THE INFLUENCE OF PARA-CHLOROMERCURIBENZOATE ON VARIOUS CHEMICAL AND CULINARY ATTRIBUTES OF BOVINE LONGISSIMUS DORSI MUSCLE

By

JERRY D. GRESHAM

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1965

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, 1971

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MUSCLE

Thesis Approved:

John J. Guenther
Thesis Adviser

Lowell E. Walters

George V. Odell

D. D. Durham
Dean of the Graduate College

788303

ACKNOWLEDGMENTS

The author expresses his sincere appreciation to Dr. John J. Guenther, Professor of Animal Science, for his guidance and counsel during the course of this study and the preparation of this thesis.

Grateful acknowledgment is also extended to Dr. L. E. Walters, Professor of Animal Science, and Dr. G. V. Odell, Professor of Biochemistry, for their cooperation and assistance in the preparation of this thesis.

Special appreciation is extended to Dr. R. D. Morrison, Professor of Mathematics and Statistics, for his assistance in the statistical analysis.

The author also wishes to acknowledge the partial financial support of this study by the Market Quality Research Division, Agricultural Research Service, United States Department of Agriculture under Cooperative Agreement 12-14-100-9348(51).

Further appreciation is also extended to Mrs. Juanita Chin-Choy, laboratory technician, and Mr. Roy Escoubas, graduate student in Food Science, for their invaluable assistance in the collection of the data.

The writer also wishes to express thanks for the understanding and continuous encouragement from his wife, Carolyn, and son, Darryl, during this program of graduate study.

TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	3
	Structure of Muscle	3
	Macro-Structure	3
	Micro-Structure	4
	Composition of Myofibril	5
	Myofibrillar Proteins	7
	Myosin	7
	Actin	9
	Tropomyosin	9
	Troponin	10
	Theory of Muscular Contraction	11
	Mechanism	11
	Actin-Myosin Interaction	12
	Onset of Rigor	14
	Myofibrillar ATPase Activity	15
	Cation Effect on ATPase	17
	Enzymatic Inhibition by Organic Mercurials	19
	Tenderness and Culinary Attributes	20
	Protein Solubility	20
	Contraction and Tenderness	21
	Variation in Tenderness	22
III.	MATERIALS AND METHODS	23
	General	23
	Procedure	23
	Extraction of Experimental Material	23
	Preparation and Injection	24
	Cooking and Tenderness Evaluation	24
	Sampling for Chemical Evaluation	27
	pH Determination	27
	Extraction of Myofibrillar Protein Fraction	27
	Protein Determination	29
	Enzyme Assay Conditions	30
	Inorganic Phosphate Determination	30
	Calculation of ATPase Activity	31
	Protein Solubility	32
	Statistical Analysis	33

Chapter | Page

IV. RESULTS AND DISCUSSION 34

Uniformity Study 34
Cooking and Tenderness Evaluation 34
Tenderness Evaluation 45
Inhibitor Study 51
Objective 51
Results of Tenderness Study 51
Results of ATPase Study 54
Results of pH Study 59
Results of Protein Solubility Study 62

V. SUMMARY AND CONCLUSIONS 68

LITERATURE CITED 70

APPENDIX 75

LIST OF TABLES

Table		Page
I.	Analysis of Variance for Cooking Evaluation By-Side	37
II.	Analysis of Variance for Cooking Evaluation Pooled	38
III.	Analysis of Variance for Shear Force By-Side	47
IV.	Analysis of Variance for Shear Force on Pooled Basis . . .	49
V.	Analysis of Variance on the Effect of PCMB on Shear Force	52
VI.	Analysis of Variance on the Effect of Time on Calcium and Magnesium Activated Myofibrillar ATPase Activity . .	55
VII.	Analysis of Variance on the Effect of Location on Calcium and Magnesium Activated Myofibrillar ATPase Activity	57
VIII.	Analysis of Variance on the Effect of Time on pH	60
IX.	Analysis of Variance on the Effect of Location on pH	61
X.	Analysis of Variance on the Effect of Time on the Solubility of Myofibrillar Protein	64
XI.	Analysis of Variance on the Effect of Location on the Solubility of Myofibrillar Protein	66
XII.	Mean Values for Initial Steak Weight, Cooked Steak Weight, and Cooking Loss by Steak Location	76
XIII.	Mean Values for Cooking Time, Percent Cooking Loss, and Cooking Rate by Steak Location	77
XIV.	Mean Values for Shear Force	78
XV.	Mean Values for Influence of PCMB on Shear Force	79
XVI.	Mean Values for Influence of PCMB on Myofibrillar ATPase by Time and Location	80

Table | Page

XVII. Mean Values for Influence of PCMB on pH by Time and Location 81

XVIII. Mean Values for Influence of PCMB on Myofibrillar Protein Solubility by Time and Location 82

LIST OF FIGURES

Figure		Page
1.	Proposed Structure of the Sarcomere	6
2.	Proposed Interdigitating Model for Muscle Contraction	13
3.	Proposed Interaction of Actin and Myosin	16
4.	Proposed "Hinged" Structure of Myosin Head	16
5.	Schematic Illustrating Steak Locations and Sampling Sites	26
6.	Extraction Procedure for Myofibrillar Protein	28
7.	Design of Experiment	35
8.	Relationship of Cooking Variables	44
9.	Relationship of Cooking Time and Cooking Rate	46
10.	Mean Shear Values by Core Position	50
11.	Mean Shear Value by Steak Location	50
12.	Relationship of Shear Values for Control and Treated Muscles by Steak Location	53
13.	Influence of Time on Myofibrillar ATPase Activity of PCMB Treated Muscles	56
14.	Influence of Time on Myofibrillar ATPase Activity of Control Muscles	56
15.	Influence of Location on Myofibrillar ATPase Activity of Control Muscles	58
16.	Influence of Location on Myofibrillar ATPase Activity of PCMB Treated Muscles	58
17.	Influence of Time on pH	63
18.	Influence of Location on pH	63

Figure | Page

19. Influence of Time on Protein Solubility 67

20. Influence of Location on Protein Solubility 67

CHAPTER I

INTRODUCTION

During recent years, the beef industry has progressively faced stiffer competition from producers of synthetic food products for part of the food dollar. Since these synthetic products may be produced and sold to the consumer at a lower cost, it is imperative that measures be taken to improve the quality and lower the cost of retail block beef.

While in post-mortem rigor, the beef muscle is considered in its low point in market quality for such attributes as tenderness and juiciness. The development of this condition may be characterized by hydrolysis of ATP and the interaction of the primary muscle proteins, myosin and actin, into the major protein constituent of "dead" muscle, actomyosin. It was felt that the study of this interaction might offer some insight into their relationship to quality of block beef.

Prior research indicated that the control of myosin ATPase was possible, but the isolation and purification of myosin is a lengthy procedure. Therefore, it would be of benefit to use a procedure not requiring this great length of time and muscle sample size.

This study was directed towards determining the adenosine-triphosphatase activity of the myofibrillar protein fraction, of which myosin is the major component. The assay system to be utilized

was the same as for a purified myosin system. Previous work had indicated that para-Chloromercuribenzoate was an efficient inhibitor of myosin ATPase and should have the same effect on a myofibrillar fraction. Also, the relationship of myofibrillar ATPase activity to tenderness of the muscle was to be determined.

CHAPTER II

LITERATURE REVIEW

The review to follow is divided into the following general areas: structure of muscle, characteristics and properties of the myofibrillar proteins, theory of muscle contraction, and culinary attributes of the Longissimus Dorsi muscle.

Structure of Muscle

Macro-Structure

All muscles within a meat animal are characterized by an external boundary of connective tissue known as the fascia or epimysium. The function of this layer of connective tissue is to bind the individual bundles of muscle fibers and groups of muscles into place. It is composed mainly of fibrils of collagen, which are highly inextensible as soon as they become taut. Another important epimysial component is elastin. Elastin is comprised of wavy fibrils of rubber-like protein and is concentrated primarily in the arterial walls and ligaments where rapid elastic recovery from stretch is necessary. The thickness and composition of the fascia is generally determined by the stress the muscle must undergo. The greater the stress, the thicker the fascia and the higher the concentration of elastin in the connective tissue. The muscles of locomotion (i.e., semimembranosus, biceps

femoris) would be expected to have a thicker fascia than the muscles of support (i.e., psoas major and psoas minor).

Within the fascia, the muscle consists of primary bundles of fibers bound together by a thinner layer of connective tissue known as the perimysium. These bundles are collected together into larger bundles by septa, which originate from the epimysium or fascia-sheath. From the epimysium emerges the endomysium, fine extensions which surround the individual muscle fibers. The muscle fiber is the smallest unit composing the "macro-structure" of meat.

Micro-Structure

Muscle fibers are composed of microscopic myofibrils which are generally 1 to 2 microns thick. These fibrils are separated from one another by the fine network of tubules of the sarcoplasmic reticulum. These tubules meet at points along each fibril to form the triads from which transverse tubules pass from one fibril to another. These tubules terminate upon contact with the muscle cell membrane, or sarcolemma (Porter, 1961). This system of tubules serves to transmit the nervous impulse from the sarcolemma to the individual fibrils (Huxley, 1957). The sarcoplasm is the intercellular fluid of muscle which contains glycogen, the glycolytic enzymes of the glycolysis pathway, adenosine triphosphate (ATP), phosphocreatine (CP), and certain amino acids and peptides.

The myofibrils (or fibrils) are the functional contractile units of the muscle cell. The composition and structure of these myofibrils give rise to the cross-striations of striated muscle. Electron micrographs indicate these fibrils consist of alternate dark (A) and

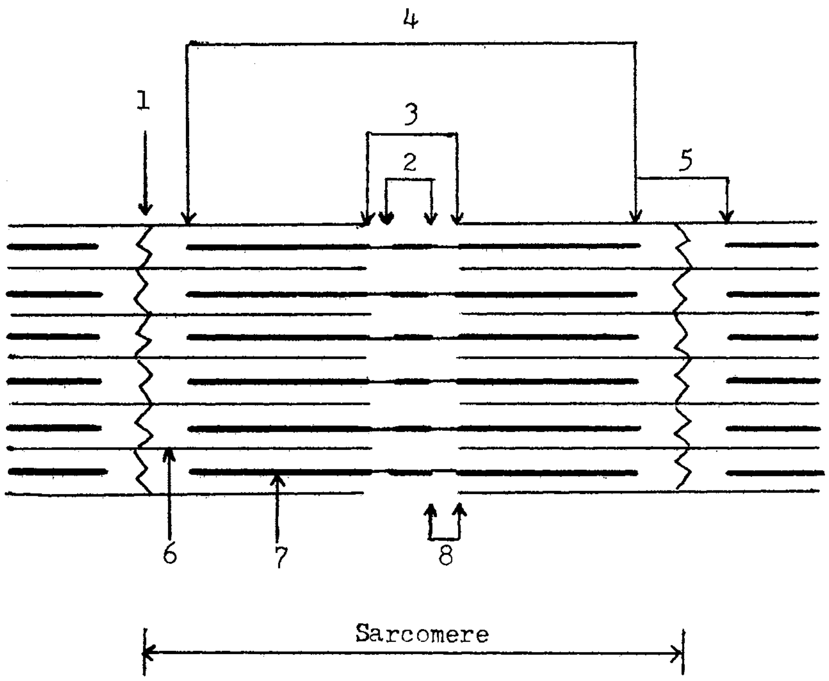
light (I) bands. A clear H-zone is distinguishable in the middle of the dark band. A thin line, known as the Z-line, may be detected transversing the light bands at regular intervals. The longitudinal repeat unit from one Z-line to the next is known as a sarcomere (Figure 1).

Further investigation led to the discovery that the fibril is composed of two sets of filaments, a thick set which runs from edge to edge of the A-band and a thin set running from each Z-line to the middle of the A-band. A fixed muscle may show the thin filaments touching in the H-zone. However, normal relaxed muscle will show a definite, well-defined H-zone (Huxley and Hanson, 1960).

The myofibril, as mentioned previously, is the structure of muscle involved in the contractile process. It exists in its most specialized form in striated muscle where it occupies 60% to 70% of the whole cell mass and a similar proportion of the muscle volume. It is reported that the three major proteins of the myofibril are myosin, actin, and tropomyosin. These three proteins make up approximately 80% of the total protein content of the myofibril (Perry, 1965). These proteins have repeatedly been found to be essential to muscle contraction and relaxation. There are other minor proteins in the myofibril, troponin and α-and β-actinin. These proteins are involved in the contractile process, but they are of lesser importance than the three major proteins listed.

Composition of Myofibril

To fully understand the complexity of the muscle cell and its functional components (myofibrils), it is necessary to review the



1: Z line (tropomyosin, troponin, -actinin)
2: M line
3: H or pseudo H zone
4: A band
5: I band
6: Thin filament (actin, α-actinin, β-actinin, tropomyosin, troponin)
7: Thick filament (myosin)
8: L line

Figure 1. Proposed Structure of the Sarcomere (Huxley, 1969)

physical and chemical properties of the various protein components. This discussion will be limited to the characteristics of the more important components of the myofibrils.

Myofibrillar Proteins

Myosin

Even though the most elementary property of myosin would appear to be its molecular weight, this has been a very controversial subject and not yet fully resolved. The molecular weight of myosin has been reported in a range of 420,000 (Mommaerts and Aldrich, 1958) to 1,500,000 (Snellman and Erdos, 1948). Kielly and Harrington (1960), utilizing Archibald experiments, proposed a value of 619,000 on rabbit skeletal myosin. It was thought that possibly the high value reported by Kielly and Harrington was due to the high salt concentration required for the fractionation procedure leading to aggregation of the protein. Gellert and Englander (1963) determined a value of 520,000 with ammonium sulfate fractionation. It appeared that the method of extraction had an effect on the apparent molecular weight of the molecule. Rickansrud (1969), using the analytical centrifuge for sedimentation studies, reported a value of 485,000 for bovine myosin.

From the apparent high molecular weight of the myosin molecule, it was thought that the molecule could contain subunits which could be separated and identified. Kielly and Harrington (1960) and Kielly and Barnett (1961) suggested that the myosin molecule consists of three similar polypeptide chains of molecular weight 200,000 each. This value would be in agreement with the molecular weight of 600,000

reported by the ammonium sulfate fractionation procedure; however, it is above our best estimate of 475,000 to 490,000. Locker (1954) reported one C-terminal isoleucine of 300,000 molecular weight. Further studies with the electron microscope by Slayter and Lowey (1967) indicate that the myosin molecule is composed of two identical, coiled polypeptide chains. It has also been shown that myosin may be composed of other fragments. Under mild conditions of tryptic digestion, myosin is converted into two major fragments, the light and heavy meromyosins (Szent-Gyorgyi, 1953). Lowey and Cohen (1962) also suggest that the myosin molecule may be broken into two fragments by tryptic digestion. These fragments are designated as light meromyosin fragment 1 (LMM Fr. 1) and heavy meromyosin (HMM) (Lowey and Cohen, 1962; Slayter and Lowey, 1967). The LMM Fr. 1 is obtained by alcohol fractionation from LMM and is the major product of LMM which was determined to be approximately 30% of the total myosin digest. The HMM was determined to compose the other 70% of the total myosin digest.

Further digestion of the HMM produces subfragments designated as HMM S-1 and HMM S-2 (Mueller and Perry, 1961; Slayter and Lowey, 1967). The HMM S-1 was shown to be the globular head of the myosin molecule by electron microscopic studies of Gergely (1964). Additional investigations by several workers have shown that the HMM S-1 is the site of enzymatic activity of the myosin molecule (Frederickson and Holtzer, 1968; Lowey and Cohen, 1962; Slayter and Lowey, 1967). Further degradation of HMM S-1 by Locker and Hagyard (1967) indicated there are three small subunits of molecular weight near 20,000 in the globular head of myosin.

In summary, myosin is the principal protein present in the myo-

fibril; has a molecular weight of just under 500,000; and is composed of two identical, α-helical coiled polypeptide chains terminating in a globular head which contains the active sites for enzymatic activity.

Actin

The second most abundant protein in the muscle myofibril is actin. It may exist in either of two forms, globular (G-actin) or fibrous (F-actin) (Gergely, 1964). The G-actin molecule was shown to have a bound nucleotide in the form of ATP which will polymerize to form a fibrous (F) protein aggregate, containing bound ADP. Each molecule of G-actin apparently binds one Ca^{++} ion very tightly in addition to one molecule of ATP leading to polymerization to the F-actin form. This rate of binding appears to be approximately 1.0 to 1.5 moles of bound Ca^{++} per 60,000 molecular weight of protein.

An actin filament consists of subunits which appear to be identical and spherical in shape with a molecular weight of near 68,000 for each spherical unit (Hanson and Lowey, 1963). These subunits are the G-actin units which are polymerized to F-actin filaments. Huxley (1960) determined the molecular weight of a G-actin subunit to be between 60,000 and 70,000.

The function of actin in the muscle contractile system will be discussed in the following section.

Tropomyosin

The difficulty in obtaining a pure sample of actin has been reported by several authors. The difficulty is apparently due to contamination with other proteins. One of the major contaminating

proteins is tropomyosin (Bailey, 1948), the third major component of myofibrillar protein. The molecular weight of tropomyosin has been determined to be approximately 70,000 (Holtzer et al., 1965; Woods, 1965). It is a highly charged molecule and polymerizes readily under ionic conditions essentially opposite to those prevailing for actin (Hayashi, 1967). Tropomyosin also complexes with actin, and this property leads to its persistence in actin preparations (Drabikowski, 1962). Because of this close association with actin, it is reasonable to expect that tropomyosin would and apparently does occur in the thin filaments of the myofibril (Page and Huxley, 1963). It has been proposed that the function of tropomyosin is to stabilize the structure of the thin filaments by inhibiting depolymerization of the F-actin polymer (Szent-Gyorgyi and Kaminer, 1963).

Troponin

Troponin is a globular protein that promotes the aggregation of tropomyosin and has been shown to be an essential component of native tropomyosin (Ebashi and Kodama, 1966). Troponin alone has no apparent influence on the process of superprecipitation of highly purified synthetic actomyosin, but if combined with tropomyosin, it shows essentially the same action as that of native tropomyosin. Since troponin does not appear to have any direct effect on actin or myosin, its effect is considered to be exerted through tropomyosin bound to F-actin. While neither seem to be of major importance alone in muscle, the presence of both proteins is essential for the physiological action of calcium ions in the regulation of the contraction-relaxation cycle of muscle (Ebashi and Kodama, 1966; Stewart and Levy, 1970).

Ebashi et al. (1968) suggest that the major role of troponin in muscular contraction is to serve as a binding site for calcium. Also, it has been reported that the binding rate of troponin for calcium was not affected by addition of any of the other contractile proteins, nor the addition of ATP. In addition, although troponin contains sulfhydryl groups, it was shown that PCMB did not have any effect on the calcium binding capacity of troponin (Ebashi et al., 1968). Therefore, we may conclude that while troponin is not directly involved in the contraction-relaxation cycle of muscle, it may exert its influence by serving as the binding site for calcium. Since calcium is necessary for contraction, it would appear troponin serves by a depressing action on the interaction of actin and myosin. This troponin effect is abolished by the addition of calcium to the medium (Ebashi et al., 1968). Stewart and Levy (1970) reported a tropomyosin-troponin complex was responsible for relaxation of muscle by facilitating the binding of the Mg-ATP complex which inhibits ATP hydrolysis. The addition of calcium overcomes this substrate inhibition and causes the muscle to contract. The removal of the tropomyosin-troponin complex apparently desensitizes the system to calcium, thus prohibiting rapid contraction.

Theory of Muscular Contraction

Mechanism

The sliding-filament theory is presently the most widely accepted description of the mechanism by which striated muscle contracts (Huxley and Hanson, 1954). A proposed structure for this mechanism

is presented in Figure 1 (Huxley, 1969).

According to this interdigitating filament model (Figure 2), the contractile material consists of series of partially overlapping arrays of actin and myosin filaments which form the myofibrils. When the muscle changes length, the sliding model supposes that the length of the filaments remain essentially constant, but the overlapping units slide past each other—the actin (I band) being drawn into the myosin (A band). At lengths below complete overlap of the filaments, the I filaments overrun each other, and their number doubles in the center of the A band (Huxley and Hanson, 1957).

One of the foremost problems in the study of muscular contraction has been the mechanism of the actin-myosin interaction. There is considerable evidence now that a cross-bridge does indeed exist between the myosin molecule and the actin molecule. This was first shown by electron microscope studies of Huxley (1957). These bridges appeared to be projections on the myosin filaments and were not observed on the actin filaments. The only function of these projections appeared to be an attachment to the actin filament. Thus, it was proposed that these projections probably represent the HMM subunit of the myosin molecule which contained the sites for enzymatic activity.

Actin-Myosin Interaction

The interaction of actin and myosin is evidenced by the enzymatic behavior of myosin. Purified myosin, in the presence of concentrations of magnesium and calcium ions similar to those expected in muscle during activity, has relatively low ATPase activity (Mommaerts

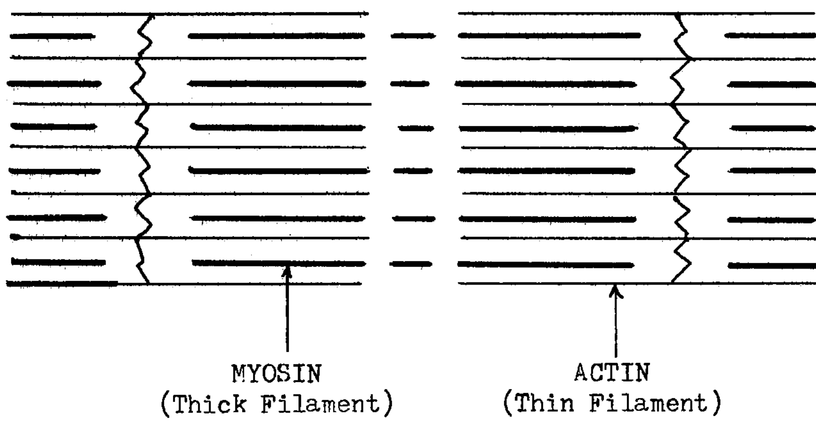


Figure 2. Proposed Interdigitating Model for Muscle Contraction (Huxley, 1969)

and Green, 1954). However, at the same concentrations and in the presence of actin, the ATPase activity is increased to the amount required for muscular activity. Therefore, it would appear this increased activity could be due to contact between the actin and myosin filaments. Weber and Herz (1963) reported that the ATPase activity of unpurified actomyosin and of myofibrils can be regulated in vitro by the concentration of calcium ions, a change in concentration from $10^{-7}M$ to $10^{-5}M$ being adequate to increase myosin activity to actin-myosin activity 20-fold. Huxley (1964) presented evidence that a release of calcium ions from the sarcoplasmic reticulum activated muscular contraction, and a rebinding of the calcium ions to the sarcoplasmic reticulum permitted relaxation. Perry and Grey (1956) reported that the ATPase activity of purified actomyosin was insensitive to the absence of calcium and continued high when calcium was withdrawn. Ebashi (1964) reported that calcium sensitivity could be restored to this purified system by adding tropomyosin and troponin.

Onset of Rigor

"Rigor mortis" is the loss of extensibility of a muscle. When a muscle loses ATP and goes into rigor, it becomes rigid and inextensible, a phenomena associated with the attachment of a large number of the cross-bridges of myosin to the actin filament. Since neither the subunit repeats nor the α-helical repeats of the myosin and actin filaments are the same, such an attachment can take place only if some part of the structure alters its configuration from the resting state.

Muscles in rigor show a high proportion of the whole mass of each of the cross-bridges moving away from the position it occupied in the resting muscle (Figure 3). There now appears to be an attachment to the subunits of the actin filament resulting in the formation of actomyosin, principal protein of "dead" muscle. However, this attachment does appear to be flexible, and the cross-bridges are able to swing around the thick filaments (Huxley, 1969). This phenomena was explained by Huxley and Brown (1967). They reported the globular part of myosin (cross-bridge) was attached to the backbone of the myosin filament by a linear region of the molecule which would be bonded at one end and not along its entire length. Hence, the "head" of the molecule is "hinged" to the thick filament (Figure 4). If the end of the cross-bridge is allowed to move to a new position by attachment to actin, it is not necessary to change the orientation of the globular region, but only to move the complete globular region to the attachment site on the actin molecule.

Myofibrillar ATPase Activity

Studies on myosin ATPase are numerous; however, one drawback is the time required to isolate a pure myosin sample. Whereas it takes approximately 72 hours to isolate a myosin sample, the myofibrillar fraction can be isolated in a period of one to two hours. Thus, it would appear to be advantageous to study the myofibrillar ATPase system by use of mercurical inhibitors for possible practical applications (i.e., determination of condition and state of post-mortem rigor mortis). Inhibition of the myofibrillar ATPase system should inhibit the formation of the actomyosin complex, resulting in

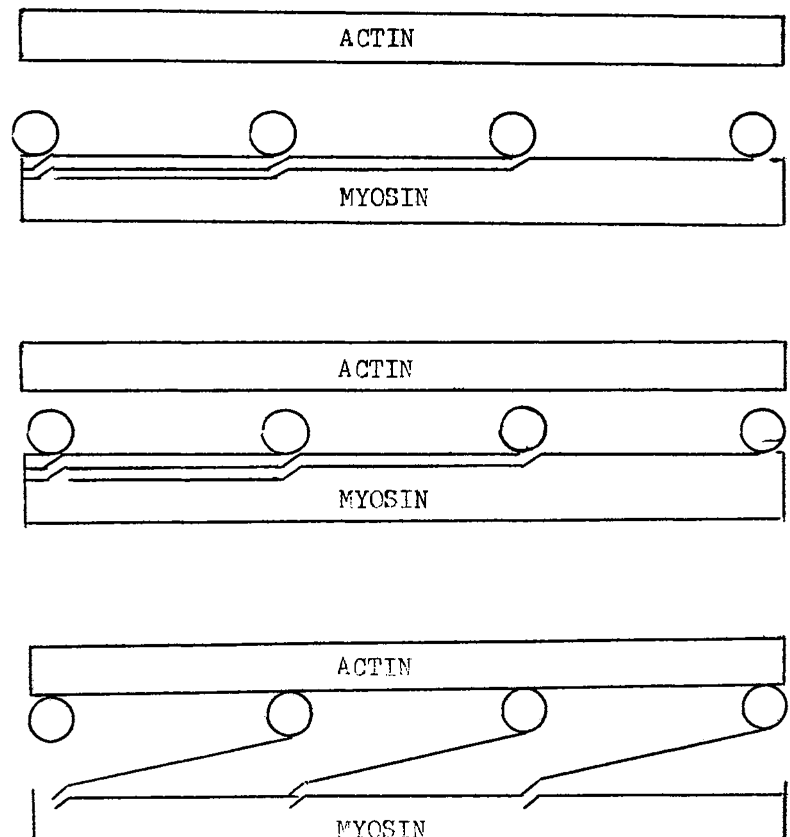
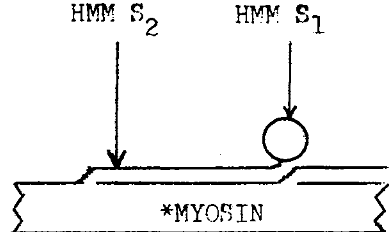
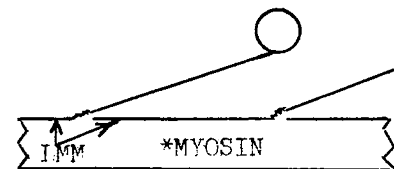


Figure 3. Proposed Interaction of Actin and Myosin (Huxley, 1969)





*Backbone of Myosin Filament

Figure 4. Proposed "Hinged" Structure of Myosin Head (Huxley, 1969)

existence of free myosin and actin. Preventing the myosin-actin interaction would greatly enhance the palatability of the retail product. Therefore, a quick, accurate determination of the state of the actomyosin complex could serve as a valuable tool in marketing retail beef.

Perry and Grey (1956) reported results of a study on the ATPase activity of a fresh muscle tissue homogenate. They reported that the activation of a myofibrillar ATPase by magnesium was highly dependent on substrate concentration. Maximum activity was obtained when the concentration of ATP and magnesium were about equal. An inhibition of ATPase activity was observed when the concentration of ATP exceeds that of magnesium. Calcium was also found to activate myofibrillar ATPase; however, increased activity was observed in the presence of magnesium. Excess ATP has less inhibitory effect with calcium as the activating metal than with magnesium.

Cation Effect on ATPase

Calcium (ionic radius 0.99Å) is virtually the only cation that will stimulate myosin ATPase activity (Schaub and Ermini, 1969). However, the interaction of actin with myosin causes the enzymatically active center of myosin to become accessible to stimulation by a wider range of bivalent cations. Magnesium (ionic radius 0.65Å) will now stimulate ATPase activity as well as many other bivalent cations (i.e., Co^{++}, Mn^{++}, Zn^{++}, Cd^{++}). The ATP-metal ion complex is thought to be the true substrate in the enzymatic reaction with the active site of the protein (Weber, 1959). From the results of Schaub and Ermini (1969), it appears that the "ionic size" of the metal ion seems

to effect the ATPase activity of myosin, and myosin with other protein, and not the chemical properties of the metal ion. It was found that myosin ATPase was stimulated by metal ions with an ionic radius of approximately 1.0Å (i.e., calcium). Actomyosin ATPase was stimulated by metals with an ionic radius of approximately 0.6Å (i.e., magnesium).

It is known that high concentrations of ATP, in excess over Mg^{++} concentrations, inhibit the rate of hydrolysis of ATP by actomyosin. Perry and Grey (1956) attribute this inhibition to a competition between the Mg-ATP complex, the assumed substrate, and free ATP for the active site on the enzyme. Therefore, the rate of hydrolysis of ATP by actomyosin depends on the concentration of ATP as well as that of Mg^{++}. At a concentration of 1mM ATP, the rate of cleavage is activated by Mg^{++} in concentration lower than that of ATP, but it is inhibited if the concentration of Mg^{++} exceeds that of ATP. At a higher concentration of ATP (6 to 8mM), the rate of hydrolysis is more complex. When the concentration of ATP is 8mM, Mg^{++} will activate ATPase in a concentration of 0.001mM to 0.1mM. Between 0.1mM and 0.5mM, Mg^{++} will inhibit ATPase, then activate again between 0.5mM and 1.0mM (Weber, 1959). The highest rate of hydrolysis was when the Mg^{++}:ATP ratio was 1. If calcium is added (0.1mM) to the above system, the inhibition in the intermediate concentration of Mg^{++} does not occur, and the maximal activation is near 0.5mM. Therefore, maximum hydrolysis of ATP by actomyosin, with Mg^{++} as activator, occurs at very low concentrations of Ca^{++}. In the absence of Ca^{++}, the Mg^{++} ion creates a dual effect. It will activate to a small extent at very low concentrations and inhibit at higher

concentrations. Weber suggests that Mg^{++} activates actomyosin ATPase by forming a substrate Mg-ATP, but the hydrolysis of the substrate is inhibited when additional Mg^{++} is bound to the other site of the enzyme.

Enzymatic Inhibition by Organic Mercurials

There is considerable evidence in the literature of the location of the enzymatically active site of the myosin molecule. It is generally accepted that the active site is in the HMM S-1 fragment (Frederickson and Holtzer, 1968; Lowey and Cohen, 1962; Slayter and Lowey, 1967; Gazith *et al.*, 1970). The importance of the sulfhydryl groups to enzymatic activity has been shown by the use of sulfhydryl blocking agents (Sreter, 1966; Gazith *et al.*, 1970). Thus, the sulfhydryl groups are apparently located in or near the active site of the myosin molecule and are essential to enzymatic activity. Since the hydrolysis of ATP is necessary for muscular contraction (Huxley, 1969), it would seem reasonable that the blocking of the sulfhydryl groups would reduce ATPase activity and inhibit muscle contraction since there would be no source of energy. One method of accomplishing this is by mercurial compounds. Mercurial compounds are not specific for a particular enzyme, hence, would be expected to react with all -SH groups in a system. One of the more commonly used compounds in muscle research is para-Chloromercuribenzoate (PCMB), an organic mercurial compound. The blocking of the -SH groups should prevent the formation of actomyosin. Both actin and myosin possess -SH groups of differing degrees of reactivity and function. In the complexing of actin and myosin to form actomyosin, it is the -SH groups of

myosin which are important (Bailey and Perry, 1947). Drabikowsky and Gergely (1963) reported that mercurials released ATP from G-actin, thus causing depolymerization of the F-actin to G-actin. The concentration of PCMB is quite important for its effect on ATPase activity. Sreter (1966), using PCMB, reported a difference in red and white muscle myosin ATPase activity. The same authors also reported an increase in ATPase activity by addition of PCMB with twice the concentration of PCMB required for maximal activation of red muscle myosin ATPase as with white muscle. Gazith et al. (1970) reported that blocking of -SH groups by PCMB could be reversed by removal of the blocking groups by dialysis against β-mercaptoethanol or dithiothreitol with no loss in ATPase activity.

Tenderness and Culinary Attributes

Protein Solubility

The relationship of solubility of the myofibrillar proteins and tenderness has been investigated by several workers. Wierbicki (1954) reported a positive relationship between extractability of muscle proteins and tenderness with an increase in tenderness and total nitrogen extracted over a storage period of 4 to 15 days. This indicates that an increase in protein solubility is related to the resolution of rigor.

Cook (1967) found that stretching muscle fibers during the onset of rigor mortis led to a significant increase in the solubility of protein per gram of post-rigor tissue as compared to non-stretched muscle fibers. This indicates the contractile state of the myofibrils

greatly enhances their solubility. Similar results were reported by Khan (1964) who found that the solubility of the myofibrillar proteins could be increased by inhibiting the complete interdigitation of actin and myosin.

Davey and Gilbert (1968) reported a relationship between the ultimate pH of bovine longissimus dorsi muscle and myofibrillar protein extractability. Their results showed that the maximum percentage extractability was 57% for meat of ultimate pH 5.45, increasing to 72% for meat of ultimate pH 6.30.

Contraction and Tenderness

There is considerable evidence that tenderness decreases post-mortem. Locker (1960) suggested the toughening that occurred might have been due to shortening of the muscle during the onset of rigor mortis. Locker's results also showed that this shortening was greater for muscles excised immediately post-mortem than for those left on the carcass during chilling. The relationship of contraction and tenderness was also reported by Gothard et al. (1966), who examined sarcomere length with a phase microscope and filar micrometer. Their results indicate that longissimus dorsi muscle at 8 days post-mortem was less tender in the more contracted muscles than with the less contracted muscles.

Herring (1968) studied the relationship between enzyme ATPase activity and tenderness. Also, Herring reported that the onset of rigor mortis was associated with an increase in actomyosin ATPase activity with either calcium or magnesium as activating cation. This activity was also shown to decrease with post-mortem aging of the

muscle, but never to the level found in the fresh muscle samples. Herring also found that actomyosin ATPase was lower for less tender muscle than for more tender muscle at 0 hours post-mortem.

Variation in Tenderness

Ramsbottom, *et al.* (1945) reported a variation in tenderness of the longissimus dorsi muscle with the anterior end being less tender than either the middle or posterior end. Howard and Judge (1968) found the lateral half of the longissimus dorsi muscle was more tender than the medial half. Tuma *et al.* (1962) and Alsmeyer *et al.* (1965) found bovine longissimus dorsi muscle to be more tender in the medial half than in the lateral half.

CHAPTER III

MATERIALS AND METHODS

General

Experimental material for this experiment consisted of seven, Choice grade, steers of similar background and weighing approximately 500 kg. each. The steers were delivered to the OSU Meat Laboratory approximately 24 hours prior to slaughter and withheld from feed and water for this period. The animals were inspected and prepared for slaughter according to Federal Meat Inspection Regulations. The animals were humanely stunned with a Remington captive-bolt stunning device, shackled, and bled. The carcasses were processed according to standard procedures and presented for sampling within 15 minutes of stunning.

Procedure

Extraction of Experimental Material

Based on the results of a previous pilot experiment, it was decided to utilize only the longissimus dorsi muscle for this experiment. Since the purpose of this study was to evaluate the relationship of certain myofibrillar protein properties to tenderness of block beef, the muscle samples were removed as "blocks". The experimental unit consisted of the longissimus dorsi muscle from

each side, removed from the seventh rib to the fourth lumbar vertebra. This resulted in the muscle section weighing approximately 4.0 to 4.5 kg. and measuring 50 cm. in length. Immediately upon removal from the carcass, samples were removed from each end of both muscles for initial pH determinations. The muscles were alternately designated as treatment and control and then prepared for injecting with the test solution.

Preparation and Injection

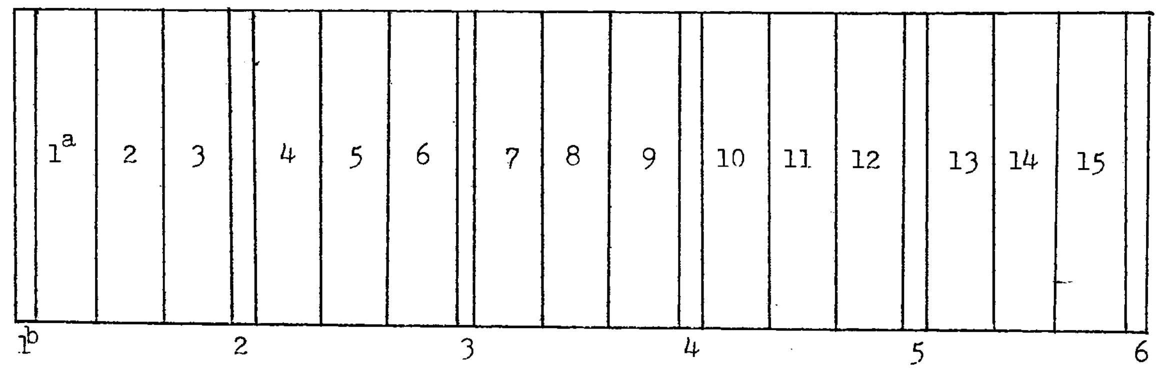
Both muscle blocks were taken to a processing room and trimmed of excess fat. Samples were removed from each for later chemical evaluation. The muscles were then weighed, and the control muscle was wrapped in two layers of "TITE" freezer paper and stored in a $4^{o}C$ cooler. After preparation of the injection solution, the muscle blocks were placed on a metal oven rack over a sink in order to allow any uninjected solution to drain. The treatment muscle was then injected with para-Chloromercuribenzoate (PCMB) to a final concentration of 5.0×10^{-4}mM in a volume of 15% of its original weight. PCMB utilized for this experiment was obtained from Calbiochem. The solution was injected with a 50 cc. hypodermic syringe fitted with a 15 cm. needle. After injection, the muscle was again weighed in order to calculate the percent injection of each muscle block. The muscle was then wrapped and stored the same as the uninjected control muscle block.

Cooking and Tenderness Evaluation

Both control and treated muscle blocks were stored for 96 hours

and then removed for cooking and tenderness evaluation and sampling for biochemical studies. Upon removal from the cooler, both muscles were weighed and percent cooler shrink was calculated. In order to maintain the muscles in as near the same condition as possible, only one muscle was removed at a time for evaluation. After weighing, a 2.5 cm. slice was removed from the posterior and anterior end of each muscle and discarded. Another 0.5 cm. slice was removed for pH determination and for chemical evaluation. All samples removed for later chemical evaluation were wrapped in foil and frozen at $-10^{\circ}C$. After quick freezing, they were stored at $0^{\circ}C$ until required for chemical analysis.

The muscle blocks were then cut into individual slices approximately 2.5 cm. in thickness and prepared for cooking. Slices were cut beginning with the anterior end of each muscle block (Figure 5). Each slice was individually weighed, and a cooking thermometer (Premium Instruments, Model 390G) was inserted into the center of each slice from the medial edge. The slices were placed into an oven preheated to $149^{\circ}C$. The slices were cooked to an internal temperature of $71^{\circ}C$. Each slice was timed and removed individually when the desired internal temperature was attained. After removal from the oven, the slices were blotted and weighed. The percent cooking loss and cooking rate of each muscle slice was calculated. The slices were then mechanically cored with a 1/4 horsepower drill equipped with a 1.9 cm. diameter coring device. All slices were cored medial to lateral with the anterior face dorsal, just as it had been in the oven. The cores were then sheared with a Warner-Bratzler shearing device, and results were expressed as units of resistance to



[a]Steak location
[b]Chemical sampling site

Figure 5. Schematic Illustrating Steak Locations and Sampling Sites

shear force. All steaks were cored and sampled within three minutes of removal from the oven, resulting in a minimum change in temperature.

Procedure for evaluation of treatment and control muscle blocks was identical.

Sampling for Chemical Evaluation

Samples for chemical analysis were removed from the muscle blocks at the same time the muscle was sliced for cooking evaluation. Samples were all approximately 0.5 cm. thick and weighed 50 to 75 grams each. Samples were removed from both the posterior and anterior extremities of each muscle block. A sample was also removed every third slice during the slicing procedure. This gave a total of six samples from each muscle block for chemical evaluation (Figure 5).

pH Determination

The pH of each muscle sample was determined by mixing 50 ml. of glass distilled water with a 10 gram muscle sample. The pH was determined with a Corning Model 10 pH meter. All determinations were made in duplicate.

Extraction of Myofibrillar Protein Fraction

Extraction of the myofibrillar protein fraction was accomplished according to the procedure outlined in Figure 6.

Muscle samples for chemical analysis were removed from the freezer and allowed to thaw overnight at approximately $1^{o}C$. From this tissue was obtained duplicate 4 gram samples for extraction. The tissue samples were finely minced and then extracted in a Sorvall

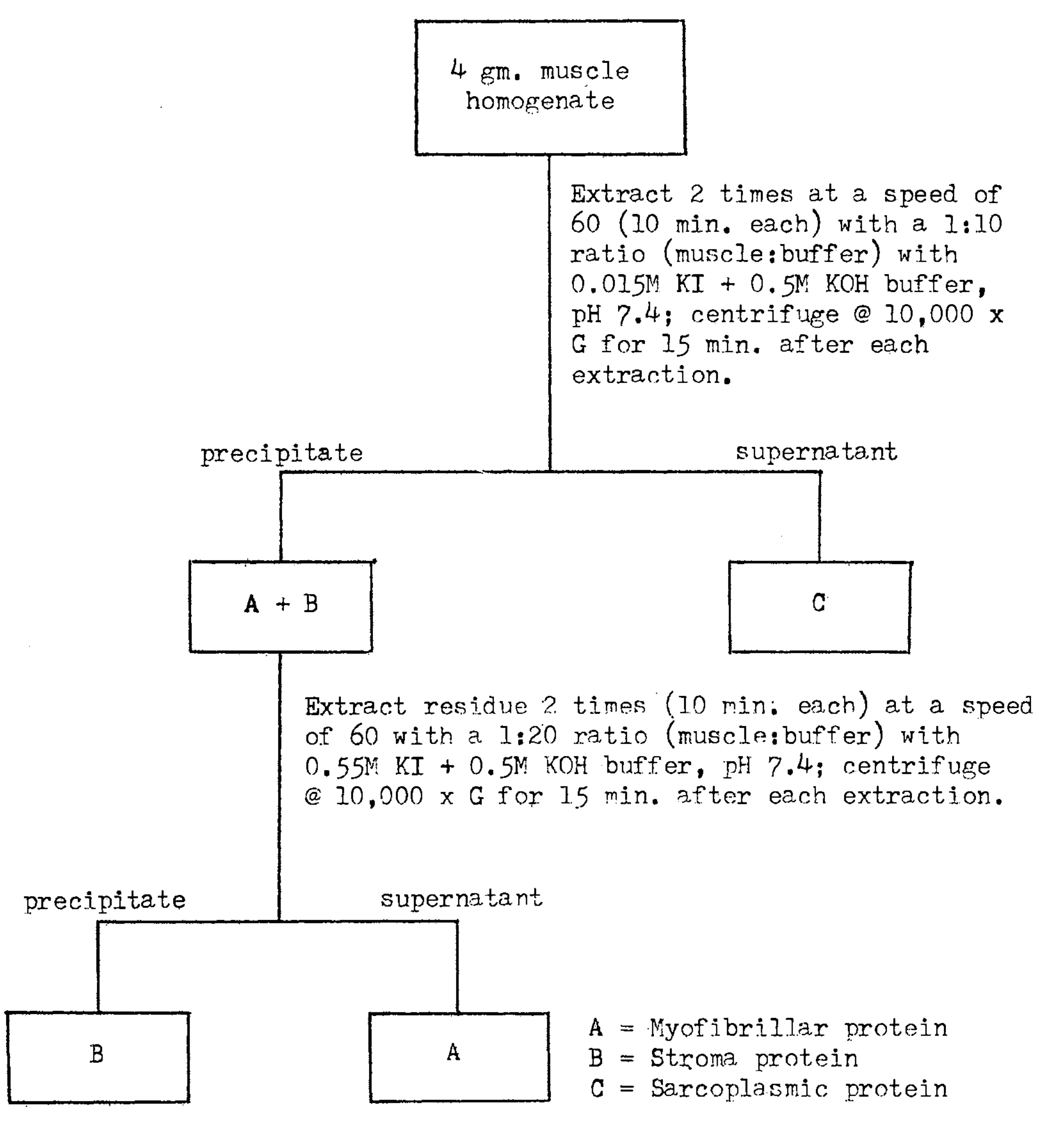


Figure 6. Extraction Procedure for Myofibrillar Protein

Omni-Mixer. Care was taken to insure that all extractions were made at 0°C. The sarcoplasmic protein fraction was obtained by extracting twice with a 1:10 (muscle:buffer) ratio with a 0.015M KI + 0.5M KOH buffer, pH 7.4, for 10 minutes with a rheostat setting of 60. After extraction, the homogenate was centrifuged at 10,000 x G for 15 minutes in a Sorvall Automatic Refrigerated Centrifuge, Model RC2-B. The sarcoplasmic protein fraction, found in the supernatant, was then discarded. The precipitate was resuspended and extracted twice in a 1:20 ratio (muscle:buffer) with 0.55M KI + 0.5M KOH buffer, pH 7.4. Centrifugation was accomplished as before, and the myofibrillar fraction was obtained in the supernatant. The precipitate residue containing the stroma protein fraction was discarded. The myofibrillar fraction contained approximately a 25 ml. volume which was then dialyzed against 50 volumes of 0.55M KI for 21 hours.

Results of previous work indicated that after dialysis it was necessary to concentrate the myofibrillar protein fraction for the desired assay procedure. Previous lyophilization and resuspension proved both time consuming and harmful to the viability of the protein. Therefore, the protein extract was concentrated by use of Aquacide I (MW = 70,000) obtained from Calbiochem. The fraction was reduced to a volume of approximately 10 to 12 ml.

Protein Determination

The protein concentration of the myofibrillar solution was determined using the biuret method (Layne, 1957). Crystalline bovine serum albumin (Pentex Inc.) was used as the standard.

Enzyme Assay Conditions

Based on the results of previous work, it was determined to use two incubation systems for evaluation of myofibrillar ATPase activity. They were termed: (1) $CaCl_2$ high ionic strength ($\mu = 0.6$) and (2) $MgCl_2$ low ionic strength ($\mu = 0.06$). These two systems were selected in order to evaluate the post-mortem state of the protein solution since Ca^{++} should activate myosin ATPase, and Mg^{++} should activate actomyosin ATPase. The composition of the assay systems was based on those described by Baril et al. (1966) and Kielley (1955) with modifications as described by Rickansrud (1969). The contents of the reaction mixture for the $CaCl_2$ high ionic strength system was: 1.4 ml. of 0.6M KCl + 0.01M Tris-HCl (pH 7.5), 0.2 ml. of 0.05M $CaCl_2$ in 0.6M KCl (unbuffered), 0.2 ml. of 0.02M ATP in 0.6M KCl buffered with 0.01M Tris-HCl (pH 7.5), and 0.2 ml. myofibrillar protein extract in 0.55M KI. The contents of the $MgCl_2$ low ionic strength system was: 0.6 ml. of 0.15M KCl + 0.2M Tris-HCl (pH 7.5), 0.2 ml. of 0.05M $MgCl_2$, 0.2 ml. of 0.02M ATP + 0.01M Tris-HCl (pH 7.5), 0.8 ml. deionized, distilled water, and 0.2 ml. myofibrillar protein extract in 0.55M KI. Total volume of the reaction mixture was 2.0 ml. for both systems. Incubation time was 10 minutes at $0^{\circ}C$. Activity was terminated by addition of 1.0 ml. 20% TCA. All assays were then filtered through Whitman No. 50 filter paper to remove any suspended materials.

Inorganic Phosphate Determination

Inorganic phosphate was determined by the method of Fiske and Subbarow (1925). Rickansrud (1969) reported many sources of error

and variation between duplicates in inorganic phosphate determinations which led to the following described procedure. After filtering in the cold room, the assay tubes were transferred to an ice bath in order to maintain a constant temperature. A 2.0 ml. aliquot was removed and transferred to a test tube maintained at room temperature. The volume was then brought to 3.6 ml. by addition of 1.6 ml. of distilled water at 25°C. This served to bring the assay to 25°C without prolonged standing at room temperature. Then, in rapid succession, the following reagents were added to each tube separately: (1) 0.4 ml. of 10N H_2SO_4, (2) 0.8 ml. of 2.5% ammonium molybdate, (3) 0.4 ml. of Fiske-Subbarow reagent, and (4) 4.8 ml. distilled H_2O. Color was allowed to develop for 10 minutes from the addition of the Fiske-Subbarow reagent in a 30°C water bath. The optical density (O.D.) was read at a wavelength of 660 mμ on a Gilford Model 240 Spectrophotometer. The O.D. was read against a reagent blank which resulted in the readings being equal to a change in optical density of the solution. A standard was prepared daily to include the concentration range of 0.1 μmole to 1.0 μmole inorganic phosphate from a stock solution of KH_2PO_4 (1μM/ml.). The procedure for the standard was the same as for the assay.

<u>Calculation of ATPase Activity</u>

Myofibrillar ATPase activity was expressed as micromoles of inorganic phosphate per milligram protein per minute, calculated from the following formula:

$$\mu M\ Pi/min./mg.\ protein = \frac{K(1.5)(d.f.)(O.D.)}{(10\ min.)(mg.\ protein/ml.\ of\ fraction)}$$

where the proportionality constant $K = \frac{1\ \mu M\ Pi}{O.D.}$ is calculated from the daily inorganic phosphate standard, 1.5 is the fraction of the total volume from which the 2.0 ml. aliquot for phosphate analysis was taken, d.f. is the dilution factor to put the myofibrillar fraction on a 1.0 ml. basis (which is 5 since a 0.2 ml. sample was used), and mg. protein/ml. of fraction refers to the protein concentration of the myofibrillar preparation. The concentration of the protein was determined by: (E)(O.D.) where the proportionality constant $E = \frac{1\ mg.\ protein}{O.D.}$ as calculated from the daily BSA standard.

Protein Solubility

Solubility of the myofibrillar protein fraction was determined by the concentration of protein based on a gram of wet tissue weight. This was necessary in order to determine if there was a treatment effect on the extractability of the protein.

Protein concentration was determined by the biuret method (Layne, 1957). A 0.4 ml. aliquot of the extract was added to 3.6 ml. biuret reagent, and 30 minutes was allowed for color development. The reciprocal of the daily BSA standard was calculated, then multiplied times 2.5 to convert to a 1 ml. system. This value was then multiplied times the O.D. reading for each sample to give mg. protein per ml. The total volume of the extract (in ml.) was then multiplied times the mg. protein per ml. value. This gave the total mg. per 4 gm. tissue, which could be converted to a 1 gm. wet tissue basis by dividing by 4. This procedure may be summarized by the following formula:

$$\text{mg. protein/gm. wet tissue} = \frac{(\frac{1}{E})(2.5)(O.D.)(V)}{W}$$

where $\frac{1}{E}$ is the reciprocal of the BSA standard, 2.5 is the conversion factor to a 1.0 ml. system, O. D. is the optical density, V is the total volume of extract (ml.), and W is the weight of sample (gm.).

Statistical Analysis

The statistical analysis of this experiment was accomplished by a split plot design with nested or hierarchal classifications. Animals served as replications, muscles as the main plot, and the steak location and core position as the sub-plots. Since the animal was the only random effect and all other effects were fixed, the mathematical model for the analysis was mixed. All analyses were accomplished according to Snedecor and Cochran (1967).

CHAPTER IV

RESULTS AND DISCUSSION

Uniformity Study

Cooking and Tenderness Evaluation

The experimental design for the cooking and tenderness test is presented in Figure 7. This experiment was designed as a split-plot with mixed models. The mathematical model for the cooking evaluation is as follows:

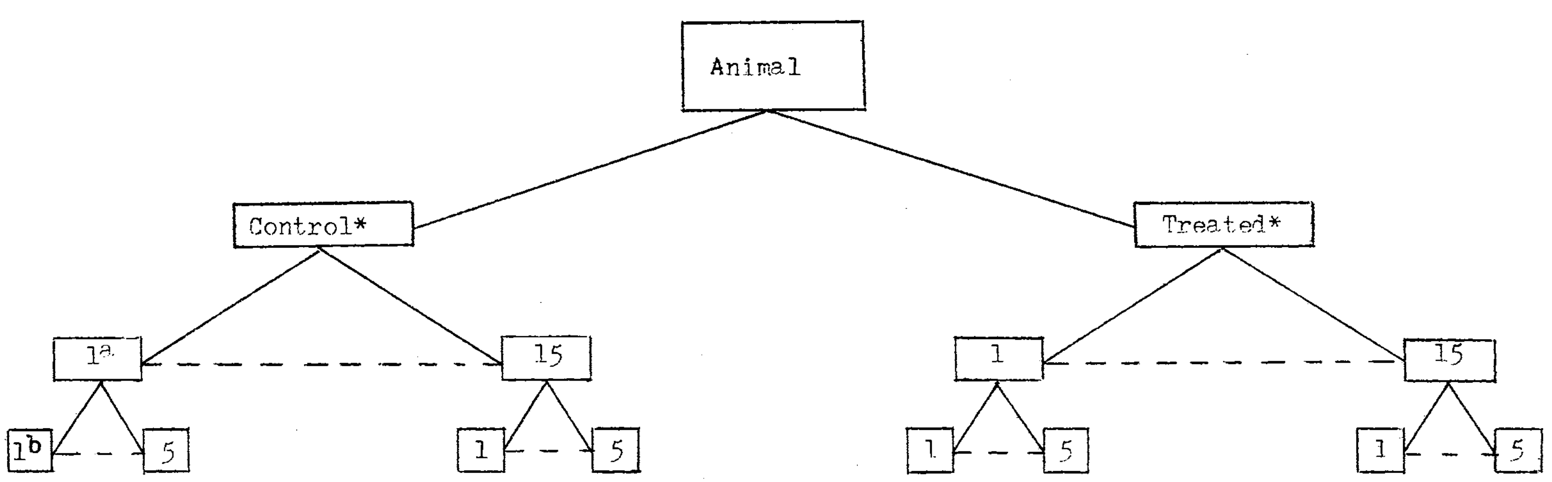
$$X_{ijk} = \mu + \alpha_i + \beta_{ij} + \gamma_{ijk} + \varepsilon_{ijk}$$

where,

X_{ijk} = any independent observation
μ = population mean
α_i = variation due to animal (random)
β_{ij} = variation due to side (fixed)
γ_{ijk} = variation due to steak location (fixed)
ε_{ijk} = experimental error

It should be noted that "animal" was the only random effect. All other effects were fixed. Consequently, in the analysis of variance, animal is the source of the error terms used to test the null hypothesis, such as variation in shear value, cooking rate, etc.

As indicated in the experimental design (Figure 7), "side" (left or right) served as either "treatment" or "control". The designation of a particular side as control or treatment was fixed at the outset of the experiment. As sides were fixed, it was



*Alternate treated and control sides
[a]Steak location (1 = anterior end; 15 = posterior end)
[b]Core position (1 = medial; 5 = lateral)

Figure 7. Design of Experiment

essential to first establish the equality of variance between the sides of the test animals. This was accomplished by a homogeneity analysis, utilizing the control longissimus dorsi muscle of the seven experimental animals.

In Tables I through IV are presented the analyses of variance from the homogeneity study accomplished on the cooking and shear data from the seven control sides. The statistical procedures used for these analyses were as outlined by Snedecor and Cochran (1967) for testing the equality of two variances.

In Table I, the analysis of variance for the six variables studied in the cooking tests are presented. Each analysis in Table I is "by-side", either left or right. Both analyses are presented together in order to illustrate the differences between sides. The source of variation, degrees of freedom, and their respective mean squares are identified "by-side". Unequalities of variance are indicated by common superscripts. Statistically significant effects are indicated by numbers. All tests of significance were accomplished within each side with the animal x steak interaction serving as the error term.

A more efficient statistical procedure would be to analyze the data on a pooled basis. Obviously, this would increase the precision of the analysis of variance by increasing the degrees of freedom in the error term, thereby enabling the identification of smaller differences in main effects. In addition, a pooled analysis would permit a direct assessment of any side effect. The pooled analysis of variance for all cooking data appears in Table II.

The analysis for variation in initial steak weight is presented

TABLE I

ANALYSIS OF VARIANCE FOR COOKING EVALUATION BY-SIDE*

Source	d.f.	Initial Weight	Cooked Weight	Cooking Loss	Cooking Time	Percent Loss	Cooking Rate
Total	103						
Left Side	44						
Right Side	59						
Animal-in-left	2	558.6[1]	564.4[1]	180.2[2]	220.8[2]	244.6[2]	27.2
Animal-in-right	3	9975.9[2]	4621.4[2]	2671.3[2]	3704.7[2]	295.0[2]	591.0[2]
Steak location in left	14	307.8[a,1]	284.3[b]	43.7[c]	42.1[d]	13.2	20.4
Steak location in right	14	1259.2[a]	862.1[b]	202.4[c]	115.0[d]	28.3	38.0
Animal x steak in left[3]	28	141.6	160.0	35.3	39.1	12.2	13.3
Animal x steak in right[4]	42	1369.0	604.7	322.8	212.0	28.6	26.6

[1]Significant ($P < 0.05$)

[2]Significant ($P < 0.01$)

[3]Error term for left side

[4]Error term for right side

[a,b,c,d]Unequal variance indicated by common superscript

*All values denote mean square

TABLE II

ANALYSIS OF VARIANCE FOR COOKING EVALUATION POOLED*

Source	d.f.	Initial Weight	Cooked Weight	Cooking Loss	Cooking Time	Percent Loss	Cooking Rate
Total	104						
Side	1	10718.8	1423.0	4430.9	1914.1	381.5	88.2
Animal-in-side[1]	5	6209.0	2772.8	1994.9	2311.2	274.8	365.5
Steak location in side	28	783.5	573.2	123.1	78.6	20.8	29.2
Steak	14	1029.9	638.8	159.1	86.7	20.3	30.7
Steak x side	14	537.1	507.6	87.0	70.5	21.2	27.6
Animal x steak in side[2]	70	885.4	426.8	350.7	125.6	22.0	20.8

[1,2]Error term

*All values denote mean square

in Table I. As indicated, there was a significant heterogeneity of variance between the steak weights of the two sides. This unequality can be explained only by technician error in slicing the steaks. This is highly probable since all cutting was accomplished manually. In future work, it would be advisable to use mechanical slicers which should give a more nearly uniform slice. Also, there is a significant variation ($P < 0.05$) in steak weight in the steaks from the left side. An increase in initial steak weight would be apparent from the anterior end to the posterior end of the muscle due to the natural conformation of the muscle, with the longissimus dorsi being larger at the posterior extremity. However, the steak variation in the right side is not significant, but any actual variation could be hidden by the large variance of the error term.

There was also a significant variation ($P < 0.05$) among animals of each side. This animal variation would be due to variation in muscle size of each animal.

When the data for initial steak weight were pooled (Table II), no significant variation in steak size was noted. It is pointed out, however, that the heterogeneity of variance shown for initial steak weight prohibits pooling of the data. Hence, the analysis presented in Table II is not valid.

In the pooled analysis, animal is confounded with side; consequently, the large mean squares associated with animal effect is incorporated into the animal-in-side term, and the latter is utilized to test the side effect. The error term (animal x steak-in-side) for testing steak location also contained the animal variation; hence, a large mean square was also observed for this value.

In the analysis of variance for cooked steak weight (Table I), results were similar to those for initial steak weight. A heterogeneity of variance was detected between the cooked steaks from the two sides. This could be directly related to the error associated with the cutting of the steaks. There was a significant animal effect ($P < 0.05$) in cooked steak weight.

The pooled analysis indicates no difference between the left and right sides. However, the animal-in-side mean square is obviously larger than the side mean square. Therefore, there is considerably more variation among animals of each side than between the two sides (Table I).

Cooking loss was determined by the difference in weight of the steak prior to and immediately after cooking. The "by-side" analysis for cooking loss (Table I) indicates a significant difference ($P < 0.05$) between sides for the steak location effect. This effect may be explained by the variation in the initial steak weight, assuming all steaks will cook uniformly. The animal variation is significant at the 1% level for both sides, indicating a large animal effect on cooking loss.

The pooled analysis (Table II) indicates no significant difference for any observed effect.

The analysis for cooking time (Table I) signifies an unequality of variance between the two sides for the steak location effect and thus suggests that the steaks from the two sides did not cook at the same rate. This result may also be explained by the difference in the initial steak weight of the various steaks. It is reasonable to assume that steaks of varying weight will not cook at the same rate.

Again, there was a significant ($P < 0.01$) animal effect on the cooking time for the steaks of both sides.

The pooled data (Table II) show no significant variation for any effect. Moreover, the animal-in-side mean square is larger than the side mean square, thus reflecting the animal variation observed in the side analysis.

Percent cooking loss is expressed as a percentage of the initial steak weight. Results show this to be the first example of homogeneity of variance between the two sides (Table I). It is noteworthy to compare the difference between this variable (percent cooking loss) and the previous four variables analyzed. The earlier discussed variables all contained dimension (i.e., grams or minutes) with absolute value. Since all steaks varied in weight, each unit (steak) studied was unequal to every other unit. However, by expressing cooking loss as a percent, a dimensionless value based on a common unit (gram) is obtained. Because of this uniformity in unit of evaluation, the heterogeneity of variance previously observed is eliminated. The animal effect was still significant ($P < 0.05$); however, the mean squares for animal effect approach equality for this variable.

In studying the results for initial weight, cooked weight, cooking loss and cooking time, it was apparent that the mean squares for the right side were consistently larger than those for the left side. By eliminating the heterogeneity of variance for steak location, similar results were obtained on a complete muscle basis. Thus, pooling the side analyses and testing for a side effect is valid for the percent cooking loss variable. Results exhibited in Table II,

however, indicate no significant effect for variation in percent cooking loss. This implies no difference between the two sides, at least for this particular variable.

It is interesting to note that the side and animal-in-side mean squares approach equality when the difference in variance has been eliminated (Table II).

Cooking rate was calculated by dividing the cooking time by initial steak weight. These results are expressed in min. per gram of initial weight. Even though this factor has dimension, it is still based on a uniform unit (gram). Due to this uniformity, a homogeneity of variance is observed (Table I). Results are quite similar to the values reported for percent cooking loss. However, the animal effect in the right side was significant ($P < 0.01$), while the animal effect in the left side was not significant. This variation is also reflected in the pooled data where the animal-in-side mean square is considerably larger than the side mean square. No effects were significant in the pooled analysis for cooking rate.

In summary, all factors having dimension and not based on a uniform unit of value (i.e., gram) exhibited a heterogeneity of variance between the two sides. This was believed to be related to the variation in initial steak weight. Yet, when the results were placed on a common unit, as for "percent" or "rate", the unequality of variance was not noted. Therefore, to pool the results, it would seem apparent that each variable must be calculated on a consistent, uniform unit of measure. A significant difference in variance was observed for all variables based on a steak unit (i.e., varying weights). However, no unequality of variance was noted when each

steak was adjusted to a common unit of measure. This observation was based on results of the percent cooking loss and cooking rate studies.

Though no statistically significant results were noted by the analyses of variance for the cooking data, it is worthy to note trends indicated by the tables of means shown in the Appendix, Tables XII and XIII. These data are illustrated in Figures 8 and 9.

The analysis of variance for initial steak weight suggested no significant variation in steak weight by location (Table II). When the means for all seven animals were plotted (Figure 8), there was an apparent trend of increasing steak weight from the anterior to the posterior end of the muscle. This would be expected due to the natural conformation of the longissimus dorsi muscle. It should be noted that steak 15 was considerably smaller than the previous nine steaks (5 through 14). This probably resulted from the difficulty in manually slicing the rather flexible 2-inch section at the end of the muscle. This might be avoided in the future by using machine slicing.

The means for cooked steak weight are plotted in Figure 8. A similar trend holds for cooked weight as for initial weight. It is evident that as initial steak weight fluctuates, the same change is observed for cooked steak weight.

Cooking loss and percent cooking loss are plotted in Figure 8. Results suggest that initial steak weight and cooking loss followed similar trends. However, from location 7 to location 15, there is less variation in cooking loss than in initial or cooked steak weight. This indicates there may be a location effect on cooking loss, but this trend was not statistically significant (Table II). Trends for cooking loss and percent cooking loss were quite similar.

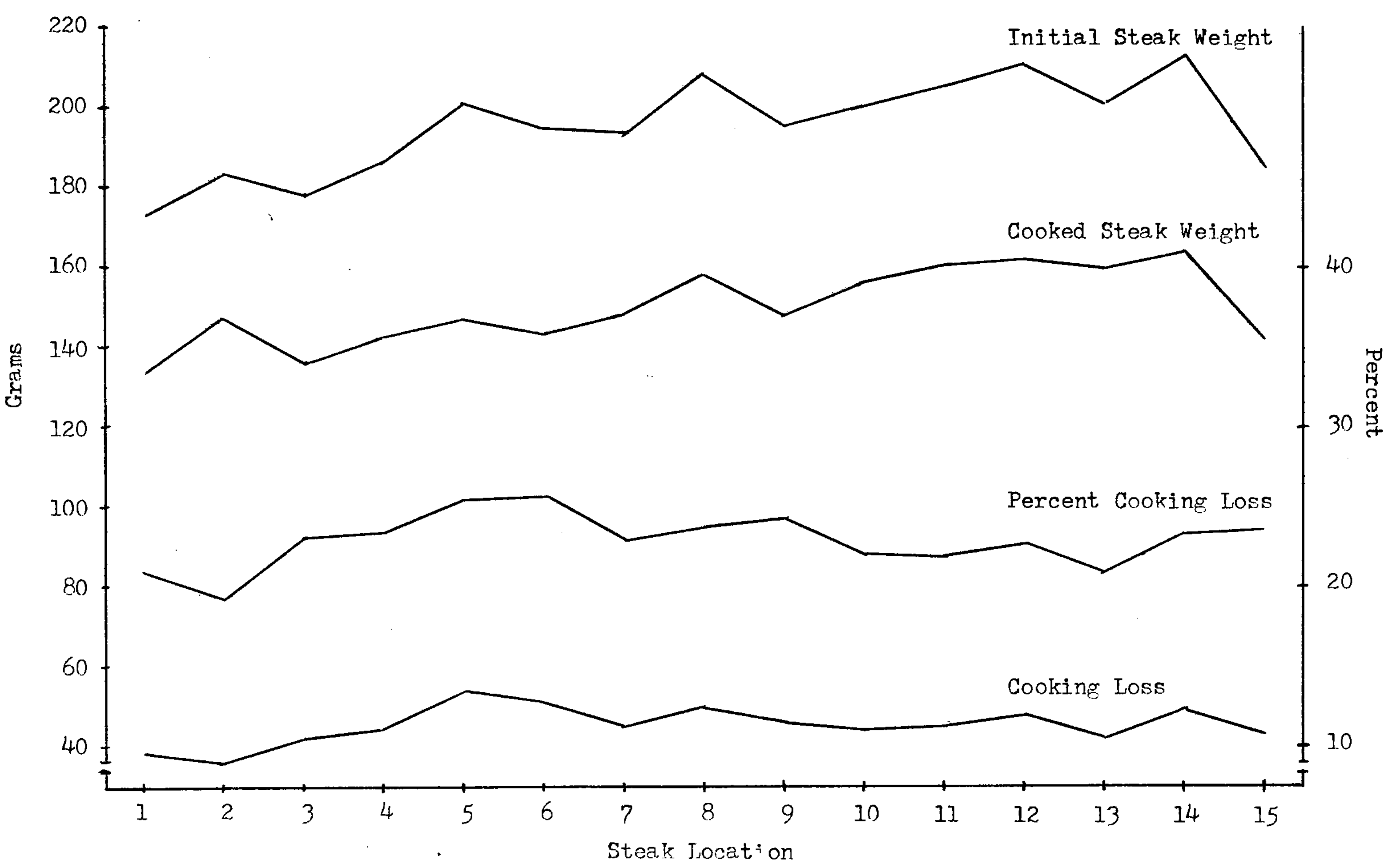


Figure 8. Relationship of Cooking Variables

Figure 9 represents the means for cooking rate at each location in total minutes and in minutes per gram of initial steak weight. By comparing these results with those in Figure 8, an inverse relationship between steak weight and cooking rate may be gleaned. As the initial steak weight increased from anterior to posterior, the cooking rate, both as total minutes and minutes per gram, decreased. This would indicate that steak weight and cooking time are not proportional.

Tenderness Evaluation

The experimental design for the tenderness evaluation is presented in Figure 7. The mathematical model for any single shear value is identical to that presented for the cooking evaluation except for the additional δ_{ijkl} term for core position. The error term may now be represented as ε_{ijkl}.

The analyses of variance for shear force in the homogeneity trial are presented in Tables III and IV. Table III is the "by-side" analysis, and Table IV is the pooled analysis. The mechanics of the two tables are as outlined for the cooking variables in Tables I and II.

The "by-side" analysis indicates a heterogeneity of variance of the two sides for the animal x steak, steak x core position, and animal x steak x core position effects. Consequently, it would not be legitimate to pool the "by-side" analyses. Any analysis should be conducted on a by-side basis. When examing the analysis, it can be observed that there are three error terms, each containing the random "animal" effect. These error terms were utilized in the tests of significance for shear force as indicated in Table III. The only

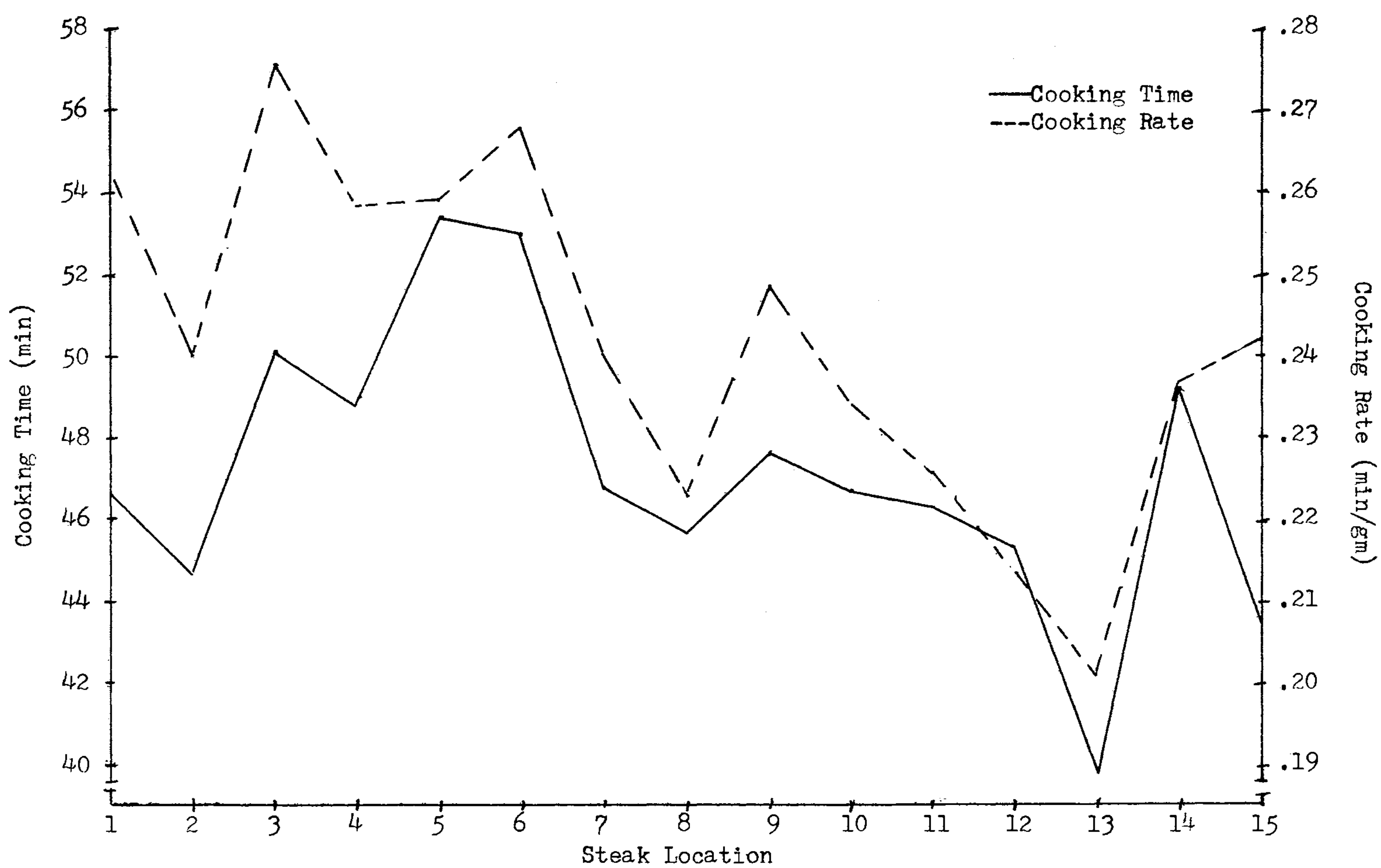


Figure 9. Relationship of Cooking Time and Cooking Rate

TABLE III

ANALYSIS OF VARIANCE FOR SHEAR FORCE BY-SIDE

Source	d.f.	M.S.
Total	523	
Left Side	224	
Right Side	299	
Animal-in-left	2	944.8[1]
Animal-in-right	3	1817.8[1]
Steak location in left	14	14.6
Steak location in right	14	17.9
Animal x steak in left[2]	28	11.6[a]
Animal x steak in right[3]	42	24.8[a]
Core position in left	4	12.8
Core position in right	4	36.0
Animal x position in left[4]	8	5.5
Animal x position in right[5]	12	13.2
Steak x position in left	56	4.1[b]
Steak x position in right	56	10.2[b]
Animal x steak x position in left[6]	112	3.8[c]
Animal x steak x position in right[7]	168	11.1[c]

[1]Significant ($P < 0.005$)

[2,4,6]Error term for left side

[3,5,7]Error term for right side

[a,b,c]Common superscript indicates unequality of variance

significant effect detected was the animal effect ($P<0.05$). This indicates an immense animal effect on shear value. The coefficient of variation for the left side was calculated as 0.1427 and the right side as 0.2174.

Table IV shows the "by-side" analysis pooled, ignoring the heterogeneity of variance. It should be noted that by pooling, a significant ($P<0.01$) core position effect was revealed. This indicated a significant variation in tenderness of the longissimus dorsi muscle from the medial to lateral side. The coefficient of variation of the pooled data was calculated as 0.1972.

The table of means for shear force is given in the Appendix, Table XIV. Figure 10 represents the average shear values for all seven animals plotted by core position. An increase in shear force from the medial to the lateral side of the muscle may be noted. As indicated in Table IV, there was a significant ($P<0.01$) variation in shear force by core position. However, the Least Significant Difference (LSD) test (Snedecor and Cochran, 1967) indicates no difference between any of the means. Similar results were reported by Howard and Judge (1968).

Figure 11 represents the overall means for the seven animals by steak location. The analysis of variance indicates no significant variation from the anterior end to the posterior end of the muscle (Table IV). This is again illustrated by plotting the means (Figure 11). It would be noteworthy to compare Figure 11 with Figure 9. These results suggest an area of high resistance to shear from location 4 to location 7. This corresponds to the area of the ninth to the eleventh rib on the live animal. A similar trend was

TABLE IV

ANALYSIS OF VARIANCE FOR SHEAR FORCE ON POOLED BASIS

Source	d.f.	M.S.
Total	524	
Side	1	348.6
Animal-in-side[3]	5	1468.5
Steak location in side	28	16.3
Steak	14	11.1
Steak x side	14	21.3
Animal x steak in side[4]	70	19.5
Position in side	8	24.4[1]
Position	4	39.8[2]
Position x side	4	9.0
Steak x position in side	112	7.2
Steak x position	56	7.2
Steak x position x side	56	7.2
Animal x steak x position in side[5]		
Animal x position in side	300	8.3

[1]Significant ($P < 0.01$)

[2]Significant ($P < 0.005$)

[3,4,5]Error terms

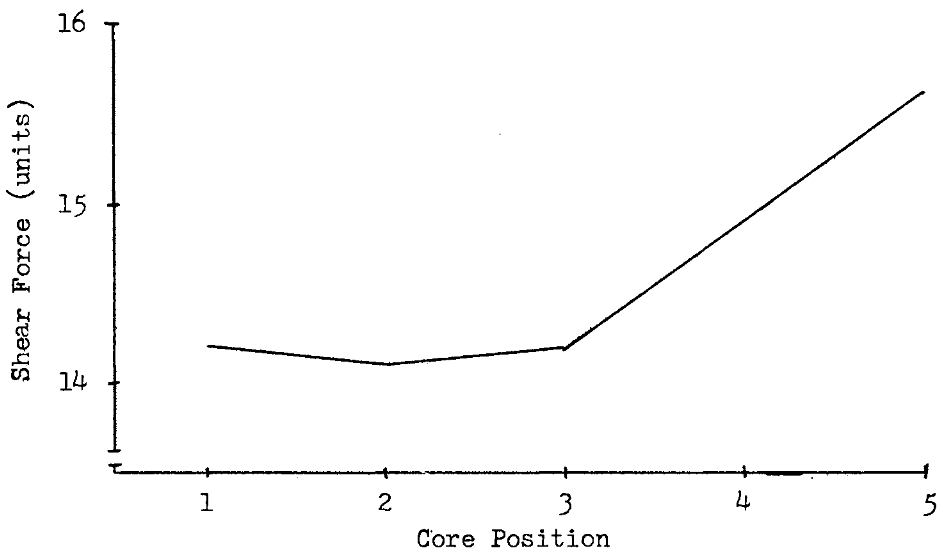


Figure 10. Mean Shear Values by Core Position

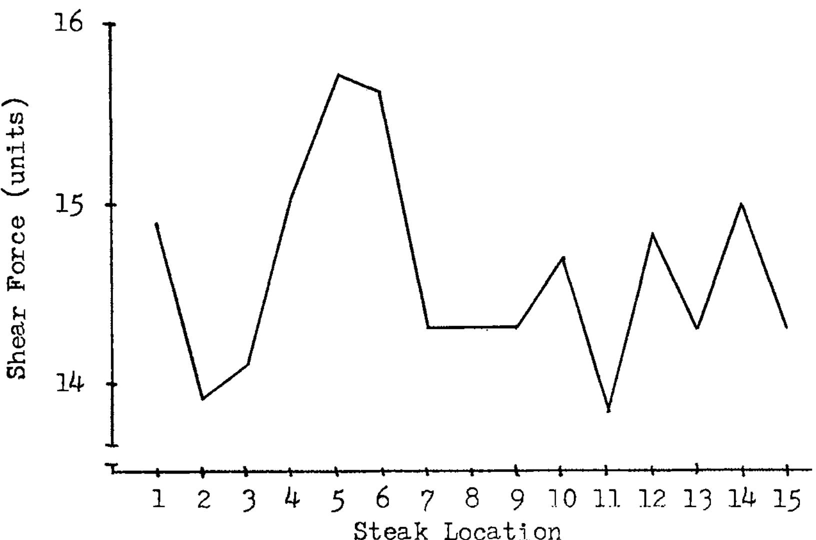


Figure 11. Mean Shear Value by Steak Location

shown in Figure 9 for the cooking rate and cooking time results. This would indicate a relationship between tenderness and the culinary characteristics of this area, worthy of further study.

Inhibitor Study

Objective

The objective of this study was to determine the influence of one particular level of para-Chloromercuribenzoate (PCMB) on shear force and various chemical attributes of bovine longissimus dorsi muscle.

Results of Tenderness Study

The results of the analysis of variance for tenderness are presented in Table V. As indicated, there was a significant ($P < 0.005$) position effect for shear force, similar to the uniformity trial. However, there was no significant effect of the inhibitor on tenderness of the longissimus dorsi muscle. It should be noted, however, that there was only one degree of freedom to test the effect of treatment.

The means for the shear data are presented in the Appendix, Table XV, and are plotted in Figure 12. It was anticipated that the inhibitor might cause a decrease in shear force of the treated muscles. However, as noted, the PCMB treated steaks averaged 4.4 units greater for one animal and 1.3 units less for the other. This indicates the need for more animals prior to making any conclusions about the effect of PCMB on tenderness.

The plot for shear values by location for the control and treated

TABLE V

ANALYSIS OF VARIANCE ON THE EFFECT OF PCMB ON SHEAR FORCE

Source	d.f.	M.S.
Total	299	
Animal	1	31.2
Treatment	1	176.8
Animal x treatment[2]	1	608.0
Steak	14	27.7
Treatment x steak	14	25.3
Animal x steak Animal x treatment x steak[3]	28	14.1
Position	4	39.6[1]
Treatment x position	4	5.2
Steak x position	56	9.1
Treatment x steak x position	56	8.5
Animal x position Animal x treatment x position Animal x steak x position Animal x treatment x steak x position[4]	120	7.8

[1]Significant ($P<0.005$)

[2,3,4]Error terms

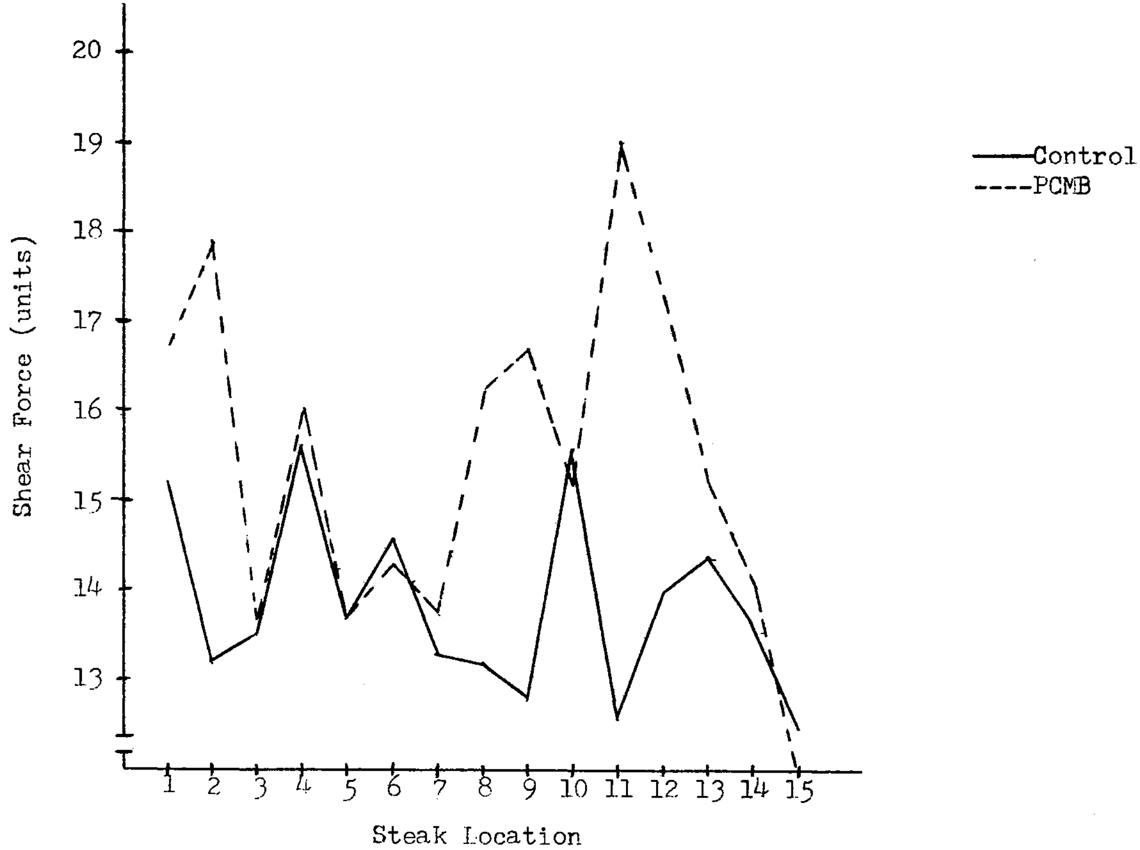


Figure 12. Relationship of Shear Values for Control and Treated Muscles by Steak Location

muscles shows results similar to those obtained in the uniformity trial (Figure 11). No discernible trends in shear force were observed along the muscle.

Results of ATPase Study

The analysis of variance for influence of PCMB on muscle adenosinetriphosphatase (ATPase), pH, and protein solubility were accomplished by time (to determine effect of storage and/or freezing) and by location. Locations were those sampling sites specified in Figure 5. Times studied were fresh (0 hours), 96-hour storage (96 hours) and 96 hours + 24 hours frozen (96 F), indicated by times 1, 2, and 3, respectively.

The analysis of variance for ATPase activity by time is presented in Table VI. There was a significant ($P<0.05$) time effect on the magnesium activated system.

The table of means for ATPase activity is presented in the Appendix, Table XVI. The data, plotted in Figures 13 and 14 indicate no trend for the calcium system. However, there was a considerable increase with time in the magnesium system. Since magnesium activates actomyosin ATPase, this indicates possible formation of the acto-myosin complex in both treated and control muscles.

The analysis of variance for myofibrillar ATPase activity by location is presented in Table VII. No significant difference in ATPase activity was observed with respect to location along the muscle.

The table of means for ATPase activity is presented in the Appendix, Table XVI. These data are also plotted in Figures 15 and 16.

TABLE VI

ANALYSIS OF VARIANCE ON THE EFFECT OF TIME ON CALCIUM AND MAGNESIUM ACTIVATED MYOFIBRILLAR ATPase ACTIVITY

Source	d.f.	Mean Square	
		Calcium	Magnesium
Total	23		
Animal	1	411.4	1598.0
Treatment	1	1051.0	385.6
Animal x treatment[2]	1	686.4	75.9
Time	2	138.6	2145.5[1]
Treatment x time	2	372.4	109.4
Animal x time Animal x treatment x time[3]	4	719.4	132.9
Residual error	12	211.3	613.7

[1]Significant ($P < 0.05$)

[2,3]Error terms

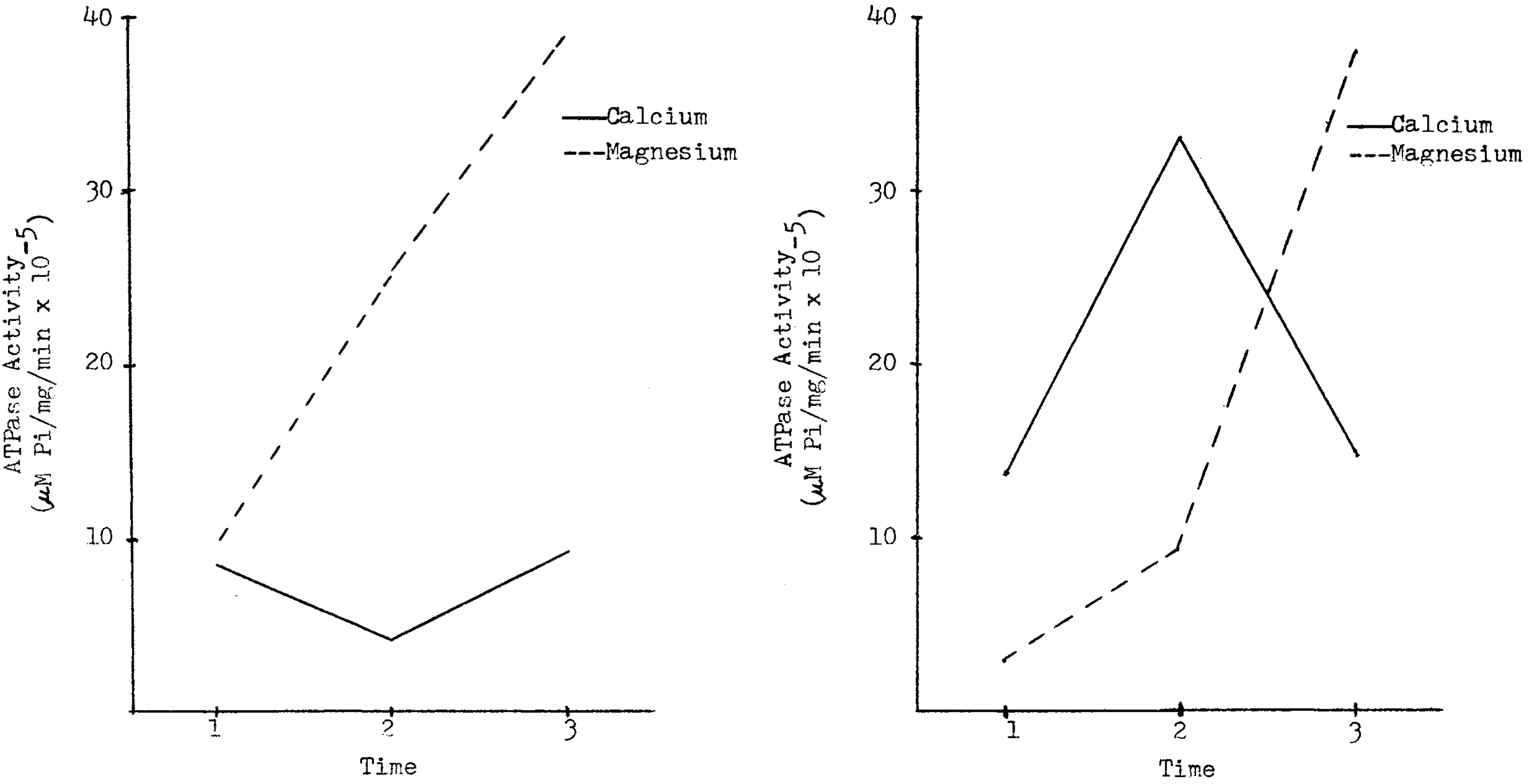


Figure 13. Influence of Time on Myofibrillar ATPase Activity of PCMB Treated Muscles

Figure 14. Influence of Time on Myofibrillar ATPase Activity of Control Muscles

TABLE VII

ANALYSIS OF VARIANCE ON THE EFFECT OF LOCATION ON CALCIUM AND MAGNESIUM ACTIVATED MYOFIBRILLAR ATPase ACTIVITY

Source	d.f.	Mean Square	
		Calcium	Magnesium
Total	47		
Animal	1	350.4	2419.4
Treatment	1	876.1	1616.7
Animal x treatment[1]	1	2442.2	667.4
Location	5	156.8	518.6
Treatment x location	5	774.8	1362.5
Animal x location Animal x treatment x location[2]	10	1134.1	1110.4
Residual error	24	715.9	1036.2

[1,2]Error terms

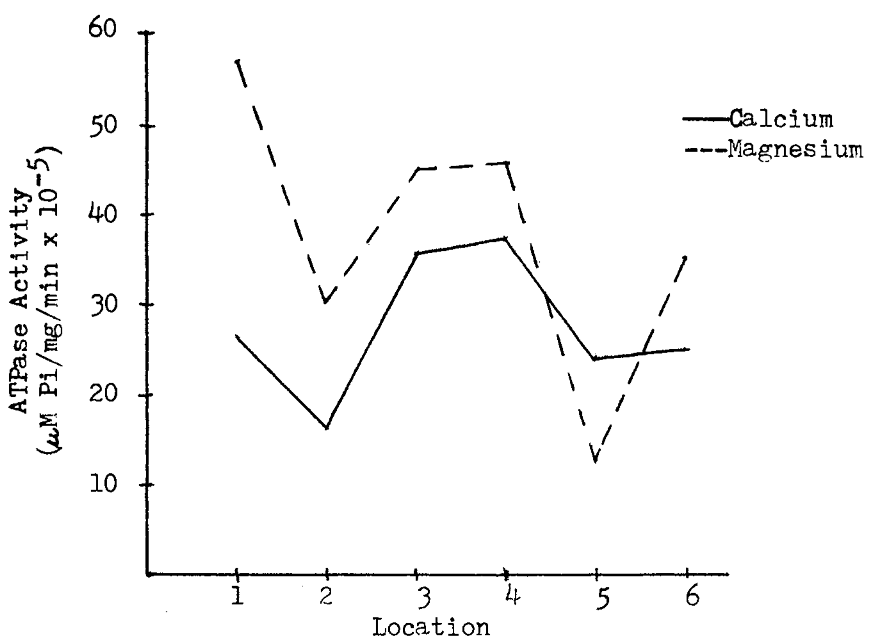


Figure 15. Influence of Location on Myofibrillar ATPase Activity of Control Muscles

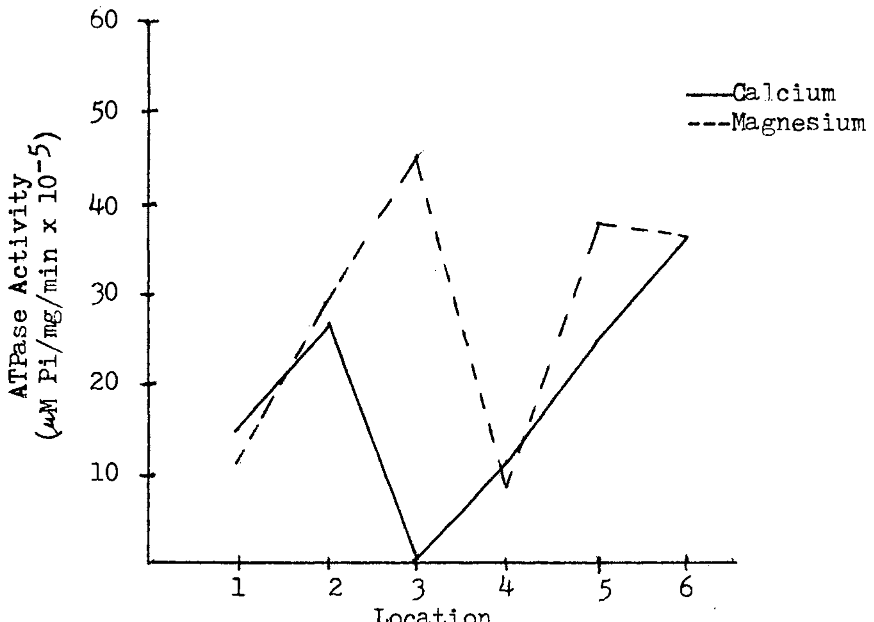


Figure 16. Influence of Location on Myofibrillar ATPase Activity of PCMB Treated Muscles

No detectable trends were indicated in the ATPase activity by location results. There is reflected a slight increase in Mg^{++} activation over the Ca^{++} system for both control and treated muscles, however.

It is apparent that the myofibrillar ATPase results show no trend which might be associated with the onset of rigor in post-mortem muscle. However, it is pointed out that the enzyme assay used to test myofibrillar ATPase activity was about the same as that developed by Rickansrud (1969) to assess purified myosin ATPase. These results would suggest that this procedure, though very efficient for purified myosin, was not sufficiently discerning to test ATPase activity of the "entire" myofibrillar fraction. Additional research would be in order to develop an ATPase assay system for the myofibrillar protein fraction. This, however, was considered to be beyond the scope of the present study.

Results of pH Study

The analyses of variance for the effect of PCMB on pH are presented in Tables VIII and IX.

Table VIII includes the analysis for the three time periods and shows a significant ($P < 0.005$) time effect on pH. This result might be expected due to the rapid decline in muscle pH associated with the onset of rigor.

Table IX is the analysis of variance for pH by location within the muscle. It is apparent there was no significant effect, at least at the 5% level on pH along the muscle block. These results are not unexpected since the minimum pH should have been reached prior to freezing of the sample.

TABLE VIII

ANALYSIS OF VARIANCE ON THE EFFECT OF TIME ON pH

Source	d.f.	M.S.
Total	23	
Animal	1	.0020
Treatment	1	.0113
Animal x treatment[2]	1	.0368
Time	2	3.2299[1]
Treatment x time	2	.0078
Animal x time Animal x treatment x time[3]	4	.0397
Residual error	12	.00018

[1]Significant ($P < 0.005$)

[2,3]Error terms

TABLE IX

ANALYSIS OF VARIANCE ON THE EFFECT OF LOCATION ON pH

Source	d.f.	M.S.
Total	47	
Animal	1	.4720
Treatment	1	.0420
Animal x treatment[1]	1	.2269
Location	5	.0094
Treatment x location	5	.0032
Animal x location Animal x treatment x location[2]	10	.0209
Residual error	24	.00012

[1,2]Error terms

The means for the pH analysis are presented in the Appendix, Table XVII and plotted in Figures 17 and 18 for the control and treated muscles. The initial pH for the control muscles averaged 6.45. After 96 hours, the pH had dropped to an average of 5.27, which is near the value of minimum pH associated with deep rigor. After freezing, the average pH was 5.32.

The average initial pH for the treated muscles was 6.33, slightly lower than the control muscles. However, after 96 hours storage, the pH had dropped to 5.27, identical to control muscle. The pH after freezing for the PCMB treated muscles was 5.30, comparable to the control sections.

The means for pH by location are plotted in Figure 18. The values for the control muscle ranged from 5.48 to 5.57. A small increase in pH was noted from location 1 to location 6. This could be a location effect, though not statistically significant. Finally, the average pH values by location tended to be slightly higher for the treated samples, ranging from 5.57 for location 1 to 5.62 for location 6.

Results of Protein Solubility Study

The analyses of variance for myofibrillar protein solubility are shown in Tables X and XI.

Table X contains the analysis of variance for protein solubility for the three time periods. As shown, there was a significant ($P < 0.005$) animal effect on protein solubility. This indicates a considerable animal variation among the animals studied, especially since there is only one degree of freedom in the error term. There

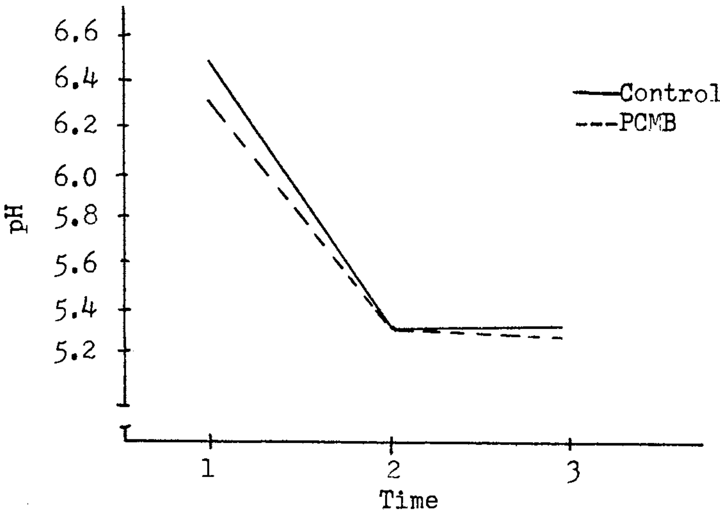


Figure 17. Influence of Time on pH

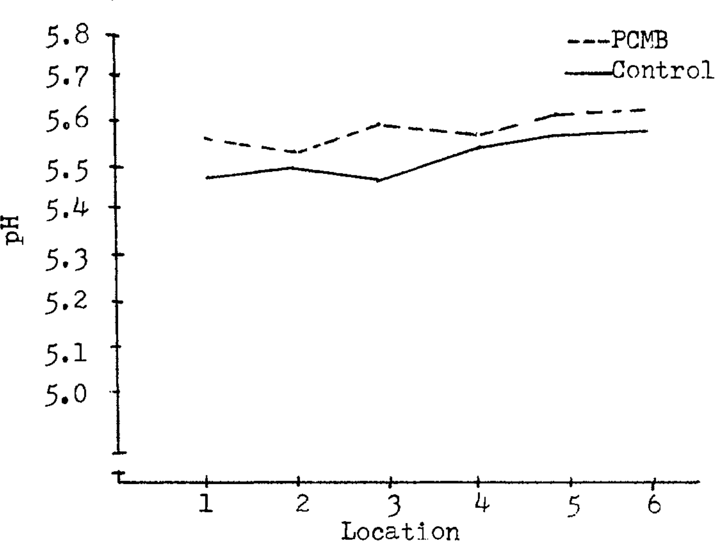


Figure 18. Influence of Location on pH

TABLE X

ANALYSIS OF VARIANCE ON THE EFFECT OF TIME ON THE SOLUBILITY OF MYOFIBRILLAR PROTEIN

Source	d.f.	M.S.
Total	23	
Animal	1	12672.4[1]
Treatment	1	46.1
Animal x treatment[2]	1	68.1
Time	2	9125.7
Treatment x time	2	3298.0
Animal x time Animal x treatment x time[3]	4	5457.6
Residual error	12	1342.6

[1]Significant ($P < 0.05$)

[2,3]Error terms

were no other significant effects.

Table XI reflects the analysis of variance for protein solubility by sample location. As indicated, there was a significant ($P < 0.005$) location effect on protein solubility. Also a significant treatment x location interaction was noted. However, the large mean square for the location effect and a small mean square for the treatment effect indicate this interaction may be due to the location effect.

The means for the protein solubility data by time are plotted in Figure 19. For the control sides, the protein concentration in the extract at 0 hours was 166.5 mg. per gm. wet tissue weight. Protein concentration increased to 172.4 mg. per gm. after 96 hours storage, then decreased to 128.7 mg. per gm. after freezing. Inverse results were obtained with the treated samples. For the PCMB treated muscles, a value of 214.9 mg. per gm. was obtained at 0 hours, which then dropped to 143.2 mg. per gm. after 96 hours storage. After freezing, the solubility of the myofibrillar fraction was only 117.6 mg. per gm. wet tissue.

The means for protein solubility by location are plotted in Figure 20. As reflected by the results for the control muscles, there was a considerable increase in solubility at location 3 and 6. Positions 1, 2, 4, and 5 were rather consistent in solubility. When the means for the treated sections were plotted (Figure 20), a gradual increase in protein solubility was noted from site 1 to site 6.

TABLE XI

ANALYSIS OF VARIANCE ON THE EFFECT OF LOCATION ON THE SOLUBILITY OF MYOFIBRILLAR PROTEIN

Source	d.f.	M.S.
Total	47	
Animal	1	28.3
Treatment	1	479.9
Animal x treatment[3]	1	4223.0
Location	5	1859.5[1]
Treatment x location	5	715.9[2]
Animal x location Animal x treatment x location	10	152.9
Residual error	24	254.7

[1]Significant ($P<0.05$)

[2]Significant ($P<0.005$)

[3,4]Error terms

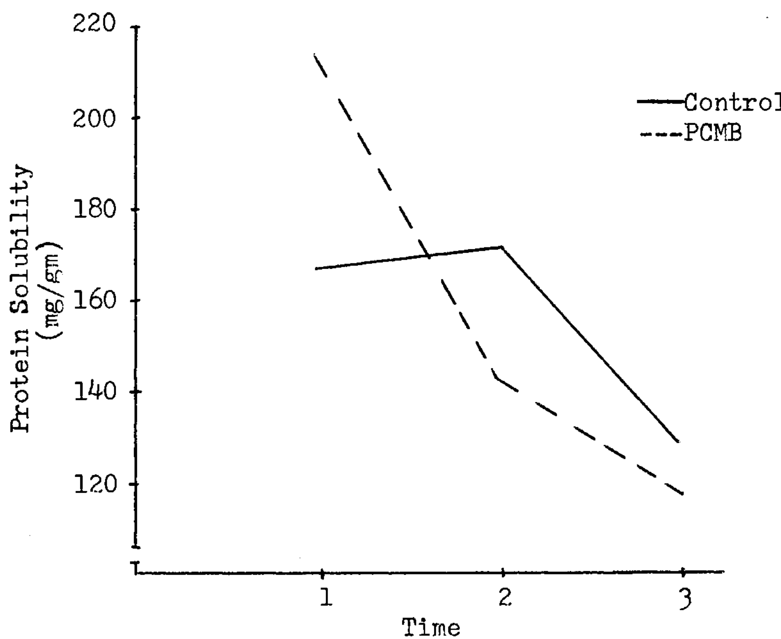


Figure 19. Influence of Time on Protein Solubility

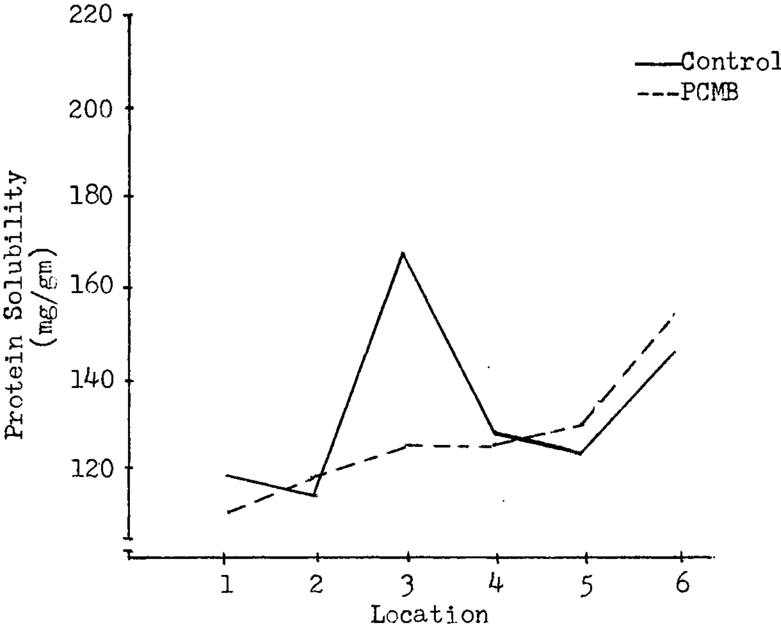


Figure 20. Influence of Location on Protein Solubility

CHAPTER V

SUMMARY AND CONCLUSIONS

The objective of this study was to establish the influence of a particular level of para-Chloromercuribenzoate on tenderness and certain chemical attributes of bovine longissimus dorsi muscle. This was undertaken in order to determine if a detectable inhibition of the rigid actomyosin complex could be produced.

Experimental materials for this study were obtained from the right and left longissimus dorsi muscles taken from U. S. Choice steers.

The results of the uniformity trial indicated a heterogeneity of variance between the sides of the animals studied. Thus, pooling of the data would not be statistically valid, and the individual results obtained from each side should be analyzed separately on a "by-side" basis.

The only significant effects detected in the homogeneity trial were a significant ($P < 0.05$) core position effect and a significant ($P < 0.05$) variation among animals within each side. Examination of the means indicated an increase in resistance to shear force from the medial to lateral side of the muscle. However, the analysis for difference between means reflected no significant variation between any of the five core positions.

Results of the PCMB injection study indicated an increased

resistance to shear force for the treated muscle in one animal and the opposite effect for the other. This indicated that other levels of inhibitor should be tried to more completely assess this effect.

Results of the ATPase study suggest that the assay system utilized in this experiment to test myofibrillar ATPase was inadequate. Moreover, the enzyme activity detected in this study was below the acceptable level for a precise analysis. Nevertheless, the results obtained with the magnesium ATPase system indicated that PCMB, at the level used in the test, did not inhibit myofibrillar ATPase activity. This suggests that there was no retardation in the formation of the actomyosin complex post-mortem. This is suggested by the marked increase in ATPase activity obtained with the PCMB treated 96-hour and 96-hour frozen samples over that of the 0-hour samples.

The PCMB injection appeared to have no statistically significant effect on either muscle pH or myofibrillar protein solubility.

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APPENDIXES

TABLE XII

MEAN VALUES FOR INITIAL STEAK WEIGHT, COOKED STEAK WEIGHT, AND COOKING LOSS BY STEAK LOCATION

Steak Location	Initial Weight			Cooked Weight			Cooking Loss		
	Left	Right	Avg.	Left	Right	Avg.	Left	Right	Avg.
*1	160.7	181.8	172.7	127.3	139.8	134.4	33.3	42.0	38.3
2	175.0	189.3	183.1	144.3	148.5	146.7	30.7	40.8	36.4
3	172.3	182.5	178.1	136.0	136.0	136.0	36.3	46.5	42.1
4	183.7	188.3	186.3	145.7	139.8	142.3	38.0	48.5	44.0
5	176.7	220.0	201.4	139.0	153.8	147.4	37.7	66.3	54.0
6	183.0	201.8	193.7	142.7	142.5	142.6	40.3	59.3	51.1
7	192.0	194.3	193.3	151.0	145.8	148.0	41.0	48.5	45.3
8	193.7	218.8	208.0	155.0	160.5	158.1	38.7	58.3	49.9
9	182.0	204.0	194.6	139.3	154.9	148.1	42.7	49.3	46.4
10	203.0	198.3	200.3	164.3	150.3	156.3	38.7	48.0	44.0
11	189.7	216.0	204.7	157.0	161.5	159.6	32.7	54.5	45.1
12	189.7	226.3	210.6	153.0	169.3	162.3	36.7	57.0	48.3
13	179.7	217.5	201.3	139.7	174.5	159.6	40.0	43.0	41.7
14	182.0	236.8	213.3	137.0	184.3	164.0	45.0	52.5	49.3
**15	188.0	182.0	184.6	152.3	134.3	142.0	35.7	47.8	42.6

* Anterior end of muscle

** Posterior end of muscle

TABLE XIII

MEAN VALUES FOR COOKING TIME, PERCENT COOKING LOSS, AND COOKING RATE BY STEAK LOCATION

Steak Location	Cooking Time			Percent Cooking Loss			Cooking Rate		
	Left	Right	Avg.	Left	Right	Avg.	Left	Right	Avg.
*1	42.7	49.5	46.6	20.2	21.2	20.8	26.5	26.1	26.3
2	39.0	49.0	44.7	17.5	20.8	19.4	22.3	25.3	24.0
3	44.7	54.3	50.1	21.1	24.4	23.0	25.9	28.9	27.6
4	39.0	56.3	48.8	20.8	25.4	23.4	21.3	29.2	25.8
5	43.3	61.0	53.4	21.3	28.7	25.5	24.5	26.9	25.9
6	45.7	58.5	53.0	22.0	28.4	25.6	24.9	28.2	26.8
7	46.3	47.0	46.7	21.4	23.9	22.8	24.3	23.7	24.0
8	38.0	51.5	45.7	19.9	26.5	23.6	19.6	24.2	22.2
9	45.7	49.0	47.6	23.4	24.9	24.3	25.0	24.8	24.9
10	43.0	49.5	46.7	18.9	24.2	22.0	21.1	25.1	23.4
11	38.0	52.5	46.3	17.2	25.3	21.8	20.3	24.4	22.6
12	38.3	50.5	45.3	19.4	25.1	22.6	20.1	22.3	21.4
13	41.3	38.5	39.7	22.6	19.7	20.9	23.1	17.8	20.1
14	50.0	48.8	49.3	24.8	22.0	23.2	27.6	20.7	23.7
**15	38.3	47.0	43.3	19.0	26.9	23.5	20.5	27.1	24.2

*Anterior end of muscle

**Posterior end of muscle

TABLE XIV

MEAN VALUES FOR SHEAR FORCE

Steak Location	Left Side *3	4	6	Side Avg.	Right Side 1	2	5	7	Side Avg.	Steak Avg.
1	19.6	8.4	14.7	14.2	19.6	18.9	7.2	15.6	15.3	14.9
2	19.0	7.4	11.6	12.6	17.3	19.2	7.9	14.8	14.9	13.9
3	17.3	9.1	11.9	12.8	16.4	21.2	7.6	15.0	15.1	14.1
4	18.0	10.9	13.3	14.1	19.6	18.3	7.3	17.8	15.7	15.0
5	19.2	11.1	14.5	14.9	21.7	20.9	9.6	12.9	16.3	15.7
6	17.1	12.3	13.6	14.4	22.4	18.4	9.6	15.5	16.5	15.6
7	17.4	11.5	13.1	14.0	20.0	15.5	9.1	13.5	14.5	14.3
8	17.4	10.9	13.3	13.9	14.2	21.9	9.4	13.0	14.6	14.3
9	18.6	11.1	12.1	13.9	17.1	18.3	9.1	13.6	14.6	14.3
10	19.3	10.4	15.2	14.9	13.5	19.1	9.6	16.1	14.6	14.7
11	19.6	10.7	12.5	14.3	12.7	20.4	8.4	12.6	13.5	13.8
12	17.9	12.0	12.6	14.2	16.9	19.8	9.2	15.4	15.3	14.8
13	14.9	12.7	11.1	12.9	14.9	21.1	7.8	17.6	15.4	14.3
14	15.4	10.8	10.4	12.2	23.3	19.5	8.9	16.9	17.2	15.0
15	13.0	10.7	11.3	11.6	22.1	21.9	7.2	13.8	16.2	14.3
Avg.	17.6	10.6	12.7	13.7	18.1	19.6	8.5	14.9	15.3	14.6
Core Position										
1	17.9	10.1	12.8	13.6	16.2	18.9	9.6	14.2	14.7	14.2
2	17.0	9.4	12.3	12.9	18.4	19.6	8.0	14.2	15.0	14.1
3	17.0	11.6	12.5	13.7	18.4	17.4	7.8	14.7	14.6	14.2
4	18.2	10.4	12.7	13.8	18.6	20.6	8.1	15.5	15.7	14.9
5	17.9	11.8	13.4	14.4	18.9	21.6	9.1	16.1	16.5	15.6

*Denotes animal number

TABLE XV

MEAN VALUES FOR INFLUENCE OF PCMB ON SHEAR FORCE

Steak Location	Control	PCMB
1	15.2	16.7
2	13.2	17.9
3	13.5	13.7
4	15.6	16.0
5	13.7	13.7
6	14.6	14.3
7	13.3	13.8
8	13.2	16.2
9	12.8	16.7
10	15.6	15.2
11	12.6	19.0
12	14.0	17.3
13	14.4	15.2
14	13.7	14.0
15	12.5	10.8
Avg.	13.8	15.4

TABLE XVI

MEAN VALUES FOR INFLUENCE OF PCMB ON MYOFIBRILLAR ATPase BY TIME AND LOCATION*

	Control		PCMB	
	Calcium	Magnesium	Calcium	Magnesium
Time				
1	13.6	2.6	8.5	9.9
2	33.2	9.5	4.2	25.2
3	14.9	38.0	9.2	39.0
Location				
1	26.3	56.7	13.5	11.1
2	15.6	30.4	27.0	18.3
3	35.4	45.0	0.0	45.5
4	37.1	45.3	10.7	8.1
5	23.3	12.9	24.0	37.6
6	24.8	35.7	36.2	36.0

*All values expressed as μM Pi/mg./min. x 10^{-5}

TABLE XVII

MEAN VALUES FOR INFLUENCE OF PCMB ON pH BY TIME AND LOCATION

	Control	PCMB
Time		
1	6.45	6.33
2	5.27	5.27
3	5.32	5.30
Location		
1	5.48	5.57
2	5.49	5.52
3	5.47	5.59
4	5.33	5.56
5	5.56	5.60
6	5.57	5.62

TABLE XVIII

MEAN VALUES FOR INFLUENCE OF PCMB ON MYOFIBRILLAR PROTEIN SOLUBILITY BY TIME AND LOCATION*

	Control	PCMB
Time		
1	166.5	214.9
2	172.4	143.2
3	128.7	117.6
Location		
1	119.0	110.1
2	114.5	118.0
3	168.3	125.4
4	128.8	125.5
5	123.5	130.1
6	147.3	154.4

*All values expressed as mg. protein per gram wet tissue weight

VITA

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Jerry Dwayne Gresham

Candidate for the Degree of

Master of Science

Thesis: THE INFLUENCE OF PARA-CHLOROMERCURIBENZOATE ON VARIOUS CHEMICAL AND CULINARY ATTRIBUTES OF BOVINE LONGISSIMUS DORSI MUSCLE

Major Field: Food Science

Biographical:

Personal Data: Born in Altus, Oklahoma, August 4, 1943, the son of Mr. and Mrs. John Gresham. Married Carolyn Perrin on July 23, 1965.

Education: Graduated from Altus High School, Altus, Oklahoma, in May, 1961; received the Bachelor of Science degree from Oklahoma State University in May, 1965, with a major in Animal Science; completed requirements for the Master of Science degree in May, 1971.

Experience: Employed part-time at the Oklahoma State University meat laboratory; served in the U. S. Army from June, 1965 to August, 1969; Graduate Assistant in Animal Science, Oklahoma State University, 1970-1971.