 0.01$

VITA

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Master of Science

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