SOLUBILITY AND EMULSIFYING CHARACTERISTICS OF INTRACELLULAR BEEF MUSCLE PROTEINS

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ABSTRACT

SOLUBILITY AND EMULSIFYING CHARACTERISTICS OF

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By Gerald Ray Hegarty

It has been thought for many years that the salt soluble proteins of muscle have been primarily responsible for the characteristics of meat required for the manufacture of acceptable sausage products. However, the role of the remaining intracellular proteins and the fundamental reasons why certain protein fractions have superior water-holding and emulsifying properties have not been clarified. Certain age and sex groups of beef animals have been assumed to yield meat which is more acceptable for the production of sausage products, however, there is little scientific basis for these beliefs.

It was the object of this research to study the solubility behavior of the intracellular proteins during a seven day period post mortem, and compare the protein alterations occurring during this period between several age and sex classes. In order to do this, a relatively rapid and accurate procedure was developed to chemically partition the intracellular proteins of beef muscle. This protein fractionation procedure was also utilized in an attempt to study the relationship of muscle protein solubility to tenderness. The emulsifying properties of the major intracellular proteins were studied in model systems using purified muscle protein preparations.

Results indicated that considerable variation in protein composition existed between muscles of the same animal. Muscles considered in this

research were the infraspinatus and the longissimus dorsi. The solubility of the sarcoplasmic protein fraction exhibited little variation during the first seven days post mortem. Fibrillar protein solubility varied considerably in the same period for the three classes of animals studied. The general trend was a high degree of solubility at 0 time, followed by a sharp decrease to 24 hours and a gradual increase to seven days. The behavior of bulls in this respect deviated somewhat from heifers and cows. Fibrillar protein solubility, in the case of longissimus dorsi muscles of yearling bulls was found to be highly correlated with tenderness measured by two methods ($r = -0.69$ for shear and $r = 0.59$ for panel). Water-holding capacity was significantly correlated with tenderness as measured by the shear ($r = 0.49$).

Emulsifying capacity and emulsion stabilizing characteristics of muscle proteins were studied under varying conditions of pH and ionic strength. Emulsifying capacity of all the major intracellular proteins was found to increase as protein concentration decreased. Emulsifying capacity of the proteins studied were ranked from greatest to least as follows: actin ($u = 0$), myosin, actomyosin, sarcoplasmic, and actin ($u = 0.3$). In general, myosin and actomyosin produced emulsions with the most desirable stability characteristics, however, at pH 5.5 the sarcoplasmic fraction produced the most acceptable emulsion from a stability standpoint. Actin produced very undesirable emulsions under all conditions.

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By

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INTRODUCTION

Differences in physical characteristics of meat are due primarily to the variation in amount and character of protein constituents, which in turn vary with species, age, sex, and muscle. This variation in protein makeup is reflected by differences in tenderness, juiciness, color, freezer drip, storage shrinkage, cooking losses, emulsion forming ability, and water-holding capacity of meat.

The proteins of muscle are classed into three major types: sarcoplasmic proteins which are soluble in water or weak salt solutions, fibrillar proteins which are soluble to various degrees in salt solutions, and the stroma proteins which are insoluble in either salt solutions or water. The literature abounds with information concerning the partition of muscle proteins of guinea pigs, rabbits, rats, embryonic chickens and fish, but such information concerning meat animals is rather scarce and not in complete agreement. It has been thought for many years that the salt soluble portion of muscle proteins is primarily responsible for desirable sausage processing characteristics. However, fundamental reasons underlying this belief have not been established.

Hansen (1960), Swift et al. (1961), and Sherman (1961b) have established that fat incorporated into a sausage product is dispersed in small droplets and enveloped with a layer of protein material, producing, essentially, an oil in water emulsion, stabilized by muscle proteins. Swift et al. (1961) utilized crude muscle protein fractions to study fat emulsifying characteristics in model systems. However, much work remains to be done in this area.

For many years the amount of stroma or connective tissue of meat was believed to be the exclusive source of variation in the tenderness of meat. Wierbicki et al. (1954), Kamstra and Saffle (1959), Carpenter et al. (1961) and many others, have shown that the remaining protein constituents may be in some cases closely related to tenderness of meat. Research in this area could provide some further insight into the controversial subject of meat tenderness.

It was the object of this study to:

(1) Develop a simple and relatively rapid method for the routine partition of muscle proteins and apply this to a study of post mortem changes in protein solubility, pH, and water-holding capacity of beef from different age groups and sexes.

(2) Apply the partition techniques and water holding-capacity data to a tenderness study of a group of beef animals closely controlled in regard to age, grade, sex, and feeding, and which exhibited fairly large variations in tenderness.

(3) Study fat emulsifying capacity and relative emulsion stabilizing properties of various purified intracellular muscle proteins in model systems.

REVIEW OF LITERATURE

Structure of Muscle

In order to provide for a full appreciation of the functional and morphological relationships of muscle proteins, it is necessary to present a brief gross and microscopic picture of muscle structure. Maximow and Bloom (1954) presented a comprehensive outline of muscle structure. They recognized two distinct kinds of muscle in vertebrates: smooth muscle and striated muscle. Generally, smooth muscles contract independently of voluntary control, while the striated muscles are subject to voluntary control. Cardiac muscle, though striated, contracts independently. They stated that a muscle fiber is generally considered the functional unit of a muscle. In striated muscle these are large multinucleated cells. The thickness of the fiber varies from 10 to 100 microns and depends on the type and age of the animal and the particular muscle. Fibers are relatively long, some of which extend the full length of a muscle. The striated fibers are covered with the sarcolemma, a thin structureless membrane which completely invests the fiber. Muscles are formed of parallel muscle fibers held together by connective tissue. The muscle fibers combine to form the primary bundles, and several primary bundles combine to form secondary bundles.

Bailey (1944) provided a more detailed explanation of some important structural characteristics of muscle. He stated that fibers of striated muscles are composed of numerous fibrils one micron in diameter, arranged parallel to each other and to the fiber axis. The fibrils are the ultimate morphological units of muscle. In the electron microscope the

fibril appears to be composed of thinner threads called filaments. The fibril is composed, primarily, of actin, myosin and actomyosin which are the proteins responsible for muscle contraction. The fibrils also contain some minor protein constituents, the function of which is not clear. The fibrils are imbedded in the sarcoplasm. The sarcoplasm is made up of a complex mixture of proteins which correspond to myogen, so named by Von Furth in 1895. Contained in the sarcoplasm is also particulate material, namely the mitochrondia and microsomes common to practically all cells of animal tissue.

Bailey (1944) also described the fibers optically, as characterized by striations running throughout their whole width, the isotropic I bands alternating with the anisotropic A bands. The difference of refractive index in the two bands makes the A layer appear dark in ordinary light and the I layer bright, while in polarized light the effect is reversed.

Ramifying throughout and between the cells (fibers) is a framework of connective tissue fibers which are attached ultimately to the tendon. According to Maximow and Bloom (1954), connective tissue at the periphery of the muscle, called epimysium, projects into the spaces between the bundles of fibers as perimysium. Thin fibrous networks which enclose the fibers within the primary muscle bundles are called endomysium. Bailey (1944) indicated that this tissue consists primarily of the proteins, collagen and elastin, and is termed the extracellular protein. The protein components of the sarcoplasm and the fibril are intracellular.

Maximow and Bloom (1954) described smooth muscle cells as short (15 to 500 microns), spindle shaped, and containing a single centrally located mucleus. Smooth muscle cells do not have a distinct membrane corresponding to the sarcolemma of striated muscle. The arrangement of smooth muscle cells in respect to one another varies with their function.

Muscle Proteins

a. Myogen

The sarcoplasmic protein fraction (myogen), which can be extracted with very dilute salt solutions, was divided by Weber and Meyer (1933) into an albumin portion and a globulin portion. The globulin portion which precipitated upon dialysis or standing in solution was denoted as globulin X. Bate-Smith (1937) obtained a fraction he called myoalbumin by a technique which he called analytical denaturation. According to Bailey (1944), neither the uniformity nor the native state of these two protein fractions has been proven, nor have biochemical functions been attributed to them. He stated that most probably several native and denatured protein constituents of the myogen group contribute to globulin X and myoalbumin. The whole myogen complex is relatively unstable, and on standing becomes turbid and slowly precipitates.

According to Mommaerts (1950), myogen, although a relatively constant unit in preparative work, is far from homogenous. Myogen consists of an extremely complicated mixture of biologically active proteins among which are found most of the enzymes of the glycolytic cycle along with such proteins as myoglobin. He stated that since none of these, according to present knowledge, is directly involved in the structural and

mechanical changes of muscle tissue, the attention of most muscle researchers has been focused on the proteins that form the contractile structure.

b. Actomyosin

In 1930 Edsall extracted minced muscle with salt solutions of high ionic strength. These extracts had properties ascribable to solutions of fibrous molecules. Edsall termed this protein extract muscle globulin. Muscle globulin was further studied by v. Muralt and Edsall in 1930 and found to produce double refraction of flow (flow birefringence). Considering this property, they suggested that muscle globulin was composed of rod shaped particles.

Edsall's protein generally became known as myosin. After several years of research in this area involving studies of the swelling, X ray diffraction, and elastic properties of myosin threads, Weber (1934), Asbury and Dickinson (1935), and various other workers concluded that the A band of muscle is composed of myosin.

In 1941 Needham et al. found that ATP diminished both the viscosity and flow birefringence of myosin solutions. Shortly after this, Schramm and Weber (1942) showed for the first time that myosin solutions are polydisperse, containing a slowly sedimenting component with a low birefringence of flow (L-myosin) and several rapidly sedimenting components with high flow birefringence (S-myosins).

Straub (1942), considering the findings of previous workers, announced that the myosin of earlier workers was in reality a complex of two fibrous proteins, actin and L-myosin. Bailey (1944) stated that

after this discovery, the complex of actin and L-myosin was termed actomyosin which corresponded to the S-myosin of Schramm and Weber. According to Bailey (1944), it is now generally accepted that ATP dissociates actomyosin into its two components, resulting in the properties observed by Needham et al. (1941). In connection with this ATP dissociation, Engelhardt and Ljubimova (1939) discovered that actomyosin preparations possess ATPase activity. Banga (1941) found this property to belong solely to the myosin part of the molecule.

Considering their observation in the ultracentrifuge of several S-myosins (actomyosins), Schramm and Weber (1942) indicated that actin and myosin combine step by step to form actomyosins of quite different sedimentation constants and hence, various molecular weights. Bailey (1944) stated that artificial actomyosin can be prepared by mixing solutions of actin and myosin. He also pointed out that artificial actomyosins appear to sediment faster in the ultracentrifuge than natural actomyosins, and in the electron microscope the fibers of artificial actomyosin dc not appear as fine as the natural fibers.

From electron microscope studies conducted by Ardenne and Weber (1941), the length of natural actomyosin particles was found to vary from a few thousand A. to several microns and in thickness from 50 to 250 A.

Bailey (1944) summarized the solubility properties of actomyosin. He stated that actomyosin is the least soluble of the muscle proteins; the threshold for salting-in is by far the highest, and for salting-out the lowest. Actomyosin gel is insoluble in water and begins to dissolve at an ionic strength of about 0.3.

c. Myosin

Myosin is a remarkable protein that possesses several properties that are unique for a protein. Bailey (1954) indicated that myosin possesses the solubility properties of globulins, although the salting in threshold of globulins in general at pH 7 is lower (ionic strength = 0.04 vs. 0.3). Myosin can be prepared in a purified state and appears electrophoretically homogenous in the pure state. In 0.5 M KCl it gives a water clear solution that shows no birefringence.

Bailey (1954) also pointed out that myosin is relatively easily denatured. Freeze drying, dehydration with organic solvents, and mild heat all cause its denaturation. Depolymerizing solvents, such as urea or guanidine-HCl induce a change to a salt insoluble form.

Molecular weight determinations by Portzehl (1950), using both the ultracentrifuge and the osmotic method, showed the value for myosin to be approximately 850,000. Work by Tsao (1953b) indicated that the myosin framework is built up of five units of particle weight 165,000. Tsao depolymerized myosin with 6.7 M urea and studied molecular weight of the fragments by osmotic pressure and fluorescence polarization measurements. Bailey (1951) found that the molecule does not possess an identifiable N-terminal residue and thus may be cyclic. According to Weber and Portzehl (1951), all molecular measurements published so far indicate a very long, thin particle with a high axial symmetry. According to ultracentrifuge studies of Weber (1950), the particles are 22-23 A. wide and 100 times as long. Mommaerts (1951), by measuring the angular dissymmetry of light scattering at two wave lengths, found the myosin molecule to be rod shaped and possessing a molecular length

of 1500 A. Recent work by Kielley and Harrington (1960) using equilibrium ultracentrifugation techniques indicated that the molecular weight of the myosin molecule is 619,000. Depolymerization of myosin with guanidine-HCl resulted in the formation of molecules with an average weight of 206,000. These workers proposed a model for the myosin molecule made up of two light components and one heavy component, with the heavy component folded back upon itself. Kielley and Harrington's model has a length of 1650 A and a diameter of 22 A.

Engelhardt and Ljubimova in 1939 announced that ATPase, the enzyme that liberates inorganic phosphate from ATP, could not be separated from myosin and seemed to be identical with it. Bailey (1954) believed there was no doubt that ATPase was either closely associated with myosin, or was part of the molecule itself. Perry (1951) found that the activity is 20-2000 times less than that of other pure enzymes. Bailey (1954) pointed out that the Q_p value of an average preparation is only 3000-6000 (Perry, 1951), compared to a Q_p of 100,000 for a purified inorganic pyrophosphatase. He also stated that acidification inactivates the enzyme, as also does any treatment which blocks SH groups. However, loss of solubility does not always indicate the loss of enzymatic activity in the case of myosin ATPase.

The effect of ions on ATPase activity is extremely important and at the present time no less confusing. Bailey (1954) stated that there are essentially only two points of agreement, that certain bivalent metals are necessary for the action of ATPase, and that calcium is the most powerful activator at alkaline pH.

Mommaerts and Seraidarian (1947) made a thorough study of the influence of ions on ATPase activity. At pH 7 both myosin and actomyosin split ATP optimally in 0.3 M KCl; in the presence of 0.001 M $CaCl_2$ the potassium optimum is shifted to lower concentration, and the activity is three times as great. In the presence of 0.1 M KCl the optimum is at pH 9 when calcium is the activating ion. The effect of magnesium is always to suppress the activation by other ions. There is evidence that actin rich myosins are activated by magnesium ions at neutral pH provided potassium ions are absent.

Perry (1951), utilizing the intact myofibril, made the following observations of ion effect on ATPase activity: as noted with myosin and actomyosin, there is an optimal potassium concentration for activation by calcium ions. The activation by magnesium can be just as great as that by calcium, but it occurs at a much lower concentration. Magnesium is strongly inhibitory above the optimum concentration.

Kielley and Meyerhof (1948) discovered the presence of another ATPase in the sarcoplasm that is separate from the myosin ATPase and is activated by magnesium.

Ions also have a profound influence on other properties of myosin. In fact, according to Szent-Gyorgyi (1951), the colloidal state of most hydrophylic colloids is influenced by their ionic environment. The amount of potassium or sodium absorbed by a protein molecule affects the charge, thereby altering its isoelectric point as evidenced by titration curves presented by Szent-Gyorgyi (1951). According to Szent-Gyorgyi, any substance causing a shift in the isoelectric point will alter the

solubility characteristics of a protein. In the absence of salts the isoelectric point of myosin is at pH 5.4. The addition of 0.025 M KCl shifts the isoelectric point to pH 7. Maximal precipitation now occurs at neutral reaction, while the protein becomes soluble at its former isoelectric point, pH 5.4. At the highest potassium concentration studied, 0.8 M, the protein was again soluble at neutral reaction, in fact, the isoelectric point was shifted down to pH 3.0. According to Szent-Gyorgyi (1951), myosin does not distinquish between potassium and sodium ions. He also stated that the reaction of myosin with other ions is not so clear. Every ion induces new and specific changes in the protein which modify its affinities toward other ions. Secondly, the effect of anions is probably as important as that of the cations with which they are added.

d. Actin

Prolonged extraction of muscle with salt solutions extracts a mixture of myosin, actin and actomyosin. Straub (1942) extracted relatively pure actin by removing the sarcoplasmic proteins and myosin by a treatment with a KCl phosphate solution, denaturing the remaining protein except actin, with butanol and preparing an acetone powder of actin from the residue. Actin obtained in this manner was in the globular (G) form, its viscosity was low and no flow birefringence was shown. Addition of a salt or acid resulted in the rapid formation of fibrous (F) actin.

Szent-Gyorgyi (1951) summarized the solubility properties of actin as follows: while actin is soluble in water or low concentrations of neutral salt solutions, these solutions do not extract it readily from

the muscle. The isoelectric point of actin is pH 4.7. The fact that actin is present in the muscle in fibrous form may explain the difficulties encountered in its extraction. If actin is depolymerized by agents like KI, it is readily extracted.

The molecular weight of the actin monomer is approximately 70,000 as determined by Tsao (1953a) by osmotic pressure measurements. Actin contains small amounts of phosphate which in G actin solutions has been found to be associated with ATP by Straub and Feuer (1950). They have suggested that the G ---> F transformation involves the reversible change of ATP to ADP in the actin molecule. Weber and Portzehl (1951) pointed out that heavy metal compounds prevent the transformation of G ---> F actin, supposedly by their combination with SH groups which are necessary for polymerization.

e. Tropomyosin

Tropomyosin, discovered by Bailey (1946), is the most recent addition to proteins which form the muscle fibril. Tropomyosin has many properties in common with myosin. Bailey (1954) summarized these properties. The amino acid composition is similar, as are some of the solubility properties and the isoelectric point. Most significant of all, is that neither protein possesses appreciable amounts of N-terminal residues. This would indicate that some type of cyclic chain structure is present. Under the electron microscope the tropomyosin fibrils appear to be about 3000 A. long and 250 A. broad. True molecular weight is thought to be about 50,000.

Muscle contraction and Rigor Mortis

The morphological configuration of the actin and myosin components during contraction is controversial. According to one of the present views presented by Copenhaver and Johnson (1958), there are two types of myofilaments (thick myosin filaments in the A band and thin actin filaments in the I band and part of the A band), and it is thought that contraction is accompanied by a sliding of the thin filaments along the thick filaments. Another viewpoint outlined by these authors is that only one type of myofilament exists (an actin filament throughout a sarcomere accompanied by myosin in the A band). Contraction in this case is accomplished by an altered molecular arrangement which draws the I band material into the A band.

Copenhaver and Johnson go on to explain that when the nerve to a muscle is stimulated, there is a change in electrolyte balance along the sarcolemma. As a consequence of nerve stimulation, the actomyosin complex absorbs ATP and certain cations (K and Mg). In the process, ATP is converted to ADP with the release of free phosphate ions to supply energy for the process.

The mechanism of rigor mortis is, understandably, closely associated with the process of contraction. Bate-Smith (1948) summarized the two most popular theories for this phenomenon. The first theory advanced by Szent-Gyorgyi (1947) emphasizes intimate molecular processes. Resting muscle, i.e., before and shortly after death, contains myosin in the globular form and dissociated from actin. Muscle is maintained in this state because the contractile proteins are prevented from combining

by potassium ions. When potassium (by diffusion) and ATP (by enzymatic breakdown) are removed from myosin as occurs when muscle dies, actin combines with myosin to form actomyosin which causes the muscle to be extremely inextensible.

According to Bate-Smith's theory, rigor mortis involves the association of the ultimate filaments of a muscle fiber (this filament represents a unit containing a number of actomyosin molecules packed side by side). This association is stabilized by weak cross linkages. These cross linkages which account for the decreased extensibility of muscle are formed as a result of total removal of ATP.

Extractability and Fractionation of Muscle Protein

Deuticke (1932) was the first to report that muscles which had been fatigued by stimulation, frozen, and pulverized, imparted less protein to an extracting solution than those freshly extracted. Weber and Meyer (1933) obtained very similar results and in addition found that the decrease in protein extractability for muscle stored over twenty four hours resided largely in the myosin fraction and to a smaller extent in the globulin X fraction.

Bate-Smith (1934a) studied the effects of a series of extracting solutions. He found that with ammonium and lithium chlorides of adequate strength, no differences could be observed between the behavior of fresh and rigor muscle.

As pointed out by Bailey (1954), the most direct explanation of this early work was that stimulation and rigor involve a change of state which is reflected in a loss of solubility in some salt solutions but

not all; or in the light of recent knowledge, they involve the combination of myosin and actin to give a less soluble complex. In freshly minced relaxed muscle, the ATP acts as a specific dissociating agent. In rigor or fatigued muscle, extraction is facilitated by salts which depolymerize the complex. Bailey concluded that, considering the large amount of recent work on the theory of contraction and rigor, this is probably an over simplified explanation.

From the preceding discussion of rigor, it might be thought that the disappearance of ATP from the muscle is largely responsible for an "in vivo" aggregation of myosin and actin which retards extractions. According to Bailey (1954), this is incorrect. While ATP hastens the rate of solution, it does not increase the final yield, except when the extracting solution has an ionic strength above 0.5. Crepax (1951) indicated that the action of ATP on the extractability of the muscle proteins is that of strengthening the dissociating action of the electrolyte on the binding forces which hold the proteins in place in the muscle. The characteristic decrease in extractability of contracted muscles is not due to the hydrolysis of ATP which accompanies these contractions.

Bailey (1954) stated that extractability is not solely determined by solubility. He indicated that this is probably because the dissolution of F actin or F actomyosin threads, several microns long, is seriously impeded in a mechanical way by the insoluble components of muscle. The extractability of myosin and actin depends, in part, on the mutual combination of these proteins and the hindrance to diffusion by the

surrounding insoluble muscle structures. A relaxed muscle, freshly minced, will yield free myosin, even on coarse grinding, but further comminution and stronger salt solutions will bring out large amounts of actomyosin. Homogenization must be continued to break mechanically not only the surrounding structures but to disperse further the concentrated thixotropic actin gel inside.

Bailey (1954), considering the above facts, made the following conclusions on muscle protein extractability. At any particular stage of rigor the extractability of the intracellular protein fraction appears to be determined by pH, ionic strength of extracting solution, type of extractant and by adequacy of grinding.

Jacob (1947) studied the effects of pH on the extractability of sarcoplasmic proteins. He found that pH 7.7 was optimal for extraction of all muscle proteins within the phosphate buffer range. At all pH values a precipitate formed on dialysis. The quantity of precipitate varied considerably above pH 7.8, was least at 7.6 and become more abundant as pH fell; however, the precipitate formed above pH 7.1 was soluble in 0.5 M KCl and showed double refraction of flow.

Weber and Meyer (1933) and Bate-Smith (1934a) were the first to quantitatively partition the proteins of muscle. However, conflicting results were obtained (table 1).

Recent workers have obtained more reliable values. Herrman and Nicholas (1948) fractionated embryonic rat muscle. Muscle protein was divided into three fractions by a single procedure. One fraction was insoluble in 0.5 M KCl at pH 7.5 (stroma).

Table 1. Protein composition of muscle as reported by several authors*

Authors	Muscle	Protein N as % total protein N				
		Sarco-plasmic	Soluble fibrillar	Residual intracellular	Total fibrillar	Stroma
Weber & Meyer	Rabbit	44	39			17
Bate-Smith	Rabbit	16	54	15	69	15-17
Hasselbach & Schneider	Rabbit	28	52	4	56	16
Robinson	Chick	33	40	22	62	5
Dyer et al.	Cod	21	70	6	76	3

*From Bailey (1954)

A second fraction, most of which was myosin, was soluble in 0.5 M KCl but was precipitated at 0.16 M KCl. The remaining proteins which did not precipitate at 0.16 M KCl were designated as soluble proteins (myogen).

Hasselbach and Schneider (1951) were the first to attempt the direct estimation of actin. The muscle was coarsely ground and actin free myosin plus the sarcoplasmic proteins were first extracted with 0.6 M KCl containing pyrophosphate (action somewhat similar to ATP) at pH 6.3. The pyrophosphate dissociates actin and myosin, and the myosin diffuses out, leaving the actin associated with the stroma protein. The tissue was then homogenized in 0.6 M KCl yielding a turbid, viscous extract of actin.

Dyer et al. (1950) utilized the Waring blendor to disperse muscle tissue for extraction by a salt solution. This method displaced the previous classical methods of extraction which consisted of repeated (6-9 times) grinding with sand, shaking and centrifuging.

Dyer et al. also designed a plastic baffle plate to prevent foaming in the blendor and subsequent denaturation. These workers tested the efficiency of many salt solutions for extraction of soluble protein. It was concluded that the most important point in the extraction of protein was sufficiently fine subdivision of the muscle fibrils, and when that was obtained by use of the blendor, the type of salt used for extraction was not critical. They used a one to twenty ratio of tissue to extractant solution. The effect of temperature of extraction was quite marked in the case of fish that had been frozen with much lower extractions at 25°C than at 5°C. With fresh fish, temperature was unimportant.

These workers confirmed Bate-Smith's (1934b) results that a constant fraction of 88% of the myosin would be precipitated by a one to ten dilution. It was found that maximum extraction occurred between pH 7 and 9 and in salt solutions of three to five percent.

Robinson (1952) developed a standard method for the extraction and estimation of several protein fractions in embryonic chicks. The sarcoplasmic proteins were extracted from homogenized muscle by dilute salt solutions and the myofibrillar proteins were extracted in strong salt solutions and precipitated on dilution. Another fraction insoluble in strong salt solutions but soluble in dilute alkali formed a third fraction and the final extracellular residue a fourth fraction. Seagran (1958b) used a similar procedure to fractionate the muscle of the king crab. However, he extracted the entire myofibrillar fraction with 0.1 M NaOH instead of a strong salt solution.

Khan (1962) developed a technique for the routine fractionation and estimation of major protein fractions in chicken muscle. He also compared

different buffer systems for efficiency of extraction and found that KCl-borate and KCl phosphate buffers of pH 7.3 - 7.5 and ionic strength of 1.0 gave the maximum extractability. In one-year-old chicken meat, stroma-, myofibrillar-, and sarcoplasmic-protein nitrogen, respectively, contributed 13, 42, and 30% of total nitrogen in breast muscle and 27, 30, and 22% in leg muscle.

Fujimaki (1962) carried out chromatographic fractionation of muscle protein preparations on DEAE - cellulose and cellulose - phosphate. Effluent diagrams for the sarcoplasmic fraction from rabbit muscle showed four main peaks, each of which showed two or three peaks in ultracentrifugal analyses. He also fractionated actin solutions prepared from acetone powder. In addition to a purified actin peak, he obtained another major peak thought to contain the so called "inactive actin" and a smaller peak believed to be a prosthetic group of actin (a nucleotide).

Protein studies applied to meat research

An explanation of some of the basic concepts of meat hydration and a comprehensive review of previous work in this area has been presented by Hamm (1960). He stated that the "true hydration water" of muscle is the amount of water that attaches to protein by monomolecular and multimolecular adsorption. This water is bound directly to polar groups of proteins and makes up about 4 - 5% of the water in muscle. The physical properties of this fixed, bound water are different from those of free water. This bound water has a lower vapor pressure and a lower dissolving power than normal water.

Hamm (1960) further stated that studies of muscle tissue by different physical methods showed that most of the water in muscle is, chemically speaking, free. It seems to be free water mechanically immobilized by the network of the cellular protein membranes and protein filaments. Hamm concluded that the considerable changes of the water-holding capacity of meat caused by changes of protein charges (by pH, ions, etc.) are not due to any changes of true hydration water fixed to the polar groups of meat proteins.

He stated that the amount of free water "immobilized" within the tissue is strongly influenced by the spatial structure of muscle. Tightening this spatial structure (a network of proteins) decreases immobilized water, and loosening the protein structure has the opposite effect. This so-called "stero effect" (Hamm, 1959) is extensively influenced by changes of protein charges. The presence of certain ions or adjustment to certain pH values greatly affects the spatial protein arrangement and consequently affects water-holding capacity. The water-holding capacity of meat means the ability of meat to hold its own or added water during application of any force (pressing, heating, grinding, etc.).

Schon and Stosiek (1958a), (1958b) found an inherent difference in water-holding capacity between different species, age, and sexes of meat animals. The reasons for these differences have not been elucidated. They utilized the adductor and longissimus dorsi muscles, and found that pork had a greater water-holding capacity than beef. There were no differences between sexes in the case of pigs, however, the water-holding capacity of cattle increased in the order of steer to heifer to cow. The

behavior of bull meat in this respect was very erratic and could not be placed in the above series.

Bendall and Pedersen (1962) carried out an extensive experiment to investigate the causes of soft watery pork, a condition quite commonly associated with pork muscle. They prepared fibrils from normal and watery pork and compared the water retention at various ionic strengths. Normal and watery fibrils were also titrated to determine isoelectric points. From the results of this study, they concluded that the fibrillar proteins of the watery fibrils are not denatured or aggregated in the usual sense, but are probably covered by a layer of denatured sarcoplasmic protein that is firmly bound to the surface of the myofilaments.

Swift and Berman (1959) and Swift et al. (1960) investigated some of the factors affecting water retention of beef. They confined their observations to variations between muscles of the same and different animals. A direct, highly significant correlation was found between water retention and zinc content, in contrast to the inverse relation found between water retention and either calcium or magnesium content. The ratio, moisture/protein content, was found to be directly related to water retention, hence, the results showed that the relative ability of the muscles to hold added moisture was predictable on the basis of the original proportions of moisture and protein present.

They found that the variation in pH over a relatively narrow range, 5.49 - 5.86, was correlated directly and closely with capacity for water retention ($r = 0.95$). Variation in pH was negatively related to protein content ($r = -0.89$).

Sherman (1961a) studied factors influencing fluid retention by ground pork. Tetrasodium pyrophosphate and alkaline polyphosphate were found to be more effective than NaCl in retaining fluid. pH was found to have a positive influence on fluid retention and the degree of solubilization of actomyosin. Fluid retention was said to depend on the degree of ion adsorption. The ionic strength of the solutions employed was important only in so far as it controlled the rate of ion adsorption by the meat. The greater the ionic strength, the greater the adsorption of ions.

In Sherman's experiment, fluid retention at 100°C appeared to be related to the concentration of actomyosin that went into solution at 0°C. The greater the concentration of actomyosin in solution, the stronger the gel of denatured protein formed upon heat coagulation. A strong gel extending throughout the meat mass improved moisture retention.

Sherman (1961c) showed that stronger coagula are developed with alkaline phosphates than with NaCl. He stated that more fluid is retained by meat at higher temperatures in the presence of the former additives. Coagulation temperatures, the nature of the coagula, and consequently the rate of release of fluid at temperatures above 60°C, depend on the nature and concentration of the additive employed.

Sherman (1961c) concluded that the most important factor in the explanation of variations in fluid retention appeared to be solubilization, or swelling of proteins, particularly actomyosin, within the meat prior to heating. This process is influenced by pH, time and temperature of the initial aging period, and in the case of added alkaline phosphates, some additional factor, possibly the ability to split the bond between actin and myosin in actomyosin.

Results obtained by Swift and Ellis (1956) showed that the effectiveness of treatments with pyrophosphates was primarily related to the ionic strength and pH of a solution applied to lean meat. The capacity of pyrophosphate for buffering was also shown to be important. They showed that pyrophosphates dissolve proteins, especially actomyosin, to an extent affected by ionic strength and pH. In general, the factors governing the moisture retention of meat treated with phosphate additives are those which influence solubilization of muscle proteins; namely temperature, time, ionic strength and pH of treatments, and those specific effects that can be exerted by ions, such as I^- and Mg^{++} ions.

Swift and Ellis (1957) investigated the effects of the ordinary curing agents and certain phosphates on color and binding of frankfurters and bologna. Shrinkage during smoking and cooking and cohesiveness as indicated by tensile strength measurements were employed as criteria of binding. Of the ordinary curing ingredients, only the action of NaCl increased the development of heat when emulsions were prepared, the retention of moisture during heat processing, and the cohesion of sausage products. The addition of phosphates increased the relative binding of sausage components, and decreased shrinkage of bologna heated to an internal temperature of 160°F. The rate at which the temperature of emulsions increased during comminution was reduced in the presence of certain phosphates.

Fukazawa et al. (1961c) undertook to clarify the role played by the addition of various phosphates in the binding quality of sausage. Phosphates studied were pyrophosphate (PP), tripolyphosphate (TPP), and hexametaphosphate (HMP). They concluded that the effect of phosphates was to

increase the amount of protein extracted from fibrils by the use of a 0.6 M KCl solution. Phosphates, especially PP, induced dissociation of myosin from natural actomyosin as shown by decrease in viscosity of actomyosin solution upon the addition of PP. Data from the ultracentrifuge supported this view. The amount of protein extracted from intact fibrils with 0.6 M NaCl was shown to increase from pH 5.6 through pH 6.4 to pH 7.0.

Bendall (1954) found that orthophosphate, Calgon, and metaphosphate tended to increase swelling and water uptake of meat. This effect was increased by addition of NaCl. The effect of the above phosphates was small comparedwith that of pyrophosphate. The effects of the orthophosphate, Calgon, and metaphosphate were probably due to ionic strength increase alone, while the pyrophosphate, in addition, facilitated the extraction of actin and myosin from the tissue because of its ability to split actomyosin into its components.

Fukazawa et al. (1961b) studied the influence of fibrillar proteins on the quality of experimental sausages. The binding quality of sausage prepared from intact fibrils, actin poor fibrils, synthetic fibrils and purified myosin was estimated by elasticity determinations. It was concluded that myosin present in fibrils exerted a great influence on the binding quality of sausage. Actin and tropomyosin did not greatly influence the binding quality of sausage. It could not be shown that the amount of nitrogen soluble in 0.6 M NaCl, pH, or water-holding capacity directly influenced the binding quality of experimental sausages.

Another study by these Japanese workers, Fukazawa et al. (1961a), dealt with the preparation of subcellular muscle preparations from muscle

fibrils free of water soluble proteins and also fibrils relatively free of actomyosin called "ghost fibrils" by the authors. It was found that sausage made from the actomyosin poor fibrils showed a considerable decrease in binding quality, whereas sausage made from water-soluble-protein-free muscle fibrils showed little change in binding quality. Actin-tropomyosin poor fibrils were also prepared. These fibrils were only slightly inferior in binding quality to sausage made from whole muscle.

Sair and Cook (1938) were among the first to investigate the effect of freezing, length of storage, and pH of tissues on exudation of drip during thawing. Beef, pork and mutton were studied. pH was adjusted by injecting lactic acid or NaOH. Observations made during the course of these experiments indicated that mutton behaves like pork, in that the pH is subject to considerable variation between animals. Results suggested that the quantity of drip is related to the pH of the tissue at the time of freezing, and that the maximum total drip is associated with low pH. It was concluded that these three kinds of meat will drip to the same extent after freezing and thawing, provided they have the same pH, and that the same freezing rate was used. Sair and Cook also stated that the pH of beef is relatively constant and close to the value at which maximum drip occurs, whereas pork and mutton vary in pH from carcass to carcass, and are generally more alkaline. They claimed that this fact readily explains the small amount of drip from pork or mutton. These authors also concluded that a protein extraction procedure is not suited to a study of the nature of changes in the proteins affecting drip.

Dyer (1951) extracted soluble proteins in 5% NaCl, and followed this with an estimation of the actomyosin of the solution. The amount of acto-

myosin solubilized was used as an estimate of denaturation. It was found that the decrease in actomyosin solubility paralleled and anticipated taste panel ratings and provided a quantitative measure of the quality of frozen fish.

According to Seagran (1958a), recent work shows that drip in fish is due, at least in part, to denaturation of protein which normally tends to hold the water of muscle. He found the contractile protein, actomyosin, absent in drip from frozen and thawed rockfish. By electrophoresis and dilution techniques, he showed a definite similarity between the protein composition of drip and extracts of low ionic strength from fish muscle. From the results of this study, it was concluded that the sarcoplasmic fraction of fish muscle is not intimately associated with the origin of drip. It was suggested that drip formation and texture change resulting from freezing and thawing must be due in part to actomyosin denaturation by a dehydration process.

Hunt and Matheson (1958) used three criteria to investigate denaturation of actomyosin in freeze dehydrated beef and cod muscle. These were loss of solubility in salt solutions, loss of contractility of muscle fibers in the presence of ATP, and loss of ATPase activity. On dehydration, cod actomyosin became insoluble and the muscle fibers may or may not lose their power to contract and about one-half of the ATPase activity was lost. Muscle fibers of beef were always contractile after rehydration and they also retained about half of their ATPase activity.

In connection with studies of protein denaturation during freezing, Connell (1962b) extracted reasonably pure myosin from cod muscle by exhaustively extracting finely minced muscle with a slightly acid salt

solution containing pyrophosphate ions. He indicated that pyrophosphate dissociated the actin-myosin bonds existing in the muscle and inhibited the formation of such bonds during extraction. From this study it appeared that 70-80 percent of the myosin became non-extractable at a rate similar to that at which the total myofibrillar protein of flesh became non-extractable. The remainder of the myosin became non-extractable at a much slower rate. He cautioned the use of the term denaturation in connection with changes in extractability of the actomyosin fraction in frozen fish. He suggested that part of the myosin and actin interact very strongly during freezing in a manner similar to the interaction occurring during rigor, and that this interaction may partially account for the inextractability of myofibrillar proteins after frozen storage.

Hashimoto et al. (1959) carried out an experiment to determine whether or not denaturation of actomyosin in muscle during storage proceeded in a manner similar to that of the isolated protein. These workers followed changes in nitrogen extractability, in Weber Edsall solution, of rabbit muscle stored at 20°C. They found extractability at a high value at 0 time, dropping to a minimum at 12 hours, and in 24 hours rising to a point above that of 0 time. pH changes and water-holding capacity followed much the same pattern. ATPase activity of myosin extracted from muscle at 20°C was found to be inactivated very quickly. The course of denaturation of actomyosin in stored meat coincided completely with denaturation of isolated actomyosin.

Seagran (1956) reported on a study of changes in properties of the actomyosin fraction of fish muscle that had been subjected to frozen

storage. The properties of chemical activity, asymmetry, and solubility of actomyosin were investigated. He concluded that the freezing process produced some subtle change in the structure of actomyosin present in post-rigor muscle, yielding a more symmetrical molecule. A significant structural change was not indicated as the increase in -SH groups was very slight. He suggested a possible correlation between cold storage stability and a lipid complexed with actomyosin of fish.

King et al. (1962) investigated the effect of free fatty acids on denaturation of cod actomyosin. They found that small concentrations (0.025 ml./350 ml. of solution containing 0.45 mg. of total soluble protein nitrogen/ml.) rapidly reduced the solubility of actomyosin. The extent of insolubilization of actomyosin depended on the fatty acid used, its concentration and the length of storage of the treated solution, thus supporting the hypothesis that the accumulation of free fatty acids in frozen fish muscle causes actomyosin to become inextractable.

Connell (1962a) stated that there is good evidence that sarcoplasmic proteins survive freeze-drying virtually intact. The electrophoretic and ultracentrifuge properties of extracts of this group of proteins prepared from freeze-dried beef and cod are very similar to those of frozen controls. The amounts of sarcoplasmic proteins extractable from these species are also unchanged. Toughness developing during freeze-drying appears to be associated with a loss of the true water-holding capacity of the muscle. He indicated that it is evident that changes in actin and myosin, or their association with one another, are prime causes of textural deterioration following freeze-drying. Practically no actomyosin can be

extracted from freeze-dried cod, however, as much actin is extractable from freeze-dried cod as from a frozen control, and about half of the myosin also remains extractable after freeze-drying. Connell related the toughening during freeze-drying and subsequent storage to an increase in the number of bonds between the myofibrillar proteins. He stated that there are probably two types of bonds involved in this association: (1) actin-myosin bonds and (2) bonds between denatured myosin molecules.

Cole (1962) studied the effect of oxygen plus elevated temperature on freeze-dried beef by determining changes in solubility of actomyosin and sarcoplasmic proteins. The solubility data indicated that the sarcoplasmic proteins are much less adversely affected than those of the contractile group.

Fujimaki et al. (1961) studied the effects of gamma irradiation on the chemical properties of actin and actomyosin during aging of meat. Meats were irradiated at three stages, i.e., at slaughter, at maximum rigor (2 days) and at "rigor off" (7 days). Changes in actin and actomyosin were determined by extracting these components from the irradiated and control meats and determining sulfhydryl groups, amino acid composition, viscosity, ATPase activity and ATP sensitivity. It was found that actin was only slightly sensitive to irradiation and that actomyosin was very sensitive. Actomyosin becomes more sensitive to irradiation as the stage of rigor advances.

Hamm and Deatherage (1960b) summarized the steps of meat denaturation upon heating. In the range 20°C to 30°C, no change in hydration, rigidity, buffer capacity, solubility or charges was observed. From 30°C to 40°C,

changes in muscle proteins include two steps: an unfolding of peptide chains and the formation of new electrostatic and/or hydrogen cross linkages. In this temperature range changes in muscle proteins influence hydration, rigidity and solubility of meat. Some of these changes may be due to an increased availability of charged groups made possible by the unfolding of the peptide chains. The strongest denaturation occurs in the range of 40°C to 80°C as shown by a decrease in muscle hydration, increase of rigidity and decrease of protein solubility.

Wierbicki et al. (1954) were among the first to directly approach the study of quality attributes of meat by protein fractionation. These workers attempted to determine the amount of actomyosin in meat and relate this to tenderness. Their extracting solution was designed to dissolve actin and myosin and other proteins but not actomyosin. This solution was a citric acid buffer of pH 5.6, ionic strength of 0.48 and 0.22 M KCl. These workers also determined connective tissue by the hydroxyproline method and an alkali insoluble method. No changes were observed in content of connective tissue during aging of beef, however, tenderness did increase significantly. In a group of 48 beef animals, hydroxyproline values showed no relation to tenderness, however, a very good correlation of extractable nitrogen with tenderness was obtained (correlation coefficient was 0.507 for 46 degrees of freedom).

These workers also suggested on the basis of their study that increase in tenderness with post mortem age 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 paper, Wierbicki et al. (1956) indicated that protein-protein interactions or ion-protein interactions may be the cause of post mortem tenderization rather than classical proteolysis or dissociation of actomyosin. In their study both a buffer extract and a water extract were employed to study changes in nitrogen solubility. Both extracts were found to follow the same basic pattern, i.e., they were high at slaughter, decreased to a minimum in 12 to 24 hours and then gradually increased to five days, where they leveled off.

Kronman and Winterbottom (1960) indicated that aging beef for seven days renders the previously soluble protein less liable to extraction from the tissue, which agrees with Wierbicki et al. (1956). Using this solubility as a criterion of denaturation, the former authors concluded that from 10 to 30% of the water soluble protein may be denatured during the seven days of aging. They also found that freezing led to a decrease in extractability of water soluble protein. They stated that extractability or solubility of proteins does not provide a sufficiently sensitive criterion for protein alteration.

Weinberg and Rose (1960) attempted to study post mortem tenderization of chicken muscle by observing changes in the extractability of the contractile proteins from pre and post rigor chicken breast muscle. The amount of nitrogen extracted increased when the carcasses were held for 24 hours at 4°C, and this increase was entirely accounted for by an increase in the actomyosin fraction. They suggested that more actin was extracted from post rigor meat and that this actin combined with myosin in the extract. From the results of the study, they suggested that ten-

derization is not merely random autolysis, but results from a specific cleavage of an actin association responsible for the maintenance of the muscle matrix.

Arnold et al. (1956) studied the interactions of cations and proteins of beef during post mortem aging. They found that the total cationic shift was a movement of cations onto the meat proteins, resulting in an increased charge on the meat proteins, allowing greater hydration and improved tenderness. The absolute amounts of each cation in the meat, in the water extracts, and in the juice had no significant correlation with tenderness.

Following this work, Wierbicki et al. (1957a) studied the effects of added cations on meat shrinkage at 70°C. The cations, sodium, potassium, magnesium, and calcium were found to increase the water-holding capacity of meat, with magnesium showing the most pronounced effect. Sodium chloride, added to meat prior to freezing, reduced the amount of drip encountered on thawing.

Kamstra and Saffle (1959) attempted to evaluate the extent to which rigor contributes to toughness of meat. The normal sequence of reactions during rigor was interrupted by the infusion of a chelating agent, sodium hexametaphosphate, into hot hams. The hot hams infused with sodium hexametaphosphate showed an immediate massive contraction and then relaxation. Results showed a highly significant increase in tenderness of all hams infused with sodium hexametaphosphate.

Carpenter et al. (1961) designed an experiment suggested by the work of Kamstra and Saffle (1959) on paired hams. They attempted to interrupt the normal sequence of rigor by infusing sodium hexametaphosphate into

hot beef rounds. Previous basic studies on the contractile proteins indicated that calcium, potassium and magnesium ions influenced the formation of actomyosin. The injection of a chelating agent would supposedly bind these ions, thereby interferring with the formation of actomyosin.

The over all results of taste panel and shear showed a decided increase in tenderness for treated rounds over control rounds, however, the treated rounds were dark and flabby. The darkness was somewhat overcome by the infusion of lactic acid.

Theory of Emulsions

Becher (1957) described an emulsion as follows: "a heterogenous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, whose diameter, in general, exceeds 0.1 micron. Such systems possess a minimal stability, which may be accentuated by such additives as surface-active agents, finely divided solids, etc."

According to Holmes (1934), two mutually insoluble liquids may be emulsified by mechanical agitation, but they soon separate into two layers of the original liquid. Stable emulsions of two pure liquids can not be made. A third substance, usually colloidal, is necessary to stabilize emulsions. This is often present as an unsuspected impurity. This third substance is called an emulsifying agent and is concentrated at the interface between the two liquid phases. Soaps and proteins are among the most common emulsifiers.

Clayton (1928) stated that emulsions of oil in water (O/W) and water in oil (W/O) are common both in the laboratory and in technical practice.

Some oil/water emulsifiers are casein, albumin, agar, starch, gums, hemoglobin, pepsin, peptones, dextrin, lecithin, soaps and alkalis which may form soaps with the free fatty acids found in some fats. Finely divided solids in certain instances may promote emulsification of oil in water or water in oil.

Holmes (1934) stated that emulsions may be broken by: (1) adding an equivalent amount of emulsifying agent of the opposite type; (2) conversion of the emulsifying agent into some other compound; (3) addition of an excess of dispersed phase with violent agitation; (4) certain types of violent agitation alone; (5) electro-deposition; (6) freezing; (7) centrifuging; (8) certain types of filtration; and (9) a hot dip of metal into the emulsion.

Clayton (1928) described the preparation of an emulsion using only pure oil and water. Such a process required relatively violent agitation over very long periods of time. The oil globules in pure oil in-water emulsions have diameters of the order of 10^{-5} cm. compared with 10^{-3} cm. for colloidal suspensions. Without the presence of an emulsifying agent, emulsions of oil in water may be made up to a maximum concentration of 2 percent, however, they are usually much more dilute. The most noteworthy feature in all cases is that the purer the materials, the less stable are the emulsions. With pure oil and water, only stable emulsions of the oil in water type are known. Such emulsions are of interest theoretically, but have no practical importance.

Clayton (1928) stated that, theoretically, if oil globules were considered as rigid spheres of equal diameter, it would be possible to

pack them together in such a way that each sphere would touch twelve others. This maximum packing occurs when about 75 percent of the total available space is occupied by the spheres. Hence, such an emulsion would be 75 percent oil and 25 percent water. Practically, oil globules are deformable spheres and occur in a large variety of sizes in an emulsion. Therefore, the preceding theoretical explanation is incorrect as experiments have shown that stable emulsions containing 99 percent disperse phase may be obtained. He also stated that the oil globules of emulsions of oil in water carry a negative charge as proven by cataphoresis tests. The origin of this charge is open to question as is the more general case of the electric charge of all colloids.

Clayton (1932) stated that there can be no doubt that a protein, acting as an emulsifying agent, concentrates at the dineric interface and sometimes the act of adsorption leads to a change in the physical character of the emulsifying agent, this being "precipitated" as a fibrous or membrano-fibrous solid, no longer soluble in its original solvent.

Most of the recent research on protein denaturation at interfaces has been carried out at air-water interfaces. Bull (1947) stated that it is one of the remarkable facts of nature that when soluble, highly organized native protein molecules are placed on an aqueous surface, they promptly spread on the surface to form insoluble films whose thickness corresponds to one peptide chain, irrespective of the dimensions of the original, native protein. In order to spread on a water surface and form a stable film, a substance must possess hydrophilic and hydrophobic groups. The water side of a spread film of protein must be predominantly hydrophilic, while the side directed toward air or oil, as thecase may be, must be

predominantly hydrophobic. Otherwise, a protein monolayer would not be stable at a water-air or water-oil interface. He showed that the air side of a protein monolayer is predominantly hydrophobic.

According to MacRitchie and Alexander (1961b), protein concentration, nature of the interface, pH, ionic strength, and temperature, all influence the rate of build-up of a denatured layer, although at a particular interface for sufficient protein concentration, pH is probably the major factor. The adsorption-pH curves show a maximum near the isoelectric point, falling away steeply on either side. They stated that it is generally accepted that the process of surface denaturation occurs by adsorption in the globular form followed by an unfolding caused by the asymmetric surface forces. It is also widely held that the surface denaturation step is practically instantaneous.

MacRitchie and Alexander (1961a), in experiments with protein stabilized foams, found that the general effects of sucrose were an overall increase in foam stability and an increase in the ease of formation up to a point beyond which higher concentrations depress the foaminess.

Interfacial tension has been one of the most widely studied physical properties of emulsions. According to Becher (1957), the phenomenon of surface or interfacial tension may be explained on a molecular basis by the fact that the Van der Waals field of force acting on a molecule at the surface of a liquid is different from the forces acting on a similar molecule in the bulk of the liquid, where the forces are balanced out because their environment is the same on all sides. The value of interfacial tension will usually lie between the individual surface tensions of the two liquids involved.

Limited research has been done involving the spreading of the contractile muscle proteins on air-water interfaces. Lajtha and Rideal (1951) found that the spreading of the muscle proteins, myosin and actomyosin, depends both on the salt solution in which the proteins are dissolved and on the solution on which they are spread. The viscosity of these proteins in the monomolecular film state was compared with other proteins. Myosin was found to possess an unusually high viscosity in this state. Various phosphates were found to affect the spreading of myosin and actomyosin at interfaces. It was noted that phosphates were more effective in this respect than the other salts studied. The order of effectiveness of the phosphate compounds was ATP > ADP > hexaphosphate > triphosphate > pyrophosphate > AMP. The spreading of actin was unaffected by the addition of salts.

Cheesman and Davies (1954) stated that ATP accelerates the spreading of actomyosin but not myosin. In fact, spreading of myosin was retarded by ATP. They explained these findings by assuming that the unfolding of both actin and myosin is retarded by the combination of the two proteins. When the complex is split by ATP, the actin will be free to spread, while the unfolding of the myosin may then be delayed by the combination with ATP. This, they state is probably a manifestation of the common finding that substrates tend to stabilize their enzymes against inactivation.

According to the same authors, the tensile strength of monolayers of myosin and actomyosin spread on M KCl at pH 7.0, is greatly reduced in the presence of ATP. Although the fully unfolded myosin protein found at an interface has no ATPase activity, ATP continues to exert a plasticizing effect on the protein as evidenced by the reduction in tensile strength.

Emulsion Technology Applied to Meat Research

Swift et al. (1961) undertook a series of experiments to investigate the factors affecting the capacity of meat to stabilize emulsions. They prepared emulsions with meat slurries and with crude salt and water extracts of meat. The fat globules present in an emulsion prepared from a meat slurry were examined microscopically. They had a mean diameter of 18 microns and possessed a non-uniform polyhydral form. Histological preparations treated with a protein stain exhibited fat globules outlined by protein membranes. The rate of fat addition and the temperature of the process were found to significantly affect the amount of fat emulsified. Increased rate of fat addition, up to 1 ml./sec., caused an increase in fat emulsified while an increase in temperature from 18-46°C caused a decrease in fat emulsified from 180 ml. to 130 ml. Data from emulsions prepared from meat extracts showed that salt soluble proteins were more efficient emulsifying agents than water soluble proteins. They also found that water soluble proteins had no marked capacity as stabilizers in the absence of salt.

Hansen (1960) made an extensive microscopic study of comminuted meats, diluted comminuted meat batter, and emulsions prepared with protein solutions. Stained preparations of diluted batter and emulsions prepared from protein solutions indicated that a fat globule membrane is formed in a weiner batter. This membrane was observed only in emulsions prepared with the salt soluble extract. Results of this study also indicated that if excessive temperature rise occurs during chopping, the protein matrix may be partially denatured and broken, giving rise to an unprotected fat dispersion.

Sherman (1961b) attempted to determine if phosphates play a role of emulsion stabilization in sausages through the formation of soaps with free fatty acids. Soap formation was promoted when inedible hog fat (high in free fatty acid content) was used for the dispersed phase. If NaCl was present, fat emulsification was obstructed as any soap that was formed would be salted out. He concluded that soap formation in sausage did not occur because of the low content of free fatty acids in edible fat and the presence of salt normally used in a sausage formulation.

EXPERIMENTAL METHODS

This research was composed of two parts. Part I included two experiments, both of which involved fractionation and solubility studies of beef muscle proteins. The first of these experiments was designed to study changes in extractability, or solubility, of certain proteins during post mortem aging of carcasses from three different sex and age groups. The second experiment was designed to investigate the relationship of intracellular muscle proteins to meat tenderness. Part II was composed of an experiment designed to study the fat emulsifying properties of semi-purified intracellular muscle proteins.

General Methods

Nitrogen analysis.

All nitrogen analyses were performed by the micro-Kjeldahl method as outlined by The American Instrument Co. (1961), unless otherwise specified. All nitrogen contents were reported as mg. of protein nitrogen or non-protein nitrogen per ml. of solution, or per g. of tissue. Nitrogen analyses were made in duplicate.

pH measurement.

All pH measurements were made with a Beckman Model G pH meter. The electrodes were placed directly into the ground sample or protein solution and the observed values recorded to the nearest one tenth unit.

Non-protein nitrogen determination.

Non-protein nitrogen was determined by mixing 15 ml. of protein solution with 5 ml. of 10% trichloroacetic acid. After 15 minutes this

material was filtered through Whatman No. 1 filter paper. The filtrate was analyzed for nitrogen. This value was multiplied by 1.33 (necessary because of the TCA dilution) to give non-protein nitrogen per ml. of original solution.

Reagents.

Reagent grade chemicals and deionized distilled water were used throughout the experiment. Detailed composition of all solutions is contained in appendix A.

Centrifugation.

A model PR-2 refrigerated International Centrifuge was used throughout the experiment. Centrifuging was done at 2500 rpm (1400 X gravity) with the exception of water-holding capacity determinations.

Statistical analysis.

Simple correlation coefficients, standard errors, standard deviations and analyses of variance were calculated as outlined by Snedecor (1956).

Part I

Experimental animals.

All meat samples were obtained from carcasses of cattle fed on the Michigan State University farms and slaughtered in the Michigan State University meat laboratory. The twelve animals used in the initial fractionation study were of three different age and sex groups. Three of these were cows of varying ages and grades, five were Standard and Good grade yearling heifers and four were Standard and Good grade yearling bulls.

Twenty yearling bulls, sired by bulls selected for variation in tenderness and muscling, were utilized for the tenderness study. These bulls, of the Standard and Good grades, had received the same management and feeding treatment and were very nearly the same age.

Sampling procedures.

The infraspinatus muscle was selected for the initial fractionation study because of its convenient size and accessibility. Samples to be fractionated were taken at three periods post mortem: 0 time, generally taken within 20 minutes after death, at 24 hours, and at 7 days. Only that part of the muscle used for a particular sample (about 500 g.) was removed from the carcass. The remainder of the muscle was left on the carcass until used. The sample at 0 time was taken from the middle of the muscle, at 24 hours from the posterior portion and at 7 days from the anterior portion. The extreme ends of the muscle were not used because of increased connective tissue at the muscle attachments.

The longissimus dorsi muscle of a two inch steak taken five inches from the anterior of the short loin, was used for protein fractionation in conjunction with tenderness studies. These steaks were vacuum packaged in Cryovac bags, immediately frozen at -30°C. and stored at this temperature for approximately one month.

Sample preparation for protein fractionation studies.

All separable fat and connective tissue were removed from the muscle samples. The sample was ground twice through a 1 cm. plate and twice through a 2 mm. plate. The grinder head and plates were prechilled in all cases to prevent heat denaturation of the sample, which was ground

into a beaker and immediately covered with aluminum foil to prevent evaporation. A portion of this (about 50 g.) was frozen and analyzed later for total nitrogen and moisture. Nitrogen content (F^n) of these samples was determined by the micro-Kjeldahl method as outlined by the A.O.A.C. (1960). Fat and moisture were also determined according to the A.O.A.C. Methods.

The only exception to the above procedure occurred in the case of steaks utilized for protein fractionation in the tenderness study. They were partially thawed by removing from the freezer and storing at 4°C overnight. No drip occurred during this period. The longissimus dorsi muscle was removed from the steak and trimmed of fat and connective tissue. After being cut into small cubes, the partially frozen meat was ground and held as described above.

Fractionation procedure.

The fractionation procedure was adapted from that of Seagran (1958b) and of Turner and Olson (1959). The principal changes made in adapting these procedures were an increase in the volume of extracting solutions relative to sample size, increase in sample size, and length of time involved in each extraction.

All fractionation procedures were carried out in duplicate at 4°C unless otherwise stated. The design outlined in figure 1 was utilized for the quantitative determination of sarcoplasmic protein nitrogen, non-protein nitrogen, and total fibrillar protein nitrogen. Additional data obtained from the design outlined in figure 2 were necessary for the determination of fibrillar protein solubility. Details of these procedures are outlined below.

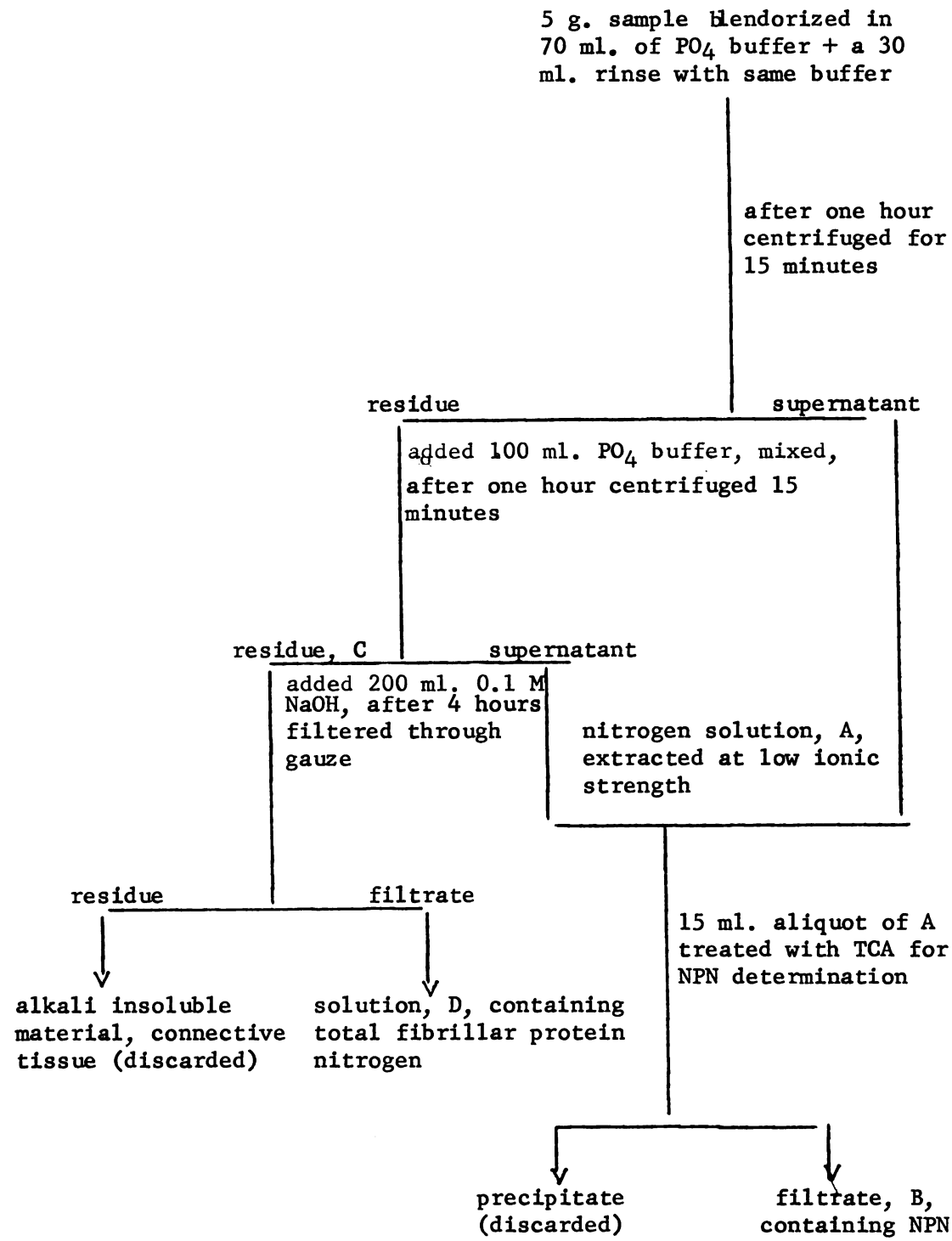

Figure 1. Scheme for the quantitative determination of sarcoplasmic protein nitrogen, non-protein nitrogen and total fibrillar protein nitrogen.

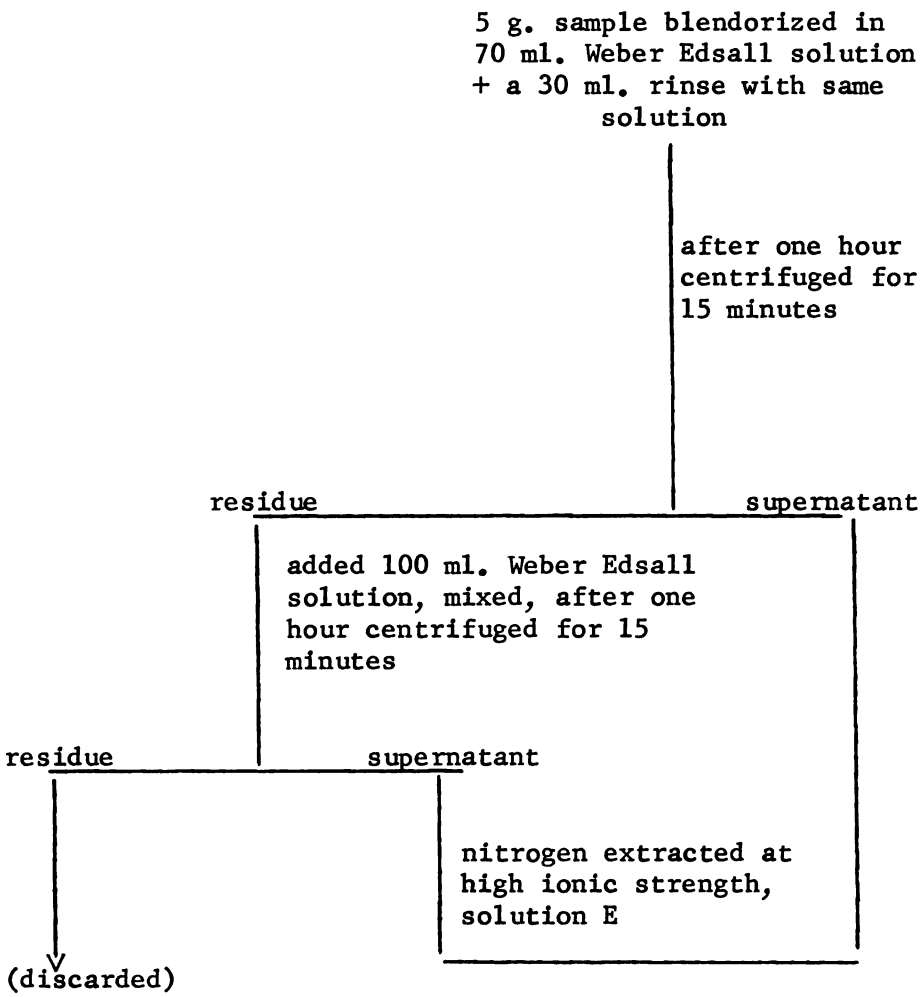

Figure 2. Scheme for the quantitative determination of fibrillar protein nitrogen solubility (complement to figure 1)

For scheme 1(figure 1), a five g. sample was weighed into a 250 ml. centrifuge tube. Seventy ml. of a phosphate buffer (pH 7.6, ionic strength 0.05) was added to the tube and the entire contents of the tube were transferred to a microblendor jar. An attempt was made to approximate the degree of comminution generally achieved in a normal bologna emulsion and at the same time to avoid protein denaturation by excessive foaming. This was achieved by blendorizing for one minute at a blendor speed of 8000 rpm (adjusted with a Powerstat transformer setting of 40). It was extremely important to control blendor speed and time, as variations in these factors caused differences in extractability. After blendorizing, the material was transferred back to its original tube. The blendor jar was rinsed with 30 ml. of extracting solution, and this too, was added to the centrifuge tube. After one hour the material was centrifuged for 15 minutes, the supernatant decanted and its volume recorded. One hundred ml. of extracting solution was again added to each tube. The tube was stoppered and shaken until complete dispersion of the tissue was obtained (about 20 seconds). After one hour the material was centrifuged, the supernatant decanted and the volume recorded as before. The two solutions obtained were combined and filtered through eight layers of gauze to remove fat and other particulate material not removed by centrifugation. This combined solution was designated as A (protein solution extracted at low ionic strength). A 15 ml. aliquot was taken for nitrogen analysis, and a 15 ml. aliquot was used for the determination of non-protein nitrogen. The filtrate resulting from the TCA precipitation was designated as B. The residue, C, remaining from the

extraction with phosphate buffer was extracted with 200 ml. of 0.1 M NaOH for four hours at room temperature. The volume of the tube contents was measured and then filtered through gauze. A very small amount of residue (alkali insoluble material, i.e., connective tissue) was retained in the gauze. An aliquot of the filtrate, D, was taken for nitrogen analysis and the remainder discarded. The procedure in figure 2 is exactly the same as the first two steps outlined in figure 1, except that the extracting solution was Weber Edsall solution (NaCl carbonate buffer, pH 9.0, ionic strength 0.67). The solution extracted by this scheme was designated E. Solutions A, B, D, and E were analyzed for nitrogen and results designated as A^n, B^n, etc. These symbols (nitrogen contents) represent the following fractions:

B^n = non-protein nitrogen

A^n = nitrogen extractable at low ionic strength

D^n = total fibrillar protein nitrogen

E^n = nitrogen extractable at high ionic strength

$A^n - B^n$ = sarcoplasmic protein nitrogen

$E^n - A^n$ - soluble fibrillar protein nitrogen

$F^n - (D^n + A^n)$ = connective tissue protein nitrogen

The above values were averages of duplicate analyses recorded to the second decimal place. Variation between duplicates for A^n, D^n, and E^n was normally from 0 - .02 mg, with an extreme range in one or two cases of .05 mg. Variation in the second decimal place was seldom observed in the case of B^n.

Tenderness measurements.

Tenderness determinations were made on the first three 1 1/2 inch steaks from the anterior end of short loins removed from carcasses which had aged for seven days. Steaks were cooked in deep fat at 141°C to an internal temperature of 63°C. They were allowed to cool 24 hours and one inch cores were submitted to a 12 member taste panel for tenderness evaluation on a nine point hedonic scale. The scores were rated from 1 (extremely tough) to 9 (extremely tender). One-half inch cores were measured for tenderness with the Warner-Bratzler shear.

Water-holding capacity determinations.

Water-holding capacity was determined according to the centrifugal method of Wierbicki et al. (1957b). Ground samples of 25 g. were heated for 30 minutes at 70°C and centrifuged at 1000 rpm. (250 X gravity) for 10 minutes. Triplicate determinations were made.

Part II

Isolation and purification of sarcoplasmic proteins.

Sarcoplasmic proteins, S, for emulsion preparation were isolated from frozen longissimus dorsi muscles of Good or Choice grade steers. The muscle was allowed to partially thaw by holding overnight at 4°C and then ground through a 2 mm plate. One hundred g. of ground muscle were mixed with 400 ml. of water or phosphate buffer (pH 7.6, ionic strength 0.05). The mixture was macerated for 20 seconds in a Waring blendor. After one hour the material was centrifuged for 20 minutes, and the supernatant collected. The nitrogen content of the buffer extracted

solution was about 0.25 mg./ml. greater than the water extracted solution, which contained about 2.25 mg. nitrogen/ml. When the phosphate buffer was used for extraction, the protein solution contained the globulin X fraction of the sarcoplasmic proteins. The phosphate buffer extract was dialyzed against water for 36 hours to precipitate globulin X. This fraction was then partially redissolved in a buffer similar to that used for the original extraction. The globulin X fraction was rather unstable as evidenced by partial insolubility of the precipitate.

Isolation and purification of actomyosin.

Actomyosin was prepared according to Szent-Gyorgyi (1951) with certain modifications found necessary in applying this procedure to frozen beef muscle. Actomyosin was prepared as outlined in figure 3, from the same type of tissue as the sarcoplasmic fraction. One hundred g. of muscle ground through a 2 mm. plate, were mixed with 400 ml. of Weber Edsall solution and stirred mechanically until the mixture attained a jellylike consistency (about two hours with previously frozen tissue). The solution was then diluted with enough Weber Edsall solution to facilitate separation of fibrous material by centrifuging for 30 minutes. The supernatant was decanted, volume measured, diluted with nine volumes of cold water, and allowed to set overnight. The actomyosin formed a precipitate which slowly settled to the bottom of the container. The clear solution above the actomyosin layer was siphoned off. The actomyosin containing layer was centrifuged for 45 minutes. The precipitate, which had a jelly-like consistency, was transferred to a 250 ml. graduate cylinder. A calculated amount of 1.2 M KCl was added to redissolve the

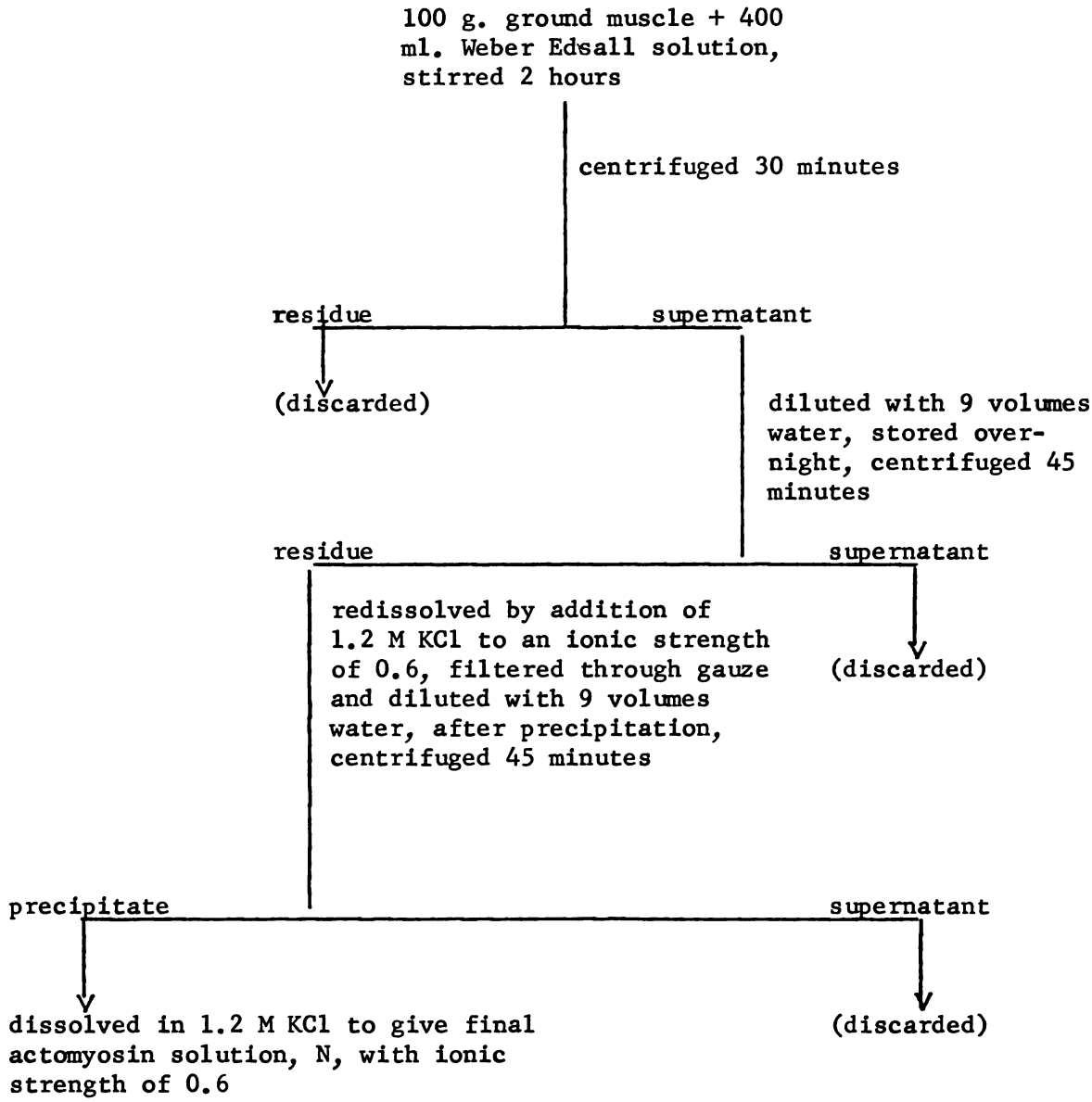

Figure 3. Scheme for the isolation of actomyosin.

actomyosin and adjust the ionic strength of the solution to 0.6. The solution was filtered through gauze and again diluted with nine volumes of cold water. After a length of time necessary for precipitation of the actomyosin (the time varied from 4-12 hours in this step), the precipitate was isolated as in the preceding step. The actomyosin precipitate was again dissolved by adding a sufficient amount of 1.2 M KCl to give an actomyosin solution N, of ionic strength 0.6. This preparation generally contained between 0.7 and 0.8 mg. of protein nitrogen/ml. Non-protein nitrogen analysis revealed that only protein nitrogen was present.

The presence of actomyosin was verified with an ATP sensitivity test. This involved the observed drop in viscosity upon the addition of two drops of 0.014 M ATP to 5 ml. of actomyosin solution containing 0.5 mg./ml. of protein nitrogen. In all cases the time required for a 2 ml. slow delivery pipette to deliver 2 ml. of actomyosin solution was reduced by approximately one half upon addition of the ATP solution.

Isolation and purification of actin and myosin.

Preparation of myosin was performed according to the method of Mommaerts and Parrish (1951), and actin was prepared as outlined by Tsao and Bailey (1953). Certain modifications were necessary to adapt these procedures to bovine muscle. Actin and myosin were prepared from the same muscle sample, i.e., the longissimus dorsi muscles of young calves. An attempt was made to extract these proteins from the neck muscles of yearling steers, but this source proved to be unsatisfactory for the isolation of myosin. It was concluded that too much unseparable fat was contained in the neck muscles and when this lipid material was carried through the

procedure it caused a large amount of surface denaturation of the myosin preparation. In fact, a very creamy emulsion was observed on the surface of the myosin preparation at several stages during the extraction.

Longissimus dorsi muscle samples were generally obtained from three months old calves within three minutes after death. All separable fat and connective tissue were removed from the sample which was ground through a 2 mm. plate. The entire myosin extraction procedure was carried out at 4°C as outlined in figure 4. Four hundred g. of ground muscle were extracted with approximately three volumes of cold KCl-phosphate buffer (pH 6.8, ionic strength 0.57). This mixture was stirred with a glass rod for 10 minutes, strained through several layers of gauze and the residue, H, pressed as dry as possible and retained for the extraction of actin.

Myosin was precipitated by a 10 fold reduction of ionic strength of the original extract, the volume of which was about 900 ml. This was achieved by dialyzing without stirring against nine volumes of water. The dialysis was carried out for 20 hours in one inch dialysis tubing. The contents of the tubing were centrifuged for 20 minutes and the resulting supernatant discarded. Assuming that 900 ml. of original extract were obtained, the precipitate was dissolved in 60 ml. of 2 M KCl and 60 ml. of 0.5 M KCl-phosphate buffer (extracting solution). The pH was in the range 6.7-6.9. This preparation was centrifuged for 20 minutes to remove any particulate material. After centrifugation, the preparation was diluted to four liters by slowly adding water over an interval of about 15 minutes with rapid stirring to promote the formation of crystalloid

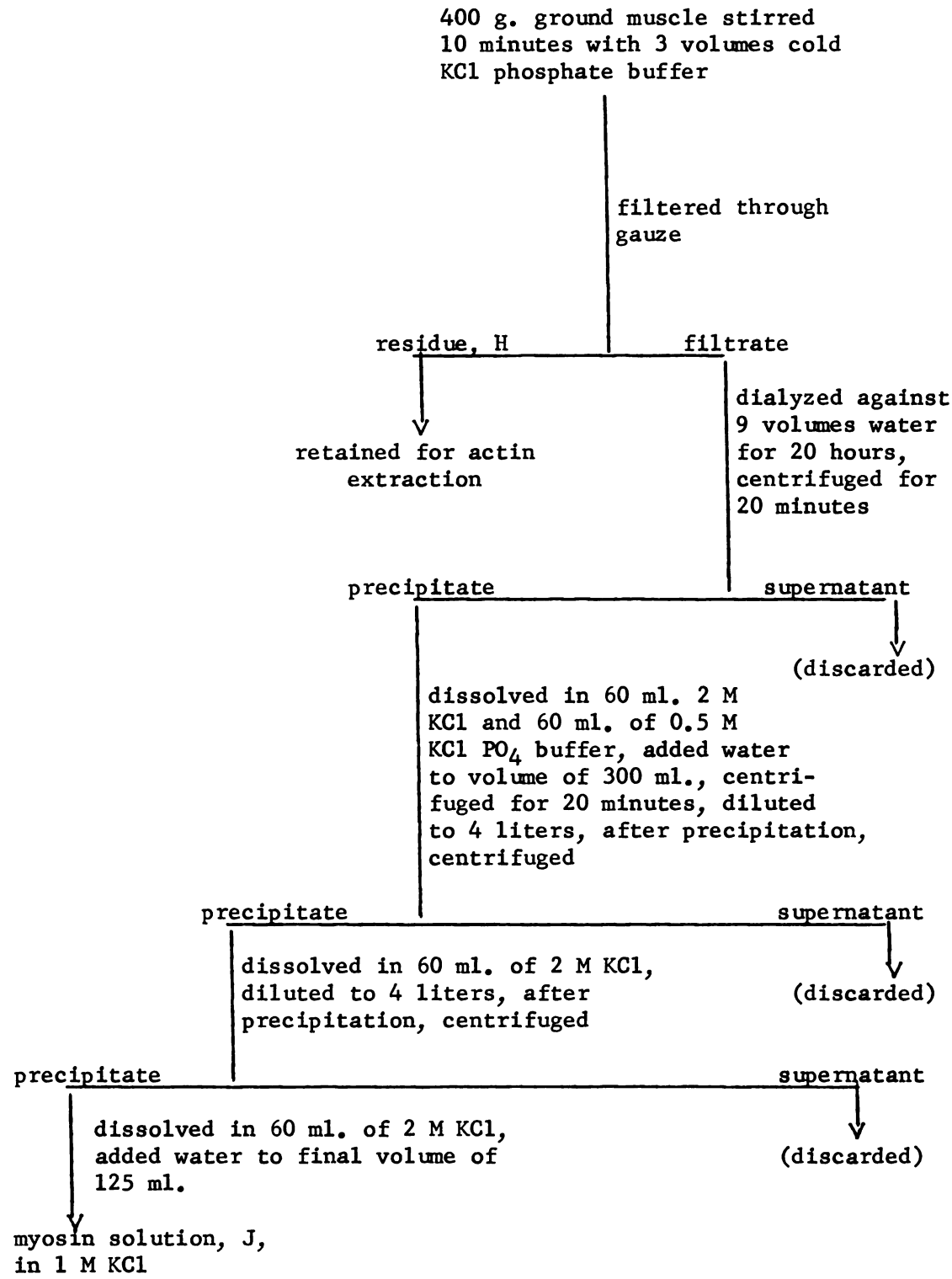

Figure 4. Scheme for the isolation of myosin.

needles. When the myosin had settled sufficiently (4-24 hours) the supernatant liquid was siphoned off and the remainder of the material centrifuged for one hour. The precipitate was then dissolved in 60 ml. of 2 M KCl and precipitated by diluting as before. Stirring was not necessary in this step and separation of the precipitate was accomplished as in the preceding step. The final precipitate was dissolved in 60 ml. of 2 M KCl and water added to a final 125 ml. volume. The ionic strength of the final preparation, J, was about 1. Protein nitrogen concentration of this solution was about 1.5 mg./ml. Mommaerts and Parrish (1951) stated that preparations of rabbit myosin prepared essentially according to the above procedure were stable for about one week. However, the preparations of calf myosin obtained in this manner began to show evidence of denaturation in 24 to 48 hours as evidenced by the appearance of insoluble shreds in the solution. It was found that the myosin preparation was more stable when stored in a salt solution of high ionic strength, i.e., 1 M KCl.

Preparation of actin acetone powder, K, was performed at room temperature according to the scheme in figure 5. The residue, H, from the myosin extraction was washed with four volumes of 0.05 M $NaHCO_3$ to adjust the pH to 7.0. This was followed by washing with 10-15 volumes of water. Each washing was carried out for 20 minutes under constant stirring with a magnetic stirrer. The washed residue was separated from the washing solution by straining through several layers of gauze. After washing with water, the residue was pressed as dry as possible and then macerated for one minute in a Waring blendor at full speed with 5 volumes of n-

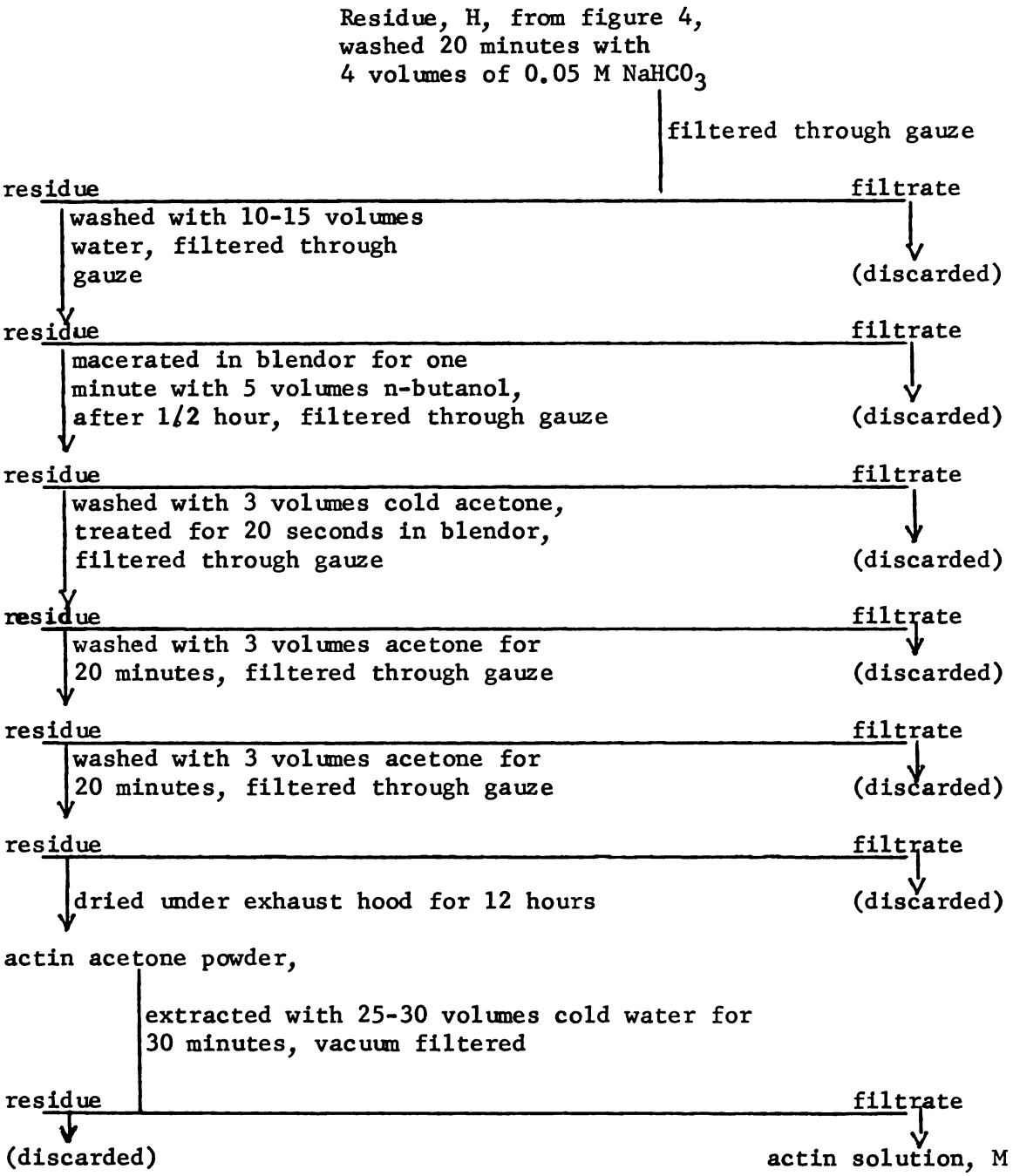

Figure 5. Scheme for the isolation of actin.

butanol. The butanol was precooled so the temperature in the blendor did not rise above 20°C. The butanol treatment dissolved the contaminating lipid material and denatured most of the remaining sarcoplasmic, myosin, and stroma proteins. After one-half hour, the butanol was strained through gauze and the residue washed with three volumes of cold acetone. The lumps of debris observed at this step were disintegrated by treating for a few seconds in the Waring blendor. The acetone was strained through gauze and was followed by two more washings of 20 minutes each in three volumes of cold acetone. The preparation was stirred occasionally with a glass rod during these washings. After the final acetone wash was strained, the residue was placed in a shallow layer in an open container and dried 12 hours under an exhaust hood. Actin was extracted from the resulting powder, K, by stirring 30 minutes at 4°C with about 25 - 30 volumes of cold water. A higher yield could be obtained if each 100 ml. of water used to extract actin contained about 20 mg. ATP. The actin solution was separated from the residue by vacuum filtration through Whatman No. 41H filter paper. The protein nitrogen concentration of the solution obtained in this manner was about 0.25 mg./ml. This was concentrated to the necessary nitrogen content by pervaporation.

Tests for confirmation and purity of myosin and actin.

An ATP sensitivity test for myosin purity was the same as that previously described for actomyosin. A pure solution of myosin would not exhibit a change in viscosity upon the addition of ATP. Therefore, any reduction in viscosity caused by ATP would indicate actomyosin contamination of a myosin preparation. All of the myosin preparations obtained

showed a slight drop in viscosity upon the addition of ATP, indicating a slight contamination with actomyosin.

Equal volumes of actin and myosin were mixed to observe the formation of "artificial" actomyosin. All actin and myosin preparations were tested in this manner. In all cases, a gel-like precipitate of actomyosin was observed upon the reduction of the ionic strength of the mixture to 0.3.

Actin extracted by the method of Tsao and Bailey (1953) is in the G form. G actin can be transformed to the viscous F form by adjusting the pH to 7 and adding 0.1 M KCl. All actin preparations responded to this test with a great increase in viscosity. In most cases, the G actin preparation, which was only slightly more viscous than water, was transformed to a semi-solid gel of F actin.

One sample of calf myosin in 1 M KCl was subjected to ultracentrifugation in a Model L Spinco ultracentrifuge at 59,780 rpm at 0°C. The protein concentration of the sample was approximately one percent. Several small fast peaks were observed which were concluded to be different sized actin-myosin polymers (figure 6). Similar small fast peaks were also evident in ultracentrifuge patterns of myosin presented by Johnson and Rowe (1960) and Kominz et al. (1959). A large, very sharp myosin peak, indicated that the preparation was predominately myosin with a degree of purity necessary for the preparation of emulsions and interfacial tension studies. The S_{20} of the myosin peak, 3.14, was slightly higher than the S_{20} of myosin for this concentration determined by the extrapolation of the values reported by Johnson and Rowe (1960).

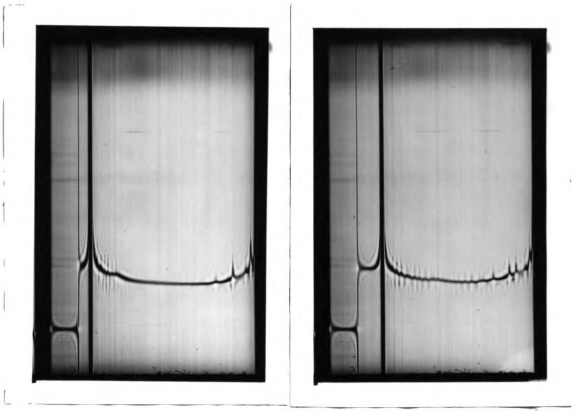

Figure.6. Ultracentrifugal sedimentation diagrams of myosin at 32 minutes and at 64 minutes (in 1 M KCl at 59,780 rpm.)

To determine the amount of active actin, i.e., actin that will react with myosin to produce actomyosin, the following procedure was employed. The myosin in 2 ml. of solution, J, (ionic strength reduced to 0.5), was precipitated by the addition of 18 ml. of water. This material was centrifuged, analyzed for nitrogen and the total nitrogen in the supernatant calculated (this value was denoted as X). In all cases, this supernatant contained no nitrogen. Next, 2 ml. of the above myosin solution, J, was mixed with an equal amount of actin solution, M (ionic strength = 0), and diluted to 16 ml. The preparation was centrifuged to remove the resulting actomyosin. The total amount of nitrogen in the supernatant from the actin-myosin mixture was determined (this value was denoted as Y). By subtracting X from Y, the amount of inactive actin and other impurities in 2 ml. of the actin preparation was determined. The amount of active actin was determined from the difference between nitrogen content of inactive actin/ml. and nitrogen content of actin solution,M,/ml. Actin contained eight percent and myosin preparations contained no measurable nonprotein nitrogen as determined by the TCA method.

Interfacial tension measurements.

Clayton (1928) described a drop volume method for determining relative interfacial tension of emulsion systems. A similar apparatus was constructed to determine the relative interfacial tension between soybean oil and the previously described protein solutions, S, N, M, and J.

A 10 ml. buret was solidly mounted in a vertical position on a ring stand. A five inch section of one-quarter inch glass tubing was formed in the shape of the letter J. The short end of the J tube was drawn to

a capillary diameter and the long end was connected by means of Tygon tubing to the delivery end of the 10 ml. buret. The J tube and the buret were filled with soybean oil by immersing the J tube in oil and applying a vacuum to the open end of the buret. For the determination of drop volume, the J tube was wiped completely free of all oil and immersed in 25 ml. of the protein solution (concentration, 0.25 mg. nitrogen/ml.) to be studied. The protein solution was contained in a water bath maintained at 25°C. The stopcock on the buret was fully opened and the drops of oil that were formed and released from the end of the capillary were counted. The oil was allowed to flow until approximately 1 ml. had been used. The volume of oil used and the number of drops corresponding to this volume of oil were recorded. From these data, volume of oil/drop could be calculated. Relative interfacial tension was calculated as follows:

$$\frac{\text{the oil drop volume in the protein solution.}}{\text{the oil drop volume in the protein solvent.}}$$

A low relative interfacial tension should have indicated good emulsifying properties of the protein in question.

Emulsion preparation and evaluation.

Emulsions were prepared in a manner similar to that described by Swift et al. (1961). The principle difference between the two methods was the stirrer speed. Swift et al. used a stirrer speed of 13,000 rpm., while the stirrer speed used in these experiments was 1750 rpm. A Lightnin Model L stirrer equipped with a three bladed propellor with a diameter of 5 cm. was used. In most cases, the emulsions were prepared in one pint Mason jars, as they proved to be more durable than glass beakers. Oil

was delivered into the emulsion at an average rate of 0.9 ml./sec. with a glass tube connected by Tygon tubing to a 300 ml. separatory funnel. All emulsions were prepared at room temperature.

To prepare an emulsion, 25 ml. of protein solution were initially placed in a jar, and the weight recorded to the nearest gram. The protein concentration of this solution was varied for studying the emulsifying capacity of each protein. After the jar and contents were weighed, stirring was begun and the oil flow started. As oil was added to the emulsion, viscosity increased to a maximum, and at a point slightly past the maximum viscosity, a sudden drop in viscosity was easily observed. This was the point at which the emulsion broke. At this point, the oil flow was shut off, stirring was discontinued, and the amount of oil added was determined by weight difference. Variation between duplicate determinations was generally within 10 g., however, in a few cases it was as high as 20 g. Emulsifying capacity was expressed as g. of oil emulsified/ mg. of protein nitrogen in the solution.

After the broken emulsion was weighed, it was centrifuged for 30 minutes. This treatment separated the broken emulsion into three distinct layers: a top layer of clear soybean oil, a bottom layer of water containing varying amounts of protein, and an intermediate layer of varying thickness consisting largely of denatured protein plus some water and oil. The bottom layer was assumed to contain protein that was not involved in the formation of an interface, as Bull (1947) and Clayton (1928) stated that proteins are denatured at a surface, i.e., an interface, and lose their solubility in the usual solvents. In other words, the proteins

which retained their solubility were not utilized in forming the emulsion. A sample of the protein solution at the bottom of the centrifuge tube was recovered and analyzed for protein nitrogen. This value was divided by the amount of protein nitrogen originally present and the resulting percentage value reported as a supplementary measure of emulsifying capacity.

Relative stability of emulsions prepared from the various protein preparations was determined by storing at room temperature and noting the degree of fat separation and loss of white color as the emulsions aged. A white color indicates small droplet size or a high degree of dispersion (Clayton, 1928). As an emulsion begins to acquire the color of its fat component, this is an indication that droplet size has increased and the emulsion stability has decreased. Emulsions for stability tests were prepared with 25 ml. of protein solution (concentration 0.5 mg. of protein nitrogen/ml.) and 200 g. of oil. When the protein in question would not emulsify 200 g. of oil, the amount of oil was reduced to a suitable level.

Depolymerization of proteins by urea.

The protein solution to be treated was dialyzed against three volumes of 8 M urea for seven days at room temperature. At equilibrium the protein was in a 6 M urea solution.

pH adjustment of protein solutions.

Emulsion stability and emulsifying capacity of proteins were studied in the acid, alkaline and neutral pH ranges. The pH of the protein solutions was adjusted by the addition of solid reagents. The pH of all protein solutions as extracted, was near neutrality, except the sarcoplasmic

fraction, which was approximately pH 5.5. An increase in pH from neutrality was achieved by the addition of Na_2CO_3 or $NaHCO_3$ and a decrease was attained by the addition of KH_2PO_4. The pH of the sarcoplasmic protein solution was adjusted to neutrality from 5.5 by the addition of K_2HPO_4 and KH_2PO_4 in a predetermined ratio. Na_2CO_3 was used to adjust the sarcoplasmic protein solution to an alkaline pH. Calculated amounts of the above materials were added to produce an increase in ionic strength of 0.05. The ionic strength of the solutions which were not changed in regard to pH, i.e., those which were studied as extracted, was increased by 0.05 with KCl in order to remove the variable of ionic strength from the pH experiments.

RESULTS AND DISCUSSION

Part I

Post mortem variation in protein composition.

Bate-Smith (1934a) developed a long procedure to partition the proteins of beef muscle and applied this to only a few animals. This procedure utilized repeated extractions with a salt solution to quantitatively recover the fibrillar proteins. Such a procedure usually furnishes rather variable results. Since he reported the results of the above experiment, very little information has been reported on the complete protein composition of beef muscle, except in connection with studies of changes during carcass aging or freezing, and in these cases usually only changes in solubility were studied. In such studies it would be beneficial if total amounts of the major nitrogen containing components were determined. If these were determined, changes in protein solubility could be reported as a percentage of the total protein fraction. Values reported on this basis would be more meaningful because the present study has shown considerable differences exist between animals in regard to the total amounts of the major protein components, especially the fibrillar fraction, in which so much interest has been directed in connection with aging and freezing studies.

The fractionation procedure outlined in the previous section could facilitate a relatively rapid and reasonably accurate determination of nitrogen components in muscle. The procedure outlined could be carried out in duplicate in one day. In order to make a complete analysis, some type of connective tissue analysis would be desirable, as connective tissue

was determined by difference in this procedure and such a determination reflects all the errors involved in all the separate determinations of the other components. Ritchie et al. (1963) reported values of collagen determined by the hydroxyproline method of approximately 1.3 g. collagen nitrogen/100 g. total nitrogen for beef longissimus dorsi muscles (1.3 percent). This differs considerably from the values for the same muscle obtained in this study (0.5 percent). Accurate determinations of such small amounts of connective tissue would be practically impossible to determine by difference.

Muscle samples of 28 animals involving three different classes of beef cattle were fractionated. Twelve of these involved the infraspinatus muscle, which is a muscle of the shoulder and of intermediate connective tissue content and 20 involved the longissimus dorsi muscle, which is of low connective tissue content. Both longissimus dorsi and infraspinatus muscles were fractionated from four of these animals which explains the discrepancy in total number of muscles and animals studied. Data obtained from these studies are outlined in tables 2 and 3. From these data there appears to be little difference between classes of animals (table 2) in regard to nitrogen composition, at least for the infraspinatus muscle. Noticeable differences occurred in the case of the fibrillar protein fraction and stroma fraction. It appears that bulls possibly have a higher percentage of fibrillar protein, which may in some way account for the supposed superiority of bull meat for the manufacture of sausage products, as, according to Hamm (1960), it has been fairly well established that the fibrillar fraction is primarily responsible for the water-holding capacity of meat. Not only do bulls have a higher percentage of nitrogen as fibril-

lar protein nitrogen, but they also have a greater amount of total nitrogen per unit of tissue weight by virtue of their lower fat content. The combination of these two factors results in a considerable increase in total fibrillar protein per unit of muscle tissue.

Table 2. Average nitrogen composition of infraspinatus muscle of three classes of beef animals expressed as percent of total nitrogen and as mg. nitrogen/g. of tissue.

	Nitrogen containing fraction				
Class of animal	Sarcoplasmic	Fibrillar	NPN	Stroma	Total
Percent					
cows (3)	16	66	10	8	
heifers (5)	18	64	9.5	8.5	
bulls (4)	18	68.5	9.0	4.5	
all animals (12)	17.5	66	9.5	7.0	
Mg. N/g. of tissue					
cows	5.10	20.97	3.23	2.37	31.67
heifers	5.95	20.74	3.10	2.81	32.60
bulls	6.01	22.85	3.05	1.38	33.35
all animals	5.76	21.50	3.11	2.20	32.57
Std. dev.	0.75	1.76	0.49		
Std. error	0.22	0.51	0.14		

The four bulls from which the infraspinatus muscle was fractionated were from the group of 20 from which the longissimus dorsi muscle was fractionated. This furnished a good comparison between muscles of the same animals. There seemed to be little similarity between the nitrogen

Table 3. Average nitrogen composition and percent total nitrogen of longissimus dorsi muscle of 20 yearling bulls.

	Type of compound				Total
	Sarcoplasmic	Fibrillar	NPN	Stroma	nitrogen
Mean (% of total nitrogen)	31	62	6.5	0.5	
Mean (mg. of N/g.)	10.70	21.22	2.22	0.16	34.30
Std. dev.	0.56	0.80	0.10		0.80
Std. error	0.12	0.18	0.02		0.18

composition of these two muscles from the same animals. The longissimus dorsi muscle contained almost twice as much sarcoplasmic protein and almost 1 mg. more total nitrogen/g. of tissue than the infraspinatus. It was possible that the value for sarcoplasmic proteins was somewhat higher than the actual sarcoplasmic protein content, due to the fact that the longissimus dorsi muscles were frozen for a short time before being fractionated. Mechanical damage to the intimate muscle structure by freezing could possibly have allowed a greater dissolution of actin and myosin in the 0.05 ionic strength buffer, ultimately resulting in a slight overestimation of the sarcoplasmic proteins. There were considerably less of the fibrillar protein fraction and the non-protein nitrogen compounds in the longissimus dorsi than in the infraspinatus.

Standard deviations and standard errors were much lower in the case of the longissimus dorsi from bulls than from the infraspinatus muscles of the mixed group. This would be expected since this was more or less a homogenous group of bulls as contrasted with a heterogenous group of different beef animals. Standard errors and standard deviations were not

calculated for each separate class of animals for the study involving the infraspinatus. Because of the small numbers involved, such data would be rather unreliable.

An analysis of variance for each of three factors was made for each class of animals, i.e., cows, bulls, and heifers, to determine if significant differences existed between samples of the infraspinatus muscle at three periods post mortem. The three factors studied were amount of sarcoplasmic protein extracted, percent of the total fibrillar protein extracted, and percent of the total water released (water-holding capacity). Data from only 11 of the original 12 animals were included in this study as all of the data were not available from one animal.

The only significant difference shown in table 4 was the smaller amount of sarcoplasmic protein extracted at 0 time from the bulls. This was followed at 24 hours and seven days by an increase in amount of extractable sarcoplasmic protein to levels greater than either cows or heifers at 24 hours and seven days. This was unexpected as such differences have not been previously reported for any species. The variation in amount of sarcoplasmic protein is probably due to a small amount of actin and myosin being extracted with the sarcoplasmic fraction. There undoubtedly was a small amount of myosin and actin dissolving in the 0.05 ionic strength buffer used for the extraction of sarcoplasmic proteins, however, it would seem that the amounts of actin and myosin dissolving in this buffer would be greatest at slaughter before any appreciable amount of these proteins combined to form actomyosin. No significant differences in regard to amount of sarcoplasmic protein were noted between individual

Table 4. Means and standard errors for amount of sarcoplasmic protein extracted at three periods post mortem and for three classes of animals (mg. N/g. tissue) (1)

Class	Period			Standard error
	0	24 hours	7 days	
cows	4.73	5.47	5.50	0.17
heifers	5.75	6.16	6.62	0.53
bulls	4.70*	7.10	7.70	0.53

(1) The means underlined with the same line do not differ significantly.
* $P < 0.05$

animals in each class. In each class the trend was an increase in extractability of sarcoplasmic proteins as the carcass aged up to seven days, however, the increase was not significant except in the case of bulls from 0 time to 24 hours.

Changes in fibrillar protein solubility are presented in table 5. Periods were not listed in chronological order because Duncan's Multiple Range Test (1955) requires that the means be listed in order of increasing size. The changes observed in this table are of particular importance because of the relation this protein fraction has to water-holding capacity of meat. In the case of cows and heifers, fibrillar protein solubility was greatest at slaughter, decreased sharply to 24 hours and then increased slightly to seven days. Bulls again proved to be the exception. At 0 time fibrillar protein extractability was at its highest level, followed by a sharp decrease to a low at 24 hours. The solubility then rose at seven days to a level almost as high as at slaughter. It is also interesting to note that the solubility of the fibrillar proteins was higher for bulls than the other two classes at all periods studied. With-

out exception, fibrillar protein solubility was highest at slaughter and was followed by a sharp decrease with the onset of rigor mortis. A similar sequence of events also occurs in other species, according to Bailey (1954).

Table 5. Means and standard errors for percent of total fibrillar protein which was soluble at three periods post mortem among three classes of animals (1)

Class	Period			Standard error
	24 hours	7 days	0	
cows	20.90	22.87	49.70**	2.58
heifers	19.24	27.52	54.98**	3.13
bulls	24.87**	51.73	57.40	3.79

(1) The means underlined with the same line do not differ significantly.
**P < 0.01

Several workers (Turner and Olson, 1959; Hamm, 1960) have related water-holding capacity to fibrillar protein solubility. In the present research, there seemed to be very good agreement in this respect (comparing data in tables 5 and 6). For cows and heifers, water-holding capacity (which is inversely related to percent water released, which is reported in the present study) followed a similar pattern to fibrillar protein solubility, i.e., it was highest at slaughter, dropped to a low at 24 hours and gradually increased to seven days but never reached the capacity observed immediately after slaughter. The water-holding capacity for cows and heifers at slaughter was significantly higher than at the other two periods. No significant differences in respect to water-holding capacity were observed between periods post mortem in the case of bulls, even

though a decrease was noted at 24 hours. For bulls, the water-holding capacity followed the pattern for fibrillar protein solubility very closely; water-holding capacity was high at slaughter, decreased to 24 hours and recovered in seven days to its original value at slaughter.

Table 6. Means and standard errors for percent of total water released at three periods post mortem and for three classes of animals (1)

Class	Period			Standard error
	0	7 days	24 hours	
cows	45.67*	47.20	49.60	0.61
heifers	45.76*	47.62	48.90	0.83
bulls	46.57	46.57	48.03	0.82

(1) The means underlined with the same line do not differ significantly.
*$P < 0.05$

Information obtained on differences between classes of beef animals in respect to the above three factors would be of interest. As the numbers of animals in all classes were not equal, an exact analysis of variance would have involved a series of rather difficult and time consuming calculations. According to Snedecor (1946), an approximate analysis of variance would provide a reasonably accurate test providing interaction was non-significant. Interaction was judged by inspection to be non-significant, and the approximate analysis was performed. The non-significance of interaction was confirmed by the small F values obtained from the approximate analysis of variance presented in tables 7, 8 and 9.

Table 7. Analysis of variance (approximate) of sarcoplasmic protein among three classes of animals and three periods post mortem.

Source	Sums of squares	Degrees of freedom	Mean square	F value
classes	2.7	2	1.35	3.65*
periods	3.9	2	1.95	5.27*
interaction	2.0	4	0.5	1.35
between	8.6	8		
within	30.7	24	1.28 X .29(1) = 0.37	
total	39.3	32		

(1) 0.29 is the factor used to correct the error term for the F test.
* $P < 0.05$

Table 8. Analysis of variance (approximate) of percent of total fibrillar protein which is soluble at three periods post mortem among three classes of animals.

Source	Sums of squares	Degrees of freedom	Mean square	F value
classes	305	2	152.5	2.09
periods	1600	2	800.0	10.98**
interaction	223	4	55.7	0.76
between	2128	8		
within	6038	24	251.4 X .29(1) = 72.9	
total	8162	32		

(1) correction factor
**$P < 0.01$

Table 9. Analysis of variance (approximate) of percent of total water released among three classes and three periods post mortem.

Source	Sums of squares	Degrees of freedom	Mean square	F value
classes	0.5	2	0.25	0.15
periods	12.3	2	6.15	3.72*
interaction	2.1	4	0.42	0.25
between	14.9	8		
within	136.8	24	5.70 X .29(1) = 1.65	
total	151.7	32		

(1) correction factor
* $P < 0.05$

The only difference between classes of beef animals (tables 7, 8 and 9) was in regard to the amount of sarcoplasmic protein. In the analysis of variance, (table 7), this was barely significant at the 0.05 level, however, when Duncan's Multiple Range Test (1955) was applied to the means (table 10), the difference was not significant. The discrepancy could no doubt be traced to the approximate analysis of variance.

The approximate analysis of variance also furnished a test for overall differences between periods post mortem for percent water released, amount of sarcoplasmic protein and percent soluble fibrillar protein (table 11). The means listed in tables 10 and 11 for these factors were not true means, but each was merely the average of three means (one mean for each class of animal). Differences between periods for percent water released and amount of sarcoplasmic protein were barely significant at the 0.05 level. These differences were not considered important because

an exact analysis was not performed. In the case of soluble fibrillar protein, there is little doubt that a definite difference does exist and that solubility is greatest immediately after death.

Table 10. Means and standard errors of three factors, over three periods post mortem, for three classes of animals. (1)

Factor	Class of animals			Standard error
	Cows	Heifers	Bulls	
% water released	47.49	47.47	47.00	0.74
mg./g. sarcoplasmic protein nitrogen	5.57	6.17	6.50	0.35
% fibrillar protein soluble	31.16	33.91	44.67	4.95

(1) The means underlined by the same line do not differ significantly.

Table 11. Means and standard errors of three factors, including 11 animals, for three periods post mortem and showing significant differences. (1)

Factor	Periods			Standard error
	0	7 days	24 hours	
% water released	46.00	47.06	48.84*	0.74
mg./g. sarcoplasmic protein nitrogen	5.05*	6.60	6.24	0.35
% fibrillar protein soluble	54.03**	34.04	21.67	4.95

(1) The means underlined by the same line do not differ significantly.
* $P < 0.05$
** $P < 0.01$

There is the possibility that the changes in muscle protein behavior observed at different periods post mortem in this study are not exactly those that would occur in the uncut infraspinatus muscle under similar conditions. Paul and Bratzler (1955) reported that the tenderness of a

muscle could be altered by cutting the muscle before rigor had occurred. Locker (1960) reported that merely loosening a muscle from either its origin or insertion resulted in a decrease in meat tenderness. Considering these facts, the normal changes occurring during and after rigor may have been altered after the section of the infraspinatus was removed for analysis immediately after slaughter.

Table 12 contains the mean muscle pH and standard error at three periods post mortem for each class of animals. The pH behavior was similar for all classes and followed a definite pattern, i.e., pH was highest at slaughter (near neutrality), dropped to a low at 24 hours (pH 5.6) and remained at this pH for considerable time. This pH pattern was in agreement with normal post mortem pH changes in beef muscle as outlined by Bate-Smith (1948). In this study the bulls did appear to attain a slightly lower ultimate pH than the other two classes.

Table 12. Means and standard errors for pH of three classes of beef animals at three different periods post mortem.

Class	Period		
	0	24 hours	7 days
cows (3)			
$\bar{x}$	6.56	5.87	5.83
$S_{\bar{x}}$ (1)	0.13	0.10	0.06
heifers (5)			
$\bar{x}$	6.50	5.72	5.82
$S_{\bar{x}}$ (1)	0.06	0.05	0.05
bulls (3)			
$\bar{x}$	6.60	5.53	5.52
$S_{\bar{x}}$ (1)	0.07	0.02	0.07

(1) Standard error

Tenderness study

This work was originally designed to investigate muscle protein characteristics in relation to their utilization in sausage products, however, during the course of the research an excellent opportunity was presented to study the relationship of intracellular protein characteristics with tenderness. It was decided to use the previously described fractionation techniques to study protein characteristics in relation to tenderness.

Wierbicki et al. (1954) were among the first to carry out an experiment to investigate the role of intracellular muscle protein in tenderness variation. These workers reported post mortem changes in protein extractability that appeared to be related to tenderness. Kamstra and Saffle (1959) produced definite differences in tenderness of paired hams by altering the normal course of rigor and thereby changing some protein solubility characteristics. The American Meat Institute Foundation (1960) stated that the ratio of sarcoplasm to myofibrils is apparently directly proportionate to the amount of work the muscle is required to do. From the present study, it appeared that this ratio was inversely related to the amount of work a muscle performs. The corresponding ratios obtained in this research were 18/68.5 for the infraspinatus and 31/62 for the longissimus dorsi. It is commonly accepted that the muscles of the shoulder, of which the infraspinatus is a good example, perform more work than the muscles of the back, e.g., longissimus dorsi. If a relationship between this ratio and the work performed by a muscle actually existed, be it direct or inverse, there was the possibility that this ratio could be related to tenderness. Consequently, this aspect was investigated.

Table 13 shows the tenderness measurements, water-holding capacity and amounts of the more important nitrogen containing fractions of the longissimus dorsi of the 20 bulls involved in the tenderness study. The only large variations between animals in this table were the values for tenderness and fibrillar protein solubility. Total nitrogen, sarcoplasmic protein, fibrillar protein, water-holding capacity, and non-protein nitrogen were remarkably constant from animal to animal as shown by small standard deviations and standard errors. Non-protein nitrogen for individual animals was not shown in table 13. The mean (2.22), standard deviation and standard error are shown in table 3.

Simple correlation coefficients were calculated between tenderness (measured by shear and panel) and sarcoplasmic protein nitrogen/total fibrillar protein nitrogen, soluble fibrillar protein nitrogen/total fibrillar protein nitrogen, and percent of total moisture released (table 14). Tenderness as measured by both shear and panel, was highly correlated with fibrillar protein solubility ($r = -0.69$ and 0.59 respectively). Correlations of the other factors with tenderness were all very close to the 0.05 level of significance, however, only one r value was significant at this level. This was the correlation between water-holding capacity and tenderness as measured by the shear ($r = 0.49$). Hamm (1960) reported that a correlation existed between water-holding capacity and tenderness, but that the relation appeared only if the differences of water-holding capacity of meat were relatively great. In other words, a high correlation between water-holding capacity and tenderness will usually be found only in extreme cases.

Table 13. Tenderness, water-holding capacity and nitrogen composition of the longissimus dorsi of 20 bulls.

Bull No.	Tenderness		Water-holding capacity	Nitrogen composition mg. N/g.			
	Shear	Panel	% released	Sarcoplasmic	Fibrillar	Soluble fibrillar	Total nitrogen
247	10.60	5.4	47.6	10.1	22.2	8.20	34.5
23	12.30	4.1	46.5	10.4	21.6	10.60	34.3
3	9.05	7.3	45.3	10.2	20.4	13.40	34.1
37	9.27	7.4	45.4	10.1	20.9	15.0	33.5
32	12.50	4.7	46.3	10.7	21.3	8.2	35.3
15	9.68	6.0	46.6	11.3	20.7	14.6	34.8
25	9.82	6.8	43.9	11.1	20.7	8.0	34.4
50	7.33	6.9	42.1	11.9	21.3	17.4	35.3
20	10.64	5.4	43.6	10.2	20.6	11.2	35.4
700	7.41	7.7	46.3	10.8	19.6	11.6	34.5
361	8.29	7.6	44.3	10.1	20.9	12.4	32.3
22	8.37	6.3	44.1	10.1	20.5	14.2	34.1
21	7.78	6.8	43.3	10.7	21.5	15.0	34.1
672	9.40	5.8	45.6	10.7	23.3	9.6	33.3
47	7.30	7.3	45.0	11.5	21.3	18.2	34.8
6	9.58	5.8	46.2	10.2	21.4	12.2	33.9
720	9.38	6.5	46.7	11.2	22.2	10.2	35.2
29	8.83	6.5	44.7	11.8	21.0	14.0	35.0
42	10.91	3.5	47.1	10.4	21.6	8.2	33.4
12	8.57	7.0	47.1	10.4	21.4	11.4	33.8
Mean	9.35	6.24	45.4	10.7	21.2	12.2	34.3
Std. dev.	1.51	1.16	1.48	0.56	0.80	3.04	0.80
Std. error	0.34	0.26	0.33	0.12	0.18	0.68	0.18

Table 14. Correlation coefficients for various factors related to tenderness (tenderness measured by shear and panel)

Factor	Tenderness	
	Shear	Panel
Sarcoplasmic N / Total fibrillar N	-0.43	0.41
Soluble fibrillar N / total fibrillar N	-0.69**	0.59**
percent of total moisture released	0.49*	-0.40

* $P < 0.05 = 0.444$
** $P < 0.01 = 0.561$

Part II

The effect of ionic strength on emulsifying capacity of actomyosin is presented graphically in figure 7. The similarity of the curves for protein solutions in 0.3 M KCl and 0.6 M KCl indicated that ionic strength, at least in this range, had no apparent influence on the emulsifying capacity of actomyosin. An ionic strength of 0.3 or 0.35 was used in many instances throughout this experiment because this approximated the ionic strength of the aqueous phase of a normal sausage batter.

The shape of the above curves was characteristic of emulsifying capacity curves for meat proteins which were studied in this experiment. Without exception, the proteins studied exhibited a greater emulsifying capacity as the protein concentration was reduced. This observation may be of practical significance to the sausage industry as the increased efficiency for stabilizing emulsions at reduced concentrations may partially compensate for the post mortem reduction in protein solubility shown in part I of this research.

The emulsifying capacity curves (figures 8 and 9) indicated that the sarcoplasmic fraction had a higher emulsifying capacity in the absence of salt. When protein concentration was plotted against total grams of oil emulsified, a marked difference was observed. The curve in case of the water solution was practically linear while a sigmoid type curve was obtained in case of the salt containing protein solution. In the presence of 0.6 M KCl the total grams of oil emulsified remained constant for a range in protein concentration from 0.3 mg. nitrogen/ml. to 1.0 mg. nitrogen/ml. In this range approximately 210 grams of oil were emulsified, regardless of protein concentration.

Swift et al. (1961) reported that the water soluble protein fraction in the absence of salt was incapable of stabilizing an emulsion and Hansen (1960), using histological techniques, found that the water soluble protein fraction was not observed at the oil-water interface of a sausage emulsion. The data in figure 8 tended to contradict these findings, however, in stability studies discussed later, it was found that emulsions stabilized with the water soluble proteins in salt solution were considerably more stable at normal pH's than emulsions stabilized with this protein fraction in the absence of salt.

The behavior of myosin and actomyosin could not be studied at low ionic strength or in pure water as they were insoluble under these conditions. However, actin once extracted from muscle tissue was completely soluble in water. Curves representing the emulsifying capacity of myosin in 0.3 M KCl and actin in both 0.3 M KCl and water are presented in figure 10. Obvious differences occurred in the emulsifying capacities

of these fractions. The presence of salt greatly depressed the effectiveness of actin. The emulsifying capacity of myosin was about midway between the above mentioned actin solutions. This relationship was always observed regardless of protein concentration.

About seven percent of the nitrogen in muscle is TCA soluble, i.e., non-protein nitrogen. Swift et al. (1961) were of the opinion that non-protein nitrogen compounds had no role in the formation or stabilization of emulsions. In the present study the phosphate buffer extracted sarcoplasmic fraction was subjected to dialysis which removed practically all of the non-protein nitrogen and caused the precipitation of the globulin X fraction. Curves of emulsifying capacity of this fraction before and after dialysis arepresented in figure 11. From these curves it appeared that neither globulin X nor the non-protein components was involved in emulsion formation. A noticeable difference was observed only at the lowest concentration, where the emulsifying capacity of the non-dialyzed fraction was the highest. A water extracted sarcoplasmic fraction was also dialyzed to remove non-protein nitrogen. A complete curve was not determined in this case, but approximately identical emulsifying capacities for dialyzed and non-dialyzed protein solutions at several protein concentrations indicated that non-protein compounds had no effect in forming emulsions.

The effect of pH on emulsifying capacity was studied with the actomyosin and sarcoplasmic fractions (figures 12 and 13). The pH effect in this regard seemed to be quite small in that the only noticeable difference due to pH occurred in the case of actomyosin (figure 13), in which

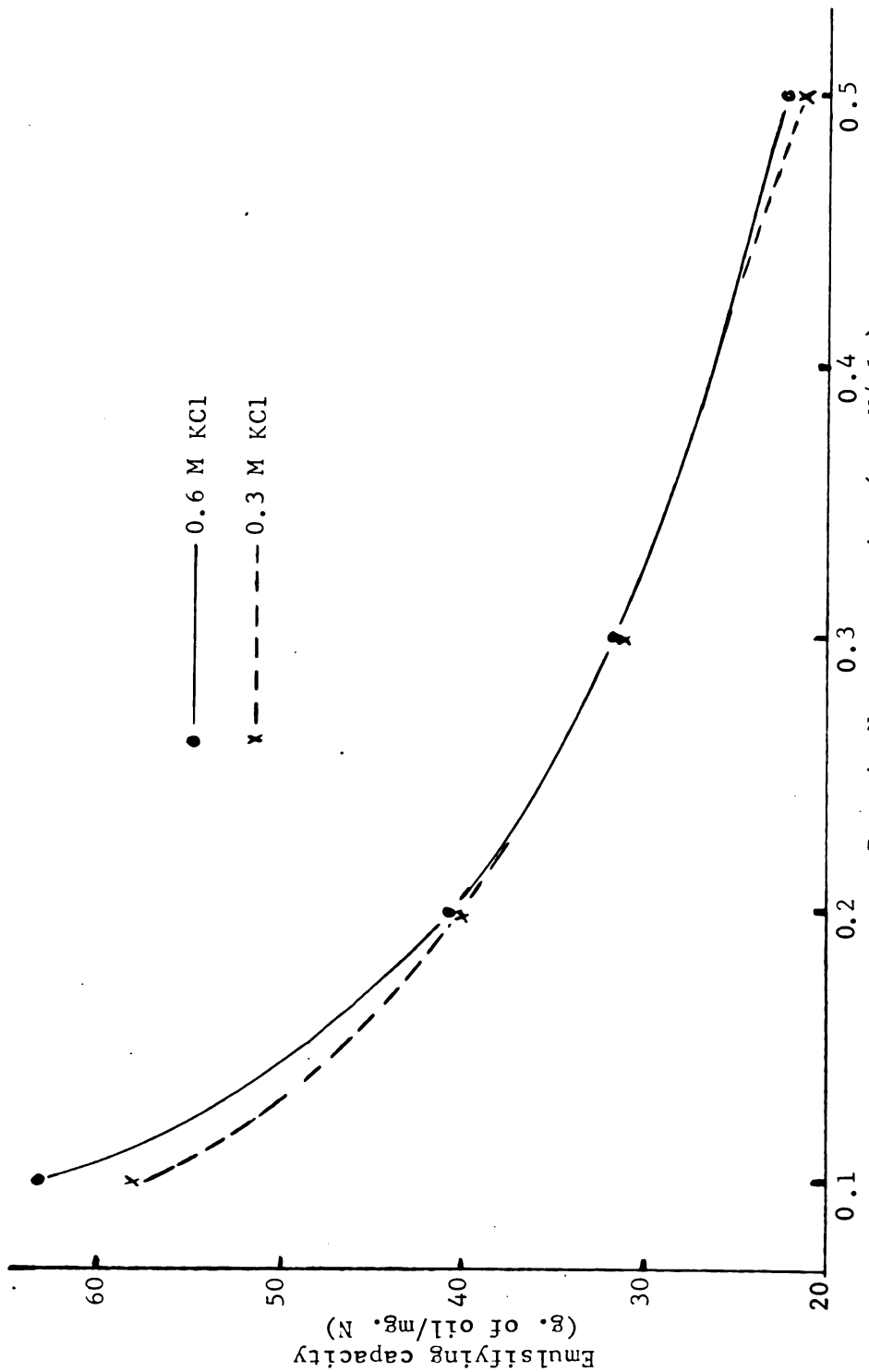

Figure 7. Emulsifying capacity of purified actomyosin vs. concentration of protein N in aqueous phase (Protein in 0.6 M and 0.3 M KCl)

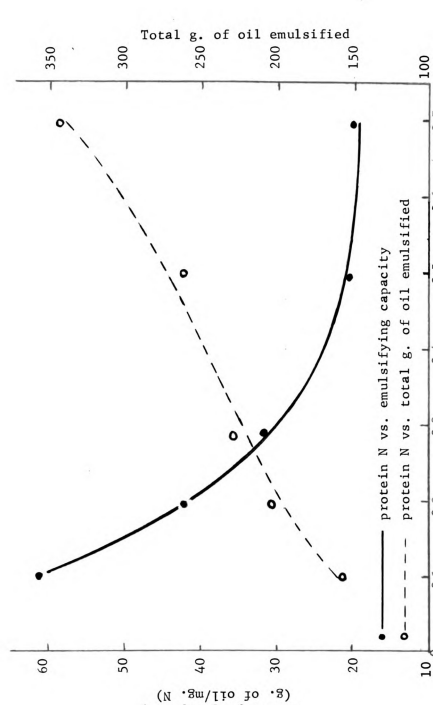

Figure 8. Emulsifying capacity of water soluble sarcoplasmic protein and g. of oil emulsified vs. concentration of protein in aqueous phase (u = 0)

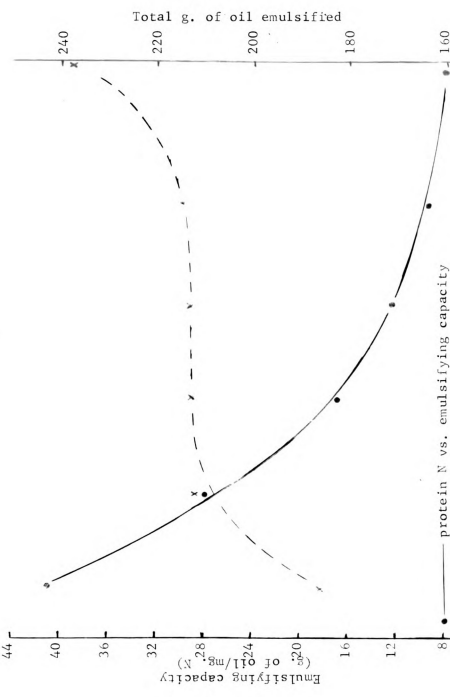

Figure 9. Emulsifying capacity of water soluble sarcoplasmic protein and g. of oil emulsified vs. concentration of protein N in aqueous phase (protein in 0.6 M KCl).

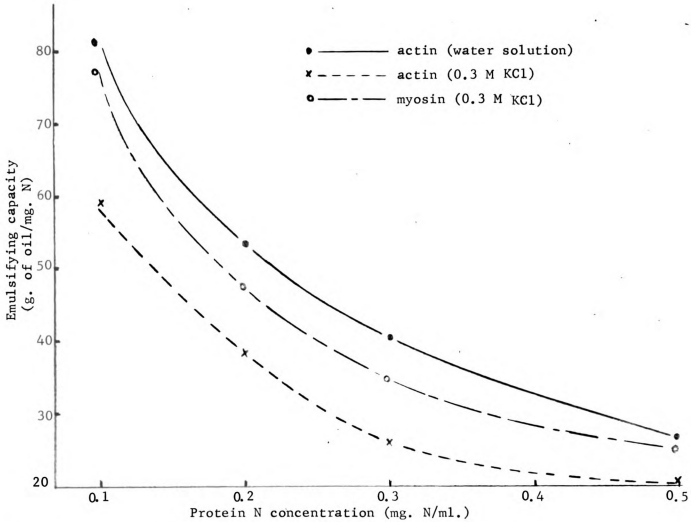

Figure 10. Emulsifying capacity of actin and myosin vs. concentration of protein N in aqueous phase.

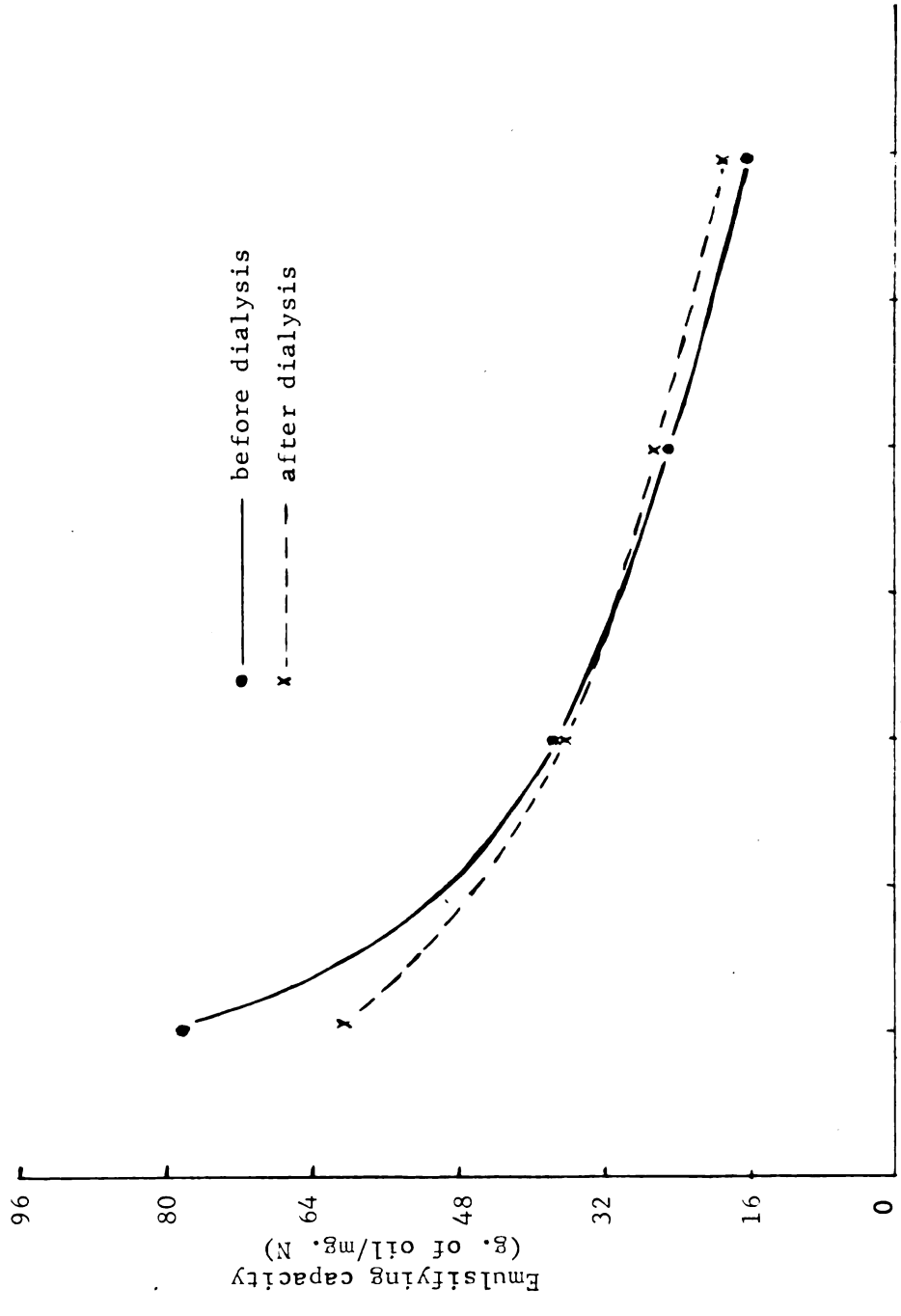

Figure 11 Emulsifying capacity of sarcoplasmic proteins (buffer extracted, u = .05. pH = 7.6) before and after dialysis vs. concentration of protein N in aqueous phase.

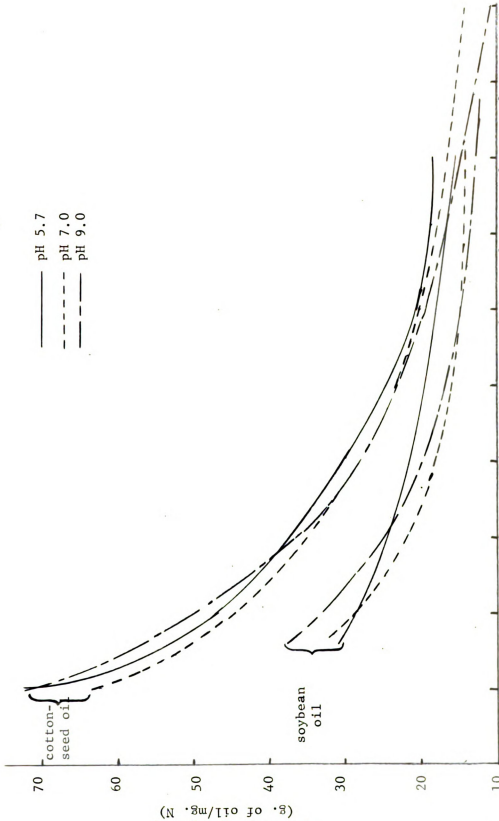

Figure 12. Emulsifying capacity of water soluble sarcoplasm proteins vs. protein N concentration of aqueous phase as affected by pH and kind of oil.

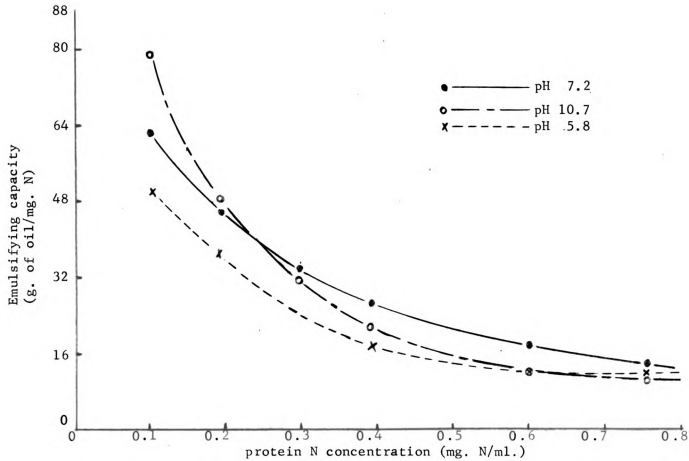

Figure 13. Emulsifying capacity of purified actomyosin vs. concentration of protein N in aqueous phase as affected by pH (u = 0.65)

case the emulsifying capacity was lowered slightly at low pH. At pH 5.8 actomyosin is approaching its isoelectric point which probably has some effect on its ability to form at an interface.

Data obtained from pH studies involving two kinds of oil (soybean and cottonseed) are presented in figure 12. At lower protein concentrations apparent differences between the two oils were observed, however, the relative effect of pH for each oil did not differ, indicating that results obtained from one oil could be applied to another oil.

Information on stability of emulsions prepared from the various protein fractions at various pH's and ionic strengths is presented in tables 15 and 16. Actomyosin and myosin were studied at only one ionic strength, 0.35. The low emulsifying capacity of actin, myosin and actomyosin at low pH necessitated a reduction in the amount of added oil. These proteins produced very unstable, short lived emulsions at low pH. Actomyosin and myosin at near neutral pH's produced very similar emulsions which were superior to emulsions stabilized by any other proteins at any pH or ionic strength. Myosin and actomyosin also produced fairly stable emulsions at alkaline pH's, although these were not as stable as those near neutrality.

Actin produced emulsions with extremely low stability at all pH's and ionic strengths studied. The possibility remains that actin in the fresh muscle may act differently than the actin utilized in this experiment. The preparation of an acetone powder constitutes a rather harsh treatment for protein extraction, however, more than one third of the actin obtained in this manner was of the active type, i.e., it combined chemically with myosin.

Table 15. Characteristics of emulsions prepared with actomyosin and sarcoplasmic protein fractions.

Type of protein (conc. =0.5 mg. N/ml.)	pH	Solvent	g. of soybean oil	Emulsion characteristics			
				Texture	Color	Viscosity	Time stable
Actomyosin	5.4	0.3 M KCl 0.05 M KH_2PO_4	160	coarse	yellow	intermediate	7 days
Actomyosin	6.7	0.35 M KCl	200	smooth	white	very high	> 3 weeks
Actomyosin	8.0	0.3 M KCl 0.05 M $NaHCO_3$	160	smooth	white	very high	> 3 weeks
Actomyosin	10.7	0.3 M KCl 0.05 M $NaHCO_3$	200	smooth	white	very high	2 weeks
Sarcoplasmic	5.5	Water	200	smooth	yellow	low	3 days
Sarcoplasmic	5.6	0.35 M KCl	200	smooth	yellow	low	2 weeks
Sarcoplasmic	6.7	0.30 M KCl 0.0157 M K_2HPO_4 0.0035 M KH_2PO_4	160	grainy	yellow	low	12 hours
Sarcoplasmic	7.0	0.0157 M K_2HPO_4 0.0035 M KH_2PO_4	200	fairly smooth	yellow	low	2 days
Sarcoplasmic	9.0	0.017 M Na_2CO_3	200	smooth	white	intermediate	10 days
Sarcoplasmic	10.0	0.30 M KCl 0.017 M K_2CO_3	125	fairly smooth	yellow	very low	3 hours

Table 16. Characteristics of emulsions prepared with actin, myosin and crude protein fractions.

Type of protein (conc.=0.5 mg.N/ml.)	pH	Solvent	g. of soybean oil	Emulsion characteristics			
				Texture	Color	Viscosity	Time stable
Actin	5.7	0.05 M KH_2PO_4	150	very smooth	yellow	low	< 36 hours
Actin	7.2	water	200	"	"	"	"
Actin	7.2	0.35 M KCl	200	"	"	"	"
Actin	8.0	0.05 M $NaHCO_3$	200	"	"	"	"
Actin	8.0	0.05 M $NaHCO_3$ 0.30 M KCl	200	"	"	"	"
Myosin	5.3	0.30 M KCl 0.05 M KH_2PO_4	125	coarse	yellow	intermediate	3 days
Myosin	6.8	0.35 M KCl	200	smooth	white	very high	> 4 weeks
Myosin	8.0	0.30 M KCl 0.05 M $NaHCO_3$	200	smooth	white	high	2 weeks
Crude extract	6.7	0.30 M KCl 0.003 M Na_2CO_3 0.01 M $NaHCO_3$ 0.05 M KH_2PO_4	200	smooth	yellow	intermediate	24 hours
Crude extract	7.6	0.30 M KCl 0.003 M Na_2CO_3 0.01 M $NaHCO_3$	200	smooth	yellow	intermediate	24 hours

A great deal of variation was observed in the stability behavior of emulsions prepared from the sarcoplasmic proteins. At low pH, i.e., 5.5, (approximately the pH of post rigor fresh meat) and in the presence of 0.35 M KCl, the sarcoplasmic fraction was superior to any other fraction in regard to emulsion stability. If no salt was present at pH 5.5, the emulsion broke in about two or three days which was very similar to those emulsions prepared with sarcoplasmic protein solutions at pH 7.0 and at ionic strength 0.05. With these protein solutions at pH 9.0 and ionic strength 0.05, emulsions had more desirable qualities and were stable for about 10 days. Proteins at alkaline pH's and ionic strength's of 0.35, produced very unstable emulsions which broke in a few hours. Emulsions prepared with crude protein extracts (containing primarily sarcoplasmic and actomyosin proteins in approximately equal proportions) were typical of emulsions prepared with sarcoplasmic proteins in regard to stability characteristics.

Actomyosin and myosin in their native states are probably completely utilized in the interface of oil in water emulsions. The protein nitrogen in the aqueous phase recovered from a broken emulsion that was stabilized by actomyosin or myosin, probably consisted of previously denatured actomyosin and myosin and contaminating protein of sarcoplasmic origin. As the emulsions were prepared at room temperature some of the myosin and actomyosin, which are extremely heat labile, were undoubtedly partially denatured before emulsification began and their interfacial activity thereby reduced.

The amount of protein nitrogen which retained its solubility in the aqueous phase after maximum emulsifying capacity was attained furnished additional information on the activity of the muscle proteins in emulsion systems (table 17). The pH effect in this regard was investigated in the case of

Table 17. Percent protein nitrogen remaining soluble in the aqueous phase after maximum amount of oil was emulsified.

Type of protein	pH	Ionic strength	% Nitrogen remaining soluble in the squeous phase
Actomyosin	5.8	0.65	8.5
"	7.2	0.65	11.3
"	10.7	0.65	13.7
Actin	7.6	0.30	48.4
"	7.6	0.0	59.0
Myosin	6.8	0.30	10.0
Sarcoplasmic	5.7	0.0	40.2
"	7.0	0.05	72.5
"	9.0	0.05	78.4

actomyosin and the sarcoplasmic fractions. There appeared to be a definite pH effect in the case of the sarcoplasmic fraction, however, the variation due to pH in the case of actomyosin was small. The amount of sarcoplasmic protein accumulating at the interface was twice as great at the low pH (5.7) than at the higher pH's. This was reflected in the greater stability observed in emulsions prepared with sarcoplasmic proteins at low pH. At the neutral and alkaline pH, the sarcoplasmic fraction showed a great deal of variation between duplicates which was not observed with any of the other proteins studied. The amount of actin recovered from broken emulsions was in the same range as the amount of sarcoplasmic protein recovered. This was also reflected in the poor stability qualities of emulsions prepared with actin.

The important observation in table 17 is the small amounts of protein recovered in the aqueous phase of actomyosin and myosin stabilized emulsions in relation to the actin and sarcoplasmic fractions. It appears that the

amount of protein denatured at the oil-water interface is directly related to the stability of the resulting emulsion.

Interfacial tension data have been utilized for many years to furnish an objective measurement of the interfacial activity of various emulsifying agents. Clayton (1928) reported that the method yielded acceptable results with soap stabilized emulsions, however, it was found in the current experiment that drop volume was not a valid method for the study of the interfacial activity of the muscle proteins (data from this experiment in table 18). This same conclusion was reached by Briggs and Schmidt as early as 1915 when they studied emulsifying properties of gelatin. They stated that the great elasticity and strength of the semi-solid protein film allowed the drop the expand as if it was contained in a rubber bag which was firmly attached to the delivery pipette. Such drops become very large because they are detached from the pipette with considerable difficulty.

Table 18. Relative interfacial tension of some protein solution-oil systems determined by the drop volume method.

Type of protein	pH	Ionic strength	Relative interfacial tension
Myosin	6.8	0.35	0.89
"	8.0	0.35	0.72
"		6 M urea	0.57
Actin	7.2	0.0	0.56
"	7.2	0.30	0.52
"	8.0	0.05	0.68
Sarcoplasmic	5.5	0.0	0.63
"	5.6	0.60	0.58
"	7.0	0.05	0.83
"	9.0	0.05	0.28
"		6 M urea	0.57
Actomyosin	6.7	0.35	0.82
"	8.0	0.35	0.72
"		6 M urea	0.66

SUMMARY AND CONCLUSIONS

Part I

A procedure was developed to fractionate the major nitrogen containing components of muscle. This procedure was utilized to determine the protein composition of the infraspinatus muscle of a group of 12 beef carcasses composed of bulls, heifers and cows, and also to determine the protein composition of the longissimus dorsi of 20 yearling bulls. Four of the group of 20 bulls were the same bulls from which the infraspinatus was fractionated.

Results showed that bulls had a higher percent of fibrillar protein and more total nitrogen per unit of muscle tissue than the other two groups studied. The longissimus dorsi contained more total nitrogen per unit of muscle tissue and almost twice as much sarcoplasmic protein as the infraspinatus. The longissimus dorsi contained less fibrillar protein and non-protein nitrogen than the infraspinatus.

Variation in protein solubility at three periods post mortem was also investigated in connection with the fractionation studies of the infraspinatus. A slight variation was noted in the extractability of the sarcoplasmic fraction with a general increase from slaughter to seven days. Fibrillar protein solubility was greatest at slaughter, and decreased significantly to a minimum at 24 hours. After seven days, fibrillar protein solubility had increased only slightly in the case of cows and heifers, while in the case of bulls it increased to almost the level observed immediately after slaughter. Fibrillar protein solubility was higher for bulls than cows and heifers at all times studied. Water-holding capacity followed the fibrillar protein solubility pattern very closely for each class of animals. The results obtained in this

study are possibly somewhat different from the changes that would occur in an uncut muscle.

A portion of the data obtained from the fractionation of the longissimus dorsi of the previously mentioned group of 20 bulls was correlated with tenderness measurements of an adjacent portion of the longissimus dorsi. The following factors were correlated with tenderness as measured by shear and panel: sarcoplasmic protein nitrogen/total fibrillar protein nitrogen; soluble fibrillar protein nitrogen/total fibrillar protein nitrogen; and percent total water released. Fibrillar protein solubility was highly correlated with tenderness ($r = -0.69$ for shear and $r = 0.59$ for panel). An r value of 0.49, significant at the 5 percent level, was found between water-holding capacity and tenderness as measured by the shear.

Part II

Emulsifying capacity curves for actin, myosin, actomyosin and the sarcoplasmic fraction of beef intracellular muscle proteins have been determined. Without exception, the emulsifying capacity increased as protein concentration decreased within the concentration range studied. The effect of pH on emulsifying capacity was negligible in the case of the sarcoplasmic proteins at an ionic strength of 0.05. The pH may have a slight depressing effect on the emulsifying capacity of actomyosin at low pH. The presence of salt greatly depressed the emulsifying capacity of actin. Variation in salt concentration between 0.3 and 0.6 M did not affect the emulsifying capacity of actomyosin. Non-protein nitrogen compounds and the globulin X fraction of the sarcoplasmic proteins were shown to have no influence on emulsifying capacity. In regard to emulsifying capacity, the proteins studied ranked from greatest to least

as follows: actin (ionic strength = 0), myosin, actomyosin, sarcoplasmic and actin (ionic strength = 0.3).

Stability of emulsions prepared with these same fractions and with a crude extract was also studied under various conditions of ionic strength and pH. At neutral pH and at an ionic strength of 0.35, myosin and actomyosin produced similar emulsions with excellent stability. The sarcoplasmic fraction produced emulsions which varied greatly in stability. The most desirable emulsions, from a stability standpoint, prepared with this fraction were at low pH (5.5 - 5.7) and 0.35 ionic strength, and the least desirable were those emulsions prepared with protein solutions at high pH and high ionic strength. Under no conditions did the emulsions prepared with the sarcoplasmic fractions compare favorably with myosin and actomyosin fractions at neutral pH. However, at low pH (5.5 - 5.7), which is approximately the pH of meat, the sarcoplasmic fraction produced the most stable emulsions. Emulsions prepared with actin were very unstable and under all conditions studied broke within 24 hours.

An analysis of the protein nitrogen remaining soluble in the aqueous phase after the maximum amount of oil was emulsified affords some interesting data. The amount of myosin and actomyosin accumulating at the oil-water interface was much greater than the amount of either actin or the sarcoplasmic protein. Such data are generally in agreement with the superior stabilizing qualities of actomyosin and myosin.

The fibrous structure of myosin and actomyosin is probably an important factor in emulsion stabilization. Such molecules have a great number of available polar and non-polar side groups essential for interfacial activity opposed

to the sarcoplasmic proteins which are of the globular type and are relatively spherical in shape and present a minimum of such side groups. As little is known about the structure of actin, it would be difficult to explain its behavior on this basis.

Results of interfacial tension studies carried out by the drop volume method indicated that this procedure was unsatisfactory for the study of emulsions prepared from the muscle proteins.

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APPENDIX

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

List of solutions:

1. Weber Edsall solution (for protein fractionation, figure 2) u = 0.67, pH 9.
2. Phosphate buffer (for protein fractionation, figure 1, and isolation of sarcoplasmic fraction) u = 0.05, pH 7.6.
3. KCl phosphate buffer (for isolation of myosin) u = 0.57, pH 6.5.
4. Sodium bicarbonate (for isolation of actin) u = 0.05, pH 8.2.
5. Weber Edsall solution (for isolation of actomyosin) u = 0.67, pH 9.

Composition:

Solution	Salt	Molarity	g./liter
1.	NaCl	0.60	35.04
	$NaHCO_3$	0.04	3.36
	Na_2CO_3	0.01	1.06
2.	K_2HPO_4	0.156	2.71
	KH_2PO_4	0.0035	0.475
3.	KCl	0.30	22.35
	KH_2PO_4	0.09	12.24
	K_2HPO_4	0.06	10.44
4.	$NaHCO_3$	0.05	4.20
5.	KCl	0.60	44.70
	$NaHCO_3$	0.04	3.36
	Na_2CO_3	0.01	1.06

Appendix B. Complete calculated data from infraspinatus muscle of 12 beef animals.

Animal No.	Sarcoplasmic protein nitrogen (mg./g. tissue)			Soluble fibrillar protein nitrogen (mg/g. tissue)		
	Slaughter	24 hours	7 days	Slaughter	24 hours	7 days
Cows:						
1	4.7	5.5	6.0	10.6	5.0	5.9
2	4.3	5.3	4.9	9.7	4.9	5.1
3	5.2	5.6	5.6	10.9	3.2	3.4
Heifers:						
1	6.4	7.8	7.7	10.8	5.0	3.9
2	5.0	7.1	5.0	15.0	3.8	7.8
3	5.6	6.5	5.6	10.7	2.7	7.2
4	6.6	5.0	7.1	9.9	4.1	4.8
5	5.1	4.4	7.7	10.5	4.4	4.7
Bulls:						
1	4.6	5.9	8.0	12.8	5.9	9.0
2	5.7	7.0	-	14.8	7.8	-
3	4.5	7.0	7.8	12.6	4.7	11.7
4	5.0	8.4	7.3	13.0	6.0	14.1

Appendix B. Complete calculated data from infraspinatus muscle of 12 beef animals. (continued)

Animal No.	Percent of total water released			pH		
	Slaughter	24 hours	7 days	Slaughter	24 hours	7 days
Cows:						
1	42.7	48.3	44.9	6.6	6.1	5.9
2	48.6	50.2	48.9	6.7	5.7	5.9
3	45.7	50.3	47.8	6.4	5.8	5.7
Heifers:						
1	43.9	47.5	45.5	6.45	5.8	6.0
2	44.5	47.1	47.9	6.35	5.6	5.8
3	46.8	45.8	47.7	6.70	5.85	5.8
4	48.8	52.5	47.7	6.50	5.70	5.8
5	44.8	51.6	49.3	6.50	5.70	5.7
Bulls:						
1	44.7	48.5	47.5	6.7	5.55	5.65
2	47.0	44.2	--	6.4	--	--
3	47.9	48.5	47.1	6.5	5.5	5.4
4	47.1	47.1	44.6	6.65	5.55	5.5

Appendix B. Complete calculated data from *infraspinatus* muscle of 12 beef animals. (continued)

Animal No.	Total fibrillar protein nitrogen (mg./g. tissue)	Stroma protein nitrogen (mg./g. tissue)	Total nitrogen (mg./g. tissue)	Non-protein nitrogen (mg./g. tissue)	Fat (%)
Cows:					
1	22.7	-0.9	31.3	4.0	5.0
2	19.4	2.2	30.2	3.3	8.6
3	20.8	5.0	33.5	2.4	4.9
Heifers:					
1	22.0	-1.4	31.1	3.4	5.0
2	20.5	4.3	34.3	3.4	5.7
3	20.6	3.2	33.5	3.6	6.3
4	22.6	1.7	32.6	2.5	3.3
5	18.0	6.2	31.5	2.5	3.2
Bulls:					
1	21.2	4.3	33.9	3.1	1.8
2	24.4	-0.4	33.2	2.9	2.8
3	23.2	0.6	32.6	3.1	2.3
4	22.6	1.3	33.7	3.1	2.3

Appendix C. Complete calculated data from *longissimus dorsi* muscle of 20 bulls.

Animal No.	Sarcoplasmic protein nitrogen (mg./g. tissue)	Total fibrillar protein nitrogen (mg./g. tissue)	Soluble fibrillar protein nitrogen (mg./g. tissue)
247	10.1	22.2	8.2
23	10.4	21.6	10.6
3	10.2	20.4	13.4
37	10.1	20.9	15.0
32	10.7	21.3	8.2
15	11.3	20.7	14.6
25	11.1	20.7	8.0
50	11.9	21.3	17.4
20	10.2	20.6	11.2
700	10.8	19.6	11.6
361	10.1	20.9	12.4
22	10.1	20.5	14.2
21	10.7	21.5	15.0
672	10.7	23.3	9.6
47	11.5	21.3	18.2
6	10.2	21.4	12.2
720	11.2	22.2	10.2
29	11.8	21.0	14.0
42	10.4	21.6	8.2
12	10.4	21.4	11.4

Appendix C. Complete calculated data from *longissimus dorsi* muscle of 20 bulls. (continued)

Animal No.	Non-protein nitrogen (mg./g. tissue)	Stroma protein nitrogen (mg./g. tissue)	Total nitrogen (mg./g. tissue)
247	2.2	0.0	34.5
23	2.2	0.1	34.3
3	2.2	1.3	34.1
37	2.4	0.1	33.5
32	2.2	1.1	35.3
15	2.2	0.6	34.8
25	2.4	0.4	34.4
50	2.2	-0.1	35.3
20	2.2	2.4	35.4
700	2.2	1.9	34.5
361	2.2	-0.9	32.3
22	2.2	1.3	34.1
21	2.2	-0.3	34.1
672	2.2	-2.9	33.3
47	2.4	-0.4	34.8
6	2.0	0.3	33.9
720	2.2	-0.4	35.2
29	2.2	0.0	35.0
42	2.2	-0.8	33.4
12	2.2	-0.2	33.8

Appendix C. Complete calculated data from *longissimus dorsi* muscle of 20 bulls. (continued)

Animal No.	% of total water released	pH	Tenderness	
			Shear	Panel
247	47.6	5.60	10.60	5.4
23	46.5	5.55	12.30	4.1
3	45.3	5.60	9.05	7.3
37	45.4	5.45	9.27	7.4
32	46.3	5.70	12.50	4.7
15	46.6	5.60	9.68	6.0
25	43.9	5.55	9.82	6.8
50	42.1	5.45	7.33	6.9
20	43.6	5.60	10.64	5.4
700	46.3	5.50	7.41	7.7
361	44.3	5.60	8.29	7.6
22	44.1	5.60	8.37	6.3
21	43.3	5.50	7.78	6.8
672	45.6	5.50	9.40	5.8
47	45.0	5.50	7.30	7.3
6	46.2	5.60	9.58	5.8
720	46.7	5.60	9.39	6.5
29	44.7	5.50	8.83	6.5
42	47.1	5.60	10.91	3.5
12	47.1	5.50	8.57	7.0

Appendix D. Protein concentration of aqueous phase and average amount (g.) of oil emulsified for various trials involving determination of emulsifying capacity.

Actomyosin at three pH's, u = 0.65.

Protein nitrogen concentration	g. of oil emulsified		
	pH		
	7.2	10.7	5.8
0.77	263	222	237
0.60	266	213	191
0.45	265	220	162
0.40	260	212	172
0.30	254	236	150
0.20	227	237	150
0.10	156	208	81

Actomyosin at two ionic strengths, pH 7.2

Protein nitrogen concentration	g. of oil emulsified	
	salt concentration (KCl)	
	0.6 M	0.3 M
0.5	278	270
0.3	237	240
0.2	200	204
0.1	168	144

Buffer extracted sarcoplasmic fraction before and after dialysis. u = 0.05, pH 5.8.

Protein nitrogen concentration	g. of oil emulsified	
	Before dialysis	After dialysis
0.8	341	370
0.5	316	350
0.3	278	272
0.1	185	153

Appendix D. Protein concentration of aqueous phase and average amount (g.) of oil emulsified for various trials involving determination of emulsifying capacity. (continued)

Sarcoplasmic fraction at two ionic strengths, pH 5.4.

Protein nitrogen concentration	g. of oil emulsified salt concentration (KCl)	
	0	0.6 M
1.2	-	240
0.9	-	214
0.7	348	212
0.5	262	212
0.3	233	216
0.2	205	-
0.1	153	183

Actin at two ionic strengths, pH 7.2.

Protein nitrogen concentration	g. of oil emulsified salt concentration (KCl)	
	0	0.3 M
0.5	344	252
0.3	302	194
0.2	268	190
0.1	203	147

Myosin in 0.3 M KCl, pH 6.8.

Protein nitrogen concentration	g. of oil emulsified
0.5	299
0.3	255
0.2	235
0.1	192

Appendix D. Protein concentration of aqueous phase and average amount (g.) of oil emulsified for various trials involving determination of emulsifying capacity. (continued)

Sarcoplasmic proteins at three pH's using two kinds of oil, u = 0.05.

Protein nitrogen concentration	g. of oil emulsified					
	pH 5.7		pH 7.0		pH 9.0	
	Cotton	Soy	Cotton	Soy	Cotton	Soy
1.0					300	
0.8	373	320	356		318	264
0.7	366		334			
0.6	303		323	230	300	228
0.5	270					
0.4	283	213	293	180		200
0.3					298	
0.2	231	143	225	146		180
0.1					195