SOLUBILITY AND ELECTROPHORETIC PROPERTIES OF SOME POST-MORTEM AGED BOVINE MUSCLE PROTEINS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Elton David Aberle

1965

ABSTRACT

SOLUBILITY AND ELECTROPHORETIC PROPERTIES OF SOME

POST-MORTEM AGED BOVINE MUSCLE PROTEINS

by Elton David Aberle

The solubility characteristics of intracellular bovine muscle proteins during a 14 day post-mortem aging period were investigated. Results among various sex and/or age groups were compared. Longissimus dorsi and semitendinosus muscles were used in this study. A fractionation procedure which allowed partition of the major muscle protein fractions was developed. The relationship between meat tenderness and protein solubility, as measured by the fractionation procedure, was evaluated. In addition, changes in the sarcoplasmic proteins during post-mortem aging were followed by vertical starch gel electrophoresis.

In the semitendinosus muscle, the solubility of the sarcoplasmic nitrogen was highest at slaughter and decreased as aging proceeded. Solubility of the sarcoplasmic protein did not change in the longissimus dorsi muscle. Fibrillar protein was least extractable at 24 hours post-mortem, but its solubility was significantly higher at both 7 and 14 days than at 24 hours post-mortem with both muscles. All sex and/or age groups followed this pattern of change. Negative correlation coefficients were found between fibrillar nitrogen solubility and Warner-Bratzler shear values at 24 hours, 7 days, and 14 days post-mortem (r values of -.38, -.77, -.44, respectively). The increase in amount of soluble fibrillar nitrogen during post-mortem periods of 24 hours to 7 days, 24 hours to 14 days, and 7 to 14 days was positively correlated with an increase in tenderness. The "r" values were 0.55, 0.62, and 0.82, respectively.

While 15 zones were definable in the starch gel electrophoretogram, no distinct, regularly occurring differences were evident between the two muscles or between sex and/or age groups. The mobility of certain sarcoplasmic protein components was observed to vary during aging. The amount of protein, which migrated toward the cathode, increased as post-mortem aging proceeded. The slower moving anodic zones exhibited more distinct boundaries during the latter stages of aging.

SOLUBILITY AND ELECTROPHORETIC PROPERTIES OF SOME POST-MORTEM AGED BOVINE MUSCLE PROTEINS

By

Elton David Aberle

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science

1965

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. R. A. Merkel for guidance and suggestions throughout the course of this research and for assistance in the preparation of this manuscript.

He also thanks Dr. A. M. Pearson for advice and suggestions in the writing of this thesis.

The author is grateful to Mrs. Dora Spooner for assistance with nitrogen and Warner-Bratzler shear determinations.

Special appreciation is expressed to the author's parents for their encouragement and assistance throughout his education.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

Characterization of the many intracellular muscle proteins of meat animals is incomplete. Efforts to efficiently separate and classify muscle proteins are relatively recent. With the advent of more advanced methods in protein chemistry, it is now possible to separate, classify, and characterize these proteins with greater accuracy. It is known that most of the intracellular muscle constituents are enzymatic in nature and that many different components are present. However, the changes which the proteins undergo following death are not well defined. Data concerning the effects of post-mortem aging are fragmentary and the information is incomplete.

Intracellular muscle proteins have been classified into three main types: (1) sarcoplasmic proteins, which are soluble in water or weak salt solutions; (2) myofibrillar proteins, which are soluble in high ionic strength salt solutions; and (3) stroma proteins, which are insoluble in salt solutions. Data are available on the partition of the proteins of many different species but the data concerning meat animals are not in complete agreement. There is a large amount of information available regarding the effects of post-mortem aging upon protein solubilities in fish and chicken muscle (Dyer et al., 1950; Khan, 1962; Weinberg and Rose, 1960). However, little data is evident with respect to the muscle proteins from meat animals. Those results, which are available, are often contradictory (Wierbicki et al., 1956; Hegarty, 1963).

Post-mortem aging may result in specific protein changes in muscle. Kronman et al. (1960) have employed electrophoretic and ultracentrifugal techniques in studying post-mortem age associated changes in sarcoplasm. Scopes (1964) presented a method for starch gel electrophoresis of muscle proteins, which gave separation superior to other methods. However, this technique has not been applied to muscle during post-mortem aging.

Mindful of these facts, this study was undertaken with the following objectives:

1. To develop a repeatable and rapid procedure for partition of the various beef muscle proteins on the basis of solubility.
2. To study changes in solubility of various protein fractions during post-mortem aging and to relate the changes to alterations in tenderness during aging.
3. To determine if changes could be detected in the components of sarcoplasmic protein by starch gel electrophoresis.

REVIEW OF LITERATURE

Muscle Proteins

1. Sarcoplasmic fraction

The sarcoplasmic fraction of muscle protein, also referred to as myogen, consists of that part of the muscle fiber which is soluble in water or weak salt solutions, such as 0.9% sodium chloride (Whitaker, 1959). This fraction was initially thought to be homogeneous in nature. Weber and Meyer (1933) divided myogen into globulin X, which precipitated upon dialysis, and albumin, which remained soluble. Denaturation of the proteins in a sarcoplasmic extract is quite rapid and upon neutralization most of the protein precipitates. The soluble fraction remaining was termed myoalbumin (Bate-Smith, 1937).

It is now known that sarcoplasmic protein is far from homogeneous. It contains the components of the glycolytic cycle plus many non-protein compounds. It seems probable that all of the protein components are enzymatic in nature (Whitaker, 1959). Bailey (1954) listed more than fifty enzymes, which have been found in myogen.

2. Myosin

Kuhne (1859) found that a large amount of protein could be extracted from muscle by strong salt solutions and that it precipitated upon dilution of the extract. This precipitate was originally called myosin until it was found to be composed of a complex of myosin and actin (Schramm and

Weber, 1942). Myosin is readily soluble in 0.5 M KCl, in which it yields a clear solution. It is easily denatured by freeze-drying, by heat or by dehydration with organic solvents (Whitaker, 1959). Myosin functions as an enzyme to split ATP in the contraction process, thus it is referred to as ATPase. The molecule may be fragmented by digestion with trypsin or chymotrypsin to yield two distinct components, a heavy meromyosin, which possesses the ATPase activity, and a light meromyosin having the solubility properties of myosin (Whitaker, 1959).

3. Actin

Actin has a characteristic tendency to form complexes with myosin. The protein may exist in two forms; as a monomer called globular or G-actin or as a polymer termed fibrous or F-actin (Szent-Gyorgyi, 1951). F-actin is considered to be a linear aggregation of G-actin units (Whitaker, 1959). It is the F-actin form that interacts with myosin.

4. Actomyosin

Whitaker (1959) stated that actomyosin results from a complex between actin and myosin, which forms at the moment of excitation or stimulus. This protein complex is often called myosin B. Under the proper conditions, namely the presence of ATP and magnesium, calcium or potassium ions, it is contractile (Szent-Gyorgyi, 1951). However, actomyosin as such does not exist in resting muscle. Approximately 67% of the total protein extracted from muscle, which has contracted or passed through rigor, is actomyosin (Weber and Portzehl, 1952). This protein possesses

ATPase activity and appears less labile than myosin in the uncombined state (Hunt and Matheson, 1958).

5. Tropomyosin

Tropomyosin was first isolated by Bailey (1946) and accounts for 10 to 12% of the total myofibrillar protein. Its properties are similar to those of myosin and it has been postulated to be an integral part of the myosin molecule (Whitaker, 1959). Its exact role in muscle has not been elucidated, however.

Extractability of Muscle Proteins

Deuticke (1932) reported that muscles which had been fatigued by stimulation, then frozen, and pulverized yielded less protein on extraction than those immediately extracted. The solution used was 0.2 ionic strength phosphate buffer containing one-sixth part of 0.2 ionic strength potassium iodide. Weber and Meyer (1933) observed similar results and found that the myosin fraction was responsible for the decrease in solubility.

Smith (1934) studied the effects of a series of solutions on muscle extraction and found that with 5 to 10% solutions of ammonium and lithium chloride, no differences could be detected between fresh muscle and muscle in rigor. He also stated that during post-mortem storage of rabbit muscle, the residual or non-extractable intracellular fraction increased, due in part to the protein becoming insoluble.

Bailey (1954) explained the loss of extractability by stating that stimulation and rigor involved a change of state, which is reflected in

a loss of solubility in some salt solutions, but not in all. This change of state involves the combination of myosin and actin into actomyosin, a less soluble complex. In freshly minced, relaxed muscle, ATP acts as a specific dissociating agent. In rigor or fatigued muscle, extraction is facilitated by salts which depolymerize the complex (Bailey, 1954).

As pointed out by Weber and Portzehl (1952), extractability is not solely determined by solubility of the muscle proteins. Under all conditions, it is the insoluble structures surrounding the muscle filaments, which are the main barriers to the diffusion of either F-actin filaments or F-actomyosin threads. These same authors stated that for normal muscle, an increase in ionic strength as well as the degree of comminution increases the rate of extraction. This is explained by the increased concentration gradient present at higher ionic strengths. In contracture or other abnormal states, whenever the level of ATP is diminished, a much higher ionic strength is required to split the F-actin-myosin complex. Dissociation of the complex is thought to be a prerequisite for extraction.

Dubuisson (1950) attributed the extractability of the muscle globulins, actomyosin and myosin to the firmness of the binding forces, which maintain these proteins in their natural positions, and to the dissociation power of the salts used for extraction. This is given as an explanation of the reason these proteins, particularly myosin, which are inextractable from contracted tissue by potassium chloride, become extractable by potassium iodide.

Bailey (1954) interpreted the decrease in muscle protein extractability originally observed by Deuticke (1932) as an aggregation process.

The authors pointed out that this process can be influenced in several ways. First, actin and myosin can associate through lack of ATP. A relaxed muscle, freshly minced, will yield free myosin even on coarse mincing, but further comminution and stronger salt solutions will yield large amounts of actomyosin. While addition of ATP hastens the rate of solution, it does not increase the final yield, except when the extracting solution is above 0.5 ionic strength. Secondly, the extent of the interaction is determined by physiological events, in particular the molecular mechanism which accompanies contraction. Lastly, the supporting structures may hinder the diffusion of proteins out of the tissues to varying degrees.

Dyer et al. (1950) studied the proteins of fish muscle using the method of salt extraction reported by Smith (1934). They used the Waring blendor to obtain better subdivision of fibrils. A baffle was inserted to prevent foaming, which caused incorporation of air and some denaturation of protein. Neutral, normal salt solutions were all efficient in dissolving the protein, when the blendor was used, and allowed recovery of up to 95% of the total protein present. For maximum extraction of protein, they reported an optimum salt concentration of 3 to 5% sodium chloride and an optimum pH range of 7 to 9. The temperature should be maintained below 5°C during extraction. The authors reported almost no protein was extracted below pH 5 in the presence of 5% sodium chloride. This was attributed to the fact that most muscle proteins are rapidly denatured in acid solution in the presence of salt. In addition, pH 5 is near the isoelectric point of many muscle proteins, where solubility

in water is minimal. The isoelectric point of myosin is pH 5.2, of globulin X, pH 5.2, and of myoalbumin, pH 3 (Dyer et al., 1950).

Helander (1957) conducted an extensive study on the quantitative extraction of muscle protein fractions using rabbit muscle. The effects of pH, salts, additives, ionic strength, length of extraction, and solvent volume were studied. Protein yield was greatest in the pH range of 6.5 to 9.0 with substantially the same recovery anywhere within these limits. The amount of muscle protein extracted with potassium iodide was higher than with any other salt solution tested. Extractability increased as the concentration of salt increased and was maximal at 1.1 M potassium iodide. This corresponds to a total ionic strength of the solution of 1.3. Using 1.1 M potassium iodide, the yield upon extraction was not increased by the addition of sodium pyrophosphate or ATP. Helander (1957) also found that sarcoplasmic proteins were quantitatively extracted in 2 to 3 hours using 0.03 M phosphate buffer. Extraction with 1.1 M potassium iodide was complete in 3 hours while 0.6 M potassium chloride required 10 hours. Protein yield increased with solvent volume, but there was no further increase in yield using solvent to tissue ratios greater than 10:1.

Khan (1962) developed a procedure for the routine fractionation and estimation of the major protein fractions of chicken muscle. Various buffer systems were compared for efficiency of extraction. Results indicated that KCl-borate and KCl-phosphate buffers of pH 7.3 to 7.5 and an ionic strength of 1.0 gave maximum extraction. When the fractionation was performed with KCl-borate buffer, the stroma, myofibrillar, and sarco-

plasmic fractions contributed 13, 42, and 30% to total nitrogen of breast muscle and 27, 30, and 22% in leg muscle, respectively.

Relation of Protein Extractability to Meat Tenderness

Hill (1962) studied nitrogen distribution within characteristically tough (semitendinosus) and tender (longissimus dorsi) muscles for different species. The effect of compositional differences upon tenderness or toughness was considered. Sarcoplasmic-protein nitrogen, myofibrillar-protein nitrogen, non-protein soluble nitrogen, and stroma nitrogen were determined. His analyses revealed a higher myofibrillar nitrogen content and lower sarcoplasmic nitrogen content in beef semitendinosus than in the longissimus dorsi muscle. He suggested that the higher myofibrillar-protein nitrogen content may be reflected to some extent in the greater toughness of the semitendinosus muscle. He also suggested that the greater sarcoplasmic nitrogen content and smaller myofibrillar nitrogen content may be reflected to some degree in the greater tenderness of lamb and pork muscles as compared to beef.

Husaini et al. (1950a) studied the relationship of longissimus dorsi muscle composition to tenderness of beef and reported a correlation coefficient of -.88 between tenderness and alkali-insoluble protein. No relationship was found between tenderness and total nitrogen, non-protein nitrogen, pH, moisture, lactic acid, or inorganic phosphate. In a later study, Husaini et al. (1950b) found no relationship between tenderness and alkali-insoluble protein of the longissimus dorsi muscle at 3 days post-mortem, while a low negative correlation was observed at 15 days

post-mortem. Muscle hemoglobin content, taken as a measure of muscle plasma, showed no relationship to tenderness at 3 days, but a significant correlation was seen at 15 days post-mortem. Increases in tenderness between 3 and 15 days post-mortem were also correlated significantly with muscle hemoglobin. This observation indicates the importance of muscle plasma in meat tenderness.

Wierbicki et al. (1954) were the first to relate protein extractability to tenderness changes post-mortem. The extracting solution, which was designed to dissolve actin, myosin, and other soluble proteins, but not to extract actomyosin, was a citrate buffer of pH 5.60, ionic strength 0.48 and the solution contained 0.22 M potassium chloride. Hydroxyproline content and alkali-insoluble residue were determined as indices of connective tissue content. These authors found no changes in amount of connective tissue during aging by either method. The percent total nitrogen which was extractable increased during aging and was related to tenderness at 15 days post-mortem. They concluded that increases in tenderness with post-mortem aging may be related to: (a) the dissociation of actomyosin or some similar protein changes, which increase protein extractability; and (b) redistribution of ions within muscle, thus causing increased hydration and tenderness.

In a subsequent report (Wierbicki et al., 1956), the authors reported water extractable nitrogen and potassium citrate soluble nitrogen decreased during post-mortem aging, indicating that dissociation of actomyosin into actin and myosin is not responsible for post-mortem tenderization. They

attributed this decrease to denaturation, protein-protein interaction or some other phenomenon, which altered muscle protein solubility. Post-mortem pH changes were observed, which could contribute to increased protein hydration. The authors suggested that post-mortem tenderization may be an ion-protein or protein-protein interaction that contributes to higher protein hydration.

Kronman and Winterbottom (1960) studied the effect of aging bovine skeletal muscle on certain protein properties. Post-mortem aging was shown to result in decreased extractability of the water soluble proteins in agreement with the results of Wierbicki et al. (1956). They stated that if solubility is indicative of denaturation, that from 10 to 30% of the soluble protein may be denatured during 7 days aging. They concluded that extractability or solubility of muscle proteins do not provide a sufficiently sensitive criterion for protein alteration. Measurements of specific properties such as acid-base binding ability and ultracentrifugal and electrophoretic behavior would appear to be more promising methods for elucidating post-mortem changes.

Weinberg and Rose (1960) studied post-mortem tenderization of chicken muscle by observing changes in extractability of the contractile proteins from pre- and post-rigor chicken breast muscle. An overall increase in total protein extractability was reported during aging for 24 hours at 4°C. This increase was accounted for by a larger actomyosin fraction. They suggested that more actin was extracted from post-rigor muscle, and that the actin combined with myosin in the extract. They advanced the

theory that post-mortem tenderization is not merely random autolysis, but results from a specific cleavage of an actin association responsible for maintenance of the muscle matrix.

Hegarty et al. (1963) found significant correlations between myofibrillar protein solubility and tenderness of bovine muscle ($r = -.69$ for shear and $r = .59$ for panel). However, Goll et al. (1964) reported that tenderness appeared unrelated to protein solubility. They stated that protein solubility decreased during the first 6 hours post-mortem but did not change during a 6- to 312-hour post-mortem period. Myofibrillar protein solubility decreased in muscles left attached to the carcass during aging, when compared to muscles excised immediately from the carcass at slaughter. Sarcoplasmic protein solubility also followed this pattern.

Locker (1960) attempted to detect proteolysis during aging in the longissimus dorsi muscle of beef by the method of N-terminal analysis. Protein extractability was observed to decrease during rigor and then increase slightly by the end of a 16 day aging period. A very slight increase in N-terminal groups and definite but small increases in free amino acids were observed during the post-mortem period. Because of these small changes, the author concluded that proteolysis, as such, is not a significant factor in the post-mortem tenderization of meat.

Sharp (1963) studied autolysis in rabbit and bovine muscle during aseptic storage at elevated temperature and for extended periods of time. The author observed a continuous breakdown of protein with the formation of non-protein nitrogen at rates of 20.7 and 10.3 micromoles of nitrogen

per gram per day for rabbit and beef muscle, respectively. Over a period of 6 months storage, he detected no change in the solubility of the collagen fraction. Microscopic examination of the myofibrils showed no change in their fine structure during this period. He concluded that the main autolytic effect of the muscle cathepsins appeared to be concentrated on the sarcoplasmic proteins.

Starch Gel Electrophoresis of Muscle Proteins

The use of a gel prepared from hydrolyzed potato starch as a carrier or supporting medium for zone electrophoresis was introduced by Smithies (1955). This technique was reported to give separation of the serum proteins superior to any other previously reported electrophoretic procedure. Smithies (1959) presents a comprehensive review of the technique involved and application of the method to serum proteins.

Kronman et al. (1960) studied the myogen fraction of eight different beef muscles. Water soluble protein and aldolase content were higher in the longissimus dorsi, semimembranosus and semitendinosus than in any of the other muscles studied. The electrophoretic patterns of aqueous extracts of these beef muscles were found to be comparable to those for rabbit muscle. They observed considerable variation in the relative amount of components from muscle to muscle and animal to animal, there being no apparent regularity in the variation. Extracts of the longissimus dorsi muscle were fractionated by ammonium sulfate precipitation and the fractions were further characterized. The beef muscle sarcoplasmic components were tentatively identified by comparison of solubilities,

electrophoretic mobilities, and sedimentation constants to those of rabbit muscle proteins. Major components and their approximate contribution to the total sarcoplasmic fraction were: lactic dehydrogenase, 13-26%; phosphofructokinase, 10%; aldolase, 10%; and ATP-creatine transphorylase, 10-38%.

Tsuyuki et al. (1962) were able to detect species differences in patterns obtained by starch gel electrophoresis of myogen extracts of Pacific salmon. They were able to classify sockeye, pink and chum salmon into one ancestral group and spring and coho salmon into another. Giles (1962) found distinct species differences in the sarcoplasmic muscle proteins of beef, pork, veal, mutton, lamb, and rabbit by use of starch gel electrophoresis. The species could be identified as a result of these differences. The differences found were: (a) variation in the number of bands; (b) variation in the intensities of bands, indicating differences in concentration; and (c) variation in electrophoretic mobilities of similar proteins, indicating slight differences in physical properties.

Hartshorne and Perry (1962) obtained 15 definable bands in the starch gel electrophoretogram of a sarcoplasmic extract of rabbit muscle. Some of these bands were identified with known proteins. The component migrating fastest toward the anode possessed a velocity identical with that of rabbit serum albumin. Peaks were found showing aldolase and creatine-phosphokinase activity.

Odense and Skinners (1962) compared starch gel electrophoresis of a cod muscle extract with direct vertical electrophoresis of the tissue.

Nine distinguishable bands were found in the extract while one additional band was evident in the tissue pattern. The tissue was sectioned and stained after electrophoresis. The A and I bands and cross-striations were clearly visible under the light microscope, the indication being that sarcoplasmic, water-soluble muscle albumins are removed from the tissue by electrophoresis while the insoluble structural components remain intact. They stated that this method reduces the danger of denaturing the proteins, which is inherent in the preparation of an extract.

Scopes (1964) used starch gel electrophoresis in attempting to evaluate the effect of post-mortem glycolysis with its accompanying pH fall on the properties of beef muscle sarcoplasmic proteins. By using either a discontinuous tris-boric acid, tris-diethylenetriaminepenta-acetic acid buffer system or a tris-chloride system with no discontinuity, a total of 30 bands could be observed in a pre-rigor extract. He observed few consistent differences between the pre-rigor extract pattern and that of an extract from muscle which had passed into rigor at 0°C. However, when compared with samples which had gone into rigor at 37°C, a large decrease in creatine kinase was seen in the 37° extract. Also, lesser decreases were seen in certain minor bands. The implication of these results is that certain sarcoplasmic proteins are specifically denatured by conditions of low pH and high temperature.

Neelin and Rose (1964) extracted chicken muscle proteins during post-mortem aging and examined them by starch gel electrophoresis. Proteins extracted by vigorous homogenization in dilute buffer were designated as

myogen proteins. No change occurred in this fraction derived from white muscle but one additional electrophoretic component slowly appeared in dark muscle extracts. A "sarcoplasmic" protein fraction of breast muscle, pre-rigor or in-rigor, extracted by gentle homogenization in 0.44 M sucrose lacked some constituents of their myogen fraction. However, many of the absent constituents reappeared during aging. The authors suggested that breakdown of intracellular barriers and subcellular particles may have allowed soluble proteins to escape into the extract and thus account for the additional electrophoretic components of aged muscle sarcoplasm.

EXPERIMENTAL METHODS

1. Experimental animals and sampling procedures

All muscle samples were obtained from the carcasses of 6 cattle selected from the Michigan State University herd and slaughtered in the University abattoir. Two USDA Commercial grade cows 6 and 8 years of age were used in this study. Two Choice grade steers and 2 Choice grade heifers, all 2 years old were also included in this study.

The longissimus dorsi muscle from the lumbar region of the loin and the semitendinosus muscle from the round were used in studying protein solubility. These two muscles were chosen since they possess divergent degrees of tenderness. Samples were taken at 4 periods post-mortem: zero time (within 40 minutes following death), and at 24 hours, 7 days and 14 days post-mortem. Carcasses were aged at 4°C and all muscles were left on the carcasses during aging. The zero time samples were obtained from the left side of the carcass, while all subsequent samples were taken from the right side. Muscle surfaces, which may have been exposed to the atmosphere during aging, were discarded at the time of removal. Extreme ends of the muscles were not sampled because of a higher connective tissue content. A 1 1/2 inch steak was also obtained from the longissimus dorsi muscle after 24 hours, 7 days, and 14 days post-mortem aging. These steaks were frozen at -30°C and stored until all samples had been obtained, at which time they were evaluated for tenderness.

All visible fat and connective tissue were removed from the exterior of the muscles. A muscle sample, approximately 500 g, was ground once through a 2 mm plate. The grinder head, knife, and plate were prechilled to 4°C in all cases to prevent heat denaturation. After grinding, the sample was covered tightly with aluminum foil to prevent evaporation. A portion of the ground muscle was sealed in a glass jar, frozen, and later analyzed for total nitrogen (F^n) by the macro-Kjeldahl procedure as outlined by the A.O.A.C. (1960).

2. Fractionation of muscle proteins

The fractionation procedure utilized was a modification of the method of Helander (1957) as described by Lawrie (1961). In the present study, modifications included the use of magnetic stirrers and an increase in sample size. All fractionation procedures were carried out in duplicate at 4°C unless otherwise stated. The flow sheet shown in Figures 1 and 2 was used for the quantitative determination of soluble sarcoplasmic-protein nitrogen, soluble non-protein nitrogen and soluble fibrillar-protein nitrogen.

Essentially, the procedure was carried out as follows: A 10 g sample of ground tissue was accurately weighed into an aluminum drying dish. The sample was then transferred to a microblendor jar and homogenized with 30 ml of cold phosphate buffer (pH 7.4, 0.03 M PO_4) for one minute at a speed of 3000 rpm. This gave a satisfactory dispersion of tissue in the extracting solution with a minimum of foaming during the blendorizing period. Blendor speed and time were closely controlled as variation in these factors caused differences in extractability, especially if foaming occurred.

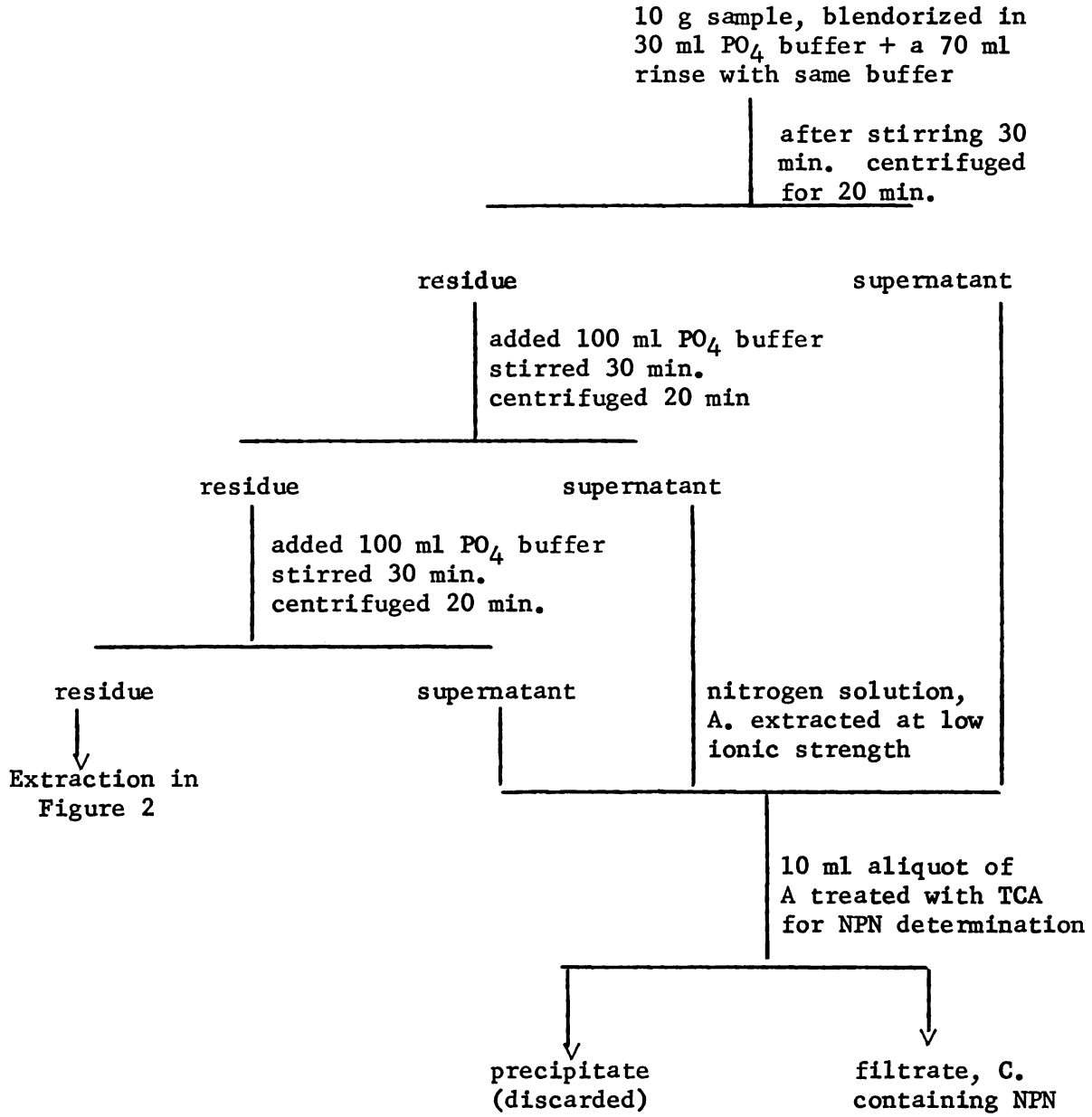


Fig. 1. Scheme for the quantitative determination of sarcoplasmic protein nitrogen and non-protein nitrogen.

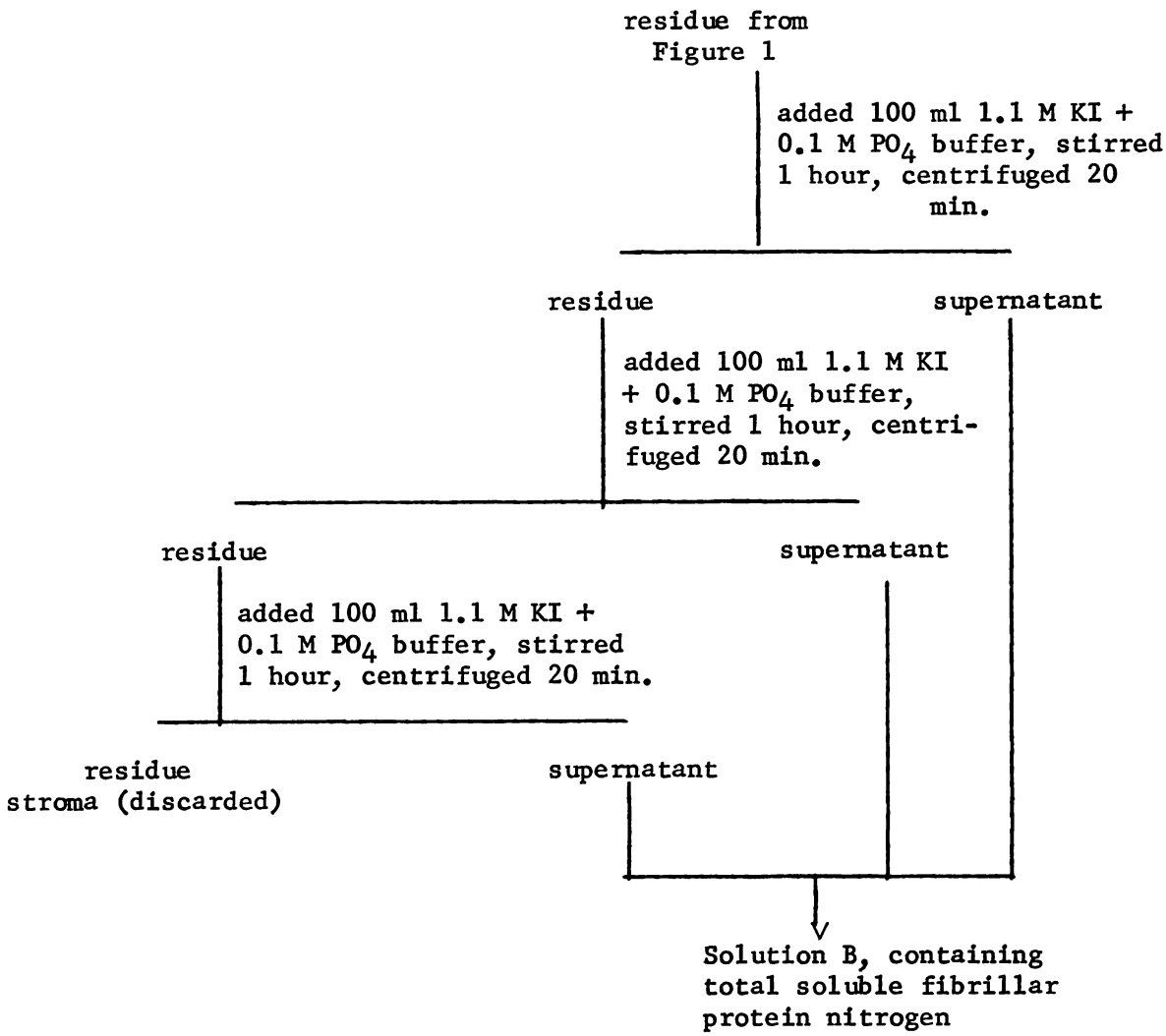


Fig. 2. Scheme for the quantitative determination of fibrillar protein nitrogen.

After blending, the material was transferred to a 250 ml centrifuge tube, the blendor jar was rinsed with 70 ml of extracting solution and added to the centrifuge tube. The material was stirred by means of a magnetic stirrer for 30 min and then centrifuged for 20 min. All centrifugation was done in a model PR-2 refrigerated International centrifuge at 4°C and 2800 rpm. The supernatant was decanted and the volume recorded. The residue was resuspended in 100 ml of cold 0.03 M phosphate buffer and the procedure repeated twice. The three supernatants resulting from this extraction were combined and filtered through 8 layers of gauze to remove any remaining fat or other particulate matter. The filtrate was designated as solution A. A 10 ml aliquot of solution A was mixed with 10 ml of 10% trichloroacetic acid solution. After 1 hour, the material was filtered through Whatman No. 2 filter paper and the filtrate designated as solution C, which contained the non-protein nitrogen. The residue remaining from low ionic strength extraction was then resuspended in 100 ml of 1.1 M KI plus 0.1 M potassium phosphate buffer, pH 7.4. It was stirred for one hour at 4°C and then centrifuged as before, the supernatant being decanted and its volume recorded. The extraction with 1.1 M KI plus 0.1 M phosphate buffer was repeated 3 times. The supernatant was retained and its volume recorded in each case. Stroma material remained as insoluble residue in the bottom of the centrifuge tube and was discarded. The three supernatants were combined, filtered through gauze and the filtrate designated as solution B. The nitrogen content of solutions A, B, and C was determined by the micro-Kjeldahl procedure as outlined by the American

Instrument Co. (1961). It was necessary to multiply the value obtained for solution C by 2 in order to correct for the TCA dilution. The nitrogen contents of the solutions were designated A^n, B^n and C^n, respectively, and were reported as mg of nitrogen per g of tissue or as percent of total nitrogen present in the tissues. These symbols (nitrogen contents) represent the following fractions:

A^n = nitrogen soluble at low ionic strength

B^n = total soluble fibrillar protein nitrogen

C^n = total soluble non-protein nitrogen

$A^n - C^n$ = total soluble sarcoplasmic-protein nitrogen

$F^n - (A^n + B^n)$ = stroma nitrogen (insoluble)

The values are a result of duplicate analyses. Variation between duplicates for A^n and C^n was normally from 0.00 to 0.02 mg, with 0.04 mg occurring in several cases. Slightly less variation occurred in B^n.

3. Tenderness measurements.

Tenderness determinations were made on the 1 1/2 inch thick steaks taken from the lumbar region of the longissimus dorsi muscle after 24 hours, 7 days, and 14 days post-mortem aging. Steaks were thawed slowly overnight at 4°C and then cooked in deep fat at 141°C to an internal temperature of 63°C. They were allowed to cool for 24 hours and 10 cores, 1/2 inch in diameter, were taken from each steak for Warner-Bratzler shear determination. Shear values reported are an average of the 10 measurements.

4. Statistical analyses.

Simple correlation coefficients, analyses of variance, standard errors and standard deviations were calculated as outlined by Snedecor (1956). Duncan's (1955) Multiple Range Method was used to test for differences among means.

5. Sample preparation for electrophoresis.

The sample for starch gel electrophoresis was obtained at the same time as the sample for protein fractionation. The sarcoplasmic extract was prepared at 4°C. Twenty g of ground muscle tissue was homogenized with 50 ml of cold, deionized, distilled water for 1 min at a blendor speed of 4000 rpm. The slurry was allowed to stand in the cold for one hour, after which it was centrifuged for 20 min at 2800 rpm. The supernatant was first filtered through gauze to remove fat particles and then through Whatman No. 1 filter paper to remove the remaining solids. In preliminary trials, the extract was centrifuged at 35,000 x g for 45 minutes rather than using filtration. This procedure did not affect the results obtained during electrophoresis so it was discontinued. The pH of the clear filtrate was adjusted to 8.6 with 1 M tris buffer (2-amino-2-hydroxymethyl-1,3-propanediol). It was then placed in cellulose tubing and dialyzed for 8 hours against a 0.01 M tris - 0.001 M citric acid buffer containing 0.5 M sucrose. This concentrated the protein extract from about 2.5 to nearly 4% protein. The sarcoplasmic protein solution was now ready to be placed on the starch gel.

6. Starch gel electrophoresis.

Starch gels were prepared similar to the method described by Smithies (1955). Dry, hydrolyzed starch was obtained from Connaught Medical Research Laboratories, Toronto. To prepare a gel, 36 g of starch was added to 300 ml of buffer in a 1000 ml beaker. Continuous rapid stirring was carried out as the mixture was heated over a Bunsen burner. As the temperature increased, the suspension first turned semi-solid and then became a viscous fluid. When the temperature reached 85°C, heating was stopped and the beaker immediately placed in a desiccator. To remove gas from the suspension, negative pressure was applied to the desiccator until the fluid boiled vigorously and bubbles were seen to come from the very bottom of the beaker (approximately 5 sec). Boiling time was kept short in order not to change the composition of the gel due to water loss.

The electrophoretic cell assembly was now completely filled with the hot gel. A slot-former was placed in position approximately 8-10 cm from one end of the cell. The formation of air bubbles was carefully avoided. The slot former (Quinn, 1963), shown in Figure 3, consisted of a piece of 15 by 2 cm plexiglass onto which 4 plexiglass projections measuring 2.5 by 6 by 12 mm were fused. These projections were spaced 12 mm apart with their long axis parallel to the long axis of the slot-former. When in position, these pieces extended into the gel to a depth of 6 cm. After the slot-former was put into position, the gel was placed in a water vapor saturated atmosphere and allowed to cool to room temperature (approximately 2 hours). The gel was now ready for use.

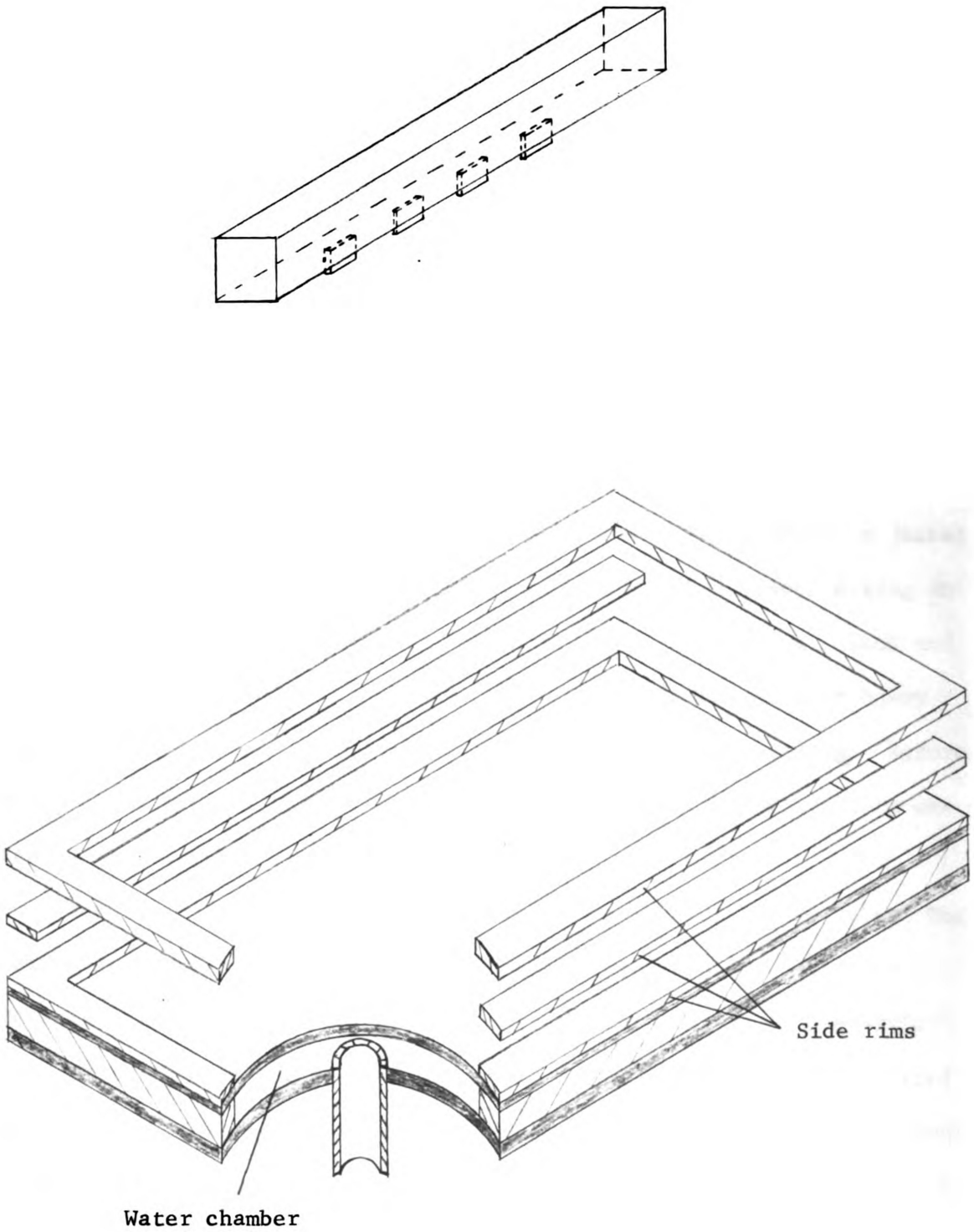


Fig. 3. Slot-former (top) and electrophoretic cell assembly.

The electrophoretic cell assembly was similar to that described by Wake and Baldwin (1961) with some modification. The assembly is diagramed in Figure 3. It consisted of a plexiglass tray 26 cm long and 15 cm wide. The sides were formed of 3 plexiglass rims each 2.5 cm in thickness. The bottom rim was fused to a sheet of plexiglass which formed the bottom of the tray. The 2 removable top rims were fastened in place with plastic tape along both sides and at the corners. A rim of plexiglass 5 mm in thickness was fused to the bottom side of the gel tray and another sheet of plexiglass was fused to this rim. Holes were drilled in the sheet and short pieces of plexiglass tubing were inserted and fused into the holes. The compartment immediately below the gel tray provided a jacket through which a constant temperature solution could be pumped during an electrophoretic run, thus closely regulating gel temperature. Each end of the assembled tray had a narrow slit into which was inserted heavy chromatographic paper cut so as to fit tightly into the opening. Before the gel was poured, the chromatographic paper was inserted so as to extend 3 cm into the gel tray.

Several buffer systems were tested in preparation of the gels. The borate buffer system of Smithies (1955) was used in the electrolyte vessels and in preparation of the gels. This contained 0.022 M boric acid and 0.009 M NaOH and had a pH of 8.6. This buffer was also tested at various pH values. The discontinuous buffer system described by Poulik (1957) was also employed. By this technique, the gels were prepared with 0.076 M tris - 0.005 M boric acid buffer and the electrolyte vessels contained 0.30 M boric acid - 0.06 M NaOH buffer. Smithies (1959) stated

that a boundary is formed between the two buffers during electrophoresis and is associated with an abrupt change in voltage which sharpens many zones. However, the optimum separation was obtained with a modification of the discontinuous buffer system of Scopes (1964). The gel buffer was composed of 0.012 M tris - 0.0018 M citric acid, pH 8.6 at 4°C. The electrode vessels contained 0.1 M boric acid - 0.06 M tris buffer, pH 8.6 at 4°C. This system of buffers was used in all subsequent electrophoretic runs.

During an electrophoretic run, the tray containing the cooled gel was suspended vertically between two electrode vessels. The chromatographic paper dipped into the electrolyte solution in the vessels and served as electrolyte bridges. These electrode vessels were constructed of 3 mm thick plexiglass and each had a capacity of 2 l. of solution. The electrodes consisted of platinum wire 16 cm in length stretched across the bottom of the electrode vessels. They were connected to an adjustable, constant voltage, DC power supply to complete the electrophoretic circuit. The complete electrophoretic circuit is shown in Figure 4.

All starch gel electrophoretic runs were performed at 4°C. The gel was pre-cooled to this temperature and ice water was continuously pumped through the tray jacket during a run in order to maintain this temperature. The slot-former was carefully removed, and the sample of sarcoplasmic extract inserted into the slot with a capillary tube. The filled slots were covered with petroleum jelly, and the entire gel was covered with Saran wrap to minimize evaporation. A sheet of plexiglass was taped over the gel, so as to keep it from falling out of the tray during the run.

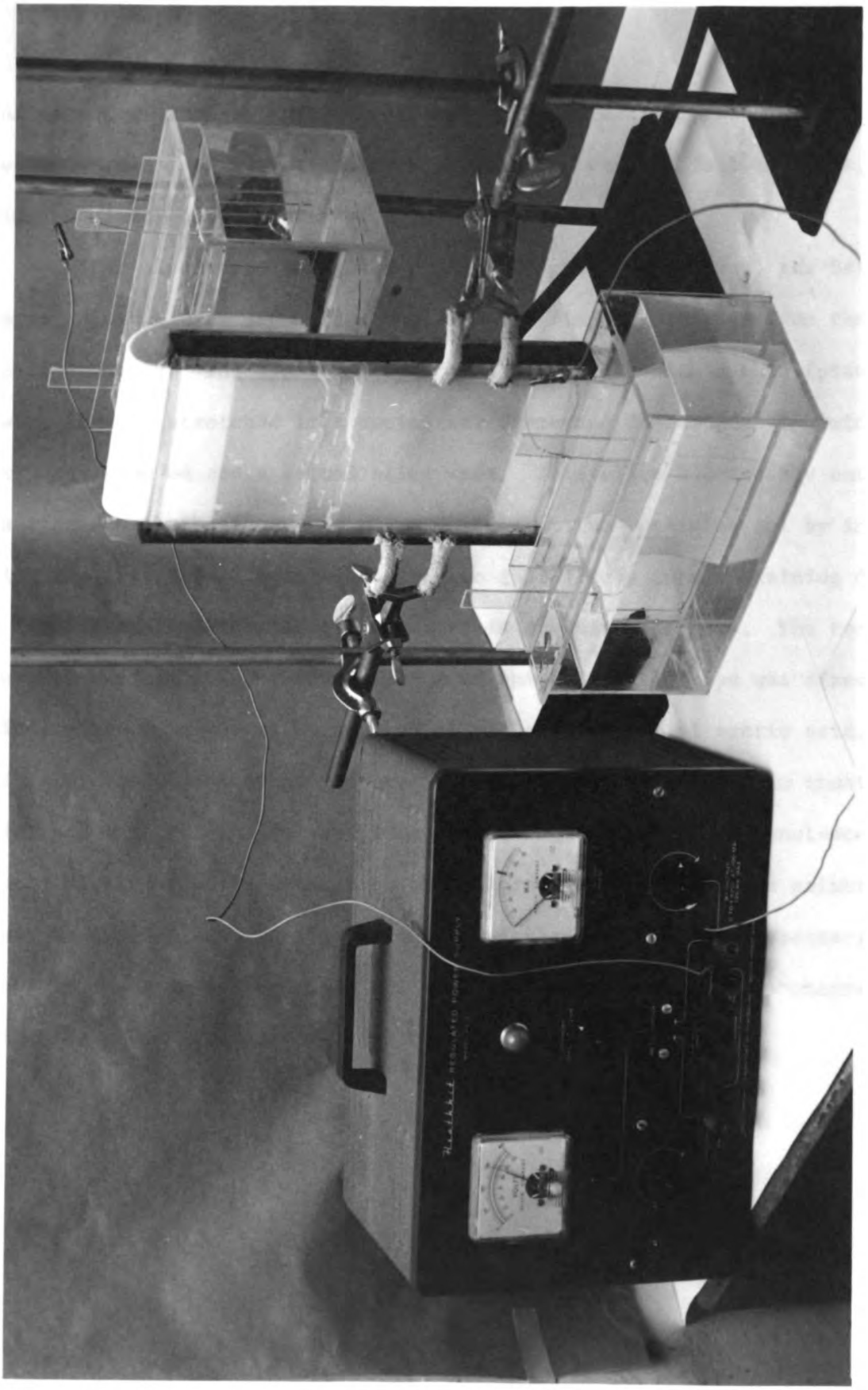


Fig. 4. Complete starch gel electrophoretic circuit.

After a few minutes, current was applied. The maximum voltage applied was 300 volts and the current was 12 ma. The current fell to 10 ma. during an electrophoretic run of 9 hours.

After electrophoresis, the protecting plexiglass sheet, the Saran wrap, plastic tape fastenings, and the top rim were removed from the cell assembly. The upper 2-3 mm of gel was sliced with a thin (piano) wire tightly stretched in a coping saw frame and discarded. The middle rim was removed and a second slice made. This slice had freshly cut surfaces on both sides. It was removed from the remaining gel by inverting the tray and allowing the slice to fall freely into a staining dish.

A protein detecting dye was used in staining the gels. The technique was that of Smithies (1955). Two g of Amido-Black 10B dye was dissolved in a mixture of 250 ml water, 250 ml methanol and 50 ml acetic acid. This dye solution was poured over the gel slice and allowed to remain for one minute. The gel was then rinsed with the water-methanol-acetic acid mixture (5:5:1) until free of background stain. The dye solution was reused. Since the gels dried out very rapidly, it was necessary to obtain a permanent record of the electrophoretic pattern by photographing the gel shortly after destaining.

RESULTS AND DISCUSSION

Muscle Protein Fractionation

The fractionation procedure outlined in the previous section was completed within 24 hours of the time of sampling. Replicate determinations showed that the method was reliable. In this procedure, connective tissue is included in the stroma fraction. Since the stroma protein is determined by difference, the values reported reflect all errors present in the other analyses.

The longissimus dorsi and semitendinosus muscles of 6 beef animals were fractionated by this procedure. The nitrogen fractions from two muscles at slaughter (zero time) are presented in Tables 1 and 2. Standard errors were calculated for the means of all animals. It is not surprising that rather high standard errors were obtained since a heterogeneous group of animals is represented. Values were not calculated for each class because of the limited number in each.

Inspection of Tables 1 and 2 reveals that the mean for total nitrogen content is slightly greater in the semitendinosus muscle. This is also true of the mean for total nitrogen content for each class (sex and/or age). The nitrogen distribution in the longissimus dorsi muscle, expressed as percentage of total nitrogen, is similar to data given by Hill (1962) for the same muscle. He reported values of 54.73% for myofibrillar nitrogen, 19.12% for sarcoplasmic nitrogen, 13.62% for stroma nitrogen, and 12.53% for non-protein nitrogen on a fat-free, fresh tissue basis.

Table 1. Distribution of nitrogen in various protein fractions of the longissimus dorsi muscle from 3 classes of beef animals at slaughter

Class of animal	Nitrogen fraction				
	Sarcoplasmic	Fibrillar	NPN	Stroma	Total
	Percentage of total nitrogen				
steers	21.6	49.0	12.8	16.6	
heifers	23.6	53.7	14.2	7.3	
cows	24.3	53.5	13.5	8.7	
$\bar{x}$ of all animals	23.2	52.0	13.5	10.9	
	Mg N/g tissue				
steers	6.90	15.63	4.10	5.30	31.93
heifers	7.39	16.78	4.42	2.61	31.20
cows	7.64	16.82	4.24	2.73	31.43
$\bar{x}$ of all animals	7.31	16.41	4.25	3.53	31.50
standard error	0.20	0.42	0.14		

Table 2. Distribution of nitrogen in various protein fractions of the semitendinosus muscle from 3 classes of beef animals at slaughter

Class of animal	Nitrogen fraction				
	Sarcoplasmic	Fibrillar	NPN	Stroma	Total
	Percentage of total nitrogen				
steers	21.7	50.8	12.0	15.5	
heifers	21.2	52.6	12.9	13.2	
cows	23.1	52.8	13.1	10.9	
$\bar{x}$ of all animals	22.0	52.1	12.7	13.2	
	Mg N/g tissue				
steers	6.96	16.32	3.88	4.94	32.10
heifers	6.80	16.90	4.14	4.26	32.10
cows	7.58	17.33	4.29	3.59	32.80
$\bar{x}$ of all animals	7.12	16.85	4.10	4.23	32.30
standard error	0.23	0.36	0.22		

If the mean values for all animals are considered, it is seen that the longissimus dorsi muscle contained more sarcoplasmic and non-protein nitrogen, less stroma nitrogen, and slightly less fibrillar nitrogen than the semitendinosus muscle. Hill (1962) reported wider variation in fibrillar nitrogen between the two muscles than was found in the present study. When considering the mean values for each class, the longissimus dorsi of heifers and cows contained less stroma nitrogen than the semitendinosus muscle. Also, the higher non-protein nitrogen content of the longissimus dorsi was quite consistent among the classes.

The between class differences in sarcoplasmic, fibrillar, and non-protein nitrogen were not tested for significance because of the small number of animals in each class. However, the amount of stroma nitrogen was higher in steers than in heifers or cows, regardless of whether nitrogen was expressed as percentage of total nitrogen or as mg nitrogen /g tissue. The higher stroma nitrogen content of steers is more evident in the longissimus dorsi than in the semitendinosus muscle. With one exception, sarcoplasmic, fibrillar, and non-protein nitrogen were lower in the muscle from steers than in heifers or cows. The semitendinosus muscle of steers contained slightly less sarcoplasmic nitrogen than that of heifers. However, the amount of variation between classes was much less for sarcoplasmic, fibrillar and non-protein nitrogen than for stroma nitrogen.

An analysis of variance was determined for the amount of soluble sarcoplasmic nitrogen, soluble fibrillar nitrogen and soluble non-protein

nitrogen to determine if significant differences existed between the samples at any of the 4 post-mortem periods. The results are presented in Tables 3, 4 and 5 using the combined data from all animals.

While the amount of sarcoplasmic nitrogen (Table 3) extracted from the semitendinosus muscle decreased steadily as aging proceeded, only the amount extractable at 14 days was significantly lower than the other periods studied. This is in accord with the results reported by Kronman and Winterbottom (1960) for the longissimus dorsi muscle and by Goll et al. (1964) for the semitendinosus muscle. They found the greatest amount of sarcoplasmic protein is extracted immediately after death. However, in the present study, the longissimus dorsi muscle did not follow this trend and the mean percentage of extractable sarcoplasmic nitrogen remained essentially constant during post-mortem aging. The large standard error indicates that wide variation existed between animals, but there was no consistent pattern in the variation.

Table 3. Means and standard errors for percentage sarcoplasmic nitrogen extracted at 4 post-mortem periods[1]

Muscle	Time				Standard error
	0	24 hours	7 days	14 days	
longissimus dorsi, % total N	23.2[a]	23.6[a]	23.3[a]	23.4[a]	4.10
semitendinosus, % total N	22.0[b]	21.8[b]	20.8[b]	18.6[a]	0.66

[1]By Duncan's Multiple Range, all means with the same superscript are not significantly different at the 0.05 level.

Changes in fibrillar protein solubility are given in Table 4. In both muscles studied, fibrillar nitrogen solubility was lowest at 24 hours post-mortem. However, fibrillar nitrogen extractability increased with post-mortem aging. The increase was significant between both 24 hours and 7 days and between 24 hours and 14 days. However, the differences in extractability between 7 and 14 days were nonsignificant for both muscles. The development of rigor mortis prior to 24 hours post-mortem probably accounts for the lower fibrillar extractability in the samples removed at 24 hours (Bailey, 1954). No indication as to the cause of increased fibrillar nitrogen solubility during aging is evident from these data.

Table 4. Means and standard errors for percentage fibrillar nitrogen extracted at 4 post-mortem periods[1]

Muscle	Time				Standard error
	0	24 hours	7 days	14 days	
longissimus dorsi, % total N	52.0[a]	50.2[a]	55.9[b]	57.2[b]	1.29
semitendinosus, % total N	52.1[ab]	48.7[a]	55.6[bc]	57.9[c]	1.44

[1]By Duncan's Multiple Range, all means with the same superscript are not significantly different at the 0.05 level.

Changes in soluble non-protein nitrogen content of the muscles are presented in Table 5. In both muscles, non-protein nitrogen increased during aging with the largest increase occurring between zero and 7 days. The non-protein nitrogen content of the longissimus dorsi muscle at zero

time was significantly lower than at any other post-mortem period. After 24 hours, there was no significant difference in non-protein nitrogen extractability. A significant increase in non-protein nitrogen occurred between zero and 7 days and between zero and 14 days in the semitendinosus muscle. The increase between zero and 24 hours and that between 24 hours and either 7 or 14 days was not significant. These results are similar to those obtained by Sharp (1963) in studying aseptic autolysis in bovine muscle.

Table 5. Means and standard errors for percentage NPN extracted at 4 post-mortem periods[1]

Muscle	Time				Standard error
	0	24 hours	7 days	14 days	
longissimus dorsi, % total N	13.5[a]	14.1[b]	14.8[b]	14.8[b]	0.30
semitendinosus, % total N	12.7[a]	13.6[ab]	14.1[b]	14.4[b]	0.40

[1]By Duncan's Multiple Range, all means with the same superscript are not significantly different at the 0.05 level.

Paul and Bratzler (1955) reported that removal from the carcass or cutting (sectioning) the muscle shortly after death resulted in decreased tenderness when compared to intact muscles aged normally on the carcass. More recently, Goll et al. (1964) found excising the muscle from the carcass at death resulted in significantly greater amounts of salt-soluble protein. Therefore, in order to eliminate any possible effects upon tenderness and protein properties, the muscles were left on the carcasses

throughout this study. In addition, the 24 hour, 7 day and 14 day samples were obtained from the right side which had passed into rigor normally; whereas, zero time samples were removed from the left side.

Trautmann and Fiebiger (1952) as cited by the American Meat Institute Foundation (1960) stated that the ratio of sarcoplasm to myofibrils is apparently directly proportionate to the amount of work that the muscle preforms. Hegarty (1963) found an "r" value of -.43 between Warner-Bratzler shear value and the ratio of sarcoplasmic to fibrillar nitrogen. It is commonly accepted that muscles of the round such as the semitendinosus are required to do more work than the longissimus dorsi muscle. Therefore, a ratio of soluble fibrillar nitrogen to soluble sarcoplasmic nitrogen was calculated for the 4 post-mortem periods. These data are presented in Table 6. The ratio decreased initially from zero to 24 hours and then increased through 14 days post-mortem. The changes are much more pronounced in the semitendinosus muscle as shown by the significant difference between the means for all periods. In view of the increased fibrillar extractability and decreased sarcoplasmic extractability, especially in the semitendinosus muscle during post-mortem aging, the changes in this ratio are not surprising.

Table 6. Means and standard errors for fibrillar to sarcoplasmic ratio at 4 post-mortem periods[1]

Muscle	Time 0	24 hours	7 days	14 days	Standard error
longissimus dorsi, F/S ratio[2]	2.24^{ab}	2.12^{a}	2.43^{b}	2.46^{b}	0.087
semitendinosus, F/S ratio[2]	2.38^{a}	2.24^{b}	2.70^{c}	3.12^{d}	0.032

[1]By Duncan's Multiple Range, all means with the same superscript are not significantly different at the 0.05 level.
[2]F/S ratio is the ratio of fibrillar to sarcoplasmic nitrogen.

Warner-Bratzler shear values for the longissimus dorsi muscle are given in Table 7. The values decreased during aging, indicating increased tenderness. The average shear value at 24 hours was 11.83; at 7 days, 8.20; and at 14 days, 6.84. The decrease in shear value of the longissimus dorsi muscle during post-mortem aging from 24 hours to 14 days was greater for steers and heifers than for cows. Approximately 70% of the total decrease in shear value occurred during the first 7 days of aging. The greatest increase in fibrillar nitrogen solubility also occurred during this time. The pattern of tenderness changes agrees with that reported by Goll et al. (1964).

Table 7. Warner-Bratzler shear values of beef longissimus dorsi at 3 post-mortem periods

Class of animal	Post-mortem period 24 hours	7 days	14 days
steers	14.28	8.94	7.35
heifers	11.09	7.31	5.66
cows	10.10	8.36	7.50
$\bar{x}$ of all animals	11.83	8.20	6.84

Simple correlation coefficients were determined between tenderness as measured by the Warner-Bratzler shear and some extractable protein fractions of the longissimus dorsi muscle. Values are given in Table 8. The amount of soluble fibrillar nitrogen was negatively correlated with shear value at all three post-mortem periods. The correlation ($r = -.77$) at 7 days was significant at the 0.05 level. These results are in agreement with Hegarty (1963) and indicate that tenderness is directly related to fibrillar protein solubility. Correlation coefficients were also calculated between the increases in soluble fibrillar nitrogen and the decrease in shear force during the periods from 24 hours to 7 days, 24 hours to 14 days and 7 to 14 days. A positive correlation was obtained in each case, 0.55, 0.62, and 0.82, respectively. However, only the correlation for the 7 to 14 day period was significant. These data indicate that the increase in tenderness (decrease in shear value) is directly related to change (increase) in fibrillar protein solubility. A low negative correlation was found between the fibrillar to sarcoplasmic ratio and shear value at 7 days ($r = -.33$). However, the values for the other two periods were essentially zero.

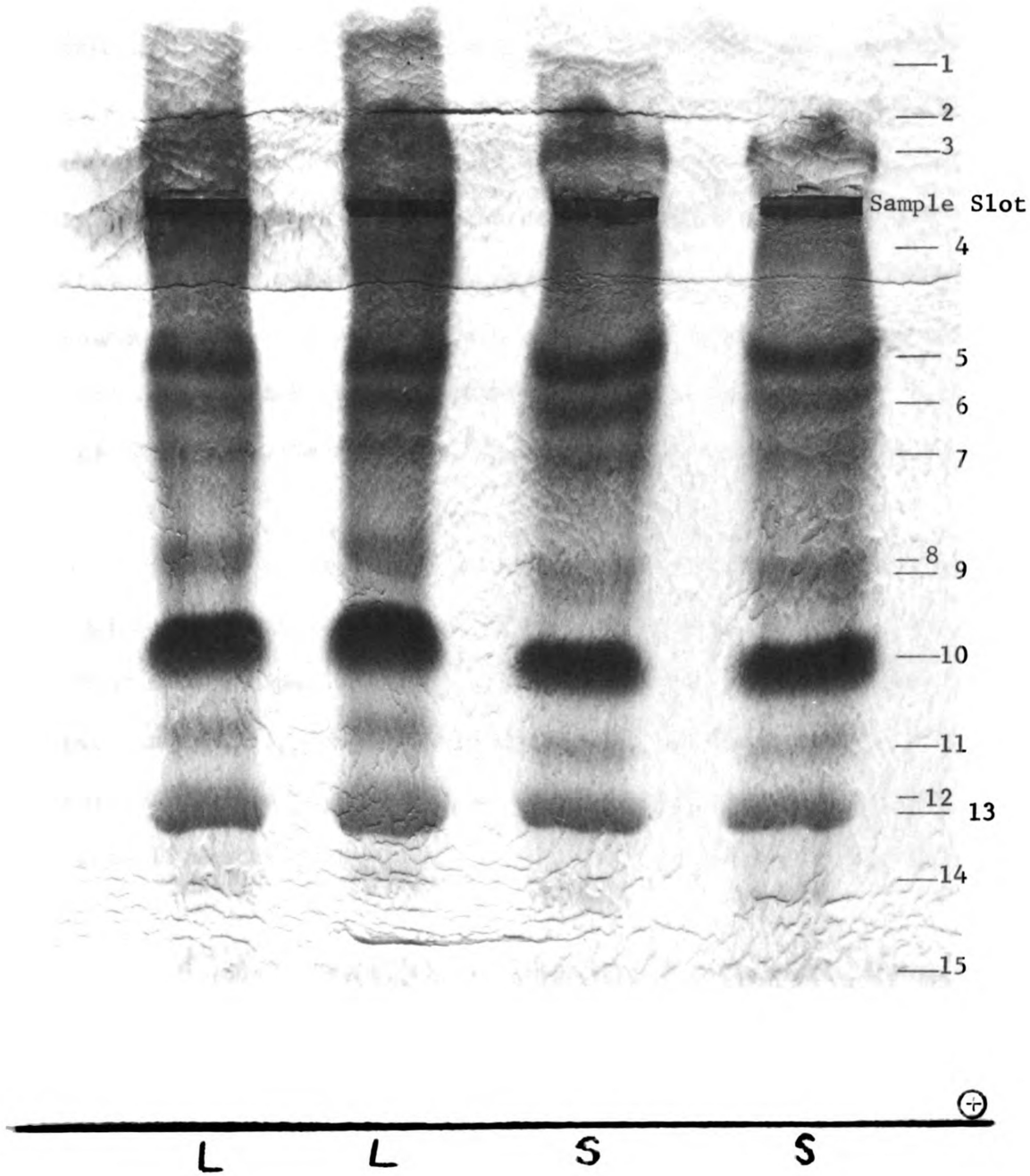
Table 8. Correlation coefficients for various factors related to Warner-Bratzler shear

Factor	Shear value (lbs)	Decrease in shear value
Fibrillar nitrogen, % total N		
24 hours post-mortem	-.38	
7 days post-mortem	-.77*	
14 days post-mortem	-.44	
Fibrillar to sarcoplasmic ratio at:		
24 hours post-mortem	0.02	
7 days post-mortem	-.33	
14 days post-mortem	0.04	
Fibrillar nitrogen, increase from:		
24 hours to 7 days		0.55
24 hours to 14 days		0.62
7 days to 14 days		0.82*

*$P < 0.05$

Starch gel electrophoresis of sarcoplasmic proteins

Figure 5 is an enlarged photograph of the starch gel electrophoretic pattern obtained with sarcoplasmic proteins extracted at zero time. Both the longissimus dorsi and semitendinosus muscles are represented. Individual zones are designated by numbers along the right margin, beginning with those zones nearest the cathode. Fifteen such zones are indicated. Since the pH of the buffers used in preparation of the gels was above the isoelectric point of most muscle proteins, the majority of the sarcoplasmic components migrated toward the positive electrode. In all cases, separation of those components migrating toward the anode was superior to separation of those moving toward the cathode.



875 - O

Fig. 5. Vertical starch gel electrophoresis of sarcoplasmic proteins extracted at zero time. Numbers below electrophoretogram designate animal and post-mortem period. L = longissimus dorsi, S = semitendinosus.

The starch gel electrophoretic patterns of the longissimus dorsi and semitendinosus muscles are quite similar. Even though the muscles are of quite divergent degrees of tenderness, distinguishable differences in the patterns of the two muscles occur only in isolated and irregular cases. In addition, a comparison of the sarcoplasmic electrophoretic patterns of animals differing widely in degree of tenderness reveals no readily apparent differences between animals. This is in contrast to suggestions made by other workers, among them Husaini et al. (1950b), which suggest that muscle sarcoplasm may be important in determining meat tenderness. Variation in electrophoretic pattern does not seem to be related to the sex of the animal as is apparent by comparison of Figures 5, 6 and 7.

Although none of the zones were identified, zone 10 of the electrophoretogram is thought to consist largely of myoglobin since a red pigment, most likely heme, is quite prominent at this position before staining with protein dye. It is difficult to distinguish between zones 8 and 9 in Figure 5. However, in other cases, these zones were more clearly visible. Zone 15 was quite variable and does not appear in all electrophoretograms.

Figures 6 and 7 show electrophoretic patterns obtained with sarcoplasmic proteins extracted from two different animals during post-mortem aging. In the zero time and 24 hour electrophoretograms of Figure 6, both zones 8 and 9 and zones 11 and 12 are clearly distinguishable. However, these zones are much less distinct at 7 days and 14 days post-mortem. It is quite possible that these zones may vary in their mobility as aging

proceeds and thus migrate at more nearly the same rate. Therefore, the 2 zones may merge as aging proceeds. In contrast, zones 11 and 12 were still fairly clear in the 14 day electrophoretogram of Figure 7. Zone 14 seemed to increase in intensity during aging, especially in Figure 6, and the amount of protein migrating toward the cathode appeared to increase as shown by a comparison of zero time and 14 day samples.

In both Figures 6 and 7, the individual zones become sharper and more clearly defined during post-mortem aging. This is especially true with all zones lying between 5 and 10. Zones 5, 6, 10 and 13 remain quite constant in their positions. However, in Figure 7, the intensity of all zones seemed to decrease during aging. This could be the result of lower sarcoplasmic extractability as aging proceeds.

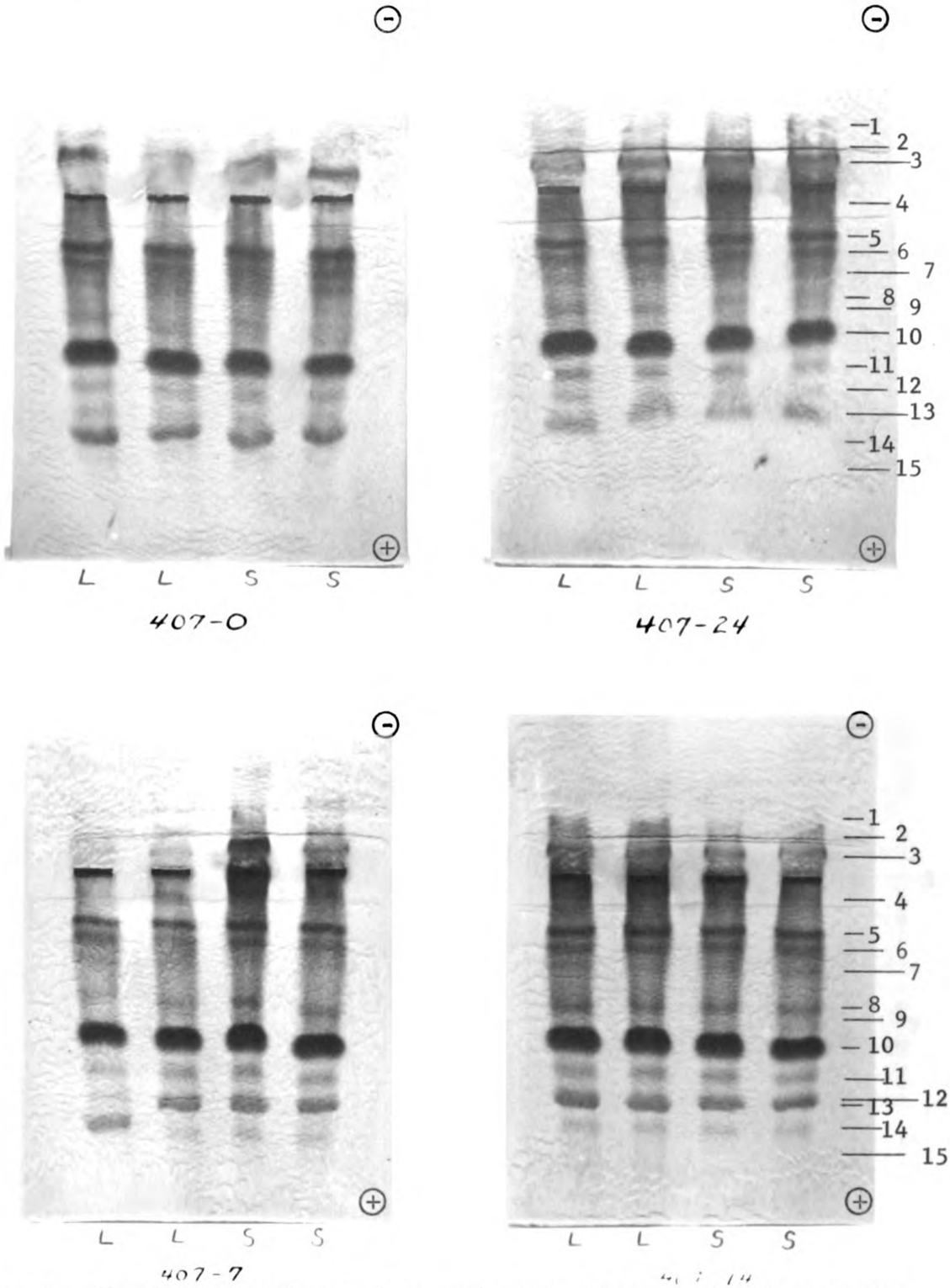


Fig. 6. Vertical starch gel electrophoresis of sarcoplasmic proteins extracted before and during aging. Numbers below electrophoretograms designate animal and post-mortem period. L = longissimus dorsi, S = semitendinosus.

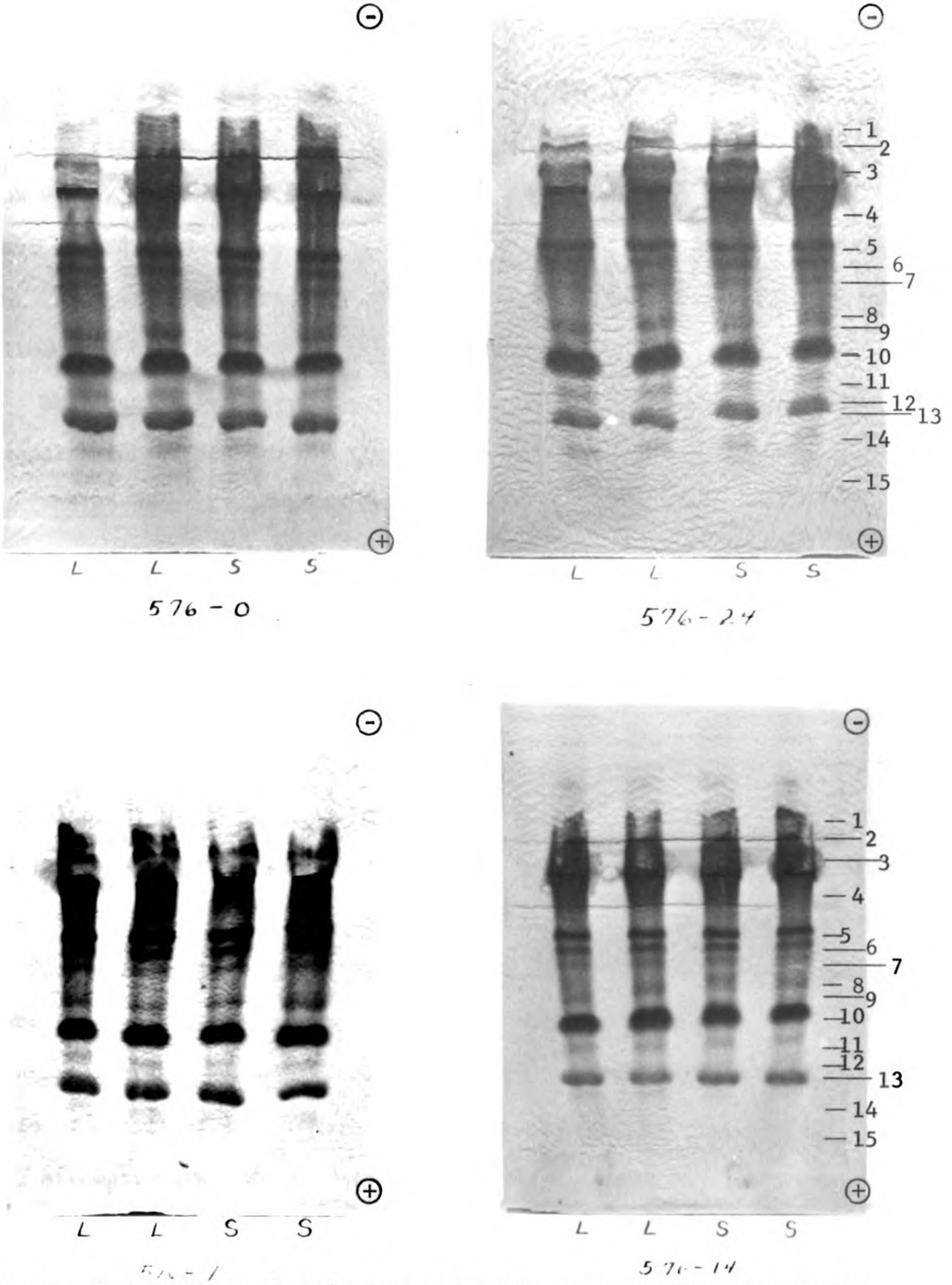


Fig. 7. Vertical starch gel electrophoresis of sarcoplasmic proteins extracted before and during aging. Numbers below electrophoretograms designate animal and post-mortem period. L = longissimus dorsi, S = semitendinosus.

SUMMARY

The nitrogen in the longissimus dorsi and semitendinosus muscles from 6 beef carcasses, including steer, heifer and cow carcasses, was fractionated into sarcoplasmic, fibrillar, non-protein, and stroma nitrogen. Changes in solubility during a 14 day post-mortem aging period were followed in both muscles using this fractionation procedure.

The longissimus dorsi contained slightly less total nitrogen, more sarcoplasmic and non-protein nitrogen, and less stroma nitrogen than the semitendinosus muscle. Steer muscle contained more stroma nitrogen and less sarcoplasmic, fibrillar, and non-protein nitrogen than muscle from heifers or cows.

The extractability of the sarcoplasmic nitrogen in the semitendinosus muscle decreased significantly during 14 days aging, however, no changes were found in the longissimus dorsi muscle under similar conditions. Solubility of the fibrillar protein was lowest at 24 hours post-mortem and increased significantly from 24 hours to 7 and 14 days for the two muscles studied. The amount of non-protein nitrogen was lowest at slaughter and increased during aging. While the amount of non-protein nitrogen at 7 and 14 days post-mortem was significantly higher than at slaughter, the greatest increase occurred during the first 7 days. In addition, the ratio of fibrillar to sarcoplasmic nitrogen increased during aging.

Attempts were made to relate the levels of the extractable protein fractions of the longissimus dorsi muscle with tenderness (Warner-Bratzler shear). Extractable fibrillar nitrogen was negatively correlated with

shear value in all cases. Positive "r" values were found between the increase in amount of soluble fibrillar nitrogen and the decrease in shear values for the periods of 24 hours to 7 days, 24 hours to 14 days and 7 to 14 days.

Starch gel electrophoresis was employed in an attempt to detect changes in the sarcoplasmic proteins during post-mortem aging. Fifteen zones were visible in the electrophoretograms. The majority of the sarcoplasmic components migrated toward the anode and their resolution was superior to that of cathodic migrating components.

The electrophoretic patterns of the longissimus dorsi and semitendinosus muscles were very similar and only minor variation occurred between animals. Changes were observed which indicate that certain sarcoplasmic components vary in mobility as post-mortem aging proceeds. The amount of protein migrating toward the cathode appeared to increase and zone 14 (migrating toward the anode) became more intense during the latter stages of aging. Also, some of the more slowly migrating anodic zones developed more distinct boundaries during aging.

BIBLIOGRAPHY

American Instrument Co. 1961. The determination of nitrogen by the Kjeldahl procedure including digestion, distillation and titration. Reprint No. 104.

American Meat Institute Foundation. 1960. The Science of Meat and Meat Products. p. 15. W. H. Freeman and Co., San Francisco.

Assoc. Offic. Agr. Chemists. 1960. Official Methods of Analysis. Assoc. Offic. Agr. Chemists. 9th ed. p. 95. Washington 4, D. C.

Bailey, K. 1946. Tropomyosin: A new asymetric component of muscle. Nature 147, 368.

Bailey, K. 1954. Structure of proteins II. Muscle. In The Proteins. (H. Neurath and K. Bailey, eds.) Vol. 2, part B, p. 854. Academic Press, New York.

Bate-Smtih, E. C. 1937. Native and denatured muscle proteins. Proc. Roy. Soc. (London) B 124, 136.

Deuticke, H. J. 1932. Kolloidzustandsänderungen der muskelproteine bei der muskeltätigkeit. Z. Physiol. Chem. 210, 97.

Dubuisson, M. 1950. Influence de la nature des ions sur l'extractibilite des proteines de muscles au repos ou contractures. Biochim. et Biophys. Acta 5, 489.

Duncan, D. B. 1955. The multiple range and multiple F-test. Biometrics 11, 1.

Dyer, W. J., H. V. French, and J. M. Snow. 1950. Proteins in fish muscle I. Extraction of protein fractions in fresh fish. J. Fisheries Research Board, Can. 7, 585.

Giles, B. G. 1962. Species differences observed in the sarcoplasmic proteins of mammalian muscle. J. Sci. Food Agric. 13, 264.

Goll, D. 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.

Hartshorne, D. J. and S. V. Perry. 1962. A chromatographic and electrophoretic study of sarcoplasm from adult and foetal rabbit muscle. Biochem. J. 85, 171.

Hegarty, G. R. 1963. Solubility and emulsifying characteristics of intracellular beef muscle proteins. Ph. D. Thesis, Michigan State University, East Lansing.

Hegarty, G. R., L. J. Bratzler and A. M. Pearson. 1963. The relationship of some intracellular protein characteristics to beef muscle tenderness. J. Food Sci. 28, 525.

Helander, E. 1957. On quantitative muscle protein determination. Sarcoplasm and myofibril protein content of normal and atrophic skeletal muscles. Acta Physiol. Scand. 41, Suppl. 141.

Hill, F. 1962. Fibre composition of tough and tender muscles of meat animals. Irish J. Agric. Research 1, 319.

Hunt, S. M. V. and N. A. Matheson. 1958. Adenosinetriphosphatase and contraction of dehydrated muscle fibers. Nature 181, 472.

Husaini, S. A., F. E. Deatherage, L. E. Kunkle and H. N. Draudt. 1950a. Studies on meat I. The biochemistry of beef as related to tenderness. Food Technol. 4, 313.

Husaini, S. A., F. E. Deatherage and L. E. Kunkle. 1950b. Studies on meat II. Observations on the relation of biochemical factors to changes in tenderness. Food Technol. 4, 366.

Khan, A. W. 1962. Extraction and fractionation of proteins in fresh chicken muscle. J. Food Sci. 27, 430.

Kronman, M. J., L. E. Weinberger and R. J. Winterbottom. 1960. The water-soluble proteins of bovine skeletal muscle. Arch. Biochem. Biophys. 86, 238.

Kronman, M. J. and R. J. Winterbottom. 1960. Post-mortem changes in the water soluble proteins of bovine skeletal muscle during aging and freezing. J. Ag. and Food Chem. 8, 67.

Kühne, W. 1859. Investigations on the movement and changes of the contractile substance. Arch. Anat., Physiol., and Med. 1, 767.

Lawrie, R. A. 1961. Studies of the muscles of meat animals I. Differences in composition of beef longissimus dorsi muscles determined by age and anatomical location. J. Agric. Sci. 56, 249.

Locker, R. H. 1960. Proteolysis in the storage of beef. J. Sci. Food Agric. 11, 520.

Neelin, J. M. and D. Rose. 1964. Progressive changes in starch gel electrophoretic patterns of chicken muscle proteins during aging post-mortem. J. Food Sci. 29, 544.

Odense, P. H. and C. W. Skinners. 1962. Direct vertical starch gel electrophoresis of cod muscle tissue. Can. J. Biochem. Physiol. 40, 1842.

Paul, P. and L. J. Bratzler. 1955. Studies on tenderness of beef. II. Varying storage times and conditions. Food Res. 20, 626.

Poulik, M. D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. Nature 180, 1477.

Quinn, J. R. 1963. Isolation and characterization of multiple bovine myoglobins. Ph. D. Thesis. Michigan State University, East Lansing.

Schramm, G. and H. H. Weber. 1942. Monodisperse myosin solutions. Kolloid-Z. 100, 242.

Scopes, R. K. 1964. The influence of post-mortem conditions on the solubilities of muscle proteins. Biochem. J. 91, 201.

Sharp, J. G. 1963. Aseptic autolysis in rabbit and bovine muscle at 37°C. J. Sci. Food Agric. 14, 468.

Smith, E. C. 1934. A scheme for the approximate determination of the proteins of muscle. J. Soc. Chem. Ind. (London) 53, 351 T.

Smithies, O. 1955. Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. Biochem. J. 61, 629.

Smithies, O. 1959. Zone electrophoresis in starch gels and its application to studies of serum proteins. In Advances in Protein Chemistry (M. L. Anson, K. Bailey and J. T. Edsall, eds.) Vol. 14, p. 65. Academic Press. New York.

Szent-Gyorgyi, A. 1951. Chemistry of Muscular Contraction. 2nd ed. Academic Press, New York.

Trautmann, A. and J. Fiebiger. 1952. Fundamentals of the Histology of Domestic Animals. Transl. and rev. from 8th and 9th German ed. 1949. Comstock, Ithaca, New York.

Tsuyuki, H., E. Roberts and R. A. Gadd. 1962. Muscle proteins of Pacific salmon. III. The separation of muscle proteins soluble in low ionic strength salt solutions by starch gel electrophoresis. Can. J. Biochem. Physiol. 40, 929.

Wake, B. C. and R. L. Baldwin. 1961. Analysis of casein fractions by zone electrophoresis in concentrated urea. Biochem. Biophys. Acta. 47, 225.

Weber, H. H. and K. Meyer. 1933. Dos kolloidale Verhalten der Muskeleiweisskorper. Biochem. Z. 266, 137.

Weber, H. H. and H. Portzehl. 1952. Muscle contraction and fibrous muscle proteins. In Advances in Protein Chemistry. (M. L. Anson, K. Bailey and J. T. Edsall, eds.) Vol. 7, p. 161. Academic Press, New York.

Weinberg, B. and D. Rose. 1960. Changes in protein extractability during post-rigor tenderization of chicken breast muscle. Food Technol. 14, 376.

Whitaker, J. R. 1959. Chemical changes associated with aging of meat. In Advances in Food Research. (C. O. Chichester, E. M. Mrak, and G. F. Stewart, eds.) Vol. 9, p. 1. Academic Press, New York.

Wierbicki, E., L. E. Kunkle, V. R. Cahill and F. E. Deatherage. 1954. The relation of tenderness to protein alterations during post-mortem aging. Food Technol. 8, 506.

Wierbicki, E., L. E. Kunkle, V. R. Cahill and F. E. Deatherage. 1956. Post-mortem changes in meat and their possible relation to tenderness together with some comparisons of meat from heifers, bulls, steers and diethylstilbestrol treated bulls and steers. Food Technol. 10, 80.

APPENDIX

Appendix A. Composition of solutions used for isolation and fractionation of proteins and starch gel electrophoresis (deionized water used in all cases)

List of solutions:

1. Potassium phosphate buffer (for protein fractionation, figure 1) pH 7.4.
2. Potassium iodide - potassium phosphate buffer (for protein fractionation, figure 2), pH 7.4.
3. Tris - boric acid buffer (for electrode vessels) pH 8.6.
4. Tris - citric acid buffer (for gel preparation) pH 8.6.
5. Tris - citric acid buffer containing sucrose (for dialysis of sample for electrophoresis) pH 8.6.

Composition:

Solution	Substance	Molarity	g/liter
1.	K_2HPO_4	0.024	4.180
	KH_2PO_4	0.006	0.816
2.	K_2HPO_4	0.0888	15.467
	KH_2PO_4	0.0112	1.524
	KI	1.1	166.02
3.	Tris $H_2NC(CH_2OH)_3$	0.06	7.268
	Boric acid H_3BO_3	0.1	6.184
4.	Tris $H_2NC(CH_2OH)_3$	0.012	1.454
	Citric acid $(COOH)CH_2C(OH)(COOH)CH_2COOH$	0.0018	0.378
5.	Tris $H_2NC(CH_2OH)_3$	0.010	1.211
	Citric acid $(COOH)CH_2C(OH)(COOH)CH_2COOH$	0.001	0.210
	Sucrose $C_{12}H_{22}O_{11}$	0.50	170.15

Appendix B. Complete calculated data from longissimus dorsi muscles of 6 beef animals

Animal No.	Soluble sarcoplasmic protein nitrogen (mg/g tissue)				Soluble fibrillar protein nitrogen (mg/g tissue)			
	Slaughter	24 hours	7 days	14 days	Slaughter	24 hours	7 days	14 days
Steers:								
568	6.6	7.1	7.8	6.7	15.8	15.2	17.7	18.6
576	7.2	7.4	8.0	8.2	15.4	15.6	18.9	19.2
Heifers:								
18	6.9	7.9	5.6	7.0	15.6	16.5	17.3	18.1
200	7.9	8.0	8.2	8.2	17.9	17.5	19.6	19.3
Cows:								
875	7.8	7.9	7.7	7.2	17.5	17.2	16.4	18.4
407	7.5	7.3	8.1	8.6	16.2	14.8	18.8	18.6
	Soluble non-protein nitrogen (mg/g tissue)				Stroma nitrogen (mg/g tissue)			
Steers:								
568	3.6	4.6	4.6	5.2	5.5	4.8	2.0	2.4
576	4.6	4.6	4.8	4.8	5.1	5.2	1.6	.6
Heifers:								
18	4.3	4.1	4.9	4.8	3.3	1.6	3.8	2.3
200	4.6	4.8	4.8	4.9	1.3	3.0	.68	.7
Cows:								
875	4.3	4.7	5.0	4.7	2.3	4.0	4.6	3.9
407	4.2	4.5	4.7	4.7	3.1	4.0	-.9	-.6
	Total nitrogen (mg/g tissue)				Tenderness (shear)			
Steers:								
568	31.6	31.6	32.0	33.0		10.44	8.30	5.48
576	32.2	32.8	33.2	32.7		18.13	9.58	9.24
Heifers:								
18	30.8	30.8	31.6	32.1		10.76	7.70	4.85
200	31.6	33.4	33.3	33.1		11.42	6.92	6.47
Cows:								
875	31.8	33.8	33.7	34.2		10.56	9.96	10.38
407	31.0	30.5	30.7	31.3		9.65	6.77	4.62

Appendix C. Complete calculated data from semitendinosus muscles of 6 beef animals

Animal No.	Soluble sarcoplasmic protein nitrogen (mg/g tissue)				Soluble fibrillar protein nitrogen (mg/g tissue)			
	Slaughter	24 hours	7 days	14 days	Slaughter	24 hours	7 days	14 days
Steers:								
568	7.4	7.1	6.6	5.6	16.1	15.2	19.2	19.4
576	6.5	7.0	6.0	6.1	16.6	16.5	16.9	19.2
Heifers:								
18	6.9	6.8	5.9	5.9	15.8	16.1	18.5	18.7
200	6.7	6.9	7.5	6.0	18.0	14.4	19.8	19.3
Cows:								
875	8.0	6.9	7.6	6.9	17.4	15.2	16.8	17.9
407	7.2	7.7	7.6	6.1	17.2	17.2	18.6	19.0
	Soluble non-protein nitrogen (mg/g tissue)				Stroma nitrogen (mg/g tissue)			
Steers:								
568	3.2	4.4	4.3	4.5	5.1	5.2	2.6	2.8
576	4.5	4.3	4.5	4.9	4.9	5.3	5.7	3.1
Heifers:								
18	3.7	4.3	4.6	4.6	5.3	4.4	3.0	2.1
200	4.5	4.5	4.7	4.7	3.2	7.4	1.0	2.7
Cows:								
875	4.1	4.4	4.7	4.6	3.8	6.9	5.4	5.0
407	4.5	4.6	5.0	5.0	3.4	2.1	1.2	2.3
	Total nitrogen (mg/g tissue)							
Steers:								
568	31.8	31.8	32.6	32.3				
576	32.4	33.1	33.1	33.2				
Heifers:								
18	31.6	31.6	32.0	31.2				
200	32.5	33.2	33.0	32.6				
Cows:								
875	33.4	33.4	34.4	34.4				
407	32.2	31.6	32.4	32.5				

ROOM USE ONLY