THE CONNECTIVE TISSUE IN PRE- AND POST-RIGOR BOVINE MUSCLE

By

LING WONG LEE

Bachelor of Science

The National Taiwan University

Taipei, Taiwan

1964

Submitted to
the faculty of the Graduate College of
the Oklahoma State University
in partial fulfillment
of the requirements
for the degree of
MASTER OF SCIENCE
May, 1967

THE CONNECTIVE TISSUE IN PRE- AND POST-RIGOR BOVINE MUSCLE

Thesis Approved:

Robert L. Henrickson
Thesis Adviser

George V. Odell

D. D. Durham
Dean of the Graduate College

ACKNOWLEDGEMENT

The author wishes to thank Dr. R. L. Henrickson, Professor of Animal Science, for aid and advice throughout this program of graduate study and the thesis preparation.

Further appreciation is extended to Dr. George V. Odell, Associate Professor of Biochemistry, for valuable suggestions during the preparation of this thesis.

The author is grateful to her husband, Che-Fu Lee for his guidance in the statistical analysis, and continuous help and encouragement throughout the study.

Finally the author wants to express her gratitude to her parents, who have made all this possible.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Tenderness, juiciness, and flavor variations influence the palatability of meat. According to many consumer-preference studies, tenderness is the most desired eating quality attribute. For centuries, Food Scientists using meat and meat products have improved these attributes to meet the consumers' satisfaction. Recent studies have indicated that post-rigor excised muscle is more tender than muscle excised pre-rigor. Emphasis was placed on the muscular contraction, since muscle fibers are considered to be the main factor contributing to tenderness differences. Little work has been done on the connective tissue from pre-rigor muscle, which, in addition to the muscle fibers, is a very important factor influencing the tenderness of meat. The objectives of this study, therefore, were to explore, by chemical methods:

(1) the quantitative differences in the amount of connective tissue between pre- and post-rigor excised muscles; and

(2) the changes in the components of connective tissue between pre- and post-rigor excised muscles.

CHAPTER II

LITERATURE REVIEW

Chemistry of Connective Tissue

Connective tissue, in muscle, is found as the endomysium which invests each myofiber and is attached to the sarcolemma by reticular connective tissue; perimysium which surrounds the fasiculi; and epimysium which contains the entire muscle.

Connective tissue can be morphologically described as a mixture of semifluid gel in which are bathed fibrous and cellular elements varying in size, shape, and relative number from site to site. Its chief components are collagenous and elastic fibers; and ground substance — mucoprotein.

Collagen

Collagenous fibers are derived from the fibroblast cell and lie entirely outside the muscle fiber. Collagen protein is a major constituent of the reticular sheath of muscle and of septa between muscles. It is insoluble in normal protein solvents. Chibnall (1946) using the techniques of absorption chromatography on ion exchange resins conducted collagen studies on one protein preparation. The amino acid

composition of collagen was obtained by Eastoe (1955) using chromatographic techniques. According to Eastoe, collagen contains a fairly well balanced proportion of positively and negatively charged side chains. This ensures a reasonably high degree of ionic reactivity with other components in connective tissue, such as mucoprotein and water. It also contains relatively high non-polar amino acids. Glycine makes up one third of the total amino acid content, while alanine, proline, and hydroxyproline together account for another third. By comparison with other proteins, the tyrosine and methionine contents of collagen are low. There seems to be little variation in the amino acid pattern between collagen from different connective tissues of the same animal.

Boedtker and Doty (1956) described the three dimensional characteristics of the collagen molecule as follows; the basic unit (monomer) of the collagen molecule — tropocollagen, can be represented as long thin rods 3000 Å in length and 14-15 Å in diameter. These rods have a molecular weight on the order of 300,000. Each molecule is made up of three intertwined peptide chains wound in a left-handed helix.

Ramachandran and Kartha (1955) reported that such helical chains wound round one another in a macro-helix along most of the molecule. This molecular structure concept of collagen has been generally accepted.

Rich and Crick (1961) constructed a collagen molecule

model, composed of the frequently observed amino acid sequence of collagen; glycine, proline-hydroxyproline. The glycine residue which comes in every third position in each of the three peptide strands lies close to the core of the three chain unit.

Piez and Gross (1960) concluded that it was the total imino acid content of collagen, which determined the degree of stability of the collagen protein.

Ramachandran et al. (1961) showed that two hydrogen bonds were situated between chains for each three amino acid residues. There are no hydrogen bonds in the structure of the intra-chain character. This is in contrast to the α-helix structure in which hydrogen binds from one loop of the helix to the next loop. In collagen all hydrogen bonds run from one chain to another nearly perpendicular to the chain axes. They are intra molecules, in a sense, because these hydrogen bonds which are found within the triple chain unit are really inter-peptide strand hydrogen bonds.

Mechanic and Levy (1959) demonstrated that peptide linkage may provide the necessary bonds to bind these chains together by demonstrating a peptide bond between glutamic acid and lysine end groups. They isolated L, L-N-ϵ(glycyl-glutamyl)-lysine, peptide by chromatographic techniques from a partial hydrolyzate of collagen.

Wood and Keech (1960) suggested that collagen fibril formation was the result of two processes; first, nucleation tropocollagen, the basic units of collagen, are aggregated

to produce the smallest fiber particles; second, in the growth phase the nuclei are converted into discrete collagen fibers by accretion of further tropocollagen molecules to their surface then collagen becomes a longer chain and forms procollagen by a process akin to crystallization.

From the above literature review, it seems that the structure of collagen protein is stabilized by a regular array of interchain hydrogen bonds together with stereochemical restrictions from the high content of proline, hydroxyproline, some unusual esters, and γ-glutamyl links.

The progressive insolubility of the collagen fiber is a result of increased binding by weak bonds between tropocollagen and the introduction of covalent linkages within individual tropocollagen molecules.

Elastin

Elastic fibers occur in the connective tissue as a loose network of fine yellow fibers that branch in various directions. They extend readily upon stretching, but return to their normal length when released. This is a rubber-like protein, resistant to boiling water, acids, alkalies, but it can be digested slowly with pepsin and trypsin.

Gotte et al. (1963) observed that the amino acid composition of elastin is unusual for an animal protein. It has a small number of hydrophilic side chains and a large amount of proline. This is the reason why elastin does not have an ordered structure. Spacing of the pyrolidine ring

of proline at fairly close intervals cause the fiber kinks produced in the peptide chain, this tends to prevent the regular helices formation.

Partridge (1955) first proved the existence of covalent linkage in elastin. Thomas and Elsden (1963) found in addition to large amount of glycine, alanine, and proline, two other amino acids; desmosine and isodesmosine. Both compounds are tetracarboxylic tetra-amino acids. These two amino acids, by virtue of their structure, could form a common linkage with up to four peptide chains. The insolubility of elastin was due to the presence of the desmosine cross-linkage.

Thomas and Elsden (1963) assumed some kind of soluble elastin to be the first product of elastin syntheses. This was later incorporated into the cross-linked structure of the elastin fibers by two processes; first, the lysine on the peptide was converted to an intermediate product; then the slow cyclisation reaction occurred.

Partridge (1966) constructed the molecular model for elastin, and showed soluble proelastin as globular protein. The hydrophobic side chains were arranged in a relatively water free environment inside the molecule. The hydrophilic elements included the side chain of lysine directed toward the surface of the elastin molecule. The cross-linked polymer arose by condensation reactions on the surface of the protein.

The chemical structure of elastin protein was

reported by Partridge (1966). He suggested that the elastin molecule is composed of two peptide chains, with ten lysine residues arranged on the outside. Eight of the lysine molecules would condense with adjacent lysine pairs on the neighboring molecules. This would bring about a structure in which each globular molecule is combined with its neighbor at four points directed toward the corners of a tetrahedron. Partridge assumed that the elasticity of the polymer would be due to distortion of the spheres to ellipsoids.

Ground Substance

Mucoprotein, a polysaccharide-protein complex, is the main constituent of ground substance in connective tissue. Usually the acid polysaccharides are present in all ground substance of connective tissue, but the amount varies considerably. Much work on acid polysaccharides of connective tissue has been reported from the laboratory of Meyer. According to Meyer's evidence (1945), two groups of acid polysaccharides exist. These have been identified in various tissues. The first group is known as the simple polysaccharides which are sulfated polysaccharides. The main polysaccharide found in organized connective tissue is the 4-sulfated chondroitin sulfate-B. In general the sulfated polysaccharides appear to have the function of regulating the consistency of the tissue in which they occur. They accomplish this by combining appreciable amounts of

water to form gels of varying strength.

A protein component which was in firm association with chondroitin sulfate in cartilage was reported by Shatton and Schubert (1954). Partridge and Davis (1958) also obtained a protein polysaccharide complex. The protein portion of this complex has a different amino acid composition from that of collagen. Mathews et al. (1963) has firmly established that chondroitin 4-sulfate occurs in cartilage covalently bounded to protein. Muir (1964) commented that, in certain chondromucoproteins, serine may be the only amino acid involved in the linkage of carbohydrate to protein.

Maxwell (1966) suggested that the possibility of entanglement seemed, at the moment, to be the most plausible mechanic binding between diffuse molecules—protein-polysaccharides in solution and in soluble fibrils—collagen. The holding of water would be attributed to the tendency of the protein polysaccharides to occupy a large domain by spreading throughout a large solution volume. This is due to the mutual repulsion of nearby chondroitin sulfate chains and the extending effect of the interchain repulsion of neighboring anionic groups along each chondroitin sulfate chain.

Connective tissue contains these characteristic macromolecules collagen, elastin, and ground substance—mucoprotein. Finally, it is important to note that the special properties of each component of connective tissue depends not only on the special characteristic of each macromolecule

and the amount of each present, but also on the way these macromolecules are structurally interrelated.

Connective Tissue as Related to Tenderness

Early in 1897 Lehmann first noted the relationship between the amount of connective tissue and tenderness. Mitchell et al. (1928) reported the differences in tenderness between beef cuts of the same animal to be due to collagen and elastin. The less tender cuts contained more collagen than the tender cuts. Morgan and Smith (1929) in a study of post-morten change in animal tissue indicated that connective tissue was a major factor contributing to toughness. Steiner (1939) however, contended that increased tenderness with aging was due to an effect upon the muscle fibers rather than upon the connective tissue. Ramsbottom et al. (1945) further indicated that there is an inverse association between the quantity of connective tissue and tenderness. Husaini et al. (1950) demonstrated that the connective tissue, as alkali-insoluble nitrogen, at 15 days post-mortem, was responsible for tenderness. But, Herschberger et al. (1951) argued, in their biochemical study of meat quality in relation to certain feeding management, that the correlation between collagen nitrogen content and beef tenderness was not significant. Histological data presented by Hiner et al. (1955) indicated that both collagen and elastin fibers influence tenderness. More recently, Wilson (1959) reported that the physical and chemical state of connective tissue

may affect the tenderness of meat. He further explained that the sarcolemma lost some of its semipermeable characteristics during the onset of rigor mortis, thus permitting a greater interchange of electrolyte. This, in turn, could greatly affect the ionic atmosphere, water binding capacity, and result in more tender meat.

The relationship between connective tissue and the tenderness of meat under different processes of cooking was reported early in 1938 by Satorius et al. He noted that heat did not have the same effect on all cuts of beef. Bell et al. (1941), using different methods of cooking, found beef lost 22 per cent collagen nitrogen upon broiling, 26 per cent collagen nitrogen upon roasting and the losses of collagen increased with longer cooking periods. Paul and McLean (1946) further studied the effect of different internal temperature on roasts from calves using three different cuts. They described the changes in microscopic appearance as swelling of the collagenous fibers in semitendenosus; and partial breakdown of the connective tissue reticulium surrounding the muscle fibers. The collagen content of cooked beef was significantly higher for commercial than for the prime grade, but not significantly different for top and bottom muscle of the round. This was substantiated by Griswold (1955). But, Cover and Hostetler (1960), in a cooking experiment, found that the collagen content of two muscles of differing anatomical locations was not related to tenderness and the moisture in moist-heat cookery was required primarily to

obtain high temperatures and not necessarily to convert collagen into gelatin. A recent report by Richey (1965) indicated that as temperature increased the meat was drier, harder, more mealy, and contained less connective tissue, but the fragmentation of muscle fiber was unchanged. McClain et al. (1965), compared the shear value and alkali-insoluble collagen of longissimus dorsi, semimembranosus, and triceps brachii muscles of yearling steer carcass. Their work specifically indicated that the absolute quantities of alkali-insoluble collagen were not related to shear value in uncooked and cooked muscle. However, differences were observed in the per cent of alkali-insoluble collagen converted to gelatin upon cooking which indicated different types or characteristics for muscle collagen.

This author concluded from the above literature review that the possibility exists for biochemical alterations in the connective tissue. This may explain at least part of the variation in tenderness of bovine muscles.

Factors Affecting Connective Tissue in Meat

Age of animal is an important factor affecting the content of connective tissue of bovine muscle. It has been a moot point for various studies. Mitchell et al. (1928) found that age does not seem to have a great effect upon the connective tissue content of muscle. Bate-Smith (1942) indicated that as the animal grew older the proportion of connective tissue protein of total body protein decreased.

But an increase in collagen concentration soon after birth was followed by a decrease with advancing age. Mackintosh et al. (1936) noted that meat from yearling steer contained less collagen nitrogen and was more tender than that from mature steer. Meyer and Lang (1946) demonstrated that in the thoracic aorta of adult human subjects collagen increased with advancing age. Coolidge and Lightfood (1948) using guinea pigs showed that a significant increase in the per cent of collagen occurred with age, and collagen is of the same general nature in all the mammalian tissues. Hanson et al. (1946) found a greater amount of elastin in a yearling steer than that in an eight year old cow. Gersh and Catchpole (1949) suggested that absolute amount of connective tissue remains relatively constant throughout life, but that small amounts of connective tissue coalesce into stronger fiber with age. Bray et al. (1951) found the moisture and ash content of elastin and collagen in connective tissue to decrease with age of animal, and that hydrated connective tissue is more easily hydrolyzed. Veal had a higher moisture content than tissue from an older animal. The fat content of muscle also increased with age. In data reported by Wilson et al. (1954), there were greater amounts of collagen in the veal than in the cow or steer samples, with no significant difference between cow and steer. Elastin varied considerably between animals of the same grade and age, but steer and cow contained less elastin than veal. Hiner et al. (1955) noted that the size of the elastin

fibers in the young veal and steer calves was smaller than in heifers and cows. As animals matured, the size of elastin fibers increased and the number of collagenous fibers increased. Lorinez and Szeredy (1959) indicated that the quality of connective tissue determines the chewing and the cutting resistance of meat, not the quantity of connective tissue. Lansing (1959) noted that elastin tissue underwent fragmentation with age while collagen did not alter perceptibly, but rather increased in amount with age in many organs. Sobel and Marmorston (1956) reported that the gel to fiber ratio in skin of fat guinea pigs decreased with age, with a steady increase in the fiber component in proportion to ground substances occurred. Woessner (1963), working with human tissue, stated that collagen and elastin proteins which were normally considered relatively inert from the view point of metabolic turnover behaved in an exceedingly dynamic fashion in the uterus. The weight of collagen and elastin increased to a maximum by age 30. These parameter remained constant for the next 20 years then declined to levels about one half of the maximum values during the period from 50-60 years of age. Goll et al. (1964) further explained that the maturation of collagen was accompanied by the formulation of stronger cross-linkage within tropocollagen molecules and that increase in thermal shrinkage temperature could conceivably alter the amount of collagen converted into gelatin during cooking, which ultimately would affect tenderness. LaBella and Paul (1965) also indicated the changes of

connective tissue with age are progressive cross-linking of peptides, which means decreased susceptibility to proteolysis, increased thermal shrinkage temperature and decreased swelling. There was no marked sex difference in solubility of collagen fluoresence.

The degree of muscular contraction during rigor affects tenderness. According to Embden (1922), a muscle frozen in liquid air is a contracted one. Ramsbottom and Strandine (1949), in their study of objective and subjective measurements of tenderness, showed that beef which was chilled in carcass form was more tender than beef which was boned and then chilled to 35°F. They also indicated that changes of muscle fiber were correlated with changes in the tenderness of beef. Casella (1950) stated the thickness of the sarcolemma and associated connective tissue, which reduced thickness when fibers were stretched, may represent another factor in addition to the contracted elements which contribute to tenderness. Marsh (1954) observed the effect of temperature on beef muscle shortening. There appeared to be less shortening in longissimus dorsi at 17°C than at 37°C. Koonz et al. (1954) stated that excising muscles before the onset of rigor mortis would induce toughness which is only partially resolved by aging. Paul and Bratzler (1955) explained that removing muscle from the carcass resulted in less tender steaks, since it interfered with the tenderizing process. Locker (1960) pointed out that the degree of contraction which a muscle enters, or the state of rigor mortis, is

highly variable among the muscles of a carcass and might be a significant indicator of tenderness. There was a correlation between the tenderness grading of the muscles, and their contraction state on the carcass. This could be due to the dominant effect of connective tissue. DeFremery and Pool (1960) explained excising the muscles pre-rigor resulted in more rapid loss of ATP, more rapid drop of pH and loss of glycogen, and induced increased muscle toughness. They indicated that the more rapid the onset of rigor mortis the less tender will be the cooked meat. Pre-rigor excision accelerated the rate of onset of rigor mortis. Cassens (1913) noted that at low temperatures shortening begins rapidly and usually immediately. Goll et al. (1964) more precisely investigated the difference of tenderness for attached and excised muscle during different periods of post-mortem. Attached muscles were found to be least tender immediately after death and gradually increased in tenderness during aging. Excised muscle was least tender six to twelve hours post-mortem, then became more tender. After 312 hours of chilling, they were less tender than muscle still attached to the skeleton. Herring et al. (1965) presented histological evidence to indicate that when muscles shortened there were corresponding decreases in sarcomere length, an increase in fiber diameter, and a decrese in tenderness. Recently Weiner et al. (1966) noticed that muscle placed in a $-24^{\circ}C$ to $-30^{\circ}C$ freezer immediately after removal from the carcass (within one hour post-mortem) and subsequent thawing adversely affected tenderness

and expressible moisture values, yet rapid chilling of the loin to 0°C produced lower shear force values and no significant difference in expressible moisture.

There are some other factors which cause the variation of connective tissue in bovine muscles: such as, marbling; exercise; nutrition; variations within cuts, between muscles, between animals; etc. Golovkine et al. (1939) found less elasticity in muscle of fattened cattle as compared to lean cattle, when both were held for the same length of storage. Hiner et al. (1955) showed that those muscles which were used more extensively by the animal, such as, the semimembranosus and the biceps femoris, had large amounts of collagenous fibers, whereas those with less activity had smaller amounts. In muscles where fatty deposits were evident, the collagenous fibers formed more of a loose network between muscle bundles, in those with less fat they appeared bunched. Mackey and Oliver (1954) have stated that cuts from the same location in different hogs showed variations in shear forces. Paul and Bratzler (1955) found in most muscles the anterior and posterior differed in tenderness. Marion and Fenton (1956) reported that when levels of nutrition increased, the weight of muscle and fat increased in the raw meat; moisture decreased; with no significant change in total nitrogen or in shear force. Lowe and Kastelic (1961) reported that collagen nitrogen was significantly different between muscles, cuts, muscles within cuts, and between animals. Tuma et al. (1962) have stated that marbling did enhance the

tenderness of steak from older animals.

Methods of Analysis

Since the components in connective tissue seem somehow to be interlocked, the solubilities of the different components change radically as they are separated from each other. There are several methods for the quantitative determination of connective tissue (collagen and elastin).

Stokes (1897) separated collagen from other connective tissue proteins by precipitation with mercuric nitrate, then precipitated the collagen from the filtrate with picric acid. Spencer et al. (1937) removed collagen as the insoluble tannic acid-gelatin complex from the autoclaved soluble fraction. The procedure of Lowry et al. (1941) is based on the theory that collagen and elastin proteins are insoluble in weak alkaline solutions while the other muscle proteins are soluble. They first extracted the collagen and elastin from the meat sample by placing the meat in 0.1 N NaOH for 18 hours, then neutralized the alkali-insoluble residue to PH 7.0 so that the non-collagenous material would not solubilize as collagen during autoclaving. Elastin was separated from collagen by boiling the 0.1 N NaOH extracted sample. This is based on the concept that elastin is not soluble in hot dilute alkaline solutions. Then elastin is determined by gravimetric method — elastin = weight of dry residue x 100/weight of sample.

Hartley and Hall (1949) used the Waring Blendor and

centrifuge technique to determine the collagen content in beef, which gave reproducible results only when applied to raw beef. Neuman and Logan (1950) described a short and simple colorimetric method, that is applicable to the determination of hydroxyproline in hydrolyzate of collagen and elastin. They first used 0.01 M $CuSO_4$ 2.5 N NaOH six per cent H_2O_2 solution as an oxidizing reagent to form the copper complex at room temperature with the hydroxyproline in the protein hydrolyzate. The excess peroxide was destroyed by heating the solution in an 80°C water bath for five minutes, acidified the mixture with H_2SO_4 and used p-dimethylaminobenzaldehyde for the development of the color complex. They mentioned that the only amino acid other than hydroxyproline which gave a color was tyrosine but this interference was so small that it could be removed by correction. Martin and Axelrod (1953) modified the method of Neuman and Logan by the application of ferrous sulfate to remove the peroxide, this modification markedly reduced the color contribution of tyrosine. Lorinez and Szeredy (1959) determined the connective tissue from the difference between total nitrogen content and nitrogen extracted with 0.05 N alkali at room temperature in 24 hours. According to Prockop and Udenfriend (1960), hydroxyproline undergoes an oxidation and decarboxylation to form pyrrole. The pyrrole can be extracted into toluene and reacted with p-dimethylaminobenzaldehyde to form a colored product.

CHAPTER III

EXPERIMENTAL PROCEDURES

Materials

The meat samples used were from five sixteen-month-old Hereford steers of similar genetic and management background, provided by the Oklahoma Agricultural Experiment Station. They were slaughtered, following the standard laboratory procedures recommended by Deans (1951) and Willington (1953), 15 hours after being received at the meat laboratory. The representative muscles, longissimus dorsi and semitendenosus, were obtained by two different treatments—pre- and post-rigor excision. The muscles designated pre-rigor were excised from the right half of carcasses one hour after slaughter, then suspended for 48 hours in vegetable oil pre-chilled and held at 34°F. Muscles designated post-rigor were excised from the left halves of the carcasses which had been vertically suspended in a 34°F cooler for 48 hours after slaughter. Steaks from both the pre- and post-rigor excised muscles were quickly frozen at -20°F.

Methods

Sampling

Muscles, with both ends trimmed, were freed of exterior fat and connective tissue. Longissimus dorsi (three inches) and semitendenosus (two inches) sections were taken from both anterior and posterior locations. Each sample was ground using an electric grinder, in a cold room, placed in glass jars tightly sealed, and stored in a $-4^{\circ}F$ freezer. They were thawed in the glass jars at room temperature, on the day of chemical analysis.

Quantitative Determination of Hydroxyproline

Sample Preparation — Sample preparation was based on the concept that connective tissue (collagen and elastin) can be separated from other proteins in meat by their insolubility in dilute alkali. Collagen then can be separated from elastin by autoclaving, in which collagen is selectively hydrolyzed into gelatin (Lowry *et al.* (1941)). The content of collagen and elastin in samples can be indicated by determining the hydroxyproline content in the acid hydrolyzates of collagen and elastin separately after autoclaving (Lilienthal *et al.* (1950)).

Duplicate three gram samples of ground meat were weighed in polyethylene centrifuge tubes, to which 24 ml of distilled water were added, and the sample mixture was stirred with an electric grinder for 30 minutes at slow

speed while at 38°F. The mixture was carefully transferred into centrifuge tubes without loss of connective tissue which might be twisted around the mixer, and centrifuged at 3,000 rpm for 15 minutes. To remove the watersoluble proteins, only the supernatant of the centrifuged mixture was filtered by suction through a layer of glass wool on a glass filter. The glass wool was transferred into the centrifuge tube. After rinsing the glass filter with 10 ml of distilled water, 24 ml of distilled water including the rinse water was added to the tube together with three ml of 1 N NaOH, then stirred with an electric grinder at slow speed for two hours.

The sample mixture was refiltered using the same layer of glass wool on top of the filter, and washed with warm water (45-48°C) to remove any formed gelatin. The sample and glass wool were transferred into a 50 milliliter Erlenmeyer flask. Distilled water (10 ml) was used to rinse the centrifuge tube and the solution poured into the beaker covered flask. Each sample was autoclaved at 120°C under 18-pound pressure per square inch for six hours. The mixture was then filtered while hot after autoclaving. For collagen, the filtrate was transferred into a 25 ml volumetric flask, made to volume with distilled water. Five milliliter of filtrate was added with five milliliter of 12 N HC1 into a glass tubing for acid hydrolysis of collagen protein. The tubing was sealed in flame and again autoclaved at 120°C under 18-pound of pressure per square inch

for eight hours. The contents in the glass tubing were transferred into a 50 milliliter beaker after autoclaving. The glass tubing was rinsed well with distilled water, and the rinsing added into the beaker then evaporated to dryness using a hood.

Distilled water (10 ml) was added to the dried beaker. The solution was then neutralized with 1 N NaOH to pH 7.0 using a PH meter. The neutralized solution was transferred into a 100 milliliter volumetric flask, and made to volume with distilled water. Aliquots of this solution were taken for colorimetric determination of hydroxyproline in collagen.

For elastin, the sample preparation followed the same steps as mentioned above. The elastic fibers entrapped in the glass wool on top of the filter were transferred into a 25 milliliter Erlenmeyer flask. Six milliliter of 12 N NaOH was added into the flask and covered with a tightly sealed glass lid. The acid hydrolysis and neutralization procedures were followed as in the preparation of collagen. The final volume of 25 milliliter of acid hydrolyzate was made up with distilled water. Aliquots were taken for colorimetric determination.

Colorimetric Determination — (Woessner (1961)) — For both collagen and elastin the procedures were as follows:

Two ml of chloramine-T was added to each aliquot of acid hydrolyzate (total volume four milliliters) for the oxidation of hydroxyproline. The mixture was shaken well and kept at room temperature for 20 minutes. Two milliliter of

3.5 M perchloric acid was added to destroy the chloramine-T and to acidify the mixture. The mixture was stirred and allowed to stand five minutes at room temperature. Then two milliliter of p-dimenthylaminobenzaldehyde were added and the tube with material incubated in a water bath at 60°C for 30 minutes. The sample tubes were cooled with tap water for five minutes. The absorbance of solution was read with a Coleman Junior Spectrophotometer at 576 mμ.

The hydroxyproline content of collagen and elastin in the sample was obtained from a standard curve.

According to Miller (1954) collagen and elastin nitrogens were obtained by multiplying the corresponding hydroxyproline values by the factors 1.365 and 8.943, respectively. Therefore,

$$\% \text{ collagen nitrogen} = \frac{\text{hydroxyproline content of collagen} \times 1.365}{\text{sample weight}}$$

$$\% \text{ elastin nitrogin} = \frac{\text{hydroxyproline content of elastin} \times 8.943}{\text{sample weight}}$$

Total nitrogen was determined according to the Kjeldahl method and duplicate moisture determinations were conducted on each sample according to the procedures stated by the Association of Official Agricultural Chemists (1965).

CHAPTER IV

DATA ANALYSIS

Quantities of collagen and elastin are respectively expressed by the ratios of collagen nitrogen and elastin nitrogen to total nitrogen.

These data are classified into anterior and posterior for location in the muscles; the muscles are of two kinds—longissimus dorsi and semitendenosus; treatments as indicated in the experimental procedures are differentiated into pre-rigor and post-rigor excised. Material from five different animals are treated as replication for the analysis (see Table III in Chapter V for the classification of data).

The statistical method applied for this study was the Factorial analysis of variance (Steel and Torrie (1960)). The model of 5x2x2x2x2 was assumed to fixed levels for factors of animal, location, muscle, treatment and protein. Since the interactions between these five factors are presumed to be of significance, the interactions were computed only to the first order interaction. The second order interactions among muscle, treatment and protein were also computed. Therefore, the residual sum of squares including experimental error and all possible interactions of second and higher orders not computed, was taken as the error sum of

squares to serve as the basis for the F-test.

The null hypotheses to be tested in the analysis of variance (Table IV) were as follows:

(1) There is no difference in the amount of connective tissue among different animals.

(2) The amount of connective tissue does not vary with different locations of bovine muscles.

(3) There is no difference between the amount of connective tissue of longissimus dorsi and that of semitendenosus muscles.

(4) Pre- and post-rigor excising of muscles does not have an effect on the amount of connective tissue in muscles.

(5) There is no systematic association between the two kind of proteins, collagen and elastin, in connective tissue.

(6) No first order interaction between each pair of factors is effective on the amount of connective tissue.

(7) No second order interaction among muscle, treatment and protein is effective on the amount of connective tissue.

Three analyses of variance tables (Tables IV, IX and X) were manipulated; the first one was considering protein as a factor with two levels—collagen and elastin; the second and third, were used to analyze the collagen and elastin as separate observations.

Interactions between factors, if significant, were further investigated by partial analysis with cross-tabling of the two factors (Tables VII and VIII) and the difference between observations means were tested by t value (Tables V and VI).

CHAPTER V

RESULTS AND DISCUSSION

The moiety designated as collagen was the alkali-insoluble and autoclave-soluble portion, the elastin was separated from collagen by its insolubility after autoclaving. Difficulties were encountered in the preliminary experiment. The sample solution after alkali-mixing, for two hours, appeared as a slurry and was difficult to filter using the glass filter with a layer of glass wool. It was then found that 0.2 mm thickness of glass wool was effective, if filtration was accomplished at slow speed.

Several methods had been tried for the acid hydrolysis of elastin residue entrapped in the glass wool after autoclaving, because it did not consist of putting the glass wool entrapped with elastin into the glass tubing as for the acid hydrolysis of collagen. To complete the acid hydrolysis in the 50 ml Erlenmeyer flask with glass lid tightly sealed was finally found most successful in obtaining reproducible results.

In the procedures of colorimetric determination of hydroxyproline, the control of time interval between each of the successive steps was critical to obtain reproducible results. For example, the hydrolyzate cannot stay longer

than 25 minutes at room temperature after adding the chloramine T for oxidation of hydroxyproline, and not longer than 20 minutes during period of incubation in 60°C water bath for the chromogen formation. The daily preparation of standard solution, as well as the reagents chloramine T and p-dimethylaminobenzaldehyde, was also important in gaining reproducible results.

Neither the total moisture nor the total nitrogen content of two bovine muscles showed apparent change by excision (Tables I and II).

The content of collagen and elastin in pre- and post-rigor excised muscles is shown in Table III. Tables IV through X were calculated using these basic data.

The significant difference in the amount of connective tissue between two representative muscles (Table IV) substantiated the work done by Ramsbottom *et al.* (1945), Mitchell *et al.* (1928), and Bate-Smith (1942). They demonstrated large variations in the amount of connective tissue among several anatomical regions of beef carcass, and indicated that the quantity of connective tissue was inversely related to tenderness.

The significant effect between muscle and protein was further analyzed, and shown in Table VII. Collagen means for longissimus dorsi and semitendenosus were 2.67 and 3.88; and elastin means were 0.88 and 3.37, respectively. By t-test, the contents of collagen and elastin between two different muscles are significantly different (Table V).

TABLE I

TOTAL MOISTURE CONTENT OF TWO BOVINE MUSCLES AS INFLUENCED BY EXCISION

Muscle	Location	Animal	Pre-rigor %	Post-rigor %
L.D.	Anterior	1	70.92	69.32
		2	71.14	71.83
		3	71.18	71.50
		4	72.54	71.76
		5	72.43	72.37
	Posterior	1	71.31	71.08
		2	72.24	70.32
		3	70.34	69.27
		4	73.25	72.91
		5	72.92	70.70
	Mean		71.83	71.11
	St.Dev.		0.97	1.22
S.T.	Anterior	1	72.29	71.49
		2	71.81	71.03
		3	72.01	72.23
		4	72.13	72.01
		5	70.40	71.20
	Posterior	1	71.17	70.86
		2	70.65	72.65
		3	71.80	71.37
		4	72.73	73.36
		5	71.46	72.13
	Mean		71.64	71.83
	St. Dev.		0.73	0.79

L. D. -- Longissimus Dorsi Muscle
S. T. -- Semitendenosus Muscle

TABLE II

TOTAL NITROGEN CONTENT OF TWO BOVINE MUSCLES AS INFLUENCED BY EXCISION

Muscle	Location	Animal	Pre-rigor N %	Post-rigor N %
L.D.	Anterior	1	3.46	3.40
		2	3.45	3.55
		3	3.50	3.50
		4	3.53	3.47
		5	3.49	3.40
	Posterior	1	3.50	3.50
		2	3.53	3.45
		3	3.40	3.51
		4	3.37	3.53
		5	3.50	3.40
	Mean		3.47	3.47
	St. Dev.		0.05	0.06
S.T.	Anterior	1	3.50	3.44
		2	2.89	2.95
		3	3.00	3.38
		4	3.46	3.50
		5	3.47	3.50
	Posterior	1	3.49	3.51
		2	2.90	3.40
		3	3.44	3.38
		4	3.41	3.40
		5	3.44	3.36
	Mean		3.30	3.38
	St. Dev.		0.26	0.16

L. D. -- Longissimus Dorsi Muscle
S. T. -- Semitendenosus Muscle

TABLE III

COLLAGEN AND ELASTIN CONTENT OF PRE- AND POST-RIGOR EXCISED MUSCLES

Muscle	Location	Animal	Pre-rigor Collagen*	Pre-rigor Elastin**	Post-rigor Collagen*	Post-rigor Elastin**
L.D.	Anterior	1	2.68	1.26	1.32	0.43
		2	3.62	2.35	3.32	0.94
		3	3.66	1.20	2.88	0.71
		4	2.31	0.30	3.68	0.74
		5	1.66	0.86	2.59	0.53
	Posterior	1	3.54	0.42	2.80	1.53
		2	3.22	1.97	2.44	0.93
		3	3.21	0.35	2.41	0.87
		4	2.09	0.91	2.49	0.60
		5	1.91	0.29	1.53	0.44
	Mean		2.79	1.02	2.55	0.77
	St. Dev.		0.75	0.69	0.72	0.33
S.T.	Anterior	1	4.00	4.71	2.59	4.88
		2	6.84	2.42	2.34	2.78
		3	6.67	2.94	3.16	5.49
		4	3.68	1.68	4.75	3.71
		5	3.71	2.53	3.48	2.00
	Posterior	1	4.23	2.12	1.40	2.31
		2	6.48	6.52	2.47	3.29
		3	3.21	2.58	4.27	4.20
		4	3.91	2.26	3.38	3.14
		5	4.47	6.14	2.45	1.71
	Mean		4.72	3.39	3.03	3.35
	St. Dev.		1.38	1.75	0.99	1.24

* Collagen expressed as collagen nitrogen/total nitrogen.
**Elastin expressed as elastin nitrogen/total nitrogen.
L.D. -- Longissimus Dorsi Muscle
S.T. -- Semitendenosus Muscle

TABLE IV

ANALYSIS OF VARIANCE

Source of Variance	d.f.	S.S.	M.S.	F
Total (corrected)	79	200.242		
Animal	4	10.423	2.606	2.4069
Location	1	0.599	0.599	0.5533
Muscle	1	68.279	68.279	63.0695***
Treatment	1	6.009	6.009	5.5505*
Protein	1	26.229	26.229	24.2278***
Location x Muscle	1	0.011	0.011	0.0102
Location x Treatment	1	1.691	1.691	1.5620
Location x Protein	1	0.644	0.644	0.5949
Muscle x Treatment	1	2.011	2.011	1.8576
Muscle x Protein	1	8.231	8.231	7.6030**
Treatment x Protein	1	3.561	3.561	3.2893
Muscle x Treatment x Protein	1	3.267	3.267	3.2893
Residual	64	69.287	1.083	

* $p < .025$
** $p < .010$
*** $p < .005$

TABLE V

TEST OF SIGNIFICANCE FOR MUSCLES

	Longissimus Dorsi		Semitendenosus
Mean	1.78		3.62
Standard Dev.	1.47		1.11
t-test		6.35	

Unit expressed as Table IV (combining numerical value).

TABLE VI

TEST OF SIGNIFICANCE BETWEEN TREATMENT

	Pre-rigor		Post-rigor
Mean	2.97		2.43
Standard Dev.	1.80		1.32
t-test		-1.55	
r-value		0.49	

Units here expressed as Table IV (combining numerical value).

TABLE VII

INTERACTION BETWEEN PROTEIN AND MUSCLE

	Longissimus dorsi			Semitendenosus			
	Mean	N	St. Dev.	Mean	N	St. Dev.	t-test
Collagen[1]	2.67	20	0.73	3.88	20	1.46	2.9868**
Elastin[2]	0.88	20	0.56	3.37	20	1.47	7.0952**

1. Expressed as collagen nitrogen/total nitrogen.
2. Expressed as elastin nitrogen/total nitrogen.
** $p < 0.01$

TABLE VIII

INTERACTION BETWEEN PROTEIN AND TREATMENT

	Pre-rigor			Post-rigor			
	Mean	N	St.Dev.	Mean	N	St.Dev.	t-test
Collagen[1]	3.75	20	1.48	2.79	20	0.88	2.5005*
Elastin[2]	2.19	20	1.79	2.06	20	1.59	0.2419

1. Expressed as collagen nitrogen/total nitrogen.
2. Expressed as elastin nitrogen/total nitrogen.
* $p < 0.02$

They are higher in semitendenosus than in longissimus and support those findings of researches referred above.

The significant association between collagen and elastin (Table IV) in connective tissue (a correlation value of 0.49), furthermore, confirmed the relationship in the amount of the connective tissue and the tenderness of meat.

The effect of treatment (pre- and post-rigor excision) was revealed as highly significant on the amount of connective tissue (Table IV). But the t-test between means of two treatments was not significant (Table VI), when collagen and elastin were combined. This is explained by the significant interaction between treatment and protein. In Table VIII the cross tabulation of treatment and protein indicated that treatment effect was significant for collagen but not for elastin.

As reported by both Koonz et al. (1954) and Paul et al. (1955) excising muscles before onset of rigor mortis induces toughness. Locker (1960) also pointed out that the state of rigor mortis, the degree of contraction of muscle fibers, might be an significant indication of beef quality. According to our data, it seems that the temperature at which the muscles enter rigor mortis affects the degree of muscle fiber contraction. The higher the temperature the more the shortening that occurs in muscle. When muscle is shortened, a corresponding decrease in sarcomere length occurs with an increase in fiber diameter. Therefore, tenderness of beef muscle decreases. Thus, excising the

muscle pre-rigor interferes with the tenderizing process, causing a more rapid loss of ATP, more rapid drop of pH, and loss of glycogen. Moreover, the stress, which was applied on the muscles attached on the carcass during rigor, seemed to have caused some resistance of muscle fibers to contraction during the onset of rigor. Since the connective tissue forms the successive layers surrounding the muscle fibers, when the muscle fiber was stretched, the thickness of connective tissue should also be changed. This was supported by Cassela (1950). In this study, the results of a significant difference in the amount of connective tissue between the pre- and post-rigor excised muscle substantiated Cassela's work. Also, the change in the amount of collagen for pre- and post-rigor excising might be due to the presence of the structurally interrelated component mucoprotein in the connective tissue. As stated by Maxwell (1966) the mucoprotein in the connective tissue binds mechanically with the collagen molecules. It is possible that the ionic state of the post-rigor excising muscles, which have a slow onset of rigor, might cause the change of the binding force of this polyanionic compound—mucoprotein with collagen molecules. The amount of collagen in the post-rigor excised muscles was thus decreased.

On the other hand, it also might be due to the changes of collagen molecular type induced by the stress applying on the fibers for the post-rigor excising muscles. Rich and Crick (1955) proposed two models of collagen, which were

designated as collagen I and collagen II. The only difference between these two types of collagen is that for the collagen II, the OH group of the hydroxyproline residues extend radially from the peripheries of three α-helix chains and thus available for the formation of hydrogen bond with C = O groups in the backbones of adjacent triple helices. Collagen II is thought to be the actual state of affair occurring in collagen. Whereas in collagen I the OH group face inwards, and maintain the tri-helical structure. According to their suggestion, one type may be transferred into the other by the application of stress to the fiber. Therefore, the stress applied on the carcass excised post-rigor caused the change of collagen type II into type I, which has the OH group facing inwards, as well as the change of atmosphere during rigor mortis. This combined effect influenced the binding force between mucoprotein and collagen molecules. Each of these factors helps to explain the decrease in amount of collagen in the post-rigor excised muscles in this study.

No significant difference was found in the amount of connective tissue between animals, nor between the locations (Table IV). It was indicated by Mackey et al. (1954) that significant variations in shear force for cuts from the same location existed in different hogs; and by Paul and Bratzler (1955), that the anterior and posterior muscle sections differed in tenderness. In this study, however, the variation in connective tissue was found not to vary systematically,

at least, with various animal and different location. This seems to support the random selection in the animal, which was meant to be controlled.

The analyses of variance shown in Tables IX and X were computed for collagen and elastin as separate observation. The significant terms were consistent with the previous analysis which took the association between collagen and elastin into consideration by paired observations in each case.

The significant interaction between treatment and protein indicated in Table IV was further emphasized by the significant treatment effect on collagen, while the effect was not significant on elastin.

TABLE IX

ANALYSIS OF VARIANCE FOR COLLAGEN
IN BOVINE MUSCLE

Source of Variance	d.f.	S.S.	M.S.	F
Total (corrected)	39	65.004		
Animal	4	7.977	1.994	2.1303
Location	1	1.235	1.235	1.3194
Muscle	1	14.556	14.556	15.5513**
Treatment	1	9.360	9.360	10.0000**
Location x Muscle	1	0.207	0.207	----
Location x Treatment	1	0.092	----[1]	----
Muscle x Treatment	1	5.236	5.236	5.5940*
Location x Muscle x Treatment	1	0.143	----	----
Residual	28	26.198	0.936	

1. Not calculated for small value.
* $p < 0.025$
** $p < 0.005$

TABLE X

ANALYSIS OF VARIANCE FOR ELASTIN
IN BOVINE MUSCLE

Source of Variance	d.f.	S.S.	M.S.	F
Total (corrected)	39	109.001		
Animal	4	4.935	1.234	----[3]
Location	1	----[1]	----[2]	
Muscle	1	61.951	61.951	47.2295**
Treatment	1	0.166	0.166	----
Location x Muscle	1	0.115	0.115	----
Location x Treatment	1	1.057	1.057	----
Muscle x Treatment	1	0.076	0.076	----
Location x Muscle x Treatment	1	3.974	3.974	3.0297
Residual	28	36.727	1.312	

** $p < 0.005$
1, 2. Not available for small value.
3. F values of less than 1 were not calculated.

CHAPTER VI

FINDINGS

From the statistical analyses significant findings were revealed as follows:

(1) No significant difference existed in the amount of connective tissue among different animals.

(2) The amount of connective tissue did not vary with different locations of muscle.

(3) There was a significant ($p < 0.005$) difference between the amount of connective tissue of longissimus dorsi and that of semitendenosus muscles.

(4) Pre- and post-rigor excised muscles were significantly ($p < 0.025$) different in the amount of connective tissue.

(5) Paired observations of the two kinds of protein, collagen and elastin revealed a significant difference in their values. A correlation of 0.49 was found which indicates the positive association between the two proteins at 0.01 significant level.

(6) The interaction between muscle and protein was found to be significant ($p < 0.01$) in its effect on the amount of connective tissue, whereas, other

interactions were not significant.

(7) No significant interaction among muscle, treatment or muscle protein content was found.

CHAPTER VII

SUMMARY

In this research, quantitative or qualitative differences in the content of connective tissue in muscles, which differed in tenderness, were determined. Five sixteen-month-old steers were used for the meat samples. The pre-rigor muscle samples were obtained from the right halves of the five animals, while the post-rigor muscle samples were from the left halves.

The analyses of the total moisture, and of the total nitrogen content were found to vary insignificantly among the samples. There were no significant differences in the amount of connective tissue due to animal or sample location. A highly significant difference was found in the amount of connective tissue of longissimus dorsi and semitendenosus muscles. The amount of connective tissue in muscles was significantly affected by pre- and post-rigor excision. A correlation coefficient of 0.49 indicated the positive association between the collagen and elastin proteins. No significant interactions among muscle treatment and protein were found except the significant interaction between muscle and protein.

Since mucoprotein is considered as another component

in the connective tissue in addition to the two components collagen and elastin, for further confirmation of these findings, studies on mucoprotein are urged.

BIBLIOGRAPHY

Association of Official Agriculture Chemists, 1965. Official Methods of Analysis. 10th ed. Association of Official Agriculture Chemists, Washington, D. C.

Bate-Smith, E. C. 1942. "Interrelations of Tenderness, Chronological Age, and Connective-Tissue Fractions of Porcine Musculature." J. Agri. Fd. Chem. 12:504.

Bell, E. F., A. F. Morgan, and A. Dorman. 1941. "Collagen Determination in Cooked Meat." Food Res. 6:245.

Boedtker, H., and P. Doty. 1956. "The Native and Denatured State of Soluble Collage." J. Am. Chem. Soc. 78:4267.

Bray, R. W., P. H. Phillips, and G. Bohstedt. 1951. "Moisture, Ash, and Fat Content of Connective Tissue From Cattle of Different Ages." J. Animal Sci. 10:394.

Casella, C. 1950. "Tensile Tone in Total Striated Muscle Isolated Fiber and Sarcolemma." Acta. Physical Acad. 21:380.

Cassens, R. G. 1963. "Ultrastructural Changes and the Significance of Zinc in Porcine Muscle Post-Mortem." Dissert. Abstra. 23:4518.

Chibnall, A. C. 1946. "The Chemistry of Connective Tissue." J. Soc. Leather Trades Chem. 30:1. (Cited by David A. Hall 1960. The Chemistry of Connective Tissue. Charles C. Thomas Publisher, Springfield, Illinois.)

Cover, S., and R. L. Hostetler. 1960. "Beef Tenderness by New Methods." Texas Agri. Expt. Sta. Bull. No. 947.

Coolidge, T. B., and L. H. Lightfood. 1948. "The Distribution of Collagen in the Guinea Pig." J. Biol. Chem. 176:477.

Deans, R. J. 1951. "A Recommended Procedure for Slaughtering Experimental Cattle." Reciprocal Meat Conference Proceedings 4:81.

DeFremery, D., and M. F. Pool. 1960. "Biochemistry of Chicken Muscle as Related to Rigor Mortis and Tenderization." Food Res. 25:73.

Eastoe, J. E. 1955. "The Amino Acid Composition of Mammalian Collagen and Elastin." Biochem. J. 61:589.

Embden. 1922. Cited by E. Lundsgäard. 1950. Proc. Roy. Soc. (B) 137:73.

Gersh, I., and H. R. Catchpole. 1949. "The Organization of Ground Substances and Basement Membranes." Amer. Jour. Anat. 85:457.

Goll, E., D. W. Henderson, and E. A. Kline. 1964. "Post-Mortem Changes in Physical and Chemical Properties of Bovine Muscle." J. Food. Sci. 29:590.

Golovkine, N. A., E. F. Zelentosov, A. P. Smirnov, and E. F. Shkolinikova. 1939. "Quality Indices for Cooled Meat." Intern. Inst. Refrig. 20 (1). 64. (Abstr.).

Gotte, L., P. Stern, D. F. Elasden, and S. M. Partridge. 1963. "The Chemistry of Connective Tissue. 8. The Composition of Elastin From Three Bovine Tissues." Biochem. J. 87:344.

Griswold, R. M. 1955. "Effect of Different Methods of Cooking Beef Round or Commercial and Prime Grades II. Collagen Fat and Nitrogen Content." Food Res. 20:171.

Hanson, H. L., B. Lowe, B. A. McClurg, and P. S. Shearer. 1946. "Physical Organoletic and Histological Changes in Three Grades of Beef During Agin." Food. Res. 3:284.

Hartley, L. M., and J. L. Hall. 1949. "Rapid Determination of Collagen in Beef by Waring Blendor and Centrifuge Techniques." Food Res. 14:195.

Herschberger, T., R. Deans, L. E. Kunkle, P. Gerlaugh, and F. E. Deatherage. 1951. "Studies on Meat III, Biochemistry and Quality of Meat in Relation to Certain Feeding Management Practices." Food Tech. 5:523.

Herring, H. K., R. G. Cassens, and E. J. Briskey. 1965. "Further Studies on Bovine Muscle Tenderness as Influenced by Carcass Position Sarcomere Length and Fiber Diameter." J. Food. Sci. 30. 6:1049.

Hiner, R. L., E. E. Anderson, and C. R. Fellers. 1955. "Amount and Character of Connective Tissue as it Relates to Tenderness in Beef Muscle." Food Tech. 9:80.

Husaini, S. A., F. E. Deatherage, and L. E. Kunkle. 1950. "Studies on Meat I. The Biochemistry of Beef as Related to Tenderness." Food Tech. 4:313.

Koonz, C. H., M. I. Darrow, and E. O. Essary. 1954. "Factors Influencing Tenderness of Principle Muscles Composing the Poultry Carcass." Food Tech. 8:97.

LaBella, F. S., and Gerald Paul. 1965. "Structure of Collagen From Human Tendon Influenced by Age and Sex." Jour. of Gerontol. 20:54.

Lansing, A. I. 1959. The Arterial Wall. Williams and Wilkins Co., Baltimore.

Lehmann, K. B. 1897. Cited by Mackintosh, et al. 1936.

Lilienthal, J. L. Jun., and K. L. Zierler. 1950. "A Reference Base and System for Analysis of Muscle Constituents." J. Biol. Chem. 182:501.

Locker, R. H. 1960. "Degree of Muscular Contraction as a Factor in Tenderness of Beef." Food Res. 35:304.

Lorinez, F., and I. Szeredy. 1959. "Quantitative and Qualitative Determination of Connective Tissue Content of Meat and Meat Products." J. Sci. Food Agr. 10:468.

Lowe, B., and J. Kastelic. 1961. "Organoleptic Chemical and Physical and Microscopic Characteristics of Muscles in Eight Beef Carcasses Differing in Age of Animal Carcass Grade and Extent of Cooking." Ames Iowa Res. Bull. 495. Iowa State University of Science and Tech.

Lowry, O. H., D. F. Gilligan, and E. M. Katersky. 1941. "The Determination of Collagen and Elastin in Tissue, With Results Obtained in Various Normal Tissues From Different Sources." J. Biol. Chem. 139:795.

Mackey, A. O., and A. W. Oliver. 1954. "Sampling Pork Loin for Cooking Test." Food Res. 19:298.

Mackintosh, D. L., J. L. Hall, and G. E. Vail. 1936. "Some Observations Pertaining to Tenderness of Meat." Proc. Amer. Soc. Animal Proc. 29:285.

Marion, J., and Faith Fenton. 1956. "Factors of Three Levels Nutrition and Age of Animal on the Quality of Beef Palatability Cooking Data Moisture Fat and Nitrogen." Food Res. 21:421.

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

Mathews, M. B., and Irene Lozaityte. 1963. "Sodium Chondroitin Sulfate-Protein Complexes of Cartilage 1. Molecular Weight and Shape." Arch. Biochem. Biophys. 74:158.

Martin, C. J., and A. E. Axelrod. 1953. "A Modified Method For Determination of Hydroxyproline." Proc. Soc. Exptl. Biol. Med. 83:461.

Maxwell, Shubert. 1966. "Structure of Connective Tissue: A Chemical Point of View." Fed. Proc. 25. pt 1. p. 1047.

McClain, P. E., A. M. Mullins, S. L. Hansard, J. D. Fox, and R. F. Boulware. 1965. "Relationship of Alkali Insoluble Collagen to Tenderness of Three Bovine Muscles." J. Animal Sci. 24:1107.

Mechanic, G. L., and M. Levy. 1959. "An ε-Lysine Tripeptide Obtained From Collagen." J. Am. Chem. Soc. 81:1889.

Meyer, K. 1945. "Mucoids and Glycoproteins." Adv. Prot. Chem. 2:249.

Meyer, V. C., and W. W. Lang. 1946. "An Increase in the Collagen Content With Advancing Age." J. Gerontol. 1:441.

Miller, M. 1954. Iowa State University Library, Ames, Iowa. Ph.D. Thesis.

Mitchell, H. H., T. S. Hamilton, and W. T. Haines. 1928. "Some Factors Affecting the Connective Tissue Content of Beef Muscle." J. Nutri. 1:165.

Morgan, T., and E. C. Smith. 1929. "Post-Mortem Changes in Animal Tissue. The Conditioning or Ripening of Beef." (Brit.) Food Invest. Bd. Special Rept., Vol. 36. H. M. Stationary Office, London.

Muir, H. 1964. Structure and Function of Skeletal Tissue. London, Butterworths. p. 170.

Neuman, R. E., and M. A. Logan. 1950. "The Determination of Hydroxyproline." J. Biol. Chem. 184:299.

Partridge, A. M., H. F. Davis, and G. S. Adair. 1955. "The Chemistry of Connective Tissue 2. Soluble Proteins Derived From Partial Hydrolysis of Elastin." Biochem. J. 61:11.

Partridge, S. M., and H. F. Davis. 1958. Chemistry and Biology of Mucopolysacharrides. London, Churchill.

Partridge, S. M. 1966. "Biosynthesis and Nature of Elastin Structure." Fed. Proc. 25 pt. 1.

Paul, P., and B. B. McLean. 1946. "Studies on Veal. I. Effect of Different Internal Temperatures on Veal Roasts From Calves of Three Different Weights." Food Res. 11:107.

Paul, P., and L. J. Bratzler. 1955. "Studies on Tenderness of Beef. II. Varying Storage Time and Conditions." Food Res. 20:635.

Piez, K. A., and J. Gross. 1960. "The Amino Acid Composition of Some Fish Collagen." J. Biol. Chem. 235:995.

Prockop, D. J., and S. Udenfriend. 1960. "A Specific Method for the Analysis of Hydroxyproline in Tissues and Urine." Anal. Biochem. 1:228.

Ramanchandran, C. N., and G. Kartha. 1955. Proc. Indian Acad. Sci. 42A:216. Cited by D. A. Hall, 1960. The Chemistry of Connective Tissue. C. C. Thomas, Springfield, Ill., U. S. A.

Ramachandran, C. N., V. Sasisekharan, and Y. T. Thathachari. 1961. Structure of Collagen at Molecular Level. Wiley, N.Y.

Ramsbottom, J. M., E. J. Strandine, and C. H. Koonz. 1945. "Comparative Tenderness of Representative Beef Muscles." Food Res. 10:497.

Ramsbottom, J. M., and E. J. Strandine. 1949. "Initial Physical and Chemical Changes in Beef as Related to Tenderness." J. Animal Sci. 8:398.

Rich, A., and F. H . C. Crick. 1961. "The Molecular Structure of Collagen." J. Mol. Biol. 3:483.

Rich, A., and F. H. C. Crick. 1955. "The Structure of Collagen." Nature. 176:915.

Ritchey, S. J. 1965. "The Relationship of Total Bound Free Water and Fat Content to Subjective Scores for Eating Quality in Two Beef Muscles." Food Sci., Vol. 30:375.

Satorius, M. J., and A. M. Child. 1938. "Effect of Coagulation on Press Fluid, Shear Force, Muscle-Cell Diameter and Composition of Beef Muscle." Food Res. 3:619.

Shatton, J., and M. Schubert. 1954. "Isolation of a Muco-protein From Cartilage." J. Biol. Chem. 211:565.

Sobel, H., and J. Marmorston. 1956. "The Possible Role of the Gel-Fiber Ratio of Connective Tissue in the Aging Process." J. Gerontol. 11:2.

Spencer, H. C., S. Morgulis, and V. M. Wilder. 1937. "A Micromethod for the Determination of Gelatin and a Study of the Collagen Content of Muscles From Normal and Dystrophic Rabbits." J. Biol. Chem. 120:257.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw Hill Book Company, New York, New York.

Steiner, G. 1939. "The Post-Mortem Changes in Beef Muscle at Various Temperature." Arch. Hyg. Bakt. 121. 193-238. Chem. Abstrac. 33:2965.

Stokes. 1897. Cited by Spencer, H. C., S. Morgulis, and V. M. Wilder. 1937. "A Micromethod for the Determination of Gelatin and a Study of the Collagen Content of Muscles From Normal and Dystrophic Rabbits." J. Biol. Chem. 120:257.

Thomas, J., and D. F. Elsden. 1963. "Constitution of the Cross-Linkages in Elastin." Nature. 197:1297.

Tuma, H. J., R. L. Henrickson, D. F. Stephens, and Rubymoore. 1962. "Influence of Marbling and Animal Age on the Factors Associated With Beef Quality." J. Animal Sci. 21:848.

Weiner, P. D., D. H. Kropb, D. L. Mackintosh, and B. A. Koch. 1966. "Effects on Muscle Quality of Processing Pork Carcass With One Hour Post-Mortem." Food Tech. 20:4.

Willington, G. H. 1953. "Recommended Procedure For Cutting Beef." Reciprocal Meat Conference Proceedings, 6:73.

Wilson, G. D., R. W. Bray, and P. H. Phillips. 1954. "The Effect of Age and Grade on the Collagen and Elastin Content of Beef and Veal." J. Animal Sci. 13:4.

Wilson, G. D. 1959. "Physical Characteristics of Muscle Tissue as Related to Imbibed Water Proc." Recip. Meat Conf. 12:97.

Woessner, J. F., Jr. 1961. "The Determination of Hydroxyproline in Tissue and Protein Sample Containing Small Portions of This Imino Acid." Arch. Biochem. Biophys. 93:440.

Woessner, J. F., Jr. 1963. "Age Related Changes of the Human Uterus and Its Connective Tissue Framework." J. Gerontol. 18:222.

Wood, G. C., and M. K. Keech. 1960. "The Formation of Fibrils From Collagen Solutions; The Effect of Experimental Conditions; Kinetic and Electron Microscope Studies." Biochem. J. 75:588.

VITA

Ling Wong Lee

Candidate for the Degree of

Master of Science

Thesis: THE CONNECTIVE TISSUE IN PRE- AND POST-RIGOR BOVINE MUSCLE

Major Field: Food Science

Biographical:

Personal Data: Born in Si-an, China, July 4, 1941, the daughter of Ji-Yiun and Shou-Ching Wong. Married to Che-Fu Lee on June 4, 1966.

Education: Attended grade school in Taichung, Taiwan; graduated from Taichung Girl's High School in June, 1959; received a Bachelor of Science degree from Taiwan University with a major in Agricultural Chemistry in June, 1964.

Experience: Employed part time at National Taiwan University's Nutrition Laboratory; Research assistant in Biochemistry Department at Naval American Medical Research Unit II, Taipei, Taiwan.