POST MORTEM CHANGES IN PORCINE MUSCLE HELD AT 25°C

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CHAPTER I

INTRODUCTION

Commercial interest in high temperature processed pork has led to the need for more information concerned with the effect of this process on the various quality aspects of pork held at room temperature. Such information is needed to determine the handling methods for a commercial operation in the event of a line breakdown or similar disorder which would cause the hot pork to remain for extended periods of time without refrigeration.

Variation in the rate and severity of post mortem change has been reported to have an effect on the subsequent quality of pork muscle. It has been generally considered that the onset of rigor at high temperatures is associated with soft, pale, and watery pork that is less tender than normal tissue. The physical state of the muscle fiber with respect to contraction has also been reported to have an effect on the tenderness. Pre-rigor excised muscle has been found to be less tender than post-rigor excised muscle. Larger fiber diameters have been associated with less tender samples and a higher degree of rigor.

This study was designed to examine the effect of holding pork muscle at room temperatures for different lengths of time post mortem as measured by shear force, fiber diameter, percent kinkiness, press fluid, and pH. The longissimus dorsi muscle was chosen because it was sufficient in size to allow eight chops of equal size to be obtained.

CHAPTER II

REVIEW OF LITERATURE

Meat may be defined as the flesh of animals used for food and is generally considered to be the striated muscles of these animals. The study of muscle is a highly complex subject that deals with histology, biochemistry, physiology, and many other related factors. The material contained in this review will pertain predominately to skeletal muscle and will generally be confined to the following: (1) Physical characteristics of muscle, (2) Chemical characteristics of muscle, (3) Influence of temperature on porcine muscle, (4) Influence of excision, cutting, and tension on selected properties.

Physical Characteristics of Muscle

Gross Muscle Structure

There are three types of muscular tissue in the body: (1) Striated or skeletal, (2) non-striated or involuntary (smooth), and (3) cardiac. Since striated muscle is the tissue that is usually considered as meat, it will be the only type discussed in this review. Striated muscles are for the most part connected to the skeleton and are often called skeletal muscles (Sisson and Grossman 1938). Skeletal muscles cover the bone structure and their size and shape are responsible for the contour of the animal's body.

Muscle tissue is usually red in color but the shade varies with age, specie, muscle, and physiological conditions. The size and shape of a muscle is determined by inheritance, but is affected by the use the muscle receives and the function for which it is used. Muscles come in many shapes but can usually be classified as triangular, fan-shaped, fusiform, etc. (Sisson and Grossman 1938). Muscle fibers may be arranged in different manners to give various properties to the muscle. A paralleled arrangement of fibers gives the greatest distance for shortening but is a relative weak arrangement, while the pennate (feather like) arrangement increases the power of a muscle but at the expense of contraction distance (Frandson 1965).

Muscles are usually attached to bones by a tendon which is dense white fibrous connective tissue. If the fibrous tissue of the muscle attachment cannot be seen with the unaided eye it is called a "fleshy attachment". Actually in the fleshy attachment, muscle fibers attach to very short tendons and the tendons attach to the bone (Frandson 1965). The least movable attachment of the muscle is usually referred to as the orgin and the most movable end as the insertion. In extermities the orgin is usually proximal and the insertion distal. Most muscles are attached to two different bones and contraction of the muscle moves one or both of the bones. Usually in limbs, a muscle is inserted just beyond the joint it moves, thereby gaining speed at the loss of power (Lockhart 1959).

Muscles have been named for a variety of features such as attachment, action, direction, situation, structure, size, and shape. They may also be grouped according to function. A flexor is located on the side of the limb that decreases in angle, while an extensor is located on the opposite side. Muscles which tend to pull a limb toward a median plane are called adductors. In any case, muscular action is

the result of contraction. It has been found that the muscle fiber in a fully stretched state can contract to 57 percent of its length (Haines 1934). Some muscles may be unable to relax sufficiently to allow full range of movement (Lockhart 1959). Muscles may be classed as agonists (directly responsible for producing the desired action), antagonists (oppose the desired action), or synergists (oppose any undesired action of agonist) depending on the specific action being considered.

The blood supply to the muscle is quite extensive and the blood vessels are usually found in the connective tissue septa of the muscle. The nerve supplying a muscle contains both motor and senory fibers with about half of them being sensory. A single motor nerve fiber controls the action of a group or bundle of muscle fibers and sends an attachment to each fiber of the group (Lockhart 1960). Each muscle is a company of neuro-motor units, with each unit composing a nerve cell. The more powerful the action required, the more units that react at one time. The smaller the part of the body to be moved, the more rapid its action, and the smaller the unit (Lockhart 1960). All efferent nerves stimulate the muscle to contract; there are no efferent nerves to cause relaxation.

The accessory structures associated with muscles are the synovial membranes and the fasciae (Sission and Grossman 1938). Synovial structures of the body include the bursae, synovial membranes, and joint capsule. The inner layer consists of a connective tissue membrane which produces synovial fluid (Frandson 1965). The fasciae are sheets of white fibrous connective tissue. There is a deep fasciae as well as a superfical layer which can usually be easily identified. The deep fasciae may be slightly adherent to the underlying structures, but in many places it is attached to the skeleton, ligaments, or tendons.

Microanatomy and Physiology of Muscle

Muscle cells are highly specialized for the function of contraction (Frandson 1965). Connective tissue is closely associated with all muscle cells to form a sort of harness, so the pull exerted by muscle contraction can be usefully applied. The connective tissue between cells serves as a path for blood vessels and nerves. Muscle is separated into different levels of organization by the connective tissue septa. Surrounding the muscle is a heavy band of connective tissue called the epimysium. From the inner surface of the epimysium, a septa of connective tissue divides the muscle into bundles or fascicula. The size of the muscle bundle determines the texture of the muscle. Thus, in muscles capable of very fine movement, the texture is fine (Walls 1960). The connective tissue surrounding a bundle of muscle fibers is called perimysium. The perimysium further sends off a very fine sheath, composed mainly of collagenous fibers that surrounds each muscle fiber. This structure is called the endomysium.

The essential structural unit of all muscle is the fiber (Lawrie 1966). The muscle fiber is an elongated, cylindrical, multinucleated cell. The fibers range from 10-100 microns and higher in diameter and vary in length with some reaching as much as 34 cm (Walls 1960). It is generally considered that in short muscles the fibers may run the entire length of the muscle while some believe that this is not the case in longer muscles. Fiber diameter will be discussed in another section in this report. In general, the larger fibers tend to be longer and are usually found in larger muscles.

Surrounding each fiber, and underneath the connective tissue of the endomysium,

is a sheath, the sarcolemma, which was once thought to be structureless but now has been shown with the aid of the electron microscope to represent a double membrane of which the components are about 50-60Å apart (Robertson 1957). Other studies by Bennett (1960) and Wang (1956) have noted that the cell boundary can be identified as, (1) the plasmalemma, the dark line limiting the cell, (2) a fuzzy shell of intermediate density closely paralleling the cell membrane, and (3) a less well defined amorphous matrix contained connective tissue fibrils. There may also be a spiral collagenous structure between the endomysium and the sarcolemma (Lorincz and Biro 1963). The sarcolemma is a major factor in the elasticity of muscle and acts as the connective between muscle fiber and the tendinous part of the muscle or tendon (Frandson 1965).

The cytoplasm of the muscle cell is called sarcoplasm and is essentially an undifferentiated mass of protoplasm. The function of the sarcoplasm is thought to be myofibril nutrition and conduction of nerve impulses. Certain formed structures are found in the sarcoplasm, the mitochondrin, the sarcoplasmic lipid bodies, and the sarcoplasmic reticulum or tubular system. A Golgi apparatus also can usually be found in the sarcoplasm. The sarcoplasmic reticulum is entirely a sarcoplasmic component not being seen within the fibrils. It has connections with the Z bands, less regularly with the M bands and is also found in close association with the mitochondria (Walls 1960).

When viewing striated muscle the cross striations appear to be a disc through the entire fiber. Only with the aid of the electron microscope was the true structure of the muscle fiber resolved. The muscle fiber is actually composed of many long threadlike structures, which measure about one micron or less in thickness and

it is on these structures, the myofibrils, that the striations actually reside. The cross striations of the entire fiber are due to the fact that the myofibrils lie in register. The bands or striations are usually given letters instead of names. The dark area is called the A band and the light area the I band. The dark line or zone in the middle of the I band is called the Z disc. The distance from one Z disc to another Z disc is called a sarcomere, which is the structural and functional unit of the myofibril. The myofibrils are responsible for contraction of the fiber and the sarcomeres are the contracting units of the myofibril. The lighter region within the A band is known as the H zone. A darker line in the H zone is called the M line.

Investigations utilizing the electron microscope revealed that the myofibrils are composed of tiny (100 - 200 Å) elongated subunits called myofilaments. These myofilaments are made up primarily of actin and myosin (some tropomyosin) which are the contractile proteins of the myofibril. The thick filaments are the protein myosin and lie in the dark or A band. The thinner filaments are actin and reach from one Z line to the edge of the H zone (light area in center of A band). The actin rods are continuous through the Z line, although twisted and joined in a complex manner to their neighbors in the next sarcomere, thus creating the illusion of a line or disc (Bendall et al. 1963). Each myosin filament is surrounded by six actin filaments in a hexagonal arrangement. The myosin filament has six rows of projections, so called feet, and each row lies directly opposite one of the six actin filaments. Recent investigations have demonstrated that actin filaments consist of two helically wound strands composed of subunits which appear to be alike and are approximately spherical (Hanson and Lowy 1963). The subunits are probably monomeric units of G-actin.

Contractile Mechanism

The contractile elements of the striated muscle are the sarcomeres of the myofibrils. As previously mentioned, the myofibrils are composed of myofilaments of actin and myosin, the arrangement of which gives the fibril its striated appearance. Contraction, according to Huxley and Hanson (1960) is caused by the filaments of actin sliding by the filaments of myosin to causing the sarcomere to shorten. The contact points between actin and myosin are the projections of the myosin filaments that lie opposite each filament. The extent of sliding in contraction is much greater than the lateral projections of the myosin feet, so these cannot remain attached to the same points on the actin filaments for more than a very small fraction of the time required for contraction. Thus, it appears that a repetitive process goes on at each actin-myosin cross-linkage site, the link being connected for part of the cycle and disconnected for the rest (Huxley and Hanson 1960).

It has been found that the A band (myosin filaments) remains constant in length when the fiber shortens, while the I band (actin filaments) shortens. When the rabbit psoas fibril shortened to about 65% of resting length, the I bands completely disappeared, and each Z line touched the end of two adjacent A bands (Huxley and Hason 1960). The H zone (light area in center of A band where actin filaments stop) widens when the fibril is stretched and closes as it contracts. Below resting length, the I substance shows no change in length until the H zone has disappeared, and then it shortens, but a dense line appears in the middle of the sarcomere as though the actin filaments were crumpling on their ends. This line is visible in intact fibrils and is probably the M line described by some previous workers (Huxley

and Hanson 1960).

Energy for muscle contraction comes directly from the splitting of ATP to ADP, and the opposite process, relaxation, occurs when this splitting is inhibited. In the absence of the Mg-complex of ATP, the actin rods attach themselves to the myosin filaments at points of overlap. This is the so-called "actomyosin" state. Thus, it is evident that a muscle may remain active for as long as the ATP is available. In the muscle system , ATP is resynthesized from ADP and glycogen.

The actomyosin system in vitro is an extremely active ATP-ase in the presence of Mg ions, and it is obvious, therefore, that the main problem of muscular contraction is to explain how this system is kept under control and prevented from splitting ATP and developing tension in the relaxed or resting muscle (Bendall et al. 1963). Marsh (1951, 1954) discovered that muscle contains a relaxing factor. He found that this factor completely inhibited the ATP-ase activity thus contraction when in the presence of Mg ions and ATP. Because the addition of Ca ions would reverse the process, it was believed that the relaxing factor acted by removing Ca ions from the system. The factor was hypothesized to be organized on the sarcoplasmic reticulum and is thought to act as a pump for removal of the Ca ion. Contraction is then caused by a wave of depolarization that floods across the membrane allowing Ca ions to enter and contraction to take place. This process is reversed as the fiber is repolarized and the reticulum recaptures the Ca ions, ATP floods back into the system and the conditions for relaxation are once more established.

Muscle Fiber Diameter

Joubert (1956) discovered that when using absolute measures no relationship ex-

isted between body weight and fiber diameter of different species of animals. The size of the fibers of different species at birth were not in the same order as when ranked at maturity. Joubert (1956) demonstrated that, in cattle, one breed (Friesian) had thicker muscle fibers than another breed (Dairy Shorthorn). It is a generally known fact that fiber diameter increases with age. Joubert (1956) described the growth of muscle fibers as a curvilinear relationship much the same as the increase in body weight. These findings were in agreement with those of Hiner et al. (1953), Tuma et al. (1962), Carpenter et al. (1963) all of whom found that maximum fiber diameter increased with age. Herring et al. (1965) revealed that muscles from E maturity carcass groups has larger fiber diameters than A maturity groups.

It had been generally accepted that muscles from the male had larger fiber diameters than muscles from the female, but Joubert (1956) postulated that these differences were due to body weight since males are usually larger than females of the same age. He reported that in newborn lambs, the females had slightly larger fibers than the males when weight was held constant. He then postulated that males might have more fibers than females of the same weight.

The level of nutrient intake was shown by Joubert (1956) to influence fiber size. Also, Yeates (1964) stated that starvation of the adult animal resulted in a skrinkage of the muscle fiber size. He discovered that with the return to an adequate level of nutrition, that the muscle fiber recovered its original dimension. Joubert (1956) found that, in ewes on a super-maintenance diet, muscle fiber size increased, but those on a submaintenance diet showed a decrease in fiber diameter in porportion to total muscle decrease.

Hiner et al. (1953) reported the greatest change in fiber diameter for all mus-

cles was between 1 and 14 months of age. They also revealed that the muscle of the neck and foreshank had the largest fiber diameters followed by those of the round, third was the chuck muscles and the longissimus dorsi muscle and fourth the tenderloin (psoas major) muscle which had the smallest fiber diameters of all the muscles studied. They also studied the relationship of fiber diameter to tenderness and found a definite curvilinear relationship in that, up to a point, an increase in fiber diameter brought about a corresponding increase in mechanical shear force. Carpenter et al. (1963) related that with an increase in maximum fiber diameter there was a decrease in desirability as judged by a taste panel. They also stated that maximum muscle fiber diameter increased with age and this increase was positively associated with the thickness of the connective tissue and muscle bundle size. Studies by Herring et al. (1965) revealed that there was a high correlation (r^2 = .82-.87) between shear force and fiber diameter with the larger fiber diameters being less tender. They further described the fiber diameter-tenderness relationship as being dependent upon either the length of the sarcomere of the amount of shortening that had occurred. This is in agreement with the findings of Gillis and Henrickson (1968) who stated that there was a decrease in fiber diameter with an increase in tension.

Rigor Mortis and Fiber Condition

Muscular contraction after death is referred to as rigor mortis. Rigor mortis may be defined as the physical and chemical changes that take place in a muscle after death. The main observed physical change is from a highly extensive elastic condition of freshly killed animals to the inextensible and rigid conditions of a muscle in full rigor. This is a direct result of the ATP being used up, without being resyn-

thesized at a rate sufficient to meet the needs of contraction, causing the actin to become bound to the myosin in an actomyosin complex. This actomyosin complex remains locked in the contracted state until rigor resolves. Along with this change in extensibility, there occurs a shortening of the individual sarcomere as rigor approaches. Another highly discussed physical phenomenon is the appearance of rigor kinks found in localized areas along some fibers.

Tension on the muscle has been shown to have an effect upon the condition of the fibers. Harrison et al. (1967) in a study of histological changes in beef during aging found that beyond two days, there was a tendency for the fibers to straighten out and have less twist and kinks. Locker (1960) concluded that the final degree of rigor in ox muscle was dependent upon the strain imposed on it in the hanging position and might be modified by cutting or excising the muscle. Herring et al. (1965) revealed that carcass position had a definite effect upon fiber diameter and sarcomere length. They found that contracted muscles had larger fiber diameters than stretched ones or those not allowed to shorten. These differences in diameter were usually associated with changes in sarcomere length. When muscles shortened, there was an increase in diameter and a corresponding decrease in sarcomere length.

Reddy and Henrickson (1967) concluded that the amount of fiber distortion was greater ($P<.01$) for pre-rigor excised longissimus dorsi muscle than for post-rigor excised muscle. However, the opposite was true for the gluteus medius muscle, thus supporting the postulation of Locker (1960) and the results of Herring et al. (1965). They also found fiber diameters were greater for pre-rigor excised muscle than for post-rigor excised which was apparently due to the shortening of the pre-rigor excised fiber. Work by Gillis and Henrickson (1968) explored the effect of induced

tension in fiber condition. They reported a decrease in fiber diameter with an increase in tension up to 1,000 grams, then a leveling off. They proposed that the muscle fiber has reached a point of physical limitation with respect to stretching at approximately 1,000 gram pull. These workers also revealed an increase in sarcomere length with an increase in tension placed on the muscle. They studied fiber distortion, termed percentage rigor, and discovered a decrease in percentage rigor with an increase in tension up to the 1,000 gram pull.

Chemical Characteristics of Porcine Muscle

pH

One of the most common indicators used to measure rigor mortis is the decline of pH. This is brought about by the anareobic glycolysis which converts glycogen to lactic acid. May et al. (1962) stated that the fall of pH was an easy measure of glycolysis, hence a measure of the time of the onset of rigor mortis. McCarthy and Mackintosh (1953) reported a pH value of 7.4 for muscle tissue of the live animal with a decrease after death due to the production of lactic acid. Lawrie et al. (1958) reported that the ultimate pH was influenced by the muscle, species, and the physiological condition of the animal. They found that porcine muscle that had not had the glycogen content depleted by ante-mortem stress, had an ultimate pH of about 5.5. They proposed that stress before slaughter lowered the glycogen content and gave an ultimate pH above 5.5. Bate -Smith and Bendall (1949) stated that the initial pH was dependent upon the severity of the death struggle whereas the ultimate pH is dependent upon the level of feeding and degree of fatigue at time of death.

They found that the ultimate pH was associated with the length of the delay phase of rigor. Hallund and Bendall (1965) reported that muscles of Landrace pigs could be divided into two groups on the basis of pH decline; one group had a slow rate and one group had a rapid rate. They also discovered that the initial pH as affected by the death struggle was an important factor in determining the rate of decline. Lewis et al. (1962) reported that stress lowered the lactic acid content in quadriceps femoris, psoas major, and longissimus dorsi muscles. Sayre et al. (1963b) stated that short term excitement and exercise immediately prior to slaughter caused a rapid post mortem glycolysis as indicated by a rapid pH decline.

Glycogen

The animal body stores glucose in the form of glycogen. The various tissues show depletion of glycogen on demand, i.e. muscle glycogen is lowered during work. When an animal is slaughtered, the oxygen supply is rapidly depleted with the result that conditions become anareobic; under these anareobic conditions, glycogen is converted to lactic acid. Sayre et al. (1963b) concluded that long and short term sucrose feeding elevated the initial level of glycogen and that this resulted in muscle that was slightly inferior to normal muscle which had a lower glycogen content. Fasting prior to slaughter lowered the initial glycogen content and reduced the rate of pH decline. Kastenschmidt et al. (1965) concluded that a warm air to cold air treatment reduced the glycogen content and reduced the rate of pH decline.

ATP

ATP is necessary in the living muscle for contraction. When the oxygen supply to the muscle is stopped, ATP is resynthesized from glycogen at the rate of three moles of ATP for each mole of glucose. ATP can be formed from ADP and creatine phosphate also. At this point, splitting of ATP and resynthesis of ATP are exactly equal. Beyond this stage, splitting overtakes resynthesis and the ATP level begins to fall causing the muscle to lose extensibility. These resnythetic mechanisms can maintain ATP at a level sufficient to give the muscle extensibility until the glycogen and creatine phosphate is depleted.

Influence of Temperature Post Mortem

The rate of post mortem change is affected by many factors and shows great variation even among the different muscles of the same animal. One of the most important factors affecting the rate of change has been reported to be the temperature. DeFremery and Pool (1960) found the time required for a 50% reduction of ATP was 50% less at 43°C as compared with 40°C. Briskey et al. (1962) discovered a 57% decrease in the total time required for rigor at 43°C as compared to 37°C.

The rate of post mortem change (especially decline of pH), the temperatures at which these changes occur, and the effects of this quality of porcine muscle have been the topics of much discussion in recent years. It has generally been accepted that the onset of rigor mortis at a low temperature and a relatively high pH results in muscle of acceptable quality, while the converse is also a widely publicized fact; the onset of rigor at high temperatures and low pH values results in muscle that is pale, soft, and exudative, the well known PSE condition. Bate-Smith and Bendall (1949) reported that the effect of temperature was a decrease in the delay

phase as the temperature was raised. Briskey et al. (1962) found that PSE resulted from both the rapid acid rigor (10 minute delay phase) and the moderately slow acid rigor. They stated that normal pork resulted when the pH remained high for 45 minutes post mortem. Lawrie (1960) discovered exudative muscles at even the highest ultimate pH values. He also related that the faster the pH drop, the more exudative the muscle. Sayre et al. (1963a) in a study in which they attempted to alter post mortem changes by diet modification and preslaughter heat treatment, found that the ultimate color texture scores were the lowest for the animals that received a heat treatment prior to slaughter. They stated that higher temperatures post mortem resulted in increased glycolysis and a rapid decline of pH with the condition of the muscle being inferior to those held at lower temperatures. Cassens et al. (1963) reported that muscle which went into rigor rapidly at a low pH and a high temperature ultimately appeared PSE. Wismer-Pedersen and Briskey (1961) concluded that when the pH dropped to about 5.4 while the temperature was about 25°C, the muscle became unacceptable from a quality standpoint. They found that this condition did not occur when the tissue was chilled rapidly. They postulated that temperatures of approximately 40°C were not critical, if the pH remained above 7.2 but that temperature did become critical at about 35°C, if the pH was allowed to go below 5.9. Also, they said that pH values below 5.6 and temperatures above 30°C became critical to the quality of the muscle. They also concluded that alteration of the chilling rate (lowering temperatures) at the onset could significantly reduce the adverse effects of these conditions.

Bendall et al. (1963) divided Landrace pigs used in their study into two groups based upon rate of post mortem change (a fast group and a slow group). They re-

ported that the group that had the slow rate of post mortem change had muscle of excellent quality while the group that was characterized by a fast decline of pH and loss of extensibility resulted in PSE muscle. They also put forth the theory that this could be prevented if the fast group of carcasses were cooled rapidly enough to allow muscle to go into rigor below 30°C. They stated that all meat allowed to enter rigor at 37°C, or above, resulted in soft, watery pork. Cassens and Newbold (1967) studied the effect of five different temperatures on the time course of rigor mortis. Duration of the delay phase increased as the temperature was decreased from 37°C to 15°C, but this phase decreased as the temperature was decreased from 15°C to 1°C. In this work it was found that rigor commenced sooner at 1°C than at 37°C. Total time for rigor at 1°C was shorter than at either 25°C or 37°C. Bodwell et al. (1966) reported, however, that pork sides held at 37°C did not consistently become pale and watery as was expected from previous reports. They stated that this might suggest that high temperature and low muscle pH per se did not necessarily cause the PSE condition.

Various attempts have been made to prevent or reduce the severity of the PSE condition. Some workers have suggested that rapid chilling is the cure for the PSE condition. Kastenschmidt et al. (1964) in a study to acertain if pre-slaughter environment could prevent the PSE condition found that the most desirable muscle resulted when the animals were subjected to a warm environment and then a cold treatment. This combination lowered glycogen levels at death and resulted in muscle that was dark, firm, and dry with superior juice retention. These workers revealed that the longissimus dorsi muscle was extremely sensitive to ante-mortem environmental temperatures. Borchert and Briskey (1964) reported that partial freezing

with liquid nitrogen was effective in preventing PSE.

Shear Force as Related to Post Mortem Conditions

The rate and severity of post mortem change has been reported to have an effect on the tenderness of the muscle. It is a common belief that tenderness decreases and shear force values increase as pH declines. Sayre et al. (1964) found that shear values were higher for muscles that went into rigor at pH values below 6.0. Lewis (1959) stated that stress, which lowered glycogen and caused a higher pH, resulted in increased tenderness and juiciness of certain muscles. DeFremery and Pool (1960) concluded that every treatment that resulted in a more rapid loss of ATP, more rapid drop of pH, and a more rapid loss of glycogen increased toughness. These same workers in 1963 revealed that a rapid rate of rigor did not induce toughness in young birds. They used three methods to block glycolysis or minimize the effects of it. These workers reported that all three treatments resulted in more tender muscle. They stated that a rapid rate of rigor and a rapid decline of pH are not the cause of toughness because samples were not tough when glycolysis was eliminated. They further speculated that the rapid rate of formation of lactic acid is involved in the development of toughness. Bush et al. (1967) using beef semitendinosus muscle found that shear values increased as pH declined at 2°C which agreed with previous findings, however, he discovered that muscles held at 16°C increased in shear force to about 6 hours post mortem then decreased as pH declined. The psoas muscle decreased in shear force as pH declined as did all muscles held at 37°C. Bush stated that the decrease in shear values at 37°C might reflect a breakdown of connective tissue. Another possibility for the occurance of rapid tenderization at 37°C could

be increased proteolysis, since cathepsin is released form the particulate components of muscle more rapidly under conditions of high temperature and low pH. Bush and workers also stated that tension development of the muscle was maximal at 2°C. He postulated that this might be similar to cold shortening.

Condition of the muscle fiber has also been reported to have an effect on tenderness. Locker (1960) reported that relaxed muscles were more tender than contracted ones. Tuma et al. (1962) in a study involved with fiber diameter discovered that after correcting for age that fiber diameter was a poor indicator of muscle tenderness. Carpenter et al. (1963) stated that with an increase in maximum fiber diameter there was a decrease in taste panel tenderness scores. Marsh and Leet (1966) reported that shortening of up to 20% had relatively little effect on tenderness. Shortening from 20% to 40% had a rapid and severe effect with shear force values reaching a peak of several times the original value. These workers concluded that further shortening above 40% had a reverse effect and at about 60% shortening the samples cleaved about as easily as they did at 20% shortening. Hiner et al. (1953) reported that the rank of muscles in fiber diameter was essentially the same as it was for the same muscles ranked on tenderness. Herring et al. (1965) discovered shorter sarcomere lengths for muscles that were allowed to shorten. These shorter sarcomeres were associated with larger fiber diameters which were highly related to differences in shear force values. They stated that when muscles shortened, there was a corresponding decrease in sarcomere length, an increase in fiber diameter, and a decrease in tenderness. Reddy and Henrickson (1967) concluded that the degree of rigor (fiber distortion) was the only variable significantly affected by pre-rigor excision of bovine longissimus dorsi muscle. They stated that in the semitendi-

nosus muscle, pre-rigor excision resulted in an increase in fiber diameter, degree of rigor, and shear force. Gillis and Henrickson (1968) discovered that with an increase in degree of rigor there was a corresponding increase in shear force. A scattergram indicated that with an increase in sarcomere length there was a corresponding increase in fiber diameter and shear force.

CHAPTER III

MATERIALS AND METHODS

Eight Hampshire gilts ranging in weight from 88 kg to 102 kg were obtained from the Oklahoma Agricultural Experiment Station swine herd for use in this study. The animals were of similar breeding, feeding, and management. All animals were delivered to the meat laboratory approximately 24 hours before slaughter. Feed and water were withheld for 12 hours before they were sacrificed. Immediately prior to stunning, the hogs were washed with warm water (37°C) to insure good contact of the electrical stunner. Each animal was stunned with a Cervin electrical tool (220 volt), shackled by one leg, raised from the floor, and bled in the normal manner.

Immediately upon the death of the animal an initial pH reading was taken on both the left and right longissimus dorsi muscles by making a small incision and placing the electrode of a Corning pH meter directly into the muscle. The animals were then lowered to the floor, skinned, eviserated, and rapidly split. A section of each longissimus dorsi (LD) muscle extending from the last lumbar vertebrae to the sixth thoracic vertebrae (about 18 inches of sample in most cases) was removed and tagged according to side. Each section of the LD was then sliced into eight two-inch chops and the chops were tagged according to location (Figure 1). Each chop was placed into a polyethylene bag, the air removed by vacuum, and the bag sealed to insure an anareobic condition.

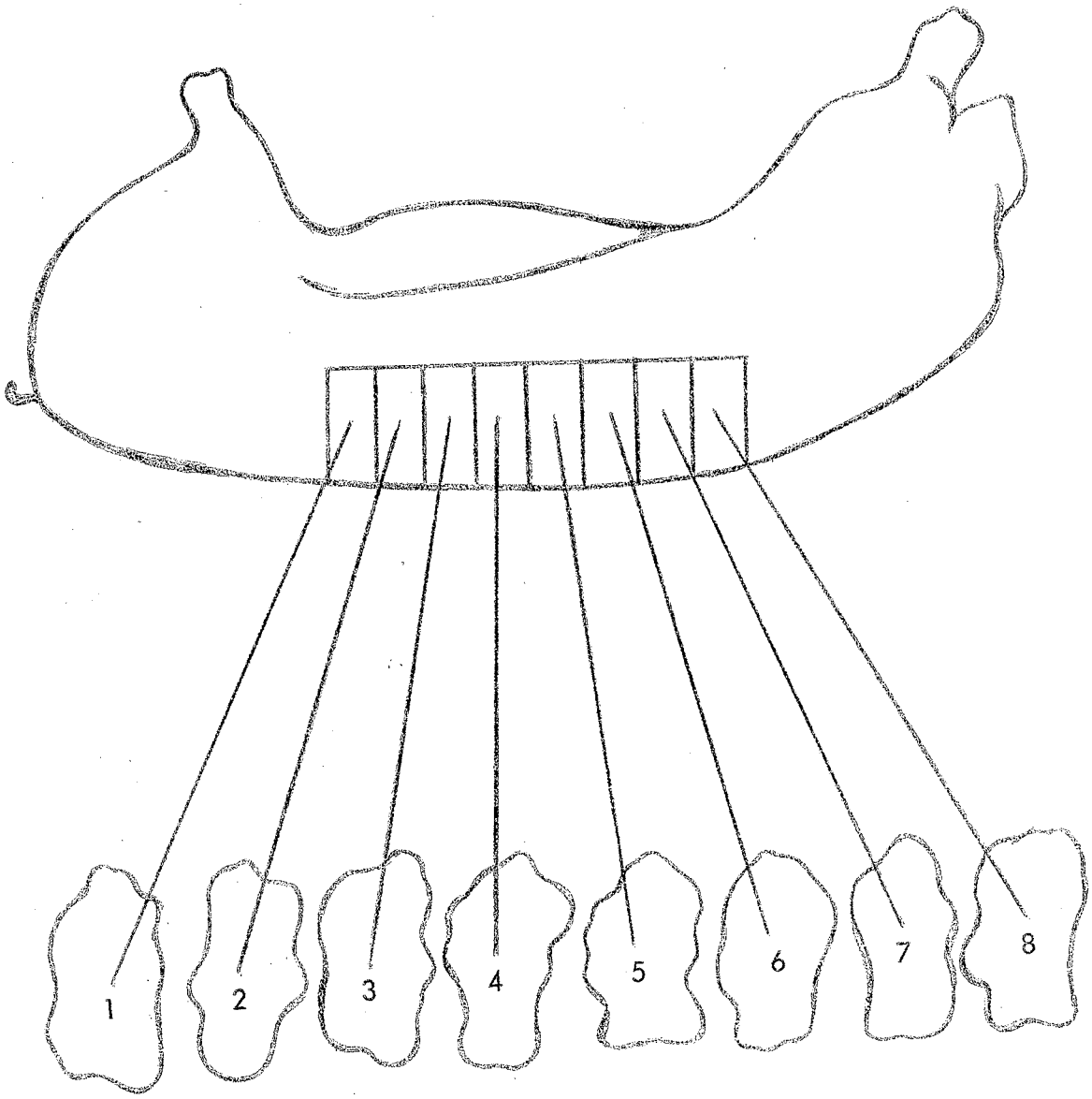


Figure 1. Location of Longissimus Dorsi Chops for Sampling.

The treatment in this experiment was the different periods of time the chops were held at room temperature (25°C) before analysis. The times were 30, 60, 120, 180, 240, 300, 360, and 480 minutes after the animal had died. At each time period the chops assigned to the treatment were removed from their respective bag, the pH recorded, and a core was removed for press fluid and histological analysis.

Methods

The change of pH was measured using a Corning pH meter. At each time interval, the chop assigned to that treatment was removed from the bag, placed on a cutting board and the pH obtained by placing the electrode directly upon the cut surface of the chop at three locations as shown in Figure 2. A one-half inch core was taken from each chop as shown in Figure 3. This core was divided for histological and press fluid analysis in the manner shown in Figure 5. The section of the sample for histological analysis was placed in a buffered 10% formalin solution and stored at 4°C for later observation.

Press Fluid

Samples for press fluid determination were taken from the one-half inch core shown in Figure 5. Each sample was cut to weigh 500 milligrams, placed on a piece of Whatman No. 1 filter paper and pressed using the Carver press for one minute at 10,000 pounds pressure. The filter paper was allowed to dry with a weight on top to keep the papers flat for ease of measurement. The total ring area was used for comparing samples instead of the mositure ring only as used in previous experiments because it was felt that moisture was contained in the meat ring. The total ring

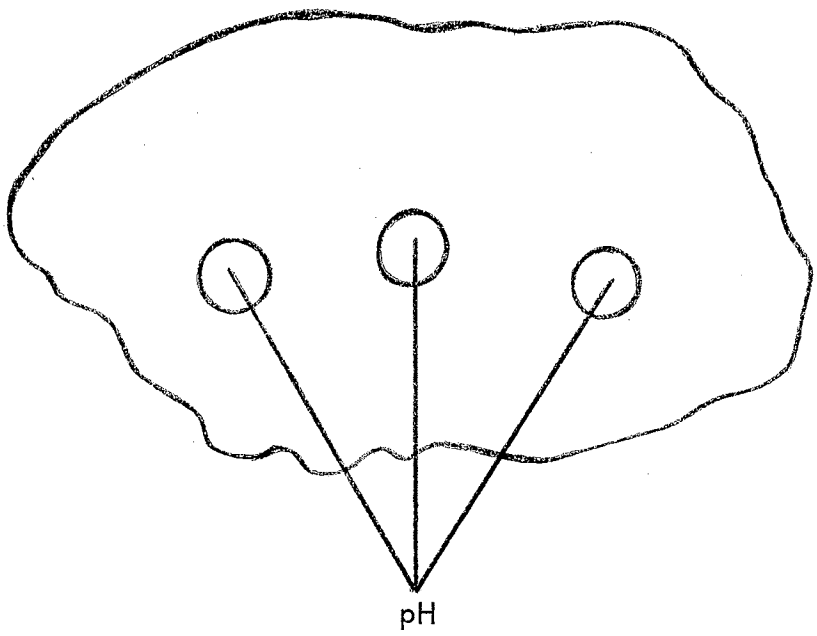


Figure 2. Location of pH Readings.

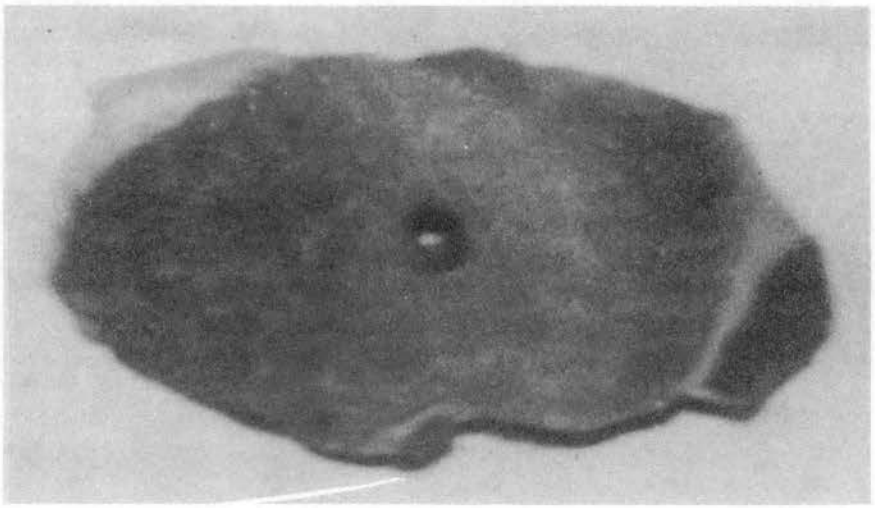


Figure 3. Location of Press Fluid and Histological Sample.

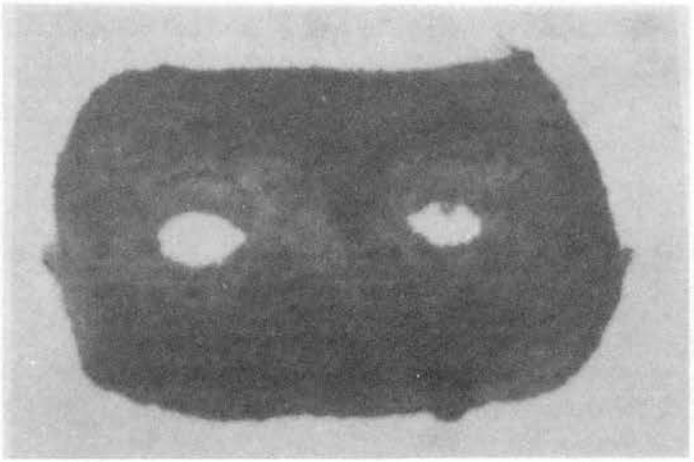


Figure 4. Location of Cores Removed for Shear Determinations.

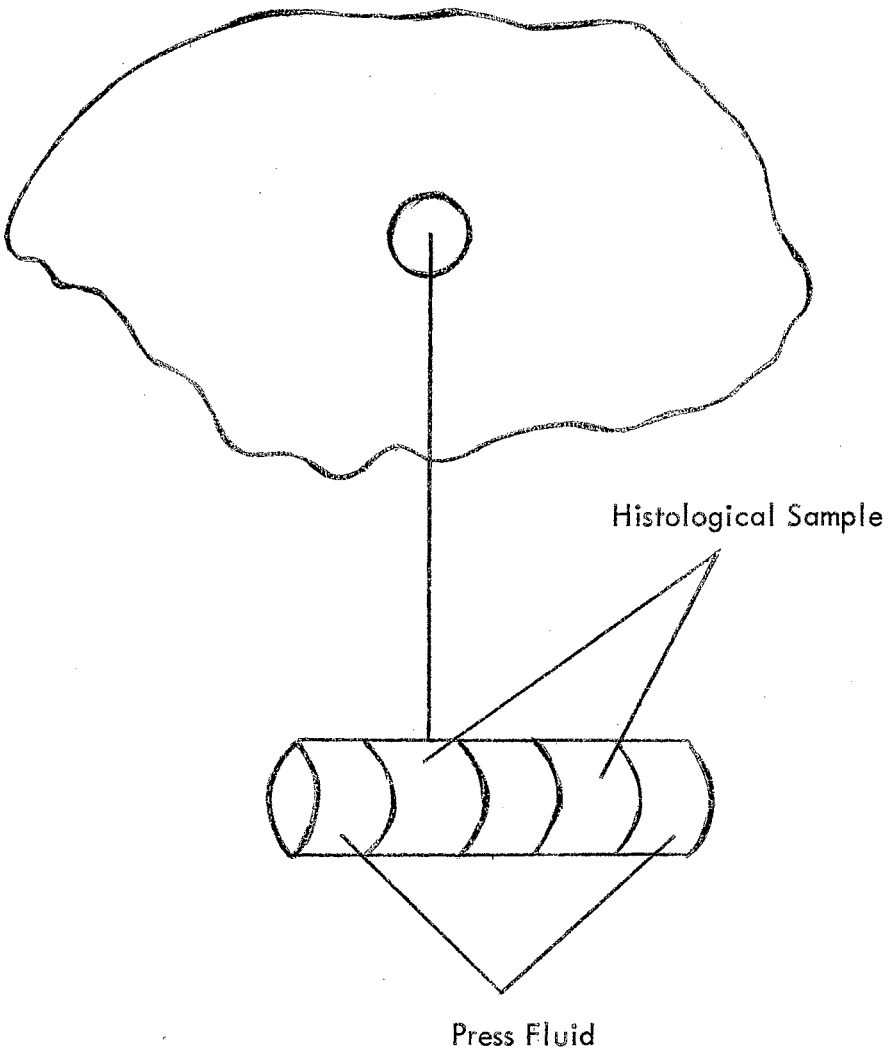


Figure 5. Utilization of Press Fluid and Histological Sample.

area was measured with a conpensating polar planimeter three times and the average of the three measurements used for the sample.

Shear Force

After removal of the core for histological and press fluid samples, the remainder of the chop was placed in a deep fat fryer with the oil preheated to 135°C until it reached an internal temperature of 70°C. The chops were then allowed to cool to an internal temperature of 4.3°C before the removal of core samples for shear force determination. These determinations were made on two three-quarter inch cores taken at locations shown in Figure 4. Each core was sheared three times using the Warner-Bratzler machine giving a total of six values for each chop. The average of the six values was used for later analysis.

Fiber Diameter

A small longitudinal section of the fixed sample was removed, placed in a fresh 10% formalin solution, and blended at a slow speed for one minute in a Waring blender in order to dislodge but not break the muscle fibers. As soon as the sample was blended, the resulting suspension was placed in a glass bottle and stored at 4°C until such time as the fibers could be measured. At the time of observation a portion of the fiber suspension was poured into a two inch diameter petri dish. The fibers were allowed to settle to the bottom, and the excess formalin was carefully decanted to prevent excess fiber movement. The petri dish was then placed on a microscope which was equipped with an ocular micrometer and a built in light source.

The diameter of 25 fibers was measured for the one petri dish, then it was emp-

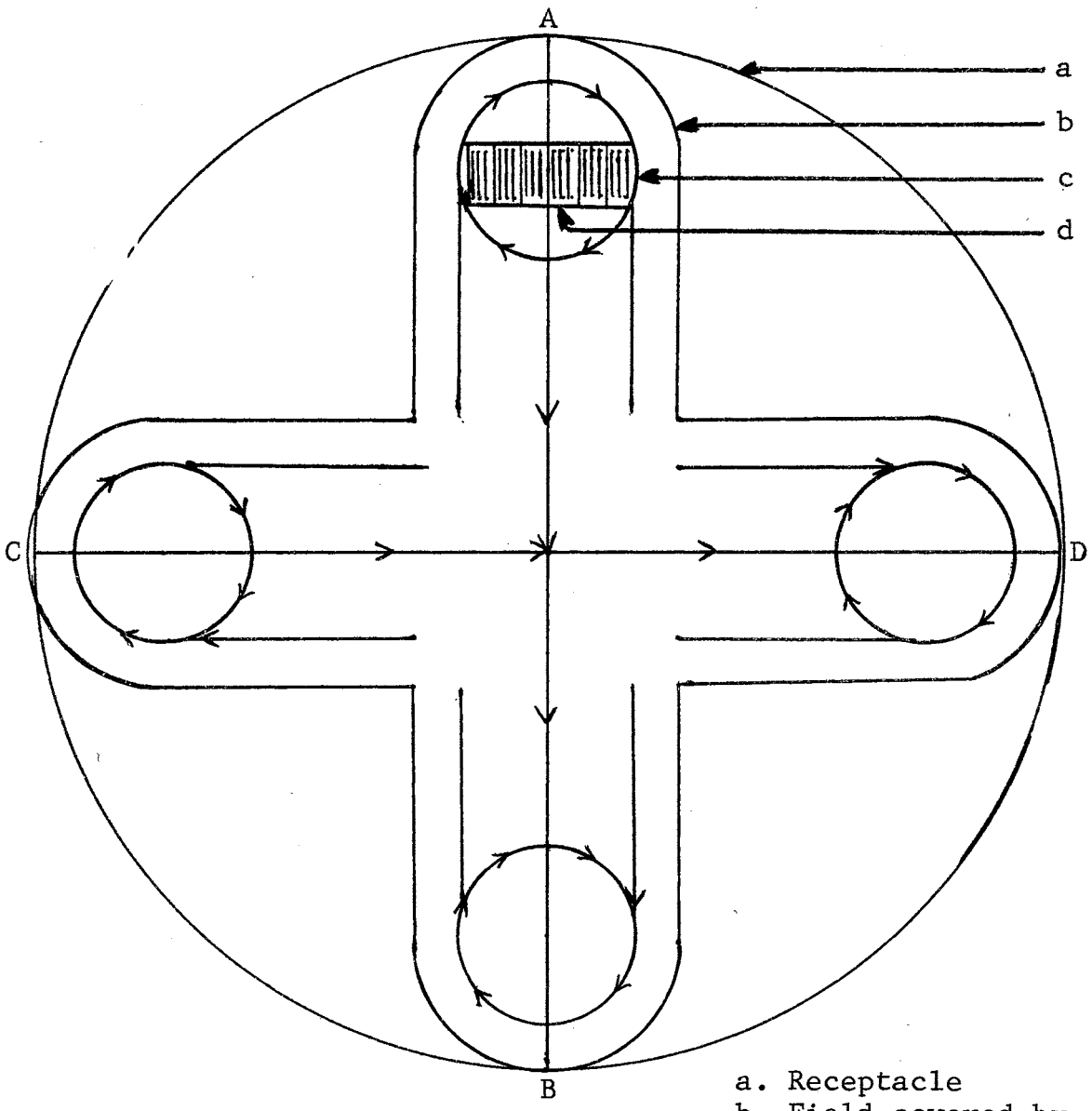
tied and the process repeated, giving a total of 50 fibers measured for each sample. The only fibers measured were those which appeared in the field of a constant course (Figure 6) and were at least the length of the field. All fibers were measured at the widest point.

Percentage Kinkiness

The amount of kinkiness was observed at the same time as fiber diameter was measured. While the fiber was under the ocular micrometer, a subjective score for kinkiness was assigned to each fiber using a scale ranging from 0-6 depending upon the condition of the fiber. A weighted score for the 50 fibers was then calculated and converted to a percentage kinkiness as illustrated in Figure 7. Photographs of the seven kinkiness classifications into which the fibers were assigned are shown in Figure 8.

Statistical Procedure

The latin square method of statistical analysis was used because earlier studies substantiated non-random distribution on many of the variables associated with the porcine LD muscle. Harrison et al. (1967) found significant differences in taste panel scores for anterior, center, and posterior sections of the pork loin. Mackey and Oliver (1954) found non-linear variations due to position along the pork loin. They stated that if no trend existed, the variation should be considered random. However, they found that a linear trend did exist in some loins. They postulated that compensation for this variation could usually be accomplished with the proper statistical design.



a. Receptacle
b. Field covered by objective lense
c. Field covered by the radius of the ocular micrometer
d. Ocular micrometer

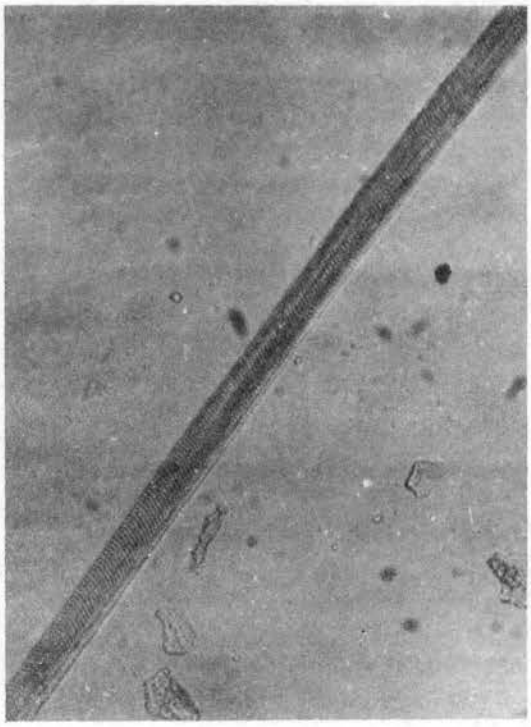
Figure 6. Pattern Used to Choose Field for Fiber Diameter Measurement (Compliments, S. G. Reddy).

No. of Fibers	Condition	Score	Sub Total
5	Straight	0	0
8	Straight +	1	8
11	Wavy	2	22
8	Wavy +	3	24
8	Twisted	4	32
7	Twisted +	5	35
3	Kinky	6	18
Total 50			139

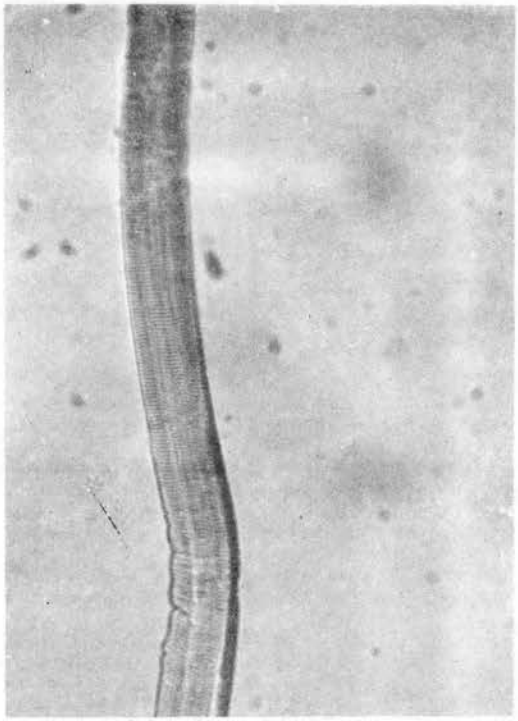
Percent kinkiness $\frac{139}{300^a}$ 46%

[a]Highest possible value 50 fibers could receive if all were scored kinky (6).

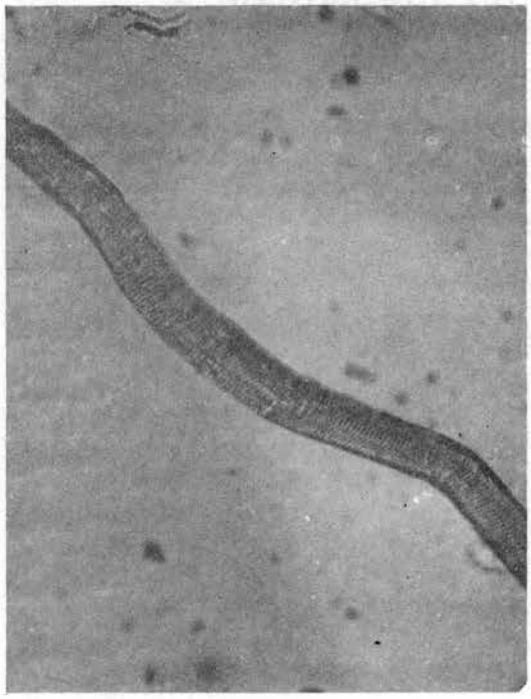
Figure 7. Procedure for Calculation of Percent Kinkiness.



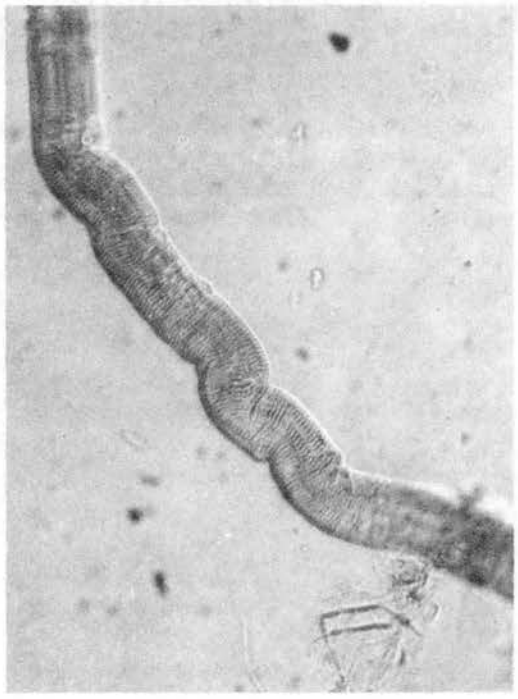
Straight Fiber



Straight Plus Fiber

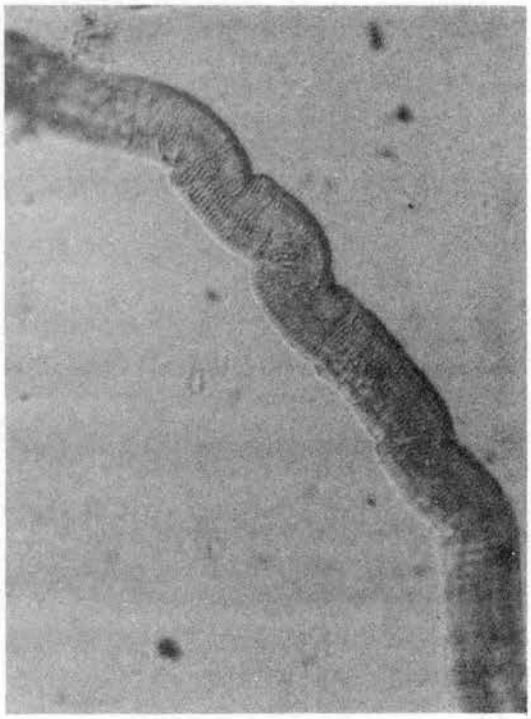


Wavy Fiber

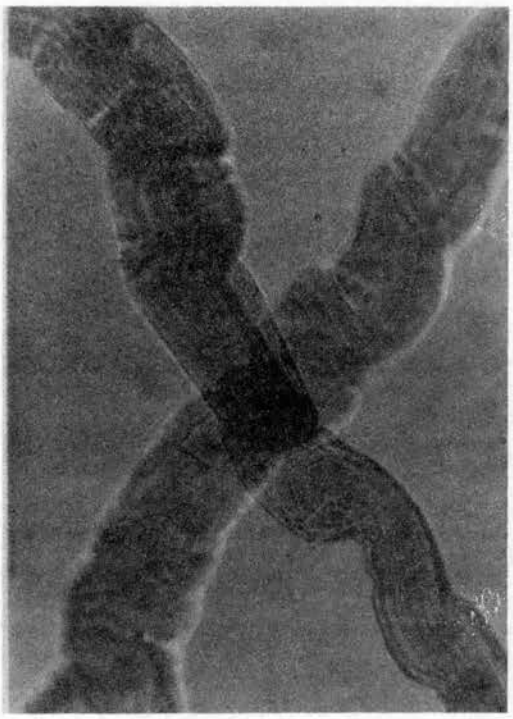


Wavy Plus Fiber

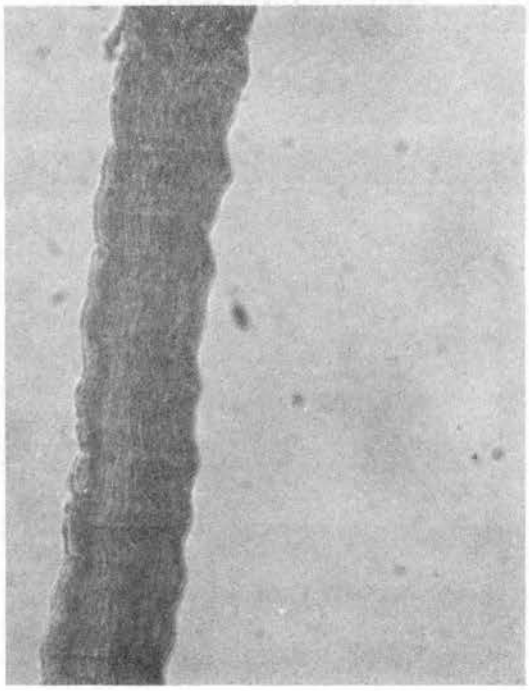
Figure 8. Representative Illustrations of the Kinkiness Scores Assigned to Muscle Fibers.



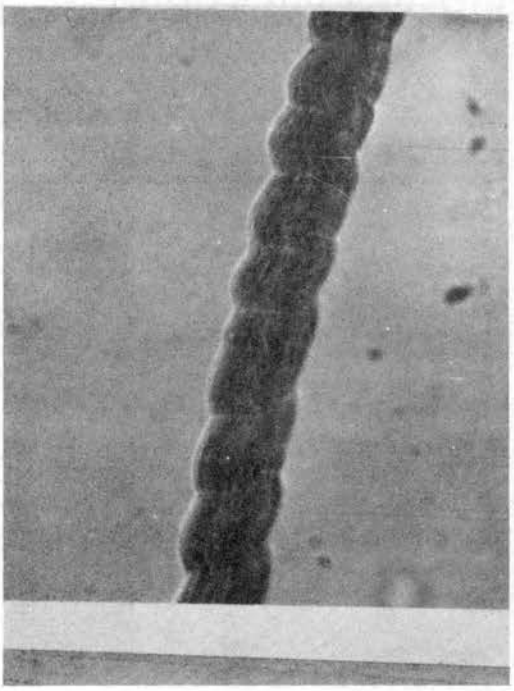
Twisted Fiber



Twisted Plus Fiber



Kinky Fiber



Kinky Fiber

Figure 8. (concluded)

The Analysis of Variance and "F" tests were used to determine if sample differences were significant and to acertain the source of variation found.

CHAPTER IV

RESULTS AND DISCUSSION

The variation among animals was found to be highly significant ($P<.01$) for all of the characteristics studied with the exception of percent kinkiness which was significant ($P<.05$). This was as expected since the greatest source of variation in biological experiments is, in most cases, the source of the experimental material itself. This large variation points up the need for large numbers of animals when dealing with many biological factors.

pH

A highly significant difference ($P<.01$) was found among the pH values due to the treatment effect (Table I). Means for pH values indicated that there was a constant decline with time post mortem. The highest value was present immediately after death and the lowest at eight hours post mortem. A highly significant ($P<.01$) difference was noted for location effect for the left side while the right side location effect was found non-significant (Table I). While some slight variation in pH would be expected due to location, the almost consistent decline noted for the left side (Table II) appeared to be due to some side by treatment interaction not isolated in the design. The failure of the left and right sides to react alike with respect to location (Table II) will be discussed in a later section of this paper. From the means

shown in Table III, it is apparent that the decline of pH becomes less rapid about six hours post mortem. The decline in pH value during the last two hours of the study was much less than in the earlier periods (Figure 9). However, it should be noted that at eight hours post mortem the ultimate pH had not yet been reached.

TABLE I

ANALYSIS OF VARIANCE FOR pH OF PORCINE LONGISSIMUS DORSI MUSCLE

Source	df	SS	MS	F	SS	MS	F
			Right Side			Left Side	
Total	63	8.12			6.99		
Animal	7	1.12	0.16	7.62**	1.15	0.16	10.93**
Location	7	0.15	0.02	1.00	0.42	0.06	4.00**
Treatment	7	6.02	0.86	40.42**	4.79	0.68	45.60**
Error	42	0.89	0.02		0.63	0.015	

*P<.05
**P<.01

Shear Force

Differences due to treatment in shear force values were found to be highly significant (P<.01) (Table IV) for the left side and approached significance for the right side. Cagle and Henrickson (1968b) found that hot slicing had a much greater effect on the shear force of porcine LD muscle from the left side than on the same muscle from the right side. It is doubtful if the variation between sides is of practical or economical significance (Table V) even though it is statistically significant

TABLE II

EFFECT OF SAMPLE LOCATION ON pH OF PORCINE LONGISSIMUS DORSI[a]

Sample Location	Right Side	Left Side
1	6.13	6.17
2	6.08	6.11
3	6.11	5.98
4	6.03	6.01
5	6.03	5.96
6	6.03	5.80
7	6.15	5.94
8	6.13	5.98

[a]Each number is an average for eight chops.

TABLE III

EFFECT OF HOLDING LONGISSIMUS DORSI AT 25°C ON pH[a]

Minutes Post Mortem	Right Side	Left Side
30	6.45	6.48
60	6.47	6.30
120	6.33	6.22
180	6.20	6.08
240	6.03	5.99
300	5.87	5.81
360	5.72	5.72
480	5.62	5.57

[a]Each value is an average of eight chops.

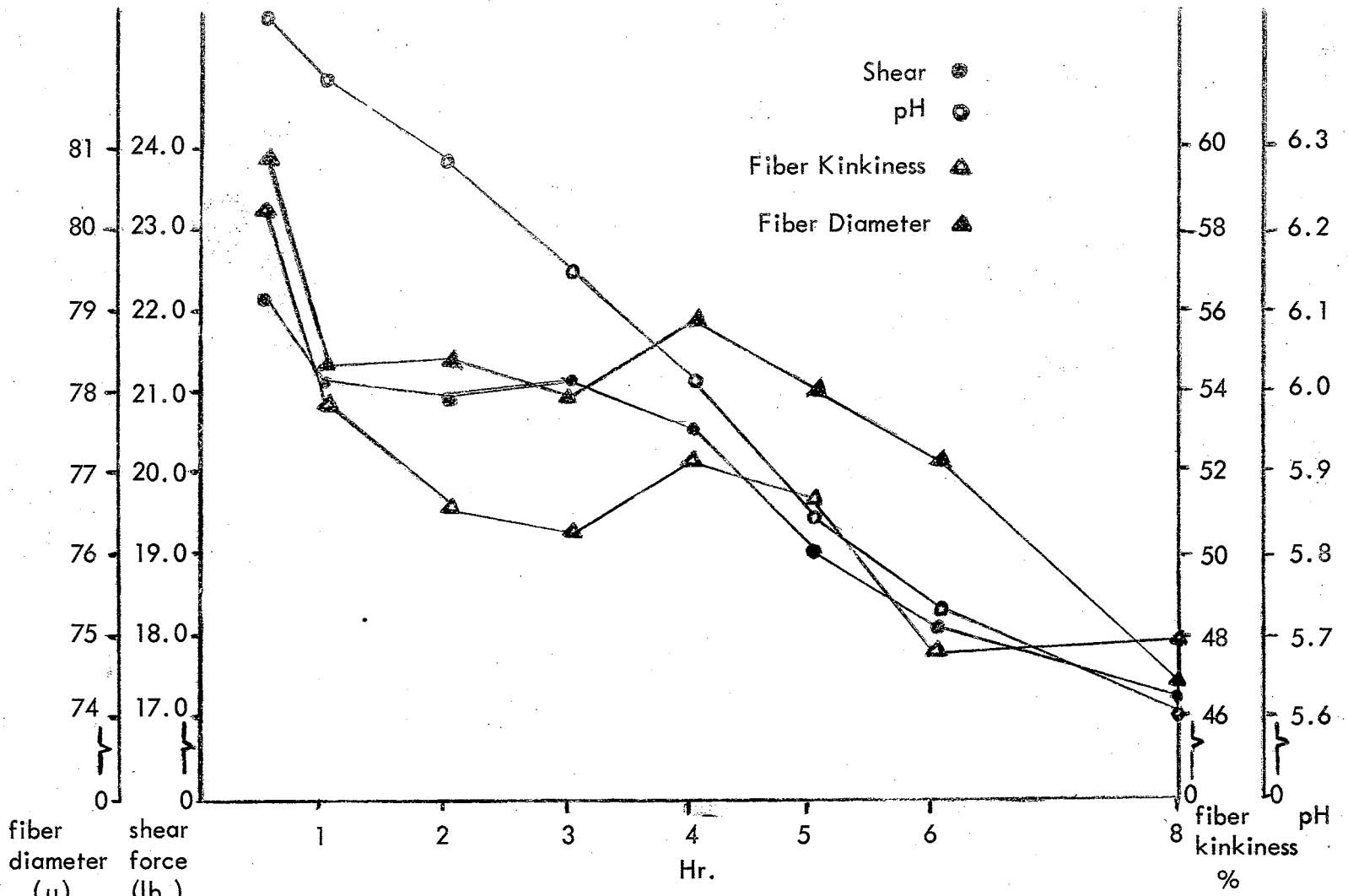


Figure 9. Relationship of pH, Shear Force, Fiber Diameter and Percent Kinkiness Changes in Porcine Muscle Held at 25°C.

($P<.01$). The "F" value of 1.65 (Table IV) for treatment effect on the right side prompted the conclusion that, although a difference was present, the number of samples and/or the technique were not sufficiently precise to pick it up. The treatment means for shear force showed an interesting relationship in that the muscle became more tender with increasing time post mortem up to eight hours (Table VI). Thus, the shear force declined as the pH declined (Figure 9), which is contrary to previous reports. However, Busch et al. (1967) reported that bovine muscle held at 37°C became more tender as pH declined. He postulated that this increased tenderness could be due to high temperature aging. Cagle and Henrickson (1968a) stated that this decrease in shear force with time might be due to the relaxation of muscle that contracted when sliced in the "near live" condition (all muscle was sliced within 10 minutes after the death of the animal). In this respect these workers found hot sliced pork loins to be less tender than loins that were sliced cold. The location means (Table V) for shear force revealed a slight variation but no linear variation was apparent as indicated in earlier reports.

TABLE IV

ANALYSIS OF VARIANCE FOR SHEAR FORCE OF PORCINE LONGISSIMUS DORSI MUSCLE

Source	df	SS	MS	F	SS	MS	F
			Right Side			Left Side	
Total	63	1,188.01			964.70		
Animal	7	599.58	79.94	7.80**	228.17	32.60	3.52**
Location	7	79.47	11.35	1.11	55.77	7.97	0.85
Treatment	7	118.29	16.89	1.65	291.28	41.61	5.94**
Error	42	430.67	10.25		398.48	9.27	

*$P<.05$
**$P<.01$

TABLE V

EFFECT OF SAMPLE LOCATION ON SHEAR FORCE OF PORCINE LONGISSIMUS DORSI[a]

Sample Location	Right Side	Left Side
1	19.19	21.11
2	19.43	21.69
3	19.78	20.64
4	20.04	20.75
5	20.32	19.13
6	21.69	20.00
7	17.98	18.73
8	19.22	19.79

[a]Each value is an average of eight chops.
[b]Pounds required to shear three-quarter inch core.

TABLE VI

EFFECT OF HOLDING LONGISSIMUS DORSI AT 25°C ON SHEAR FORCE[a]

Minutes Post Mortem	Right Side	Left Side
30	20.04	24.03
60	20.95	21.27
120	20.22	21.62
180	21.28	20.76
240	19.90	20.94
300	19.66	18.32
360	18.41	17.72
480	17.10	17.28

[a]Each value is an average of eight chops.
[b]Pounds required to shear three-quarter inch core.

Fiber Diameter

No significant difference was found for fiber diameter due to the treatment effect (Table VII). From these results, no relationship between fiber diameter and tenderness was apparent. In some cases the larger fibers were from the more tender samples while in others the exact opposite was true. No location effect on fiber diameter was apparent (Table VII and Table VIII). It may be that the state of fiber contraction has a greater effect on sample tenderness than fiber size, per se. Thus, if one fiber is larger than another because it is contracted, it may be less tender. But if the two fibers are in the same state of contraction and one is larger, there may be no difference in tenderness. It was expected from previous reports that fiber size would increase with advancing time post mortem, but evidently slicing the muscle soon after slaughter caused considerable contraction which relaxed slightly with time. This would account for the smaller fiber diameters at the later sampling periods (Table IX).

TABLE VII

ANALYSIS OF VARIANCE FOR FIBER DIAMETER OF PORCINE LONGISSIMUS DORSI MUSCLE

Source	df	SS	MS	F	SS	MS	F
			Right Side			Left Side	
Total	63	3,185.48			2,998.84		
Animal	7	1,592.88	227.55	8.08**	1,655.98	236.55	10.34**
Location	7	193.42	27.63	0.98	170.41	23.34	1.02
Treatment	7	215.86	30.84	1.09	206.66	29.52	1.29
Error	42	1,183.32	28.17		960.79	22.87	

*P<.05
**P<.01

TABLE VIII

EFFECT OF SAMPLE LOCATION FIBER DIAMETER OF PORCINE LONGISSIMUS DORSI[ab]

Sample Location	Right Side	Left Side
1	77.91	80.63
2	80.95	76.59
3	79.98	75.64
4	79.44	75.27
5	80.95	79.61
6	77.15	78.16
7	78.47	75.66
8	75.71	79.43

[a]Each value is an average of eight chops.

[b]Fiber diameter measured in microns.

TABLE IX

EFFECT OF HOLDING LONGISSIMUS DORSI AT 25°C ON FIBER DIAMETER[ab]

Minutes Post Mortem	Right Side	Left Side
30	81.06	80.63
60	79.91	76.59
120	81.19	75.64
180	77.75	77.96
240	79.31	78.40
300	78.59	77.54
360	77.31	76.91
480	74.44	74.19

[a]Each value is an average of eight chops.

[b]Fiber diameter measured in microns.

Kinkiness

Treatment variation in percent kinkiness was found to be non-significant. However, as can be seen in Table X, the "F" values are considerably greater than one for the differences due to treatment, indicating a strong possilibity for a real difference due to the treatment effect. However, more numbers would be needed to pick up such a small difference. No conclusions were drawn from the location effect or no pattern apparent from the means for percent kinkiness (Table XI). Mean values of treatment on percent kinkiness (Table XII) revealed a high value at 30 minutes post mortem, a decline in kinkiness for approximately four hours, a slight increase at four hours, and a continued decline of kinkiness to eight hours post mortem (Figure 9). This agreed closely with the pattern found for fiber diameter (Figure 9) so there appeared to be a direct relationship between kinkiness and fiber size. These findings substaniate the idea that the more kinky a fiber, the larger the diameter. No definite relationship existed between shear force and percent kinkiness but as shown in the graph (Figure 9), shear force decreased generally parallel with the decrease in percent kinkiness.

Press Fluid

Press fluid was not significantly affected by the holding period following death or the location of the sample (Table XIII). Since press fluid is a measure of the amount of liquid in a sample, it was not expected to be affected greatly by the treatment. Location effect was non-significant and the means for location (Table XIV) show only slight variation. The results in Table XV are in agreement with previous findings in that no pattern of change due to time was found.

TABLE X

ANALYSIS OF VARIANCE FOR PERCENTAGE FIBER KINKINESS OF PORCINE LONGISSIMUS DORSI MUSCLE

Source	df	SS	MS	F	SS	MS	F
			Right Side			Left Side	
Total	63	7,910.00			7,986.83		
Animal	7	1,909.28	272.72	2.92*	1,641.90	234.46	2.15
Location	7	1,102.75	156.54	1.68	481.59	68.80	0.63
Treatment	7	974.28	139.18	1.48	1,286.03	183.72	1.69
Error	42	3,923.99	93.43		4,578.03	109.02	

*P<.05
**P<.01

TABLE XI

EFFECT OF SAMPLE LOCATION ON FIBER KINKINESS[a] OF PORCINE LONGISSIMUS DORSI[b]

Sample Location	Right Side	Left Side
1	50.76	49.58
2	53.48	49.38
3	56.42	50.94
4	53.34	46.77
5	55.48	51.13
6	43.85	54.93
7	48.05	46.88
8	56.62	54.17

[a]Fiber kinkiness expressed as a percent.
[b]Each value is an average of eight chops.

TABLE XII

EFFECT OF HOLDING LONGISSIMUS DORSI AT 25°C ON FIBER KINKINESS[ab]

Minutes Post Mortem	Right Side	Left Side
30	61.99	54.77
60	51.46	55.96
120	49.83	52.19
180	46.96	53.56
240	52.50	51.92
300	53.40	48.49
360	51.62	43.47
480	51.62	43.79

[a]Fiber kinkiness expressed as a percent.

[b]Each value is an average of eight chops.

TABLE XIII

ANALYSIS OF VARIANCE FOR PRESS FLUID OF PORCINE LONGISSIMUS DORSI MUSCLE

Source	df	SS	MS	F	SS	MS	F
			Right Side			Left Side	
Total	63	63.06			71.16		
Animal	7	24.49	3.50	4.93**	29.84	4.26	5.34**
Location	7	4.69	0.67	0.94	4.39	0.63	0.83
Treatment	7	4.15	0.59	0.83	4.99	0.71	0.93
Error	42	29.73	0.71		31.94	0.76	

*$P < .05$
**$P < .01$

TABLE XIV

EFFECT OF SAMPLE LOCATION ON PRESS FLUID[a] OF PORCINE LONGISSIMUS DORSI[b]

Sample Location	Right Side	Left Side
1	9.52	9.42
2	9.99	9.64
3	9.78	9.38
4	9.11	9.23
5	9.38	9.28
6	9.21	9.85
7	9.45	10.03
8	9.64	9.60

[a]Expressed in square inches.

[b]Each value is an average of eight chops.

TABLE XV

EFFECT OF HOLDING LONGISSIMUS DORSI AT 25°C ON PRESS FLUID[ab]

Minutes Post Mortem	Right Side	Left Side
30	9.25	9.17
60	9.43	9.48
120	9.65	9.83
180	9.01	9.26
240	9.73	9.85
300	9.52	9.25
360	9.83	9.85
480	9.66	9.75

[a]Expressed in square inches.

[b]Each value is an average of eight chops.

Discrepancy of Sides

Since it has become an accepted fact that the sides of a carcass are essentially indentical, the apparent interaction of side and treatment, and side and location seemed to be due to some different treatment effect neither expected nor measured. It was found upon examining the slaughter procedure, that the animals were shackled by the left leg and suspended from the floor by the leg until death occurred from bleeding. Thus, a physical strain may have been induced in the muscles of the left side causing the fibers to partially contract. This could account for the decreased tenderness of the muscles of the left side as was found in this study.

CHAPTER V

SUMMARY AND CONCLUSIONS

Eight market weight Hampshire gilts of similar feeding and genetic background were obtained from the Oklahoma Agricultural Experiment Station herd. The animals were slaughtered and a section of the longissimus dorsi muscle extending from the last lumbar vertebrae to the sixth thoracic vertebrae was removed from each side. Each section was sliced into eight two-inch chops and held for eight time periods post mortem (30, 60, 120, 180, 240, 300, 480 minutes) at 25°C. The pH measurements were made at three locatons on each chop. Histological and press fluid samples were removed and the remainder of the chop cooked for shear determination.

The pH measurements were taken using a Corning meter with the electrode placed directly upon the cut surface of the chop. Histological testing consisted of measuring 50 fibers from each chop and assigning each measured fiber a subjective score for percent kinkiness. Shear determinations were made by shearing two three-quarter inch thick cores using a Warner-Bratzler shear device. Press fluid was determined by the filter paper Carver press technique using 10,000 pounds pressure for one minute.

Differences in shear force due to treatment were found to be highly significant ($P < .01$) for the left side and approaching significance ($P < .05$) for the right side. The shear force value was high at 30 minutes after death, remained almost constant

until about four hours post mortem at which time it declined at a rate that closely paralleled the decline of pH. Fiber diameter and percent kinkiness followed approximately the same pattern; as fiber diameter decreased, percent kinkiness became progressively smaller also. Press fluid was not significantly affected by the holding periods post mortem. The pH declined at a constant rate which agreed with previous reports.

This study suggested that holding "hot" processed pork loin muscle at room temperature for extended time periods, up to eight hours does not adversely affect the tenderness of the meat. Slicing the hot loin appeared to cause an initial decrease in tenderness. It is believed that this decrease was the result of the fibers contracting due to the release of tension caused by slicing. No definite relationship between shear force, fiber diameter, and percent kinkiness was found. The failure of the right side and left side to react the same was atrributed to the manner in which the animal was suspended during death.

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