CHEMICAL AND PHYSICAL CHANGES IN ISOLATED BEEF MUSCLE TENDERIZED BY INFUSION WITH SODIUM CHLORIDE

by

BALWANT RAI SURI

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APPROVED:

Professor and Head of Department of Food Technology
In Charge of Major

Chairman of School Graduate Committee

Dean of Graduate School

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Chapter I

INTRODUCTION

The primary quality factors of meat demanded by the consumers are tenderness, juiciness and flavor. The most essential of these three attributes is undoubtedly tenderness. If the meat is tough, it is generally objectionable, irrespective of all other qualities. Quality in terms of palatability is usually determined by the consumer whose likes or dislikes are the primary standard, for it is this consumer reaction which ultimately controls the market. Since the consumer wants continuously more meat of better quality and at the lowest possible cost, better methods for the tenderization of meat must be developed to provide yields of tender, juicy and lean meat.

The concept of tenderness involves the physical, chemical, biochemical, physiological and histological properties of the skeletal muscle. Although many contributions have been made to our present knowledge of tenderness in meat, an exact recognition of the controlling factors does not exist.

The problem of quantitative determination is relatively simple through an evaluation of those properties which

are amenable to physical and chemical measurements. But tenderness of meat cannot be so evaluated. However, taste panel techniques can effectively measure tenderness of meat and can serve as a counterpart to determine biochemical changes which may be associated with the phenomenon of tenderness.

The objective of this study was to tenderize beef muscle by the perfusion of sodium chloride, and after proving that the muscle was tenderized by this method to correlate certain biochemical properties with the changes in tenderness. Histological changes were also studied.

Chapter II

REVIEW OF LITERATURE

Tenderness is probably the most important quality attribute of meat, provided the meat is otherwise sound and fit for consumption. In fact, meat must be tender to be satisfactory. In order to understand tenderness and develop methods for its improvement, one must be familiar with the literature pertaining to the factors affecting this quality of meat, existing methodsof tenderization of meat, and the methods of measuring tenderness.

In summarizing the literature it has been desirable to organize the findings in the following categories.

Characteristics of Skeletal Muscle

Physical Structure. Muscle tissue is classified into two types: (1) skeletal, striated or voluntary muscle and (2) smooth, unstriated or involuntary muscle. In this study only the skeletal muscle will be considered in as much as a preponderate portion of marketable meat falls in this category.

Muscle cells are long and slender and for this reason are usually referred to as muscle fibers. In skeletal muscle, a great number of such fibers are massed together into bundles. The bundles are bound together by connective

tissue into larger masses. When viewed under a high-power microscope, a skeletal muscle fiber shows a series of alternating light and dark transverse bands. Each fiber is ensheeted by a thin, colorless delicate elastic membrane called the sarcolemma, and running through its entire length are a great number of fine parallel filaments--the myofibrils. The latter are embedded in the cytoplasm, which is usually referred to as the sarcoplasm. Each myofibril is constituted of a number of alternating light and dark sections. The sections in the different myofibrils lie more or less in line across the fiber--light with light, and dark with dark. It is to the combined effect of these sections that the cross-striated appearance of the whole fiber is due. The striated muscle fiber usually possesses several nuclei; these are ovoid in shape and situated just beneath the sarcolemma.

The fibers are grouped parallel to each other in bundles designated fasciculi which are surrounded by connective tissue called perimysium. Some connective tissue enters the fasciculi and is known as the endomysium. A number of these fasciculi constitute a muscle, and the connective tissue enclosing each muscle is known as the epimysium or muscle sheath (53). The integrity of the connective tissue elements of muscle: epimysium, perimysium, endomysium, and sarcolemma, continues at least to

the endomysium, and a few investigators have included the sarcolemma (53,81).

Chemistry of Muscle

Contractile Protein. In general, meat is skeletal muscle. The biochemistry and physiology of this tissue has been thoroughly described by the pioneer works of Szent-Gyorgyi and his colleagues (90,123,124,125,126,127, 128). Many properties of meat can be best interpreted and understood from the fundamental information brought together by this scientist.

In its structure, muscle consists of elongated cells, containing jelly-like protoplasm. The protoplasm is supported by a framework of connective tissue fibers which ramify through the mass of cells, thus giving the tissue as a whole the necessary rigidity and shape.

The cells contain the contractile material which make up the microscopic myofibrils; these fibrils run parallel to the cell axis and are responsible for the longitudinal striations of the muscle.

The intracellular substance of the muscle cells, the sarcoplasm, is a complex fluid containing water, proteins, glycogen (or break-down products of glycogen such as glucose and lactic acid), inorganic salts, hormones, vitamins and various enzymes.

Glycogen. A group of very similar polysaccharides with branched chains are found in the cells of all animals. Glycogen hydrolyzes only to d-glucose (dextrose) and is constructed of these sugar units joined in short chains by 1→4 linkages with the short lengths joined together in turn by 1→6 linkages to produce a branch-on-branch structure.

According to Hasselbach (59) upto 53 percent of the rabbit muscle proteins consist of myosin and actin, which interact to form actomyosin during the contraction process. According to Szent-Gyorgyi (126) myosin accounts for about 70 percent of beef muscle proteins. The remaining proteins largely consist of myogen, myoalbumin, and globulin-x (9,89,136).

The myogen fraction contains many of the enzymes of the muscle. Phosphorylase, hexokinase, glucose-1, 6-phosphomutase, aldolase, isomerase, various dehydrogenases, and phosphoglyceromutase are present in this fraction (36).

With the advance in colloid and protein chemistry the list of newly found proteins has been enlarged. The identification and classification of all of the muscle proteins is still not complete. Tropomyosin, which is considered to be a prototype of myosin, has been reported by Bailey (3,4), nucleotropomyosin by Hamoir (57), and

socalled Y-protein by Dubuisson (37).

Of all the muscle proteins myosin and actin have been studied most extensively because of their relationship with muscular contraction (126,136).

Actin exists in two forms, the globular (G-actin) and the filament (F-actin) form which is the polymer of ovoidal particles of G-actin (126). The polymerization of actin, myosin, and adenesine triphosphate is induced by salts that do not cause denaturation when added in the proper concentration (128). Usually only F-actin is able to combine with myosin. The combination of myosin and actin to form actomyosin takes place not only in vivo, but also in vitro (74,78,126). Preparations of actin in its globular form contain 0.86-1.47 percent of adenosine triphosphate (ATP), that is, approximately one molecule of ATP combines with one molecule of actin (94) or myosin.

The transformation of G-actin to F-actin is accompanied by a change of ATP to ADP (adenosine diphosphate) (95). It is assumed that <u>in vivo</u> actin-bound ADP is rephosphorylated by a phosphate donor system (94). Also urea establishes an equilibrium between the globular and fibrous actin (127).

Myosin exists in muscle as thin and long filaments, held straight by its charges. In the resting muscle, myosin and actin are separated by repulsive coulombic

forces. In excitation during contraction they unite to form the complex, actomyosin, which in the presence of ATP is unstable in its stretched form and goes over into a modification characterized by a shorter length and lower free energy (128). Muscle contraction is the folding of F-actomyosin due to action of ATP at the proper ionic concentration. The formation of actomyosin is easily detectable by a rapid increase in the relative viscosity (95,113, 126).

Adenosine triphosphate itself has a double function, a static and dynamic one. It keeps actin and myosin apart in the state of rest and makes the system elastic and supple by decreasing cohesive forces (126). Inducing contraction is the dynamic function of ATP (94). A number of enzymes are provided for the breakdown of ATP. A real landmark in muscle physiology was the discovery of Engelhard and Ljubimova (39) that the contractile protein, myosin, was effective in splitting ATP and in some way making available the energy it contained for the performance of muscular work. In addition to the ATP-ase activity myosin also possesses a pronounced deaminase activity (126), especially upon adenylic acid. A recent review considering the interactions between ATP and the structural proteins of muscle is given by Needham (97).

Myosin treated with trypsin no longer reacts typically with actin (44). According to recent discoveries made by Mihalyi and Szent-Gyorgi (90) the trypsin-treated myosin is denaturated by breaking down to particles of two different sizes showing a different sedimentation constant.

Connective Tissue

The intercellular substance is connective tissue, consisting principally of two fibrous proteins collagen and elastin. Collagen is present in the form of tough fibers which ramify in all directions to form a continuous tough membrane. These fibers come together at the terminal parts of the muscle in tendons and fascia, which attach the muscle to the skeleton or to other muscles and tissues.

Collagen itself is digested by pepsin and trypsin. It is slowly converted into gelatin by boiling and more rapidly by superheated steam. In contrast to collagen, gelatin is soluble in water. This property of collagen is partly responsible for the improvement of tenderness of meat on cooking.

Associated in varying degree with collagen in connective tissue are the fine fibers of a substance called elastin, which is quite stable and remains almost unchanged on boiling or on treatment with dilute acids or

alkalis. Elastin fibers, in contrast to gelatin, are refractive and elastic, and when massed together appear yellow. Elastin fibers are digestible by pepsin in acid solution and by trypsin in neutral solution. A specific enzyme, elastase has been reported (7), which can convert elastin from the fibrous to the globular state, liberating free amino and carbol groups. One of the resulting products has reducing properties, suggesting that elastin may be a glycoprotein (7).

The properties of different types of connective tissues depend on the proportions in which collagen and elastin are present.

Other Components of Muscle

In addition to the substantial proteins, skeletal muscle contains also other nitrogenous substances such as amino acids, adenine, adenosine, adenylic acid, urea, carnosine, xanthine, hypoxanthine, some ammonia and the already mentioned ATP and ADP.

Of the inorganic electrolytes, potassium ions predominate; the average potassium content in muscle is about 400 mg. percent. Potassium ions are of importance in preventing the precipitation of myosin (83,121,126) or actomyosin and in the regulation of the physiological functions of the skeletal muscle.

Other inorganic elements present in smaller amounts are sodium, iron, magnesium, calcium, phosphorus and chlorine.

The red pigment of muscle is myoglobin. It differs from hemoglobin, the chromoprotein of the red blood corpuscles, by its slightly different absorption spectra (111), by its lower molecular weight (126), and by its different crystal structure.

Post-mortem Changes in Muscle

The enzymatic processes which function continuously during life do not cease after the slaughter of an animal. They continue to function as autolytic reactions under different conditions in contrast to those prevailing during life. From the time of death skeletal muscle undergoes changes. At first there is a condition of extreme contracture and hardness or rigidity due to shortening of fibers. This is called rigor mortis.

The onset of rigor mortis is evidenced several hours after death and continues for a few days. There appears to be a wide variation in the time of onset and the passing of rigor in different animals even within the same species. At a given temperature, the speed at which rigor mortis sets in was found to depend on the age, sex, and other individual characteristics of the animal (120).

During rigor mortis, the contents of sarcolemma lose their transparency and the sheath (epimysium) loses its elasticity. The whole muscle fiber contracts.

A resting muscle has a neutral reaction and normally contains a considerable amount of glycogen. After death the glycogen disappears, being converted to lactic acid. This reaction can be simply represented by the equation,

$$(C_6H_{10}O_5)n + nH_2O \rightarrow 2nC_3H_6O_3$$

An extremely complicated series of transformations take place between the initial and final states. The pH of the muscle with nil or minimal amount of lactic acid is 7.4. The first direct consequence of the production of lactic acid is that the pH decreases steadily from the time of slaughter.

On the death of an animal, cell metabolism changes from the aerobic to the anaerobic type with the result that lactic acid is produced as the end product instead of carbon dioxide, which is the end product of aerobic metabolism.

The increased production of lactic acid causes a decrease in pH from the neighborhood of 7.4, the value for the living muscle, to 5.5-5.7 in the rigor (72). As the pH decreases the color changes from the purple red to a bright red.

An excellent review of post-mortem changes is included in the article by Bate-Smith (11).

von Furth (133) believed that the rigor was caused by the formation of lactic acid and consequent fall in pH. That this is not the case is shown by the fact that rigor sets in at times without any change in acidity whatsoever. Best et al. (18) mentioned this in relation to muscles poor in glycogen and made some observations on animals deprived of glycogen by administration of insulin and by feeding thyroid hormone. Nevertheless, it is a puzzling fact that when sufficient acid is produced rigor always sets in when the muscle reaches a pH in the neighborhood of 6.3 (8).

At the present time, there are two theories on rigor mortis; one has been developed by Szent-Gyorgyi (123,126); and the other by Bate-Smith (11). According to Szent-Gyorgyi (126) a resting muscle is extendible because myosin is dissociated from actin. In the muscle, after death, potassium ions, by diffusion, and ATP, by enzymatic breakdown, are removed from myosin. Actin then combines with myosin to form actomyosin which is extremely inextensible and confers on muscle the rigidity associated with rigor mortis.

Bate-Smith and Bendall (14) have more recently made further observations in connection with the causes and the

time course of the development of rigor mortis. According to these authors two factors are predominant: a) the pH of the muscle at the time of death, and b) the reserve of glycogen. They observed also that the onset of rigor coincides with the disappearance of ATP from the muscle. According to Bendall (16) the breakdown of ATP precedes the hydrolysis of creatine phosphate; only after 70 percent of the creatine phosphate had disappeared did the ATP-breakdown start. Consequently the author explained rigor shortening as a slow irreversible contraction having as an essential phenomenon the disappearance of ATP from the muscle. A parallel function of creatine phosphate in both rigor and in normal muscle contraction is to participate in the resynthesis of ATP (16).

Weber and Portzehl (137) affirmed recently that actomyosin threads or fibers, in vitro, are in a state of rigidity when ATP is split out or is absent.

According to Solov'ev (116), the destruction of ATP exceeds 90 percent at 12 hours post-mortem. These authors also found that at 24 hours post-mortem the solubility of actomyosin ceased; later on it gradually increased with time at a rate slower than that of the original drop in solubility. A significant delay of conversion of actomyosin into the insoluble state occurred during the first six hours post-mortem.

In addition, the following changes are also observed after slaughter: loss of power to contract when stimulated, increase in inorganic phosphorus, rapid breakdown of creatine phosphate, a decrease of sulfydryl groups, and liberation of carbon dioxide. These phenomena of rigor mortis have been studied by many workers, namely by Hoet and Marks (66), and especially by Bate-Smith (11,15).

All post-mortem chemical changes are more or less enzymatic in nature. In the first 6-24 hours post-mortem the muscle goes through steps of the glycolytic cycle. The glycolysis continues until either glycogen is exhausted or a fixed point in pH is reached at which the glycolytic enzyme system is completely, but not irreversibly inactivated (8). This point is at or a little below pH 5.4 (11). Thus, the level of activity found in post-mortem is to some extent related to the level of resting metabolism existing in the muscle before the animal was killed.

After the first 24 hours the enzymatic processes in meat seem to be autolytic in nature. The muscle still contains enzymes, and these together with high concentration of lactic acid formed previously in the system are responsible for the gradual physical changes in the muscle fibers. The result is that the muscle becomes

soft and pliable again.

If the meat is stored for still longer periods (over 15 days) the proteins themselves are gradually broken down into simpler parts due to an advanced autolysis process (11). The optimal autolysis of muscle of chicken, pork, horse, and cow occurs at pH 5 and at 35° C.

Autolysis

The term autolysis is usually employed synonymously with the hydrolytic breakdown of proteins by the agency of tissue enzymes. The process can be followed by determining changes in non-protein nitrogen. The early stages, in which the breakdown products still behave like proteins toward precipitants, are revealed by an increase in the amino nitrogen of the coagulable fraction.

McCarthy and King (87) and Bate-Smith (11) demonstrated that the sulfhydryl group increases during the process of autolysis. They interpreted this increase of sulfhydryl group as a sign of denaturation of proteins. McCarthy and King stated that, "There is no reason why denaturation as such should cause meat to become more tender, especially when it is borne in mind that the meat is much more drastically coagulated when it is cooked. It is obvious that the link between the chemical and mechanical events during ripening, the 'mechanochemistry' of the process, is still awaiting discovery."

Smorodintsev and Nikolaeva (114) have carried out research on the numerous aspects of autolysis of beef, primarily with a view to establishing chemical or physical coefficients by means of which deterioration can be characterized. These and other investigations have not yet reached a stage at which the precise nature of the products of autolysis and the amounts produced under the stated conditions can be specified.

Autolytic processes require some further considerations, however, on account of the increase in amino acids due to the action of these proteolytic enzymes that bring about a change in flavor of meat. Flavor of meat is an untouched field, which requires further work before any statement could be made as to what this flavor is due to, in the process of autolysis.

Changes Following Rigor

Broadly speaking, from the time of death muscle is a changing system. At first there is a rapid change which leads to rigor mortis. This is followed by much slower changes due to the action of acids and enzymes, which can be controlled to some extent by the temperature and conditions of storage, and which relax the muscle fibers. The coagulum is changed in character so that the muscle again becomes soft and pliable, and if the meat is stored for still longer periods the proteins themselves are gradually

broken down into simpler bodies. This fact is the basis of the so called ripening or tenderization process used widely in the meat industry.

The Concept of Quality and the Tenderization of Beef

The quality of a meat carcass is judged usually by its conformation, texture, firmness, color of lean and maturity; whereas the quality of cut meat is determined by its color, texture, firmness and marbling.

To the consumer, however, meat must be tender, juicy and flavorful when cooked in order to be acceptable. Generally, all fresh beef has an acceptable flavor, but there is a wide range of tenderness.

Aging. This term is applied to the practice of holding meat at cool temperatures for periods of two to four weeks, or even longer. Although aging of meat, frequently referred to as "ripening", "conditioning", "tenderizing" or "hanging" has been used for centuries, very little is known in regard to the chemical processes which take place. It is commonly believed that tenderizing during aging is the result of autolytic enzymes (40) breaking down the proteins, especially those in the tough connective tissue.

Although microorganisms themselves play no part in the aging of meat as far as tenderness is concerned, they

must be taken into account. If they are allowed to grow or multiply, they can cause moldiness, sliminess, off colors, odors and flavors. To secure satisfactorily aged meat it is necessary to:

1. Keep the microorganisms on the surface small in number by hygienic handling and holding.
2. To prevent growth of the microorganisms both on the surfaces and in the deeper tissues, by rapid cooling, holding at low temperatures, or by holding in a more or less sterile atmosphere.

Accelerated Tenderization by Electrical Stimulation. More recently Harsham and Deatherage (58) developed a method in which tenderization of the muscle and tough tissues in the flesh takes place, without loss of flavor or food value, and without significant shrinkage, within a period of 24-30 hours total time, coincident with normal processing and chilling of the carcass. This invention is based upon electrical stimulation of the fresh meat promptly after bleeding in the course of slaughtering. By this stimulation, the nerves and contractile tissues are fully or substantially exhausted. According to the authors, the latent energy stored within the muscles, nerves or tissues, is released and the meat is rendered more susceptible to the enzymatic digestion or chemical alteration capable of making the meat more tender.

Since the time required for this treatment is so short, there is no noticeable rancidification of fats, or the like. The rapidity of processing provides a commensurate reduction of inventory expenses. However, the extent of meat tenderization is very limited and the method does not offer any great promise for commercial use.

Tenderay Process. A commercial method of considerable interest is known as the "Tenderay" process. The sequence of operation is the killing of the animal, rapid chilling to prevent bone sour, aging at 60° F. for 48 hours in a 90 percent humidity controlled room. To kill bacterial and mold spores, ultra-violet lamps are placed in the aging rooms. This process has been in use for about the last 15 years in this country, but has not attained real commercial popularity. Nevertheless, it does represent a significant contribution to the studies on meat tenderization (71).

Tenderization by Freezing. The effect of freezing on the tenderness of beef was studied extensively by H iner (64), and Hankins (56). The main variations which were studied were: the rate of freezing, temperature of storage in the frozen condition and the rate of thawing. Tenderness is greatly effected by the size and the rate of ice crystals formation. However, there has been some dispute about whether freezing increases the tenderness of

meat.

In relation to rigor mortis, Steiner (119) found that only meat which has been allowed to attain maximum rigor becomes more tender on freezing. This suggests that the differences in results may rest with the methodology of the experimenters.

The effect of repeated freezing and thawing did not modify the mechanical resistance tenderness of muscle (119).

Tenderization of Meat by Plant Proteinase. The plant proteinases, such as papain, bromelin, and ficin, have been found of considerable interest as meat tenderizers. Most of the research work has been done with papain (45,61). Due to the fact that the proteolytic reaction cannot be controlled and the meat loses its structure if the action goes too far, papain has limited commercial applications.

The distribution or application of papain into meat as uniformly as possible is probably the most critical part of tenderization with the enzyme. Papain preparations are generally applied to the outside of the meat in powder form and use is often made of a fork to force the enzyme to the interior (61). The results indicate that the above methods fall short of being satisfactory. More uniform distribution of papain solutions by injection throughout

the tissues was suggested by Lowe (81).

The exact nature of the protein digestion is not known. Nevertheless, whether it be connective or fiber tissue, which is hydrolyzed, it is to be expected that the more meat is digested, the more tender it becomes. Further experimentation in the tenderization of meat by enzymes is needed if their use is to be satisfactory.

Methods of Measuring Tenderness

In order to follow the tenderization process, it is necessary to have a satisfactory measure of tenderness. It is impossible to express tenderness as clearly and as simply as certain other physical properties. There have been attempts to design instruments to measure tenderness in so-called objective methods, and some have been rather widely used. Organoleptic methods of measurement also have been used but have disadvantages such as their being time consuming, difficult to standardize, and subject to differences due to personal differences.

The shear method has gained rather wide usage as a method of measuring tenderness of meat. However, a shear machine simply cannot represent the processes of chewing; notwithstanding those equipped with cutting edges stimulating human teeth.

Various instruments have been designed for the express

purpose of duplicating the natural processes of mastication and some are complex and quite ingenious. The various principles involved in these methods may be roughly classified as cutting, tearing and squeezing. In as much as "chewing" involves all these processes it means that a single apparatus, if used alone, cannot effectively measure the tenderness of meat as experienced by the consumer. Thus, on the whole they have not provided a true measurement of tenderness and presumably measure only a few of the factors comprising the quality known as tenderness.

Some of the instruments which have been designed for shear measurements are the Warner-Bratzler (134), the United States Department of Agriculture's modification of the Warner-Bratzler shear device (25), and shear measurement devices of Volodkewich (132), the penetrometer (130), Winkler (144) and Kramer (76). The Warner-Bratzler shear machine appears to enjoy the greatest application in tenderness measurements. In part, this may be attributed to its durability and simplicity in design and function.

On the other hand, Deatherage and Garnatz (32) in a recent, comparative study of tenderness determination by both sensory panel and shear measurements on a Warner-Bratzler shear machine observe that although shear strength appears to measure fairly satisfactorily a property of

meat, these values are not closely related to the tenderness of broiled steaks as determined by a competent sensory panel and Warner-Bratzler shear machine, they presumed that shear strength and tenderness are not the same property of meat. These investigators encountered difficulties in an effort to reproduce the high correlation observed by others.

The Warner-Bratzler shear device measured the force required to draw a dull, knife-edge through a sample held against a rigid plate, the force being registered in pounds by means of a spring balance. This instrument has been adopted for most of the subsequent work in the United States, for example, that of Griswald and Wharton (50), Ramsbottom et al. (108), Hankins and Hiner (56), and Hiner et al. (64) and Black et al. (19). These papers give some evidence of the correlation between the shear values and organoleptic scoring for tenderness. Recently their shear device has been critically studied by Hurwicz and Tisher (73).

The Volodgewich machine, on which much of the results of Steiner were obtained, employs two blunt teeth as shearing edges and records the work done in cutting through the sample in terms of a load displacement curve (118). Most of the work with this machine was done in Germany. This instrument has been improved by Voledkewich (132).

An apparatus based on the same principle, but of much simpler construction is that of Winkler (143). A constantly increasing force is applied in the form of a stream of lead shots and the deformation of meat between a moving and a fixed jaw is recorded on a synchronized drum.

The penetrometer employed by Tressler, Birdseye and Murry (129), is the same type of instrument as that employed in grading jelly and simply records the greatest force required for a specially designed head to penetrate the sample of meat. Later, Tressler and Murray (130) compared this instrument with that of the Warner-Bratzler and found that the penetrometer gave the most uniform results.

The Kramer shear press, comparatively new, complicated, and consisting of carefully machined parts, employs a multipronged head which is used to shear meat or other substances through slots in alignment with the flat prongs (76). It appears that it may be capable of giving results with high precision.

The mechanical methods of measuring tenderness are subject to two main difficulties:

1. The purely instrumental one of devising a machine that will be sensitive to small differences in tenderness, and that will give reproducible results for the same cross-section of the tissue.

2. The difficulty of securing proper samples, and representative cross-section of the tissue.

The first difficulty may be ultimately solved, but the second is inherent in the sampling. This difficulty of sampling is the most serious one, since the preparation of a representative sample in the chemical sense would render it unsuitable for mechanical determinations (84,138).

The sensory panel, popularly known as the organoleptic panel, consists of a variable number of members, depending upon requirement for statistical significance. Each panel member evaluates the specified properties of a series of samples. Despite the fact that organoleptic panels are susceptible to human variabilities, they have been found to be highly suitable for detecting differences in flavors, effects of specific adjuncts, and probable consumer preference in competing similar materials. In general, trained panels have been found to be surprisingly consistent in their scores, and even with the disadvantages of the high cost of maintaining and training an expert panel and the inconvenience incurred in the gathering and the evaluation of the data, the method probably represents the best known method of evaluating tenderness.

Comparative Tenderness of Various Beef Muscles

Since one is primarily interested in tenderness of meat as it is eaten, studies of tenderness by both mechanical and organoleptic methods have been conducted with cooked meats almost without exception.

According to Lehmann (80) the mechanical strength of a muscle is directly proportional to the amount of connective tissue present. He found that the muscles that were most active had the largest amount of connective tissue, and, therefore, were the least tender. Other investigators who have reported a correlation between connective tissue content of a muscle and tenderness are Mitchell, Hamilton and Haines (92) and Mackintosh, Hall and Vail (85).

Moran and Smith (95) have investigated the relationship between the characteristics of muscle fibers and tenderness. Their observations, made on the different muscles of the same beef carcass, show that the diameter of the muscle fibers, the area of the primary bundles, and the area of the secondary bundles increase progressively in the following muscles; fillet (psoas major), rib (longissimus dorsi), outside round (biceps femoris), and inside round (semi membranosus). The number of fibers for each primary bundle decreases progressively in the same order. Since the muscles are listed in decreasing

order of tenderness it would appear that small primary bundles and small secondary bundles will be correlated with tenderness. According to Brady (22) the texture is dependent upon the size of bundles; the larger the bundles, the finer the texture. He found no significant difference in the diameters of the fibers of the following muscles: Triceps brachii (shoulder), longissimus dorsi (rib and loin), and adductor (round).

Ramsbottom, Strandine and Koonz (108) found great variation in texture of various muscles of the same carcass, texture being determined by the size of the fiber bundles (fasciculi) and the amount of connective tissue (perimysium) surrounding the bundles. In the superficial pectoral muscle there were large and well defined fasciculi with extensive perimysia, conditions associated with coarse texture. This muscle was very tough and had a high shear reading. In the psoas major, the small amount of connective tissue was not sufficient to divide the muscle into very distinct bundles, thereby forming a muscle with a smooth fine texture. This muscle had a low shear reading and was very tender. The fibers of the superficial pectoral muscle had a greater diameter than those of psoas major. Between these two extremes were found all the variations in size of bundles and the amount of connective tissues. The serratus ventralis muscle of

the chuck was an example of muscles following between these extremes. This muscle had a moderately large fasciculi and moderately thick connective tissue and rated medium in organoleptic tests and shear values. These variations in texture agree with the results of Moran and Smith (95) and also with the conclusions of Brady (22), who associated fine texture with tenderness.

Variation of Tenderness within Muscles

Ramsbottom, Strandine and Koonz (108) using a Warner-Bratzler (25) shearing device determined the tenderness in different parts of the same muscle occurring in different wholesale cuts. Sections were cut from either end and the middle of representative muscles for the purpose of making direct comparisons on differences in tenderness. Biceps femoris and lattissimus dorsi were found to be progressively more tender from the insertion end to the origin end of the muscles. The longissimus dorsi and multifidis dorsi were found to be somewhat less tender at the interior ends of the muscles.

Analysis of tenderness data obtained by organoleptic and mechanical shear tests by Weir (138) showed that the longissimus dorsi muscle of hogs was less tender in its central position that at either extreme. Mackey and Oliver (84) obtained similar results and, in addition, showed a difference between animals.

Effect of Exercise on the Quality of Beef

It is a common belief that the toughness of a muscle is directly correlated with its activity. In other words, the greater the activity of a muscle the greater its connective tissue content and so the greater is its toughness. For example, Lehmann (80) observed that "the active calf muscle" from the round of a beef carcass was more tough and high in connective tissue content than the tenderloin (psoas major), a much less active muscle.

On the basis of various observations relating activity of muscles to their tenderness, it would be believed that animals which are most active would produce meat that is less tender than that from inactive animals. This general idea, however, is not supported by the observations of Bull and Rusk (26), who showed that exercise made meat more tender. Mitchell and Hamilton (91) confirmed this finding by showing that tenderness was increased with heavy exercise. In fact, it is difficult to draw any conclusion from these experiments whether exercise has any effect on tenderness of meat.

Influence of Age of Animal on the Tenderness of Beef

Nelson and her coworkers (97) studied the effect of the age of animals on the quality of beef. A mechanical device for testing tenderness called the dynamometer was

used. Three samples were taken from the longissimus dorsi over the twelfth rib. These samples were tested without cooking. In each case the samples from the calves required more force to shear the meat than did the meat from older animals. A probable explanation for this was that lean meat from older animals had more intramuscular fat, and consequently relatively few muscle fibers.

The 9th, 10th and 11th ribs from both sides of the carcass were cooked and scored for palatability by judges. The tenderness was not greatly affected by age. The fine texture of the roasts from the calves made them seem more desirable, although the dynamometer showedthat they were slightly tougher.

Similarly Mitchell, Hamilton and Haines (92) concluded that age did not have a great effect upon the connective tissue content of muscle meat, nor a consistent effect among the different muscles of the carcass.

Effect of Cooking on Tenderness

A number of investigators have measured the effect of cooking on the tenderness of meat. The pioneers in this field are Lehmann (80), Cline (27), Cover (28), Ramsbottom et al. (108), and others (50,81,105). The general conclusion from these studies is that the cooking methods, temperatures, times, kinds or cuts of meat, quality of

meat, and methods of measuring tenderness all can have a bearing on the results obtained.

Effect of Intramuscular Fat on Tenderness

The effect of intramuscular fat on tenderness of beef muscle is not clearly understood. Nelson, Lowe and Hesler (97) found that beef from feeders was less tender than beef from similar cattle after fattening. This indicated that increased fat was a factor in improving tenderness. Hankins and Ellis (55) found no significant correlation between the fat content and tenderness of cooked longissimus dorsi muscles of beef, from which they concluded that variations in tenderness were caused mainly by factors other than fat.

Effect of Storage Period (Aging) on Tenderness

Griswald and Wharton (50) studied the effect of storage on the quality of beef. Right and left sides of three series of ten animals each, were held at 34° F. Nine days' storage was compared with 37 days' storage. The meat stored for37 days had somewhat stronger aroma and flavor, but was slightly less juicy than meat stored for nine days. Small differences were found in the tenderness and desirability of flavor of meat stored for the two periods.

Deatherage and Harsham investigated the changes in

tenderness of 14 beef carcasses during aging at 33-35° F., at intervals of 2, 6, 10, 17, 24, 31 and 38 days (33). They found "that meat from some of the animals became less tender at certain times during the aging period while some of the meat progressively increased in tenderness throughout the aging period. Tenderness of the group increased until 17 days. At 24 days there was no further improvement or a slight drop in tenderness, and finally at 31 days there was some improvement beyond the 17-day tenderness level."

"The results indicate that unless beef is to be aged at 33-35° F. beyond approximately four weeks it need be aged only two and one half weeks."

Ramsbottom and Strandine (107) studied the tenderness changes of beef cooled directly after slaughter, and of beef aged up to 12 days before cooking. They found that beef was more tender at two hours after slaughter than at two days to six days after slaughter. However, the meat was more tender after 12 days of aging than at two hours after slaughter.

Relative Tenderness of Fatty Tissue, Connective Tissue and Muscle

Ramsbottom, Strandine and Koonz (108) compared the relative tenderness of connective tissue, fatty tissue and muscle. They found that connective tissue improves

on cooking; collagen becomes more tender than elastin. On cooking, fatty tissue irrespective of the amount of connective tissue improves in tenderness, whereas the muscles become tough. Thus the final tenderness obtained on a piece of meat on cooking is the resultant of the tenderness of collagen and fatty tissue and toughness of elastin and muscles.

Effect of Boning Beef before Chilling on Tenderness

Ramsbottom and Strandine (107) have reported that boning beef before chilling has a detrimental effect on the tendernessof beef. Their study was made on round and intact sides of beef chilled to about 35° F. in 24 hours. Steaks cut from the longissimus dorsi muscle at 3, 6, 9, and 12 days after slaughter were broiled and rated for tenderness by a panel of judges. Shear readings were taken with a Warner-Bratzler shear apparatus. Their data indicated that muscles that were dissected from sides of beef before they were chilled were less tender than those which remained intact with the sides of beef until they were chilled. This tenderness difference lessened somewhat during the test. However, the beef that was chilled in sides was still considerably more tender by the 12th day than the beef which was boned before it was chilled.

These workers (107) suggested that on boning before

chilling some modification in physical and chemical changes was brought about which was responsible for this difference in tenderness. They state, further, that tenderness might have been influenced by the stimulation of muscle and nerve cells during boning.

A probable explanation for this phenomenom as stated by these authors is "that boned beef cooks faster and therefore, the chemical and physical changes do not occur to the same extent as in unboned beef. Hence the boned beef is found to be relatively tougher than the unboned beef."

Effect of pH on Tenderness

Winkler (144) investigated the relation between pH and tenderness. A mechanical device was used for measuring tenderness; pH was adjusted by injecting lactic acid or ammonia solutions. In raw pork toughness was at a maximum at pH of 5.0 to 6.0. At higher or lower pH the meat became progressively more tender. Studies with raw beef gave similar results, but there was an indication that maximum toughness occurred at a somewhat lower pH.

Relation of Protein Constituents to Tenderness

The muscle plasma quantitatively accounts for 90 percent, more or less, of the muscle substance. This may be considered as a gel of highly hydrated proteins. The

muscle proteins can be divided into three groups with respect to their solubility or extractability:

1. Corpuscular proteins, soluble in dilute salt solution (about 0.15 N) at neutral or weakly alkaline pH (myogen, globulin-X), (137). These are the proteins existing in the free state or only bound by very weak forces to the proteins responsible for the rigidity of the muscle. Some (37) have extracted this protein with distilled water.
2. Contractile proteins, consisting of interlinked peptide chains (myosin and actomyosin) which can be dissolved only by the rupture of part of these links by treatment with salt solution of higher concentration, like 0.5 M KCl solution at pH 7.5-9.5.
3. Stroma proteins are insoluble even at these salt concentration at pH 6.0-10.0. The stroma proteins consist of (a) extracellular proteins-collagen and elastin and (b) intracellular proteins, which in turn consist of soluble protein that has escaped extraction and denaturation.

The muscle plasma must possess, in rigor mortis, a structure which differs from that in the resolved state. As it is essentially composed of proteins, one must expect

extractability of these substances to differ when the muscle is in state of rigor mortis or resolution. Weber and Pertzehl (137) found that the amount of plasma proteins extracted by 0.6 M KCl solution at pH about 9 was decreased much when a muscle was in rigor, 24 hours post-mortem. That decrease was due to myosin which amounted to from 25 to 30 percent if extracted immediately, and only from 3 to 7 percent at 24 hours post-mortem. Myogen and the rest of the nitrogen practically remained unchanged. A similar finding is also reported by Bates-Smith (12).

More recently, the extractability of muscle proteins has been extensively studied by Dubuisson (37). According to his findings, if the muscle is frozen in the contracted state orcontracted by rigor mortis, the myosin and also the so-called X-protein are much reduced or absent in the extracts, and a new protein appears which has been called -myosin (37). If the muscle is allowed to relax, it yields the same extracts as normal muscle. The extractibility of proteins from muscles in the rigor mortis or contracted by monobromo acetate is only re-established when the ionic strength of the solution is equal to or greater than 0.5 (29).

Thus, it is evident that the muscle proteins are in constant dynamic changes during the whole post-mortem period. These changes seem to be mostly due to the changes

in the contractile proteins, myosin and actomyosin. It seems also that the theory of rigor mortis by Szent-Gyorgyi (127,128) stating that during the rigor mortis the actin and myosin are combined to actomyosin, which is extremely inextensible and which confers on dead muscle the rigidity associated with the rigor, is the most probable. Following resolution of the rigor, one may expect some dissociation of actomyosin into its components and consequently a different relative amount of myosin to actomyosin in the state of rigor mortis and after its resolution.

By selecting a suitable extraction solvent that would extract selectively, for example only myosin, without extracting at the samt time actomyosin, one may expect to be able to follow the changes in the muscle plasma during the resolution of rigor mortis. Since the muscle plasma improves in tenderness, such extractability study might help in the evaluation of such relationship between the tenderness and the muscle plasma.

The contractile proteins, myosin, actin and actomyosin, differ in their solubilities; based on this property their isolation and identification are usually carried out. The following table gives the summary of Weber and Portzehl (137) concerning the condition for these proteins.

Table I

Salting-in Ranges of the Contractile Proteins of Muscle

Protein	Animal	pH	Ionic strength for salting-		
			Minimum	Maximum	Salt
Actin	Rabbit	6.0-7.0	Fully soluble in water and dilute salts		
Myosin-L	Rabbit	6.5-6.9	0.04	0.3	KCl
Myosin	Rabbit	5.6	0.5	-	KCl
Actomyosin	Rabbit	6.5-6.7	0.3	0.36	KCl

The results given in the above table indicate that actomyosin is the least soluble of the contractile proteins. It starts to dissolve when the myosin has reached its solubility maximum. The lower pH moves these relative solubilities into the range of higher ionic strength. The time of extraction influences the extractibility, as reported by Portzel et al. (104). Short extraction at pH 6-6.5 gives an L-myosin largely, sometimes entirely, free of actin and actomyosin. The partially dissolved actomyosin can be removed by fractionating at ionic strength 0.3, when the actomyosin is precipitated and L-myosin remains in the solution (104).

Therefore, there is a reason to believe that the extraction solvent containing 0.3M KCl might be of use in

the study of the relation of tenderness to the changes of contractile muscle proteins occurring during the resolution of the rigor mortis. It is further expected that such an extraction solvent will be more or less specific for the extraction of myosin but not of actomyosin.

Following this consideration, citrate buffer of pH 5.6 containing 0.22 M KCl, the total ionic strength 0.48 may be selected for this particular study. The pH of the buffer selected must be approximately that of the meat muscles. The relatively high total ionic strength in the buffer has been expected to be without special influence upon the extractibility of actomyosin, since according to Hamoir (57) actomyosin does not go into the extract at pH 5-6 when 0.5 M KCl solution is used. This buffer has no dissociation power upon actomyosin. The strong linkages between actomyosin complex can be broken only by potassium iodide and pyrophosphate solutions, which are known to possess a high dissociation power upon the complex (36).

Summarization of the Literature Relating to Tenderness

There are several opinions existent on the exact mechanisms responsible for tenderizing of meat.

Since connective tissue is far less tender than the cell contents it follows that its presence in large

quantities will characterize the less tender cuts of meat. Lehmann (80) was probably the first to show a positive relationship between cuts of meat. Mitchell et al. (92,93), and Moran and Smith (95), like Lehmann, believed that connective tissue was the major element contributing to the toughness of meat and, therefore, was subject to the greatest changes during the ripening process.

Deatherage (31) showed that tenderization on aging is enzymatic and in particular proteolytic. The enzymes responsible for these post-mortem changes have been assumed, but not proven (114), to be catheptic in nature. These enzymes have an optimum pH of about 4.7 which is the range of the isoelectric point of most meat proteins. The natural anaerobic glycolysis after slaughter develops a favorable condition for the proteolytic changes because the lactic acid produced brings the meat close to a pH of 5.5-5.7. Low pH hastens tenderization by the natural enzymes present within the meat (58) especially if the temperature is high enough to permit enzymatic action.

It is generally assumed that microorganisms themselves play no part as far as tenderness is concerned. However, as referred to other organoleptic qualities of aged beef, especially those of flavor, a possibility of microbial participation might exist. It is highly possible that the contaminating yeasts, molds, and bacteria may augment the

proteolytic enzymes responsible for the tenderization of meat, or increase enzymatic activity by lowering the pH.

Deatherage and Reiman (34), in a statistical analysis of taste test data on 438 different beef loins, found evidence that at least two major factors appear to be involved in tenderness and that these factors seem to be connective tissue and muscle plasma.

It is well known that tenderness of meat increases with post-mortem age. Husaini et al. (69,70) could not find any significant changes in nitrogen fractions; however, the authors did indicate a relationship might exist between total muscle plasma and tenderness changes.

Recently, Wierbicki and Deatherage (140) have shown, "Connective tissue does not appear to contribute to increases in tenderness on post-mortem aging inasmuch as total alkali insoluble protein does not change. However, it has been pointed out that aging may be related to (a) the dissociation of actomyosin or similar protein changes and (b) redistribution of ions within muscle thus causing increased hydration and tenderness."

The connective tissue seems to be a static quantitative factor: the amount in meat reflects tenderness and does not change.

The muscle plasma seems to be a dynamic factor: tenderness of meat seems to depend upon the physiochemical

state of its plasma proteins which are capable of being changed.

It appears probable that changes in muscle plasma are more closely associated with tenderness than changes in connective tissue (140). The changes occurring in meat resulting in an improvement in tenderness seem to be largely due to some changes in the muscle plasma. These changes are assumed to be proteolytic, probably catheptic, in nature.

The contribution of intramuscular fat to tenderness as well as to juiciness and flavor is not finally established as yet. More research has to be conducted in order to understand the nature of tenderness--that quality attribute which chiefly determines the consumer acceptance of beef.

Chapter III

EXPERIMENTAL PROCEDURE

The experiment consisted, briefly, of isolating the Biceps brachii muscles from the right and left shanks of each of ten beef carcasses and perfusing one of each pair with sodium chloride solution. The tenderness of the perfused and control muscles was determined with a panel of individuals who chewed the cooked meat. The muscles were subjected to certain chemical and physical measurements, primarily to gain an insight into the fundamental nature of the tenderness of meat. The chemical analyses of physical measurements applied to each muscle and/or extract and/or residue derived from the muscle included:

Moisture

Fat

Sodium chloride

Protein

Amino acids

Sulfhydryl groups

Myoglobin

Alkali-insoluble protein

Hydroxyproline

Refractive index

pH

Since the perfusion changed the gross composition of the test muscles, it was necessary to analyze for moisture, fat, total nitrogen, and sodium chloride to express the results of both test and control samples on a comparable basis. Total nitrogen was used as a measure of the protein content of the muscles.

The amount of myoglobin reflects the amount of plasma in the muscle. Amino acids and sulfhydryl groups were determined to find if and to what extent protein breakdown occurred as a result of perfusion of salt into the muscles.

The connective tissue in the muscles, which is related to tenderness, was determined directly as alkali-insoluble protein and indirectly by hydroxyproline analysis.

Buffer extracts of the muscles were also subjected to certain analyses. The extraction, as carried out was intended to remove the myosin and actin and not actomyosin which could then be subjected to analysis to denote changes due to perfusion which, in turn, had influenced tenderness of the muscles.

The effect of holding time on the tenderness of meat and changes in chemical properties were also studied.

Finally, the test and control muscles were studied histologically.

Selection and Treatment of Test Materials

The preliminary studies were carried out with rabbits, but due to differences in composition between rabbit and beef muscles, use of rabbit was discontinued. Therefore, only beef was used.

For the test material, it was considered desirable to use an isolated beef muscle to eliminate some of the biological variations between different muscles. Also, pairs of the muscles from opposite sides of animals were used respectively for tests and controls. The muscles selected were the Biceps brachii. The main reasons for the use of these muscles were the relatively large size, the ease with which they can be isolated and the lack of difficulty in locating the arteries.

The beef muscles were obtained from carcasses which were dressed at a meat packing plant at Albany, Oregon. Complete information regarding the age, breed, and history of the animals and their carcasses was not available, but they represented lower quality beef (as far as tenderness is concerned) primarily for producing "boned-out" beef for sausages. The carcasses had been chilled in preparation for boning.

The Biceps brachii pair of muscles was isolated and removed from the right and left shanks of the carcasses. This muscle, also known as the coracoradialis or flexor

brachii, is shown in Figure I to lie on the interior surface of the humerus. It is about seven to nine inches in length and about four inches in diameter in full grown cattle.

The arteries entering the muscle were separated and tied with a thread. One of the muscles was perfused with 20 percent sodium chloride solution until the muscle increased in weight by ten percent. This gave approximately two percent of added sodium chloride in the muscle. The perfusion was made through the artery using a needle size B-D 22 attached to a graduated 20 c.c. glass syringe No. 3259 of Omega Precision Instrument Co., Englewood, N. J. The selection of right or left muscles for perfusion was made at random, the muscle from the other shank of the same animal being used as the control. The control muscle was never experimentally treated in any way.

In order to determine the effect of water during the course of perfusion, three pairs of steaks were taken. One steak from each pair was perfused with water until the steak increased in weight by 10 percent, while the other steak was kept as a control.

In another study three pairs of Biceps brachii muscles were taken from three carcasses. One muscle from each pair was perfused with water so as to increase the weight of the muscle by 10 percent. The other muscle from the same pair

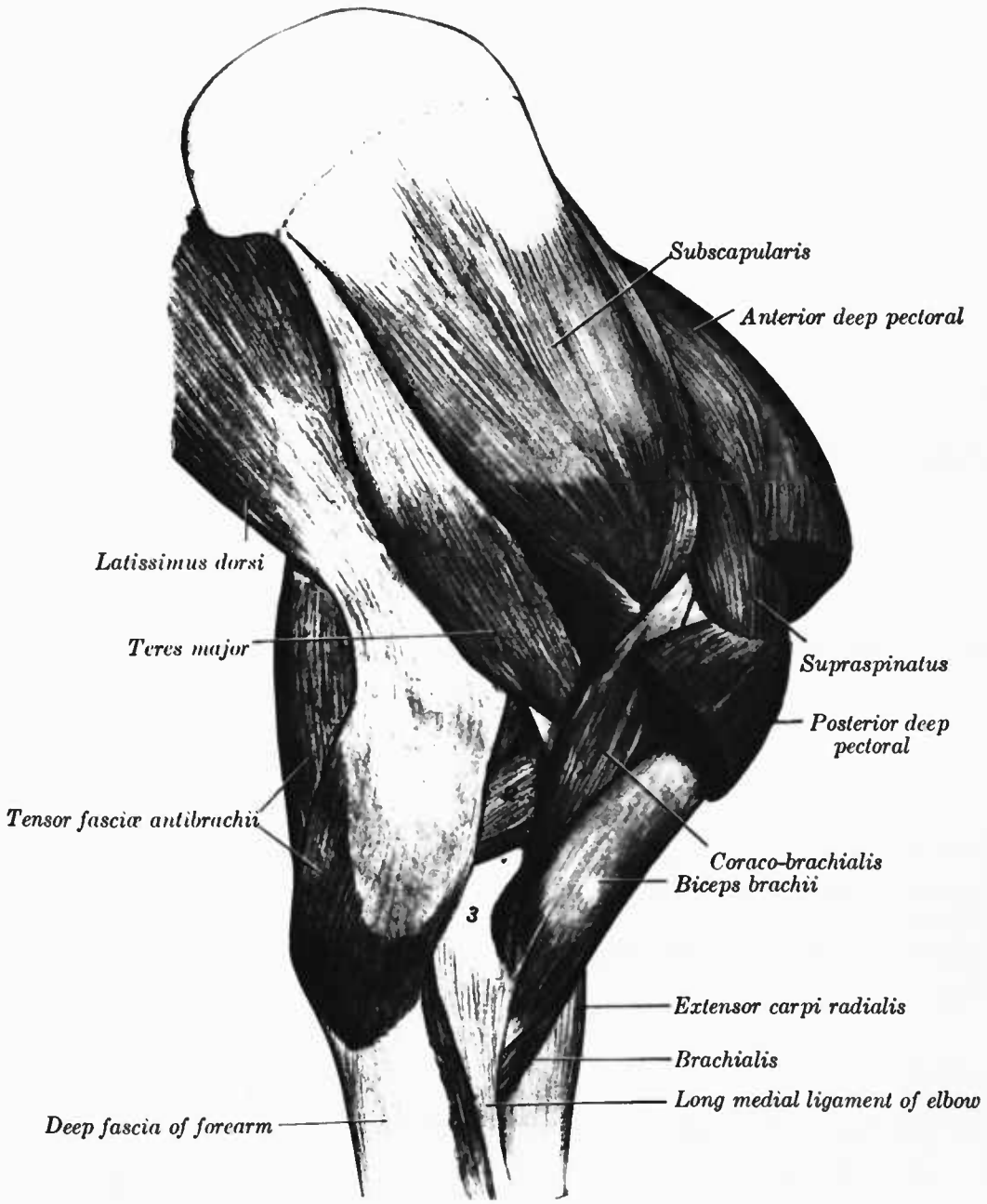

Subscapularis
Anterior deep pectoral
Latissimus dorsi
Teres major
Supraspinatus
Posterior deep pectoral
Tensor fasciæ antibrachii
Coraco-brachialis
Biceps brachii
3
Extensor carpi radialis
Brachialis
Long medial ligament of elbow
Deep fascia of forearm

Figure I

was kept as the control.

The treated and the control muscles were kept separately in pie pans after covering with aluminum foil and stored at 38° F for 72 hours after perfusion.

Design of Experiment

The paired muscles were treated according to the following statistically designed incomplete block arrangement to compare the effect of holding at 38° F after perfusion on the tenderness of muscles. The muscles were given the following treatments:

C = no perfusion

0 = 30-60 minutes after perfusion

1 = one day after perfusion

2 = two days after perfusion

3 = three days after perfusion

4 = four days after perfusion

The order of treatment was randomized as follows:

Pair	Treatment	Pair	Treatment	Pair	Treatment
1	0-3	6	2-4	11	C-3
2	1-2	7	0-4	12	1-3
3	C-2	8	0-2	13	3-4
4	C-4	9	2-3	14	1-4
5	C-0	10	0-1	15	C-1

The data were analyzed statistically. The analysis

of variance was made as follows:

		Degrees of Freedom
	Total	$tr-1 = 29$
	Blocks (unadjusted)	$b-1 = 14$
	Treatments (asjusted)	$t-1 = 5$
	Error	$tr-t-b+1 = 10$
where	$t = 6$	(number of treatments)
	$k = 2$	(number per block)
	$b = 15$	(number of blocks)
	$r = 5$	(number of replications)

The sum of squares for total and blocks was done in the usual way. The sum of squares for the treatment was obtained as:

$$\text{treatment ss} = \frac{t-1}{trk\ (k-1)} \cdot Q^2$$

$$= \frac{6-1}{6(5)(2)(2-1)} \cdot Q^2$$

$$= \frac{Q^2}{12}$$

where Q was obtained from the adjusted mean values.

The sum of squares for error was obtained by subtraction of the sum of squares of blocks and treatment from total sum of squares.

Adjusted mean values for those analysis which showed significant differences were calculated. For each treatment Q was computed as:

$$Q = kT - B_t$$

$$= 2T - B_t$$

where T = treatment total

B_t = total of the block in which the treatment occurred.

$$\text{The adjusted mean} = m \cdot \frac{t-1}{tr(k-1)} \cdot Q$$

$$= m \cdot \frac{6-1}{6(5)(2-1)} \cdot Q$$

$$= \left(m \cdot \frac{Q}{6}\right)$$

where m = mean of the entire experiment.

The adjusted mean values were plotted graphically.

Panel Testing of Meat for Tenderness

Seventy-two hours after perfusion, steaks (four to five from each muscle) approximately one-half inch thick were cut from the center of each pair of muscles and broiled lying side by side on a wire rack in a gas-heated broiling oven. The steaks were four inches below the flame and were turned over after four minutes and broiled for about eight minutes more. The steaks were cut into four equal segments. The same segment of the control and of the treated steaks were placed in paper cups. The cups were coded with three digit numbers from a random number table and presented to the judges in separate taste test booths.

Except in the statistically designed portion of the experiment, the panel consisted of 11 to 17 judges, depending upon the number of judges available. The judges were staff members and graduate students of the Food Technology Department selected on a voluntary basis. They were not screened as to tasting acuity, but were chosen on the basis of their interest in the test and availability at the time of the test. They all were familiar with the objective type of taste test with panels.

Since the test was a comparison between two samples on the basis of tenderness only, the judges were asked to chew the cooked meat and score each according to the following system:

10 = very tender
8 = tender
6 = slightly tough
4 = tough
2 = very tough

The judges were also allowed to score by appropriate odd numbers if desired. The addition of salt by the judges was permitted.

Preparation of the Muscle Samples for Chemical Analysis

On the same day that the tenderness test was conducted (three days after perfusion) the uncooked meat remaining

from each muscle after cutting the steaks was freed of all visible fat and the larger portions of connective tissue, muscle sheath, and tendons. The lean meat was ground through an Enterprise electric grinder having a No. 10 plate. The small amount of sample left in the grinder was discarded. The ground meat was mixed thoroughly and separated into individual portions for each chemical analysis. After placing into screw-capped bottles, these samples were frozen and kept at 0° F until analyzed, except those for the moisture determinations which were analyzed on the same day that the meat was ground.

In the latter part of this study, only tendons were removed and the rest of the muscle was ground as it was. The rest of the procedure followed was the same.

It was found that moisture separated from some of the ground meat in the control group and collected on the sides of the bottles upon thawing. This was not true of the perfused samples. Because of this the thawed meat was mixed thoroughly in the container with a spatula before being subjected to analysis.

Moisture Determination

Small aluminum dishes were used for moisture determinations of the muscles. These dishes were heated for an hour in an air oven at 100° C, cooled in a desiccator and weighed. Approximately two grams of ground meat was weighed

accurately into each dish. The meat was spread in the dish to provide a maximum drying surface. The pans were then heated for eight hours at 100° C in an air oven, afterwards cooled in a desiccator and weighed. The average of duplicate determinations was taken as the moisture content of muscle.

Sodium Chloride Determination

Ten grams of the ground muscle were blended with 90 ml. of water for 3 minutes in an osterizer. Exactly 25 ml. of the slurry after filtering through cheese cloth, was titrated with thiocynate using an excess of silver nitrate according to the A.O.A.C. method (p.539).

Fat Determination

Fat was determined according to the official A.O.A.C. method (p.538) on the dried meat samples.

Determination of Total Nitrogen and Protein

About 1.0 gram of the ground muscle was accurately weighed directly on a tared quantitative filter paper. The paper with the sample was folded carefully and placed into an 800 ml. Kjeldahl flask. The rest of the determination was carried out by the Kjeldahl-Gunning-Arnold method as described by Winton (146). Duplicate determinations were made with each muscle. The average of the two was taken as the total nitrogen content. The amount of total protein

was calculated by multiplying the total nitrogen by a factor of 6.25.

Determination of Myoglobin

The determination of myoglobin was made by the method essentially that of Wejrbicki et al. (140) who modified Husaini's method.

The measurement of the pigment was made spectrophotometrically and the calculations by substitutions in the Lambert-Beer Law.

$$\text{Log}_{10} \frac{I_0}{I} = D : eCL$$

Ten grams of the ground fresh muscle was blended in an osterizer with 90 ml. of distilled water for three minutes. This mixture was filtered through a cheese cloth and then through filter paper by using suction. The filtrate was quite clear. In case the filtrate was not clear, it was further filtered through the filter paper. The first few drops of the filtrate were discarded. The use of cheese cloth and the suction filter was to save time and to hasten the process so that the oxidation of myoglobin would be minimized. The absorbance of the filtrate at 540 mu was determined with a Beckman model DU quartz spectrophotometer against water as a blank.

Following the procedure of Watts and Lehmann (136) five ml. of the solution containing myoglobin pigment was

oxidized to cyanometmyoglobin by the addition of a drop of 12 percent acetic acid and a drop of five percent potassium cyanide solution. After five minutes of standing at room temperature, the solution was filtered. In order to have a workable quantity, at least 20 ml. of the cyanometmyoglobin solution was prepared.

The optical density of the color of the filtrate was measured at 540 mu.

In order to relate the percentage of cyanometmyoglobin to the fresh meat, the optical density obtained at 540 mu was multiplied by a factor 1.456 (35). This factor was obtained by dividing the molar concentration (C) by the extinction coefficient (e), in accordance with the relationship:

$$\frac{C_1}{C} = \frac{D}{e}, \text{ and } C_1 = D \times \frac{C}{e}$$

$$= D \times \frac{16,452}{11,300}$$

$$= D \times 1.456$$

Where D is the density of the color measured by the spectrophotometer, C_1 is the concentration of cyanometmyoglobin in grams per litre of the filtrate. The molecular weight of cyanide derivitive of one heme unit is 16,452 and the molecular extinction at 540 mu is 11,300.

Since 10 grams of meat were dispersed through 100 ml.- that corresponds to the dispersion of 100 grams of meat

through 1000 ml. of water - the product, D x 1.456, represents the amount of cyanometmyoglobin in grams per 100 grams of fresh meat. The data has been further calculated in milligrams per 100 grams of the fresh meat.

Amino Acids Determination

The determination of alpha amino acids was made by Folin's colorimetric method as described by Hawk, Oser and Summerson (60). The principle of the method is the development of the color between the amino acids and B-naphtha-quinone-4-sulfonic acid in alkaline solution.

According to Hiller and Van Slyke as described by Hawk (60), trichloroacetic acid in the concentration of less than five percent removed only proteins and did not precipitate partially digested protein products, so that these products together with amino acids and non-protein nitrogenous substances were retained in the filtrate. Preliminary experiment showed that the use of trichloroacetic acid was superior to sodium tungstate in removing partially digested proteins.

Ten grams of ground fresh muscle were blended in an osterizer with 60 ml. of water for three minutes. Thirty ml. of five percent trichloroacetic acid solution were added to this mixture. After standing for thirty minutes the mixture was filtered, using a Buckner funnel. This

gave a 1:10 dilution of the whole muscle. Five ml. of this protein-free filtrate were used for the colorimetric determination. The optical density was measured at 510 mu using a Beckman DU quartz spectrophotometer. The determinations were performed in duplicate with each muscle and the mean of the two determinations is reported.

Sulfhydryl (-SH) Group Determination

The method of Hellerman, Chinard and Ramsdell (62), using O-iodosobenzoic acid, as described by Bolling and Block, was used for the determination of sulfhydryl groups. The O-iodosobenzoic acid was not available. Therefore, it was synthesized by the writer by the method of Askenasy and Meyer (1). It was recrystallized three times to obtain suitable purity.

The O-iodosobenzoic acid reacts with sulfhydryl groups according to the following equation:

$$2\ RSH + OIC_6H_4COOK \longrightarrow RSSR + IC_6H_4COOK + H_2O$$

$$OIC_6H_4COOK + 2HI \longrightarrow IC_6H_4COOH + I_2 + H_2O$$

Ten grams of the ground fresh muscle were blended for three minutes in an osterizer with 90 ml. of water. The mixture was filtered through a cheese cloth. Twenty-five ml. of the filtrate were pipetted into a flask. Five ml. of a phosphate buffer of pH 7.0 and 10 ml. of 0.02 O-iodosobenzoic acid were added. After one minute a

mixture of freshly prepared solution containing about 750 mg. of KI in 1.5 ml. of water and five ml. of N HCl was added. The liberated iodine was titrated at once with a standard solution of 0.001 N $Na_2S_2O_3$, using starch as an indicator.

From the volume of the sodium thiosulfate solution used, the amount of the sulfhydryl group (-SH) in 25 ml. was found and then calculated to the mgs. of -SH per 100 grams of fresh meat. The determinations were made in triplicate on each muscle and the average calculated.

Connective Tissue Determination: The most common method for the determination of the connective tissue is the direct method where connective tissue is determined gravimetrically. This method has been used by many workers (69,70,82,141). Recently, Baker, Lampitt and Brown (5,6, 78, 79) and Wierbicki et al. (140) developed a new method where connective tissue, through its constituent hydroxyproline, can be determined colorimetrically. Both of these methods were used in the present study.

A. Gravimetric Method: Most methods for the estimation of collagen and elastin in meat are based upon their being insoluble in dilute alkali. One such method is that of Lowery, Gilligan and Katersky (69), which has been modified to overcome some of the difficulties reported by him. For

instance, a meat slurry using equal parts of meat and water, rather than one part of meat and two parts of water, gave a more homogenous slurry for further separation into aliquaot portions. Also, holding in an incubator (37° C) instead of at room temperature prevented the after alkali treatment solidification of fat.

B. Colorimetric Method: The amino acid hydroxyproline amounts to 13 to 14.5 percent of the total amino acid content of collagen, whereas the globular protein of the skeletal muscle contains no hydroxyproline. Based on this unique high content of hydroxyproline in the connective tissue, Neuman and Logan (98) developed a method for the determination of this amino acid in small quantities as a means of estimating the amount of collagen and elastin. The method was slightly modified by Wierbicki et al. (140, 141) Baker, Lampitt and Brown (5,6) for the determination of hydroxyproline in meat slurry. Wierbicki's method was adopted where fresh ground muscle was used instead of meat slurry.

The method is based upon the oxidation of hydroxyproline with sodium peroxide to a product that forms an intense red color with p-dimethylaminobenzaldehyde. The intensity of the color is directly proportional to the hydroxyproline concentration up to 20-25 milligrams per ml.; as measured by a Beckman model DU Spectrophotometer

at 560 mu. The method used was as follows:

Ten grams ofthe ground muscle were accurately weighed into a tared dry flask with a ground glass mouth which fits onto a condenser. Stannous chloride, weighing 3/4 of the weight of the protein of the sample of the meat, was added to the flask. Twenty ml. of 6 N HCl were added to the flask and the mixture was refluxed for seven hours at a temperature of 135 to 140° C. Stannous chloride, by its reducing action, prevents the formation of a humin substance. The rest of the procedure was the same as described by Wierbicki et al. (140) and Baker, Lampitt and Brown (5,6). Briefly, the method was adjusting the pH of the hydrolysate to 7.5 to 8, using 6 N NaOH and 5 drops of concentrated $CaCl_2$ solution and 5 ml. of saturated solution of $CaCO_3$, and making the total volume of the hydrolysate to 250 ml. This dilution gave 40 mg. of fresh meat per ml. of the hydrolysate. The hydrolysate was clarified and oxidized with H_2O_2 in the presence of copper. The color was developed by using p-dimethylaminobenzyladehyde and was measured by a Beckman DU quartz Spectrophotometer at 560 mu. The experiment was run in duplicate and two samples from each hydrolysate were taken for the color development.

Neuman and Logan (98) stated that connective tissue can be calculated from the hydroxyproline determination by

using a conversion factor of 7.46. The factor obtained by Wierbicki *et al.* was 8.07.

In the present study a factor of 8.16 was obtained by determining the amount of hydroxyproline in a sample obtained by combining the connective tissues isolated from the 20 muscles in the gravimetric determinations. This factor, which is very near to that of Wierbicki *et al.* was used for the calculations of the connective tissue in the muscles being studied.

pH of the Muscle: The pH of the muscles was determined, immediately after grinding the muscles, with Beckman pH meter, model G.

Preparation of Muscle Extract for Analysis

Extraction was made with a buffer solution of pH 5.6. This buffer was made just before use by mixing two solutions, A and B, which had been stored separately at 38° F. Solution A consisted of 21 grams of citric acid and 200 ml. of N KOH made up to one litre with 0.4 M KCl solution. Solution B was 0.1 N KOH. The ionic strength of this buffer is 0.48. The concentration of KCl is 0.22M at this pH.

Twenty grams of frozen meat sample were thawed and weighed into an osterizer jar and 80 ml. of the buffer were added. The jar with contents was cooled in a refrigerator

at 38° F for 15 minutes. The mixture was blended for three minutes in the osterizer, let stand for five minutes in ice water, blended for two minutes more, and let stand at room temperature for five minutes. The meat slurry was transferred to 50 ml. graduated centrifuge tubes and c centrifuged for one hour at 1800 RPM in a cooler held at 38° F. The centrifuged material was then allowed to stand for half an hour in the cooler. A thin layer of fat solidified at the top and the supernatant extract separated from the residue. In almost all cases, the supernatant liquid was very clear. The extraction was performed in duplicate on each control and treated muscle.

The time of blending was kept constant during the extraction, since, according to Hasselbach and Schneider (59), the portion of F-actin in the extract is greater, the longer the blending time. As reported by Weber and Portzehl (137), four minutes extraction results in the maximum extraction of actin and selectivity of the buffer for the extraction ofactin at this pH is maximum.

The subsequent analyses of the extract were carried out on the same day, since a cloudy precipitate was formed by keeping it overnight. The extract was kept in the refrigerator except at the time of analysis, because it was found that the color of the extract changed and the precipitate was formed by allowing it to stand even at

room temperature. This precipitate could result from a gradual spontaneous flocculation of myogen, usually observed in muscle on aging (12) or from the formation of actomyosin in the extract from the actin and myosin present.

Chemical Analysis of Muscle Extracts

Total Nitrogen: Five ml. of the extract of protein was used for the determination of protein by the macro Kjeldahl-Gunning-Arnold method. Ten ml. of the extract were used in the later studies.

Refractive Index: After filtering the extract, 10 ml. of the extract were used to determine the refractive index by the immersion type Bosch and Lomb refractometer. Sometimes, it was difficult to take the readings due to cloudiness. In that case, a small quantity of $CaCl_2$ was added to the extract which was then filtered before taking the readings.

Sulfhydryl groups: Five ml. of the extract were used for the determination of sulfhydryl groups using O-iodosobenzoic acid. The extract was diluted with CO_2 free water before making the determination.

Amino acids: A 1:10 dilution of the extract was made with 5 percent trichloroacetic acid before determining amino acids by Folin's colorometric method as described by Hawk et al. (60).

Chemical Analysis of the Muscle Residue

Total Nitrogen: About two grams of residue were exactly weighed and nitrogen was determined by the macro Kjeldahl-Gunning-Arnold method.

Amino acids: A 1:10 dilution of the residue was made with 5 percent trichloroacetic acid before determining amino acids by Folin's colorometric method.

Sulfhydryl groups: Ten grams of the residue were used for the determination of the sulfhydryl groups, using the same procedure as mentioned before.

Action of Intrinsic Enzymes on Gelatin

To determine the influence of salt on the action of pepsin, trypsin, and chymotrypsin on gelatin as the substrate, the following procedure was used:

The substrate solution was made by first adjusting a one percent C.P. gelatin solution to pH 5.4 (that of meat). To part of this solution sodium chloride was added to give a two percent solution. Each of the enzymes was made separately into a 0.2 percent solution and added to the salted or unsalted substrates in amounts to provide an enzyme concentration of 0.002 percent. Flasks containing 50 ml. of substrate and enzyme were held at room temperature for 48 hours.

Preliminary trials showed that enzyme activity was

insufficient if temperatures of 37° C or 28° C were used. At the end of the 48 hours digestion period, sulfhydryl groups and the amino acids were determined in triplicate on the salted and the unsalted samples. The mean of these three readings was recorded.

Histological Studies

In an effort to see what changes had taken place in the muscle bundles after perfusion with sodium chloride solution, histological studies were made.

Preparation of the Tissue: Pieces of muscle about 1 x 1 x 2 cms. were cut from the control and treated muscles before and after cooking. The cut sections were preserved in tubes containing a preservative which consisted of 75 parts of picric acid, 25 parts of formaldehyde, and 5 parts of glacial acetic acid. A few crystals of urea were added to this mixture. Urea helped the penetration of the preservative into the cells of the muscle sections. The sections were kept in the preservative for two weeks.

Clearing: The preservative was replaced with 70 percent alcohol containing lithium carbonate. This solution was changed every day until the solution added was not colored after 24 hours. The clearing of this solution took two months. Two small sections were cut from each original

sample of muscle and the rest of the sample was saved as a stock sample. These sections were immersed in the following solution, according to the stated periods of time and number of changes:

90 percent alcohol (30 minutes)	one change
Absolute alcohol (30 minutes)	one change
Absolute alcohol - xylol (15 minutes)	one change
Xylol (1½ hours)	two changes

Embedding: The pieces of tissue were put into a bottle of melted paraffin wax and xylol kept in the liquid state in a warm cabinet. Following this, the mixture of wax and xylol was replaced four times by fresh melted wax at two hours intervals, and kept in the warm cabinet. By this time the wax had permeated the tissue and replaced all the clearing agent. A little paper boat was then filled with melted paraffin and the piece of the tissue was placed deep into the boat. The boat was then launched in a dish of ice cold water and held upright long enough for a scum of solid paraffin to form on its surface, after which it was allowed to float to solidify the wax, thus embedding the tissue.

Sectioning and Attaching to Slides: Each embedded tissue was fixed on a wooden block and very thin sections of 10 microns (1 micron is 1/1000 mm.) were cut in a ribbon. The individual sections were carefully separated from one

another with a scalpel on a white sheet of paper. The glass slides were cleaned with alcohol, and identified by marking. A very small drop of egg albumum was rubbed on the slide until dry. (Egg albumum acts as an adhesive.) Distilled water was put on the slide and then four or five small sections ofthe tissue were added. The slide was placed in an oven at 50° C (slightly lower than the melting point of the wax), and the sections were fixed. While the slide dried the section became attached to the slide. Duplicate slides were prepared from each section of the muscle. Thus there were four slides from each control and treated muscle.

Staining: A double stain in hematoxylin and eosin (52) was accomplished by passing the slide (and the tissue) through the following solutions for the times mentioned:

Xylene	two minutes
Xylene	two minutes
Absolute alcohol	two minutes
95 percent alcohol	two minutes
70 percent alcohol	two minutes
50 percent alcohol	two minutes
Water	two minutes
Hematoxylin solution	six minutes

The slides were dipped in a bowl of water made barely alkaline with ammonia, then passed through the following

Eosin in 95% alcohol

(Two grams per 100 ml.)

95 percent alcohol

Absolute alcohol

Absolute alcohol - xylene

Xylene

Xylene - beechwood creosote

Xylene - beechwood creosote

Mounting: A drop of Canada balsam was placed over the section and a cover slip dropped gently over it to spread it evenly. The slides were then dried at 50° C for a few days.

Chapter IV

RESULTS

Tenderness of Muscles as Determined by Taste Test Panels

The results of the evaluation of tenderness with taste test panels are shown in Table II. These data are obtained from individual ballots compiled under three main headings; (1) treated more tender; (2) control more tender and (3) no difference, to show how the panels related the samples. Statistical analysis was made and the F value at 5% level of significance is reported.

The results show that the perfused muscle was significantly more tender than the control muscle of each pair.

The degree to which the perfused muscles were more tender than the controls was not determined exactly in the testing procedure used. However, on the basis ofthe scoring scale used the panels judged the control muscles as averaging about 4.51 and the perfused muscles as about 6.06.

Effect of Perfusion of Water on the Tenderness of Muscle

The three pairs of steaks in which one was kept as a control and the other perfused with water, did not show any change in tenderness. The results of the evaluation of the taste test panels is shown in Table III. The scores

Table II

Balloting for Tenderness by a Taste Test Panel

Pair No.	Total Ballots	Ballots Showing Perfused more tender	Control more tender	No difference	F value
1	15	11	2	2	9.95*
2	17	12	4	1	4.55*
3	14	11	3	0	6.30*
4	11	8	0	3	11.23*
5	14	9	1	4	6.30*
6	12	9	1	2	8.79*
7	12	11	0	1	7.97*
8	14	11	2	1	9.16*
9	12	9	2	1	5.67*
10	13	12	0	1	69.12**

*Significant at 5% level.

**Highly significant at 1% and 5% level.

obtained from the individual ballots were compiled and the tenderness scores were compared. The results indicate that the muscles perfused with water did not show any significant change in tenderness.

Table III

Effect of Perfusion of Water on the Tenderness of Muscles

Pair	Scores	
	Control	Perfused
1	5.53	5.20
2	5.73	5.66
3	4.93	4.80

Chemical Analysis of the Muscles

The chemical analysis of isolated paired beef muscles of ten animals is shown in Table IV. The results showed, as expected, that the moisture content in the perfused muscle was higher than in the control muscle of each carcass. This was due to perfusion of one muscle with sodium chloride solution. Therefore, it was necessary to use a basis upon which the constituents could be compared fairly.

Since the total nitrogen content of the right and left Biceps brachii muscles within an animal should be the same, within experimental error of determination, and since the

Table IV

Chemical Composition of M. Biceps Brachii with and without Perfusion* with Sodium Chloride Solution (Means of 10 Control and 10 Perfused Muscles)

Basis: Entire sample of muscle	Control	Perfused
Moisture, %	75.80	77.96
Fat, %	2.96	2.32
Crude Protein (N_2 x 6.25), %	21.49	17.62
Sodium chloride, %	.11	2.00
Free amino acids, mg. per 100 gm.	154.7	153.7
Sulfhydryl groups, mg. per 100 gm.	83.1	74.8
Metmyoglobin, mg. per 100 gm.	896.6	818.00
Hydroxyproline, mg. per 100 gm.	52.5	48.3
Basis: Moisture-free, fat-free, and sodium chloride-free		
Crude protein (N_2 x 6.25), %		
Free amino acids, %	.73	.87
Sulfhydryl groups, %	.39	.42
Cyano-metmyoglobin, %	4.16	4.92
Hydroxyproline, %	.249	.273
Basis: Percent of crude protein (N_2 x 6.25)		
Free amino acids	.72	.87
Sulfhydryl groups	.387	.424
Cyano-metmyoglobin	4.170	4.640
Hydroxyproline	.244	.274

experimental treatment of a muscle by perfusion would not change its total content of nitrogen, the minor constituents in which there is greatest interest were related to total crude protein.

One of each pair of muscles from each of ten beef carcasses was perfused with 60° Salometer salt solution to increase the muscles' weight 10 percent.

Amino acids, sulfhydryl groups, cyano-metmyoglobin, when presented as percent of protein, showed increases in the treated samples. To determine the extent of the increases, confidence intervals at the .95 level of confidence was determined and found to be as shown in Table V.

Table V

Confidence Intervals at .95 level of Confidence Showing Increases of Free Amino Acids, Sulfhydryl Groups, and Cyano-metmyoglobin in Perfused Muscles

Component	The increase is:
Free amino acids	1.07 to 1.99%
Sulfhydryl groups	0.41 to 1.64%
Cyano-metmyoglobin	.045 to 3.79%

Complete data for the ten pairs of muscles from which the figures in Tables II and IV were summarized are given in Appendix I, II, III, IV and V.

Connective Tissue Determination

The results of connective tissue determinations by both gravimetric and colorimetric methods is shown in Tables VI and VII. The data obtained has been examined statistically. The statistical analysis shows no difference in connective tissue between the control and the treated samples by both colorimetric and gravimetric methods at one percent of five percent level of significance. The data obtained by gravimetric and colorimetric methods are shown in Appendixes IV and V.

Table VI

Mean Values for Connective Tisue in Muscles

Basis: Fresh Experimental Samples	Control	Perfused
Gravimetric method, %	.679	.725
Colorimetric* method, %	.428	.394
Basis: Percent of Crude Protein (N_2 x 6.25)		
Gravimetric method, %	3.135	4.10
F value		2.59†
Colorimetric method, %	2.230	2.258
F value		0.008†

*By determination of hydroxyproline.

†Not significant at 5% level or 1% level.

Table VII

Connective Tissue as Percent of Crude Protein in *M. Biceps brachii* as Determined by the Gravimetric and Colorimetric Methods

Pair No.	Gravimetric method		Colorimetric method	
	Control	Treated	Control	Treated
1	5.22	5.27	1.76	1.29
2	3.64	7.35	1.75	2.89
3	3.23	3.30	2.69	2.15
4	2.33	4.40	2.04	1.26
5	1.46	2.27	1.27	2.32
6	4.54	3.81	2.04	2.53
7	3.73	3.43	2.40	1.66
8	2.85	4.49	1.89	3.18
9	2.46	4.36	3.99	2.76
10	1.89	2.32	2.47	2.54
Mean	3.135	4.100	2.23	2.258
F value		2.59†		.008†

† Not significant at 5% or 1% level of significance.

Chemical Analysis of Protein Extract

The percent protein extracted with the citrate buffer was 34.10 and 32.12 percent of the original total protein in the muscles in the control and the perfused respectively. The data obtained on protein extracted, amino acids, sulfhydryl groups and refractive index determinations is shown in Appendixes VI and VII. Since there was an increase in these constituents in the perfused muscles, a confidence interval at .95 was determined as shown in Table VIII.

Table VIII

Confidence Intervals at .95 Level of Confidence Showing Increases of Free Amino Acids, Sulfhydryl Groups and Refractive Index of Protein Extract with Citrate Buffer

Analysis	The increase is
Free amino acids	1.56 to 7.89%
Sulfhydryl groups	0.46 to 11.5 %
Refractive index	0.53 to 1.38%

Effect of Holding Period on Tenderness

The mean composition of the muscles of each treatment is shown in the Appendix VIII. The analysis of variance of the chemical analysis is shown in Table IX. These data showed that there was a great difference between the pairs of muscles, but the perfusion with salt had no effect

Table IX

Effect of Holding Period on the Tenderness of Muscles

Source of variation	d.f.	Mean Squares Taste test	pH	Myoglobin
Blocks	14	140535*	67.86*	51071*
Treatments	5	270732**	13.60	1640
Error	10	29297	9.2	1621

Source of variation	d.f.	Mean Squares Cyanomet-myoglobin	Sulfhydryl groups	Amino Acids
Blocks	14	308356*	215113**	63975**
Treatments	5	48361	6290	13473
Error	10	64552	13749	5930

Source of variation	d.f.	Mean Squares Connective tissue	Amino acids of extract
Blocks	14	557*	390*
Treatments	5	108	143
Error	10	126	113

Source of variation	d.f.	Mean Squares Sulfhydryl groups of extract	Sulfhydryl groups residue	Amino acids of residue
Blocks	14	7407**	70967**	27304**
Treatments	5	2872*	3526	2959
Error	10	693	41688	187

*Significant at 5% level.

**Highly significant.

other than to increase the amount of sulfhydryl groups in the extract.

These data were also calculated as percent of crude protein (Appendix IX) and then analysis of variance was computed as shown in Table X. These data as constrasted to data on percentage composition of the whole muscle, indicated a change in cyanometmyoglobin and amino acids of muscles and amino acids of the extract. Connective tissue, hydroxyproline, sulfhydryl groups of extract, amino acids and sulfhydryl groups of residue did not show any significant difference. However, the block effect was present in each analysis, indicating the differences between different animals.

Adjusted means were calculated for the data on taste test, cyanometmyoglobin, amino acids of muscles and extract. The adjusted mean values are shown in Table XI and Graphs 1, 2, and 3 respectively.

Table X

Effect of Holding Period on the Tenderness of Muscles as Percent of Crude Protein

Source of variation	d.f.	Mean Squares: Taste test	Myoglobin	Cyano-metmyoglobin	Amino acids
Blocks	14	140535*	1416**	2190**	186**
Treatments	5	270732**	62	230*	6
Error	10	29297	44	31	1

Source of variation	d.f.	Mean Squares: Sulfhydryl groups	Hydroxy-proline	Connective tissue
Blocks	14	66**	2286*	1517*
Treatments	5	4	136	104
Error	10	5	432	333

Source of variation	d.f.	Mean Squares: Amino acids extract	Amino acids residue	Sulfhydryl groups extract	Sulfhydryl groups residue
Blocks	14	3*	185236**	1929*	525614**
Treatments	5	6**	1352	740	2224
Error	10	1	951	260	2748

*Significant at 5% level.

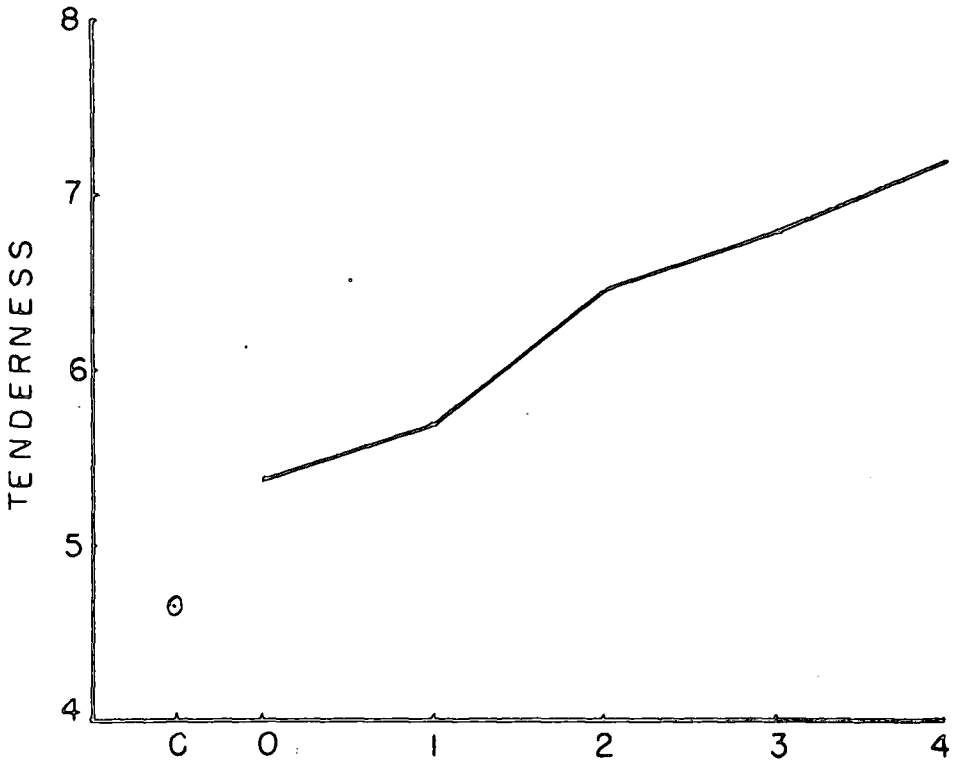
**Highly significant.

Table XI

Analysis of the Muscles Adjusted Means

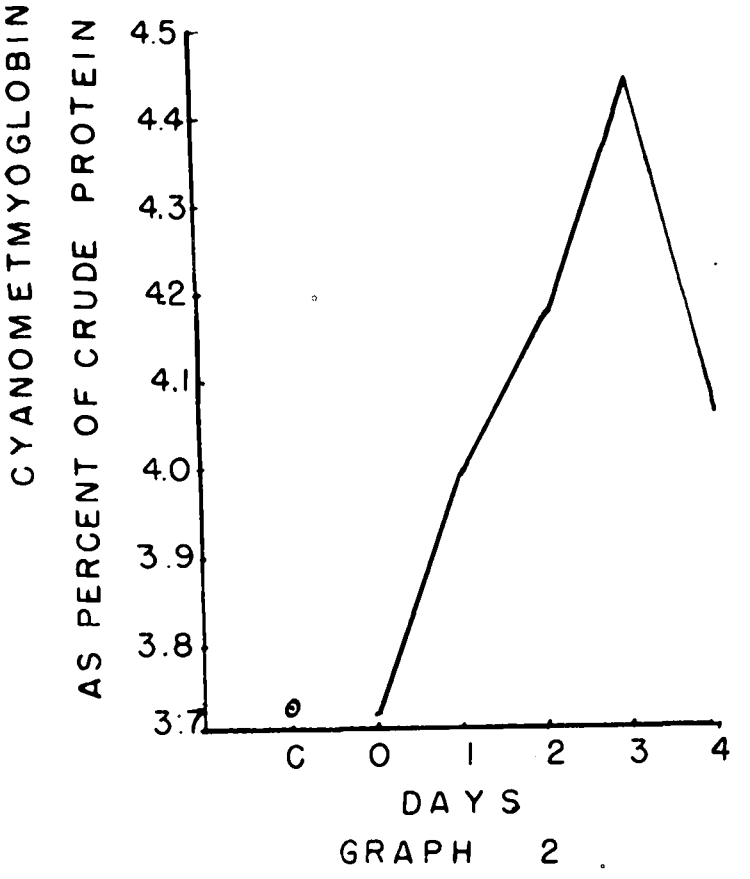
Analysis	Control	Treatment Days 0	1	2	3	4
Taste test	4.67	5.38	5.70	6.44	6.80	7.20
Sulfhydryl groups of extract mg.	8.36	7.90	7.65	6.66	8.13	5.83
Basis: Percent of Crude Protein						
Cyanometmyoglobin	3.73	3.72	3.99	4.17	4.44	4.06
Amino acids						
Muscles	.893	.927	.925	1.020	.971	.958
Extract	.283	.338	.360	.402	.382	.394

EFFECT OF HOLDING TIME ON TENDERNESS OF BEEF MUSCLES AFTER PERFUSION WITH SODIUM CHLORIDE.



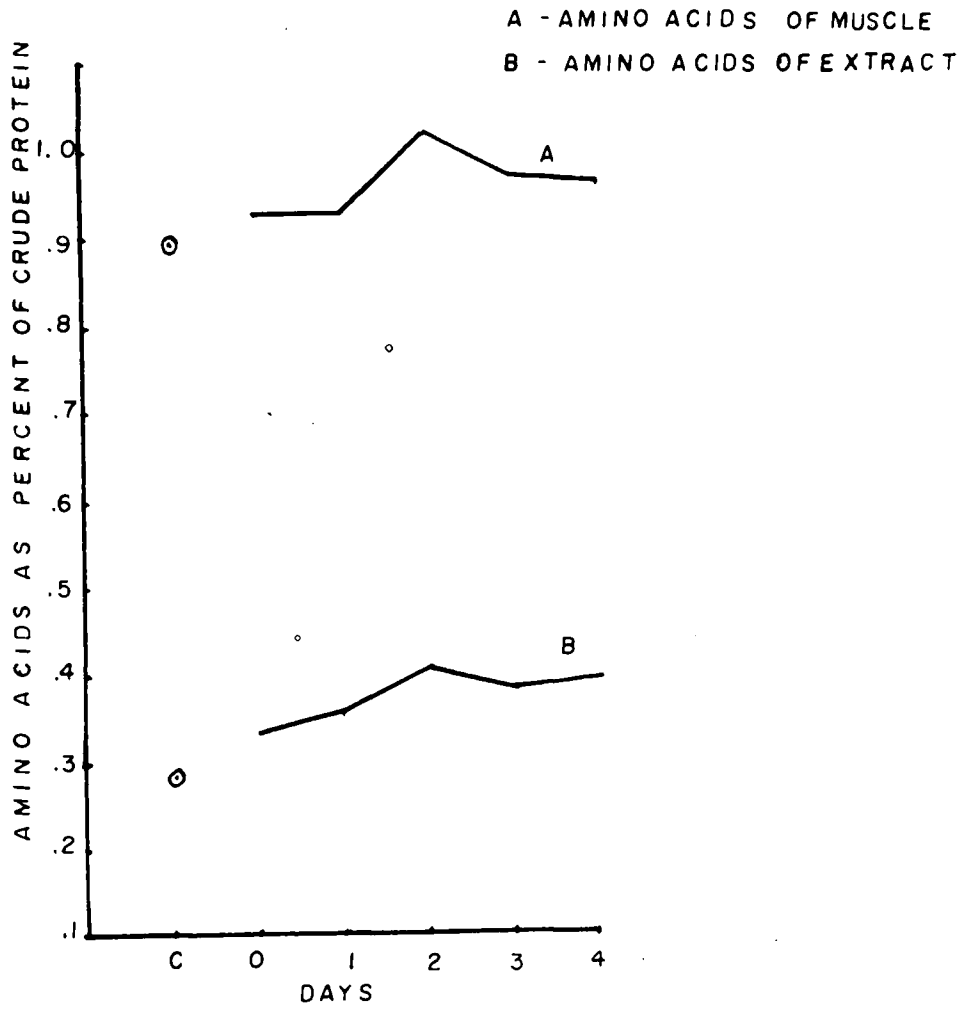
GRAPH 1

EFFECT OF HOLDING TIME AFTER PERFUSION OF SODIUM CHLORIDE IN BEEF MUSCLES ON THE CYANOMETMYOGLOBIN CONTENT.



GRAPH 2

EFFECT OF HOLDING TIME AFTER PERFUSION OF SODIUM CHLORIDE IN BEEF MUSCLE ON AMINO ACID CONTENT.



GRAPH 3

Histological Study

Each slide was observed under the 30X power lens giving the slide (300X). The photographic picture of some typical slides were taken as shown in Figures 2, 3, 4 and 5.

Figure 2 shows the cross section of the control muscle tissue (no salt treatment and uncooked). The nuclei and the connective tissue are in good condition and the bundles of the muscle show definite arrangement. (Compare with Figure 3, 4 and 5.)

Figure 3 shows the cross section of cooked control muscle tissue (no salt treatment but the meat cooked at broiler temperature for 8 minutes before the tissue was fixed for histology. Notedisarrangement of connective tissue between bundles of muscle and also note that nuclei have been dislocated, and are abnormal. The muscle bundles have shrunk in size and appear more condensed. Compare with Figures 2, 4 and 5.

Figure 4 shows the cross section of uncooked muscle tissue treated with two percent salt (20 percent salt solution and increasing the weight of the muscle by ten percent). Note that the nuclei have been damaged or destroyed and that the connective tissue between muscle bundles is almost completely broken or disarranged. The muscle bundles are loosened and expanded in size. Compare

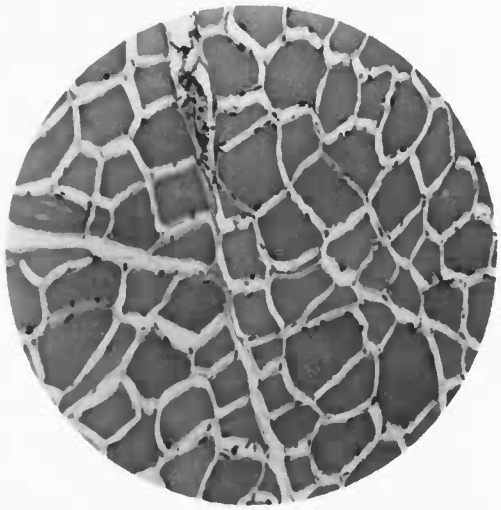
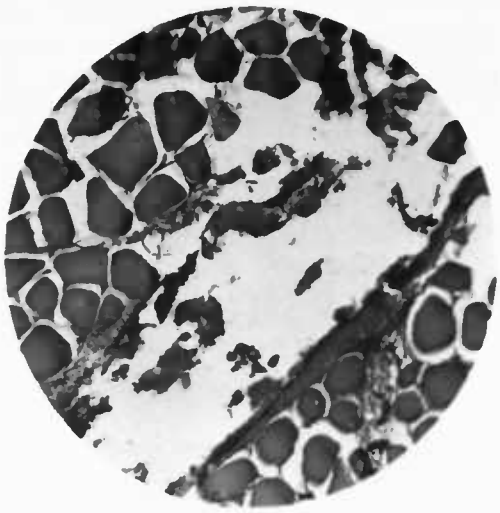


Figure 2

Figure 3

Figure 4



Is this cooked, sort-of?

Figure 5

with Figures 2, 3 and 4.

Action of Intrinsic Enzymes in Gelatin

The results of amino acids and sulfhydryl group determinations on one percent gelatin of pH 5.4 containing .02% intrinsic enzymes (pepsin, trypsin and chemotrypsin) as a substrate was compared with one containing two percent salt. These are shown in Table XII. The data indicate that the behavior of these enzymes on this substrate is not the same.

The amino acids decreased in the treated samples in the presence of pepsin while they increased in the presence of trypsin and chymotrypsin.

The sulfhydryl groups increased in the presence of salted samples in the presence of pepsin and trypsin while they decreased in the presence of chymotrypsin.

Table XII

Action of Intrinsic Enzymes on One Percent Gelatin Solution Milligrams of Amino Acids and Sulfhydryl Group Per 100 Ml. of Solution

Enzyme	Amino acids		Sulfhydryl group	
	Control	Treated	Control	Treated
Pepsin	14.30	11.91	10.56	11.22
Trypsin	12.59	12.72	8.91	8.99
Chemotrypsin	11.06	11.87	11.22	9.24

Chapter V

DISCUSSION

Tenderness tests have been made by a taste test panel on ten pairs of beef muscles Biceps brachii isolated from the right and left shanks of the carcass. The muscle perfused with salt was found to be more tender than the muscle of the same pair which was given no treatment. As one would expect, the tenderness increased with the longer holding time after perfusion. Statistical analysis of the taste tests on tenderness showed that there was a great difference among the animals. However, the adjusted means shows that tenderness increased on perfusion with salt and was greater as the muscles were held longer after perfusion.

The pairs of muscles, perfused with water and the control did not show any change in tenderness. This indicates that the increased tenderness of the muscles is due to the salt and that water has no part to play if used alone.

Tenderness is very closely related to the color of the meat. As the tenderness increased, the cyanometmyoglobin increased. The results obtained are similar to that obtained by Watts and Lehmann (136) who determined the effect of salt on hemoglobin.

The color of the intact muscle appeared to improve with the increase in the number of days after perfusion. It reached the maximum three days after perfusion and then started decreasing. This may be due to the oxidation of the myoglobin pigment into brown pigment and hence the conversion of myoglobin to cyanometmyoglobin decreased.

Connective tissue, determined by both gravametric and colorimetric methods on ten pairs of muscles (perfused and control) did not show any difference statistically. Similarly, no difference in connective tissue has been noted when the muscles perfused were held for different duration of time. A similar result has been reported by Husaini et al. (69) and Hershberger (63). The colorimetric method for the determination of connective tissue, seemed to be less time-consuming and clear cut.

Amino acids and sulfhydryl groups of both muscle and extract from the perfused muscle were higher than in those which were given no treatment. However, the statistically designed experiment to study the holding period effects, showed only increase in amino acids. The maximum increase of amino acids was found two days after perfusion. The increase in amino acid continued, but with slightly lower rate. The increase in amino acids indicates the extent of the degradation of proteins. Since there is no change in connective tissue, therefore, the degradation is in the

muscle bundles or intracellular protein.

The increase in amino acids nitrogen is similar to that obtained by Hoagland et al. (65). The increase in sulfhydryl groups in the perfused muscle is similar to the findings of McCarthy and King (87).

The increase of amino acids in the extract containing myosin and actin followed a similar pattern to that of the muscle amino acids. The amino acids content decreased slightly on three days of storage after perfusion. The reason for this decrease is due to a great variation in the muscles obtained from different carcasses of great variation in composition. However, the amino acids again showed an increase on four days storage after perfusion.

The increase in the refractive index of the extract containing the same quantity of salt in the control and perfused muscles, indicates that the salt on perfusion after storage has been able to dissolve some of the muscle proteins (intracellular). These proteins in solution result in an increase in the refractive index.

There was no significant change in the amino acids and sulfhydryl groups of the residue after extracting myosin and actin from the muscle proteins. This indicates that actomyosin and other fractions of protein besides myosin and actin do not play any important part in the change of tenderness of beef muscles.

Histological studies indicate that salt has an effect on the physical state of the muscle bundles in addition to its action as a solvent for certain protein fractions. The muscles perfused with salt and cooked, shrank and disintegrated as compared to those which were cooked but given no treatment. The latter shrank, but did not disintegrate. Thus the salt has disintegrating effect on the muscle bundles. In other words, it has been able to bring about degradation of the proteins and thus make the meat more tender.

Further, salt increases the water binding capacity of the meat proteins(122). The samples of ground muscles of both perfused and control muscles were frozen. On thawing these samples, the meat which was given no treatment showed a drip, whereas the perfused ground meat had no drip. Thus, the greater the amount of water held by the proteins, the more juicy and tender the meat is. Therefore, the increase in the hydration of the proteins seems to have resulted in the increase in tenderness of muscles perfused with salt.

These studies indicate only a few avenues of study which might throw some light on why sodium chloride tenderizes beef. Further work is necessary, and the following suggestions may be of value:

1. The extraction studies may be carried out with other buffers and through the period of rigor. There is

the possibility that other extractants may be more satisfactory than the one used.

2. The determination of the changes in the non-protein nitrogen of the extract.

3. The electrophoretic studies of the proteins extracted may be carried out. This seems to be the most promising method for a qualitative and quantitative evaluation of meat proteins in the extracts. As reported by Hamoir (57), electrophoresis appears to be more useful for the study of complicated mixtures of muscle proteins than ultracentrafugation.

4. The analysis of urea-nitrogen would be of interest inasmuch as urea is engaged in breaking up the bonds within the contractile elements (21,127). Determination of nucleotides such as adenylic acid, hypoxanthine, uric acid, and allantoin for the control and the treated muscles would be of interest. These compounds would allow some insight into the changes involved during the course of the tenderization of beef.

Chapter VI

SUMMARY

Ten pairs of Biceps brachii muscles were isolated from the right and left shanks of the beef carcasses. One muscle from each pair was perfused with twenty percent salt solution so as to increase theweight of the muscle by ten percent. This gave two percent salt content in the muscle. The other muscle from the same pair was kept as a control and was given no treatment.

The muscles were held at 38° F in the cooler for 72 hours and then t aste tests were carried out by a taste panel to determine the tenderness of the muscles. The remaining portion of the muscles was ground and kept separately in an air tight container in the refrigerator for chamical analysis.

Three pairs of muscles Biceps brachii were isolated to study the effect of water. One muscle from each pair was kept as a control and the other was perfused with water, increasing the weight of the muscle by ten percent. These muscles were also held for 72 hours at 38° F in the cooler; and then taste tests were performed by a taste test panel.

The chemical analysis included the determinations of myoglobin, cyanometmyoglobin, amino acids, sulfhydryl

groups, connective tissue and hydroxyproline in addition to the moisture, fat, protein and sodium chloride.

A citrate buffer of pH 5.6 was used to extract actin and myosin from the meat and determinations of protein, amino acids, sulfhydryl groups and refractive index, were made.

The effect of different periods of holding the muscles was studied by a statistically designed experiment.. Fifteen pairs of beef muscles Biceps brachii were isolated and perfused at random with 20 percent salt solution in a similar manner as mentioned before, according to the incomplete block design method. The time after perfusion was 0, one day, two days, three days and four days. Some of the muscles acted as control. After the prescribed time of holding, taste tests were carried out by a taste test panel. The above mentioned chemical analysis were made. In addition to these analysis, pH determinations were made and also the residue after removing the extract with the citrate buffer, was analyzed for protein, amino acids and sulfhydryl groups.

Histological studies were made on the control and the perfused muscles, before and after cooking.

Chapter VII

CONCLUSIONS

1. Beef muscles have been tenderized by perfusing with sodium chloride to increase the salt content of the raw muscle to about two percent. The tenderness increases by prolonging the holding time after perfusion, at least upto four days.

2. Muscles perfused with water in a similar manner as that of salt perfusion, did not change in tenderness. As a matter of fact perfusion of water slightly decreases the tenderness. However, this decrease is too small to show any significant difference.

3. Chemical analyses of the muscles, when presented as percent of crude protein or moisture-fat-and salt-free basis show:

a) Cyanometmyoglobin increases with the perfused muscle over the control. This increase is greater with longer holding periods.

b) The amino acids content of the muscles and the extract of the muscles, containing mainly actin and myosin, increased with the perfused muscles. This increase was greater as the time of holding the muscles after perfusion was increased. However, during this period, the increase was not the

same.

c) Sulfhydryl groups increased in the perfused muscles, but did not show any significant difference for different periods of holding after perfusion.

d) The amino acids and sulfhydryl groups in the residue left after extracting actin and myosin did not show any significant change.

4. The amount of connective tissue of the muscles did not change.

5. Histological studies showed that salt disintegrates the muscle bundles. The effect was more pronounced on the cooked muscles than on the uncooked.

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A P P E N D E X E S

Appendix I

Percentage Composition of Ten Paired Muscles

	Control				Treated			
No.	Moisture	Fat	Protein	Sodium Chloride	Moisture	Fat	Protein	Sodium Chloride
1	75.39	2.51	23.86	.11	77.31	2.13	19.93	1.90
2	77.62	1.23	22.39	.11	79.61	1.02	17.63	1.88
3	75.38	2.57	22.00	.09	77.65	2.02	16.95	1.72
4	74.58	4.40	22.17	.20	76.14	3.32	19.19	2.32
5	76.10	2.87	21.62	.11	78.22	2.25	18.43	1.99
6	75.33	2.33	20.50	.04	77.10	2.00	18.70	2.01
7	77.26	2.12	21.18	.08	79.20	2.42	15.67	2.30
8	75.84	3.41	20.47	.08	78.76	2.26	15.54	2.11
9	78.62	0.87	20.44	.14	81.23	0.47	16.60	1.96
10	71.87	7.25	20.27	.09	74.36	5.29	17.60	1.80
Mean	75.80	2.96	21.49	.11	77.96	2.32	17.62	2.00

Appendix II

Milligrams of Amino Acids, Sulfhydryl Groups and Cyanometmyoglobin Per 100 Grams of Fresh Muscle

Pair No.	Amino Acids		Sulfhydryl Groups		Cyanometmyoglobin	
	Control	Treated	Control	Treated	Control	Treated
1	146.2	160.3	73.92	75.24	1203.66	1177.90
2	139.7	197.1	69.96	58.08	733.82	707.62
3	134.3	122.1	88.44	73.92	1049.78	966.78
4	134.4	131.8	102.96	99.00	1135.68	985.71
5	140.0	137.6	84.50	72.60	710.53	605.69
6	150.4	155.1	79.20	71.28	851.76	786.24
7	164.0	132.3	71.28	55.18	575.12	506.69
8	198.9	158.5	79.86	71.28	1016.29	856.13
9	167.6	163.2	97.94	97.68	810.99	679.95
10	171.4	177.7	83.16	73.39	879.42	917.28
Mean	154.7	153.6	83.12	74.77	896.60	819.00

Appendix III

Amino Acids, Sulfhydryl Groups and Cyanometmyoglobin in the Muscle, as Percent of Total Protein

Pair No.	Amino Acids		Sulfhydryl Groups		Cyanometmyoglobin	
	Control	Treated	Control	Treated	Control	Treated
1	.613	.804	.310	.377	5.04	8.93
2	.624	.712	.313	.355	3.28	4.01
3	.611	.720	.402	.436	4.77	5.70
4	.606	.867	.464	.516	5.12	5.14
5	.648	.767	.391	.394	3.29	3.27
6	.734	.829	.386	.406	4.16	4.20
7	.774	.844	.337	.352	2.72	3.23
8	.972	1.020	.390	.359	4.97	5.51
9	.820	.983	.479	.588	3.97	4.10
10	.846	1.010	.410	.417	4.34	5.11
Mean	.725	.836	.388	.428	4.16	4.92

Appendix IV

Connective Tissue as Percent of Crude Protein Gravimetric Method

Sample No.	Control	Treated
1	5.22	5.27
2	3.64	7.35
3	3.23	3.30
4	2.33	4.40
5	1.46	2.27
6	4.54	3.81
7	3.73	3.43
8	2.85	4.49
9	2.46	4.36
10	1.89	2.32
Mean	3.135	4.100

Variations	s.s.	d.f.	M.S.	F.	Remarks
Between treatment	4.656125	1	4.656125	2.59	
Within treatment	32.411250	18	1.800625		†Not significant.

† Not significant at 5% level.

Appendix V

Connective Tissue as Percent of Crude Proetin Colorimetric Method

Sample No.	Control	Treated
1	1.76	1.29
2	1.75	2.89
3	2.69	2.15
4	2.04	1.26
5	1.27	2.32
6	2.04	2.53
7	2.40	1.66
8	1.89	3.18
9	3.99	2.76
10	2.47	2.54
Mean	2.23	2.258

Variations	s.s.	d.f.	M.S.	F.	Remarks
Between treatment	.0004	1	.0004	.0008	†Not significant
Within treatment	.8917	18	.0500		
Total	.8921	19			

†Not significant at 5% level.

Appendix VI

Chemical Analysis of Protein Extract

No.	Percent of total protein		Mg. of sulfhydryl groups		Mg. of amino acids	
	Control	Treated	Control	Treated	Control	Treated
1	7.55	5.62	6.93	6.40	16.05	13.88
2	6.31	5.29	6.60	5.45	12.98	13.28
3	6.80	5.95	6.60	5.84	12.90	12.08
4	7.00	6.11	8.74	8.12	10.05	9.00
5	6.79	5.62	8.74	7.26	11.40	10.20
6	8.39	6.92	5.54	4.62	15.04	15.51
7	7.56	5.25	5.02	3.70	16.40	13.23
8	7.35	4.83	5.41	3.83	19.89	15.85
9	7.14	5.04	5.54	3.95	16.76	16.32
10	8.40	6.09	4.09	3.56	17.14	17.77
Mean	7.33	5.67	6.32	5.27	14.86	13.71

Appendix VII

Refractive Index and Milligrams of Sulfhydryl Groups and Amino Acids as Percent of Protein Extract

Pair No.	Refractive index		Sulfhydryl groups		Amino acids	
	Control	Treated	Control	Treated	Control	Treated
1	1.33697	1.33796	.0918	.1139	.199	.262
2	1.33789	1.33827	.1030	.1046	.206	.251
3	1.33735	1.33774	.0970	.0982	.095	.102
4	1.33733	1.33949	.1249	.1330	.144	.147
5	1.33762	1.33934	.1287	.1290	.168	.182
6	1.33793	1.34086	.0660	.0668	.179	.276
7	1.33858	1.33953	.0683	.0705	.217	.252
8	1.33796	1.33877	.0736	.0793	.271	.328
9	1.33960	1.34071	.0776	.0784	.235	.324
10	1.33846	1.33983	.0487	.0585	.204	.292
Mean	1.33797	1.33925	.088	.093	.192	.207

Appendix VIII

Analysis of Muscles, Extract and Residue
Mean of Five Replications

Analysis.	Treatment					
Muscles	Control	0 day	1 day	2 days	3 days	4 days
Moisture %	75.71	77.04	76.91	77.29	77.35	75.51
Protein %	20.49	18.51	18.46	17.78	17.66	18.30
Fat %	3.40	2.76	2.38	2.99	2.96	2.07
Sodium chloride %	10.11	1.96	2.16	1.95	2.05	1.99
Taste test	4.60	6.08	5.78	6.25	6.60	6.87
pH	6.06	5.95	5.79	5.83	5.77	5.93
Metmyoglobin	.672	.632	.567	.511	.563	.562
Cyanometmyoglobin	.806	.777	.707	.761	.707	.693
Hydroxyproline	.0893	.0745	.0601	.0708	.0652	.0640
Connective tissue	.7284	.6077	.4907	.5767	.5183	.5223
Amino acids mg.	177.98	167.09	185.22	174.00	179.73	166.89
Sulfhydryl groups mg.	112.60	119.89	90.62	103.70	110.22	92.46
Extract						
Protein %	7.26	5.98	5.49	5.40	5.39	5.29
Amino acids mg.	20.80	20.09	21.03	22.34	20.18	19.60
Sulfhydryl group mg.	9.00	7.68	7.13	5.74	8.41	6.59
Residue						
Protein %	13.23	12.45	12.91	12.32	12.25	12.94
Amino acids mg.	15.57	13.62	16.57	13.37	15.95	14.15
Sulfhydryl group mg.	14.21	18.95	11.25	15.64	14.36	12.11

Appendix IX

Analysis of Muscles, Extract and Residue as Percent of Crude Protein
Unadjusted Mean of Five Replications

Analysis. Muscle	Control	0 day	1 day	2 days	3 days	4 days
Metmyoglobin	3.27	3.44	3.01	2.89	3.19	3.09
Myanometmyoglobin	3.92	4.22	3.90	4.29	4.00	3.78
Hydroxyproline	.437	.397	.325	.398	.359	.349
Connective tissue	3.57	3.28	2.67	3.24	2.93	2.85
Amino acids	.866	.903	1.004	.982	1.019	.911
Sulfhydryl groups	.546	.654	.491	.598	.625	.506
Extract						
Amino acids	.309	.338	.386	.399	.375	.372
Sulfhydryl groups	.124	.128	.132	.106	.156	.124
Residue						
Amino acids	.117	.110	.129	.110	.130	.110
Sulfhydryl groups	.107	.146	.086	.130	.117	.093