EFFECT OF CHELATING AGENTS ON POST-MORTEM CHANGES IN MUSCLE

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ABSTRACT

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by Philip David Weiner

The primary objectives of this study were to determine the effects of various chelating agents and calcium and magnesium ions upon the development of rigor mortis and the subsequent quality factors of meat. This study consisted of four experiments using rabbits and one experiment using pigs. The effect of an intravenous antemortem injection of either EDTA, CDTA, EGTA or Na Oxalate on the shortening and elasticity of the excised semitendinosus muscle from rabbits and pigs was determined. The effect of micro-injections of $CaCl_2$ and $MgCl_2$ on the shortening and elasticity of paired semitendinosus muscles from rabbits was also studied (Experiment II and III). ATP degradation, pH changes, water holding capacity, color, cooking losses, shear values and protein fractionation were determined on the longissimus dorsi and semitendinosus muscles. The relationship between ATP levels, pH values, muscle shortening, cooking losses and shear values were also determined (Experiment II and Experiment V). In Experiment IV, properties of the fibrillar protein extracts were investigated by measuring the ATPase activity and changes in turbidity and viscosity.

The intravenous injection of EDTA greatly inhibited shortening of the semitendinosus muscle in Experiment I. In Experiment II, the shortening of the semitendinosus muscle was significantly ($P < .05$) inhibited by an intravenous injection of either EDTA, Na Oxalate, EGTA or CDTA. Shortening of the semitendinosus muscle from pigs treated with EDTA was

significantly less ($P < .05$) than for control pigs (Experiment V). Increased concentrations of $CaCl_2$ injected into the muscle resulted in a definite increase in shortening during the development of rigor. However, micro-injections of $MgCl_2$ had no effect on shortening.

No consistent differences in ATP levels at either 0 hr or 24 hrs post-mortem were observed between the different treatments in either Experiment I, II or III. The ATP values for the pigs treated with EDTA were generally higher than those for controls, but the differences were not statistically significant. A definite increase in the rate of ATP hydrolysis was observed following micro-injection of $CaCl_2$ into the semitendinosus muscle.

Treatment had no consistent effect upon the elasticity or rigidity of the uninjected semitendinosus muscle at 7 hrs post-mortem (Experiment II). Muscles which were elastic after 7 hrs post-mortem had significantly higher ($P < .01$) levels of ATP than those that were rigid and inextensible. The paired semitendinosus muscles that were injected with 0.1M $CaCl_2$ were always rigid and inextensible by 7 hrs post-mortem.

The pH values of the longissimus dorsi muscle of rabbits treated with EDTA were significantly lower ($P < .05$) than those for the group treated with EGTA and the control group (Experiment II). No other significant differences in pH values were observed between treatments at either 0 or 24 hrs post-mortem. Treatment had no significant effect on either the subjective color score or water holding capacity.

No significant differences in cooking losses were observed between the different treatments in Experiment I, II and V. The shear values

of the longissimus dorsi muscles from the rabbits treated with EDTA (Experiment I and II) were lower than the control groups, but the differences were not significant. The shear values of the hams treated with EDTA were significantly lower ($P < .05$) than shear values obtained for control hams. However, the effect of treatment on tenderness varied between muscles.

There was no appreciable change in the solubility of the sarcoplasmic protein fraction during the first 24 hrs post-mortem. There was, however, a definite increase in the amount of the fibrillar protein fraction extracted at high ionic strength during the first 24 hrs post-mortem. This was accounted for by an increase in the formation of actomyosin. Muscles extracted after 24 hrs post-mortem from rabbits treated with EGTA, CDTA and EDTA had less actomyosin than the control rabbits, but the differences were not significant.

A highly significant relationship ($P < .01$) was found between ATP and pH values within 10 min post-mortem for the muscles from rabbits (Experiment II) and for the muscles from both the treated and control pigs (Experiment V). The relationship between calcium release, ATP degradation and rate of glycolysis during the development of rigor mortis was discussed. Higher ATP values at 24 hrs post-mortem in the treated pigs were significantly related ($P < .05$) to less shortening of the semitendinosus muscle, and a more tender product.

No consistent differences were observed in ATPase activity, turbidity and viscosity between the Weber-Edsall extract isolated from pre-rigor

muscle and muscle in rigor. The addition of $MgCl_2$ decreased the rate of ATP hydrolysis and extended the clear phase of the Weber-Edsall extract. $MgCl_2$ also increased the rate at which pyrophosphate reduced the viscosity of the muscle extract obtained with Weber-Edsall solution. Pyrophosphate in the presence of magnesium was as effective in clearing actomyosin as ATP. As the ATP was hydrolyzed, the viscosity of the solution became greater than that obtained before the addition of ATP. However, samples cleared with pyrophosphate retained their low viscosity characteristics indefinitely.

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INTRODUCTION

The amount and state of the various protein constituents of meat are generally considered to be of primary importance in regard to its physical characteristics. For many years the amount of stroma proteins or connective tissue in meat was believed to be the principal source of variation in tenderness. However, with an improved understanding of the structure and biochemistry of muscle derived from research on muscle contraction and current knowledge of the biochemical and physical changes occurring during rigor mortis, the importance of the fibrillar proteins on the physical properties of meat has become more apparent.

Wierbicki et al. (1954) were among the first to suggest that the initial toughening of meat after slaughter was due to the formation of the actomyosin complex. The sliding-filament model of Hanson and Huxley (1955) provides an elegant explanation of the great increase in modulus of elasticity, which occurs as rigor is completed. It is now pretty well accepted that the mechanism of contraction with the onset of rigor mortis is similar to the phenomena occurring in living fibers and in isolated myofibrils.

Several workers (Howard and Lawrie, 1956; Kamstra and Saffle, 1959; Herring et al., 1965a; Weiner et al., 1966) have shown that various pre- or post-mortem treatments of animals or muscles may have a significant effect on the quality characteristics of meat. Much of the improvement resulting from such treatments has been attributed to changes in the state of the fibrillar proteins. From work with beef, Locker (1960),

Herring et al. (1965a) and Marsh and Leet (1966) have all concluded that the state of contraction is the major factor contributing to tenderness, if the effect of connective tissue is small.

Largely as a result of studies of muscle models and their constituent proteins, changes in the physical state of skeletal muscle have been related to interactions between actin and myosin in the presence of various concentrations of adenosine triphosphate (ATP), calcium and magnesium. It is now generally accepted that contraction of skeletal myofibrils, actomyosin and glycerinated fibers requires the presence of calcium in addition to magnesium and ATP (Martonosi and Feretos, 1960). These workers also pointed out that the relaxing effect of chelating agents and of relaxing factor extract on skeletal muscle can be explained by their ability to lower the free calcium concentration of the test system. Filo et al. (1965) suggested that the properties of actomyosin which are rate-limiting for its ATPase activity during superprecipitation or clearing are the same as those that are rate-limiting in situ for the development of tension or for relaxing.

The primary objectives of this study were to determine the effects that calcium and magnesium have on the development of rigor mortis and the subsequent quality factors of meat. The more detailed objectives were as follows:

1. To determine the effects of ethylenediamine tetraacetic acid (EDTA), ethylene glycol-bis (B-aminoethyl ether) -N,N-tetraacetic acid (EGTA) and 1,2 cyclohexanediaminetetraacetic acid (CDTA) on some of

the physical and biochemical changes during rigor mortis of muscle from rabbits and pigs.

2. To study the effect of added calcium and magnesium ions on the development of rigor mortis and their relationship with the breakdown of ATP.
3. To study the clearing effect and ATPase activity of actomyosin extracts from rabbit muscle and to observe the effects of magnesium and pyrophosphate.
4. To evaluate some of the quality characteristics of meat in relation to different treatments.

LITERATURE REVIEW

Structure of Skeletal Muscle

In order to more fully appreciate the nature and function of meat proteins, a brief picture of the structure of skeletal muscle is presented. Bendall (1966) presented a comprehensive outline of the structure of muscle. He stated that a complete muscle is surrounded by a dense layer of connective tissue called the epimysium. This gross muscle is divided into bundles of various size by a thinner layer of connective tissue, the perimysium, which supports the vascular system and nerves. These bundles are composed of a large number of longitudinal muscle fibers surrounded by the endomysium, a delicate extension of fine connective tissue.

In reviewing the structure of muscle, Huxley (1960) stated that the contractile cellular unit of skeletal muscle consists of long, cylindrical and multinucleated muscle fibers. The diameter of a muscle fiber usually lies in the range of 10-100 u. Huxley (1960) pointed out that each muscle fiber extends for a considerable distance along the length of the muscle, and some even extend along the entire length. The fibers terminate in a tendonous connection to the bone or the organ that they control. Muscular contraction is brought about by the shortening of these fibers.

The striated muscle fibers are composed of three main constituents, the sarcolemma, the myofibrils and the sarcoplasm, which includes the mitochondria, nuclei and a complex of internal membranes (Bloom and Fawcett, 1962). The sarcolemma is the name traditionally applied to the delicate membrane visible with light microscopy, which completely surrounds the muscle fiber.

Porter (1961) pointed out that the complex of internal membranes within a muscle fiber is composed of two main morphologically and functionally distinct systems. He stated that one of these is the sarcoplasmic reticulum, a set of generally longitudinally arrayed, thin, irregularly shaped vesicles located in the spaces between the myofibrils. It is so arranged that each serves as a "lace-like sleeve" enveloping a particular myofibril. Marsh (1960) showed that the relaxing factor, first described by Marsh in 1952, consisted of the fragmented sarcoplasmic reticulum. Porter (1961) referred to the second system as the T-system, a set of transverse tubules about 0.03 u in diameter. These evidently originate close to the sarcolemma or even touch it and penetrate laterally or transversely into the depths of each fiber. The T-system can invade the fiber at several points on the circumference and by branching can reach every fibril within a marginal sector.

Huxley (1960) described the myofibrils as the contractile elements of striated muscle. They contain three principal proteins -- myosin, actin and tropomyosin. The myofibrils have a diameter of 1 or 2 u, are very long, are arranged parallel to the long axis of the fibers and display a pattern of striations.

According to Bloom and Fawcett (1962), myosin and actin, the two principal structural proteins of the myofibril, alternate regularly along the length of the myofibril to give it a cross-banded appearance. Myosin, the thicker of the filaments, is anisotropic (doubly refractive) and appears dark on examination with the polarizing microscope. This dark

band is called the A band. Alternating with the A bands are isotropic (singly refractive) bands called I bands. These are referred to as the thin filaments and contain the actin component of muscle, and probably tropomyosin.

Bloom and Fawcett (1962) described other bands in the myofibril. The most obvious of these, the Z band, occupies the middle of the I band. The center of the A band appears paler than the rest and is called the H band. Within this is a thin middle strip designated by the letter M. Huxley (1960) stated that the repeating unit of the myofibril, the sarcomere, is the area between two adjacent Z bands. The length of the sarcomere is usually about 2 or 3 u, and therefore, each fiber contains thousands of sarcomeres.

Huxley (1960) described the spatial arrangement of the myosin and actin filaments as viewed with an electron microscope. He pointed out that in the region where the sets of filaments overlap, each thick filament is surrounded by six thin filaments which it shares with the six neighboring thick filaments in a hexagonal array. The thin filaments tend to occupy the trigonal points in the hexagonal lattice, and thus, each thin filament has three thick filaments around it. Huxley (1960) further indicated that each thick filament in a cross-striated muscle bears a large number of regularly spaced short lateral projections often referred to as cross bridges. These always appear to lie more or less at right angles to the filament axis and can link onto the adjacent thin filaments (Huxley, 1960). The cross links between filaments represent actin-myosin linkages and correspond to the formation of the actomyosin complex.

Muscle Proteins

Sarcoplasmic proteins

In a review of meat proteins, Bendall (1964) described the sarcoplasmic protein fraction of muscle as that material which can be extracted from finely homogenized muscle with either water or very weak salt solutions. These proteins occupy most of the space between the myofibrils. They consist of a mixture of biologically active proteins such as myoglobin, all the protein components of the glycolytic system and various phosphokinases. One of these sarcoplasmic constituents, the sarcoplasmic reticulum, has been intensively studied in recent years in regards to its role as a physiological relaxing factor in muscle contraction.

Electron micrographs of a membrane fraction isolated from homogenized rabbit muscle, showing the properties of the relaxing factor, indicated that it consists mainly of the vesicles of the endoplasmic reticulum (Ebashi and Lipmann, 1962). Marsh (1960) pointed out that this fraction of muscle is capable of removing calcium from the muscle medium. From studies showing that the removal of calcium has a direct connection with the onset of relaxation of muscle in the presence of ATP, Ebashi (1960, 1961) proposed that removal of calcium may be the mechanism by which the relaxing factor acts.

Experiments using physiological concentrations of ATP (Weber *et al.*, 1963) have shown that the vesicles of the relaxing factor are capable of reducing the concentration of ionized calcium in the surrounding medium to about 0.02 uM or less. This suggests that the relaxing factor can

compete successfully with the actomyosin for the available calcium through some mechanism of calcium accumulation.

Ebashi and Lipmann (1962) and Molnar and Lorand (1962) have independently proposed the existence of an energy-requiring system for the transport of calcium ions into the vesicles. Ebashi and Lipmann (1962) also showed that a constant supply of ATP was required for holding the calcium ions in the membrane fraction. A number of workers (Martonosi and Feretos, 1964; Hasselbach and Makinose, 1964; Fratantoni and Ashari, 1965; Weber et al., 1966; Yamamato, 1966) have confirmed the presence of an "active transport" system for calcium uptake by the sarcoplasmic reticulum.

It has recently been shown that calcium uptake by the sarcoplasmic reticulum is strongly dependent on temperature (Martonosi and Feretos, 1964) and pH (Briggs and Protzehl, 1957; Brown et al., 1963). Work by Hasselbach and Makinose (1964) revealed almost complete inhibition of the calcium pump following the addition of either Salyragan, oleic acid or adenosine diphosphate (ADP).

The results of Costantin and Podolsky (1964) gave evidence that the myofibrils contain a calcium inactivating mechanism, which allows relaxation to occur. More recently, Lee (1965) presented experimental results providing direct evidence in support of the concept that electrical stimulation releases calcium from the endoplasmic reticulum, and that the cessation of electrical stimulation is followed by re-uptake of the calcium ions by this system.

Sandow (1965) has recently reviewed the excitation-contraction coupling in skeletal muscle. He concluded that calcium ions bound in the vesicles of the sarcoplasmic reticulum are released throughout the interior of the fiber in response to the inward conduction of the activity signal from the sarcolemma by way of the T-tubules. Thus, wherever there are T-tubules, the calcium ions will be released so close to the myofibril that diffusion distances would be no more than 0.5 to 1 u.

Myofibrillar proteins

Szent-Györgyi (1960) reviewed the proteins of the myofibril. He described the myofibrillar proteins as being responsible for the filamentous organization of muscle and pointed out that they directly participate in contraction. He further stated that neutral salt solutions of high ionic strength are required for the extraction of the fibrillar proteins, but after extraction some of these proteins are soluble at a lower ionic strength. Szent-Györgyi (1960) also indicated that the resistance of these proteins to extraction is partly a result of the intimate association and interaction between the various proteins within the myofilaments. He pointed out that three well identified components (myosin, actin and tropomyosin) can be isolated from myofibrils of striated muscles. Hasselbach and Schneider (1951) showed that myosin composes 36% of the protein in muscle, while actin makes up 14-16%.

Myosin

Huxley (1960) described myosin as an elongated asymmetric protein of high molecular weight. He pointed out that myosin can be extracted

with strong salt solutions ($u > 0.4 - 0.5$) in the presence of either ATP or pyrophosphate and magnesium. He further stated that the actin that is present may be removed by a stepwise reduction in the ionic strength of the solution, thereby precipitating the actin as the acto-myosin complex.

In summarizing the general properties of myosin, Szent-Györgyi (1951) stated that the dispersion of myosin in solution depends on the pH and the nature and concentration of the salt solution. He described myosin as being readily soluble in strong salt solutions, such as 0.6 M KCl, at pH 6.5 - 7.0. Under these conditions, Szent-Györgyi (1951) found that myosin showed normal viscosity at a concentration of less than 0.2%. On lowering the salt concentration below 0.3 M KCl, he showed that the viscosity increased to a point of least solubility between 0.025 and 0.050 M KCl. At salt concentrations below 0.001 M KCl, he found that myosin dissolved with a great deal of swelling. Szent-Györgyi (1951) further indicated that salt-free solutions of myosin have an isoelectric point of 5.4, but the isoelectric point will change as the salt concentration is altered.

Szent-Györgyi (1951) also pointed out that magnesium has a high affinity for myosin at a concentration of 0.11 - 0.12 M. He further indicated that bound magnesium ions will cause the adsorption of ATP, which results in the negative charge of the myosin molecule.

On studying the components of myosin, Szent-Györgyi (1953) found that digestion of myosin by trypsin for a short time breaks down the molecule into two components. He referred to these components as light

(L) meromyosin and heavy (H) meromyosin. He showed that H-meromyosin is responsible for the ATPase activity of myosin and that it possesses the capacity of combining with actin of the intact myosin molecule. More recently, Huxley (1963) was able to reconstitute filaments from purified myosin, which are very similar in appearance to the thick filaments present in muscle. His results also showed that the myosin filaments are structurally polarized and possess a center of symmetry with all heads of the molecule facing away from the center.

Szent-Györgyi (1966) stated that L-meromyosin makes up the shaft of the myosin filament, while the cross bridges are a part of the H-meromyosin.

Huxley (1960) reported that there are over two hundred cross bridges extending from each thick filament.

Perry and Catterill (1965) proposed a scheme with two separate centers on each cross bridge of the myosin molecule. They stated that one of the centers on the cross bridge is involved in enzymatic activity, while the other is involved in formation of actomyosin. Levy and Ryan (1966) described a model of the myosin molecule that required both magnesium and ATP at two different sites on each cross bridge during muscle contraction. They showed that magnesium is bound very tightly at the hydrolytic site, while it is bound less firmly at the other site.

Martonosi and Meyer (1964) suggested that magnesium participates in the binding of pyrophosphate. These workers showed that the relative effectiveness of various chelating agents to inhibit the binding of pyrophosphate to myosin increased parallel with an increase in the stability

constant of the magnesium complex. They pointed out that an interesting case was that of EGTA, which was much less effective than EDTA in inhibiting the binding of pyrophosphate to myosin. They stated that EGTA forms a weak chelate with magnesium, whereas, the stability constant of its calcium chelate is equal to that of the EDTA-calcium complex. They interpreted these results to mean that removal of magnesium from the binding site with EDTA inhibited the binding of pyrophosphate to myosin. From these and other results, they concluded that the binding site for pyrophosphate on myosin is identical with a portion of the ATPase center of myosin -- presumably the site of the interaction with the phosphate chelate of ATP.

The feature of overwhelming importance in the myosin molecule is, of course, its enzyme activity as an adenosine triphosphatase. Mommaerts and Green (1954) showed that myosin ATPase has a true optimum at pH 6.4 and a minimum at about pH 7.3. The activity increased at still higher pH values, and another apparent optimum occurred at pH 9 or higher. The enzymatic properties are very sensitive to temperature or slight acidification (Needham, 1960).

In a review, Bendall (1964) pointed out that myosin ATPase is activated by calcium ions and inhibited by magnesium ions. Muhlrad et al. (1964) suggested that magnesium inhibits ATPase activity by the formation of an unfavorable complex. Kielly et al. (1956) found that EDTA activates ATP splitting in the presence of monovalent cations. They further indicated that under these conditions, the activity is higher than in the

presence of optimal calcium ion concentrations. Szent-Györgyi (1960) suggested that EDTA activation is due to chelation with strongly bound magnesium or calcium. He further pointed out that these ions may be involved in the binding of the nucleotide base or in its fixation in an orientation favorable to the hydrolytic process.

In a review, Needham (1960) stated that there is an important difference between the ATPases of actomyosin and myosin. He pointed out that actomyosin ATPase is activated by magnesium while myosin ATPase is not. He further indicated that both the ATPase of actomyosin and myosin are activated by calcium.

According to Mommaerts and Green (1954), the enzymatic activity of isolated myosin ATPase was insufficient to explain the physiological utilization of ATP. They pointed out that the calcium concentration, for optimal ATPase activity (0.04 M), never occurs in muscle. In addition, they stated that the high concentration of magnesium present in muscle would result in inhibition of myosin ATPase activity.

Actin

Actin was first isolated by Straub (1942) from the residue left after the partial extraction of myosin from muscle. The residue was dried with acetone to denature all of the remaining proteins, except actin, and to remove the lipids. The actin was then extracted from the remaining material with distilled water. In a review of the general properties of actin, Bendall (1964) stated that actin extracted in this manner is present in the globular or G-form and has the appearance of

separate beads. He further pointed out that G-actin contains ATP, which is readily exchanged with the ATP in the medium. He then indicated that if actin is to remain in its monomeric (G-actin) form, some ATP must be present in the medium to exchange with the bound ATP.

Bendall (1964) also stated that the G-form possesses the remarkable property of polymerization on the addition of neutral salts, resulting in the formation of chain-like molecules of fibrous or F-actin. He stated that there was also a simultaneous change of the bound ATP to bound ADP with liberation of a molecule of inorganic phosphate. Thus, he pointed out that G-actin is a complex of ATP with actin, while F-actin is a complex of ADP and actin. Purified actin (free of myosin) does not possess any enzymatic properties (Needham, 1960).

In summarizing the solubility properties of actin, Szent-Györgyi (1960) pointed out that even though G-actin is soluble in water or low concentrations of neutral salt solutions, these solutions do not extract actin readily from muscle. He further stated that circumstantial evidence indicates that the structure of the chain-like molecule of F-actin is the same as the biologically active actin filaments. Szent-Györgyi (1960) thus suggested that the presence of this fibrous form of actin in muscle may explain the difficulties encountered in extraction. However, he pointed out that agents which depolymerize actin, like potassium iodide, extract actin readily.

Szent-Györgyi (1951) stated that actin, as it commonly occurs, is a calcium actinate. Even in carefully washed preparations, actin always contains about four atoms of calcium per molecule. Thus, there is one firmly bound calcium molecule for every 18,000 g of actin.

Tropomyosin

Tropomyosin is also present in small amounts in mammalian muscle (Bendall, 1964). This protein resembles myosin in its chemical constitution, but differs markedly in most of its properties (Bailey, 1948; Tsao et al., 1951). Huxley (1963) has recently demonstrated by electron microscopy that a crystal lattice very similar to that of tropomyosin exists in very thin cross sections at the level of the Z line.

In a review of muscle proteins, Bendall (1964) pointed out that tropomyosin is a highly charged protein containing 240 acidic and 150 basic groups per 100,000 g. This compares with about 160 and 140 residues respectively, for myosin and 120 and 110 for actin. Bendall (1964) also stated that tropomyosin apparently does not possess any enzymatic properties and does not combine with either actin or myosin in solution.

Stroma Proteins

The stroma proteins are retained in the residue after prolonged extraction of a well homogenized muscle preparation with strong salt solutions (Szent-Györgyi, 1960). Szent-Györgyi (1960) pointed out that this protein contains some material of a collagenous nature contributing to the structure of the sarcolemma and possibly to the Z membrane.

Interaction of Actin and Myosin

In a review on the interaction of actin and myosin, Bendall (1964) stated that when solutions of F-actin and myosin are mixed at an ionic strength of about 0.6, the viscosity of the solution rises above that of the sum of the two components. He further indicated that the change in viscosity is accompanied by a strong increase in light scattering and an increase in molecular weight. The complex formed is referred to as actomyosin. Bendall (1964) pointed out that the addition of ATP or pyrophosphate and magnesium to a solution of actomyosin at a high ionic strength results in the dissociation of actomyosin into its component parts, actin and myosin. He stated that the dissociation into actin and myosin results in an immediate fall in viscosity back to the sum of the viscosities of the two components, and also a return of the light scattering intensity to the low value characteristic of these components.

The addition of F-actin to myosin also modifies the enzymatic properties of myosin so that it becomes strongly activated by magnesium, but is no longer activated by EDTA (Bendall, 1964). Extensive work (Watanabe and Sleator, 1957; Maruyama, 1962; Weber and Herz, 1962, 1963; Watanabe et al., 1964) has been carried out with various actomyosin systems in an attempt to more fully understand the mechanism of the various components involved in muscle contraction.

Watanabe and Sleator (1957) found that glycerinated muscle fibers shortened by about half their initial length on being treated with 4 mM ATP and magnesium. Upon addition of EDTA, ATP and magnesium, the shortened fibers relaxed rapidly to approximately their initial length. These

workers pointed out that EDTA manifests its relaxing effect only in the presence of magnesium (1 mM or above) and ATP (2 mM or more). These authors further showed that the addition of calcium ions at a level less than 2 mM completely reversed the relaxing effect of 4 mM of EDTA. The shortening effect of calcium required the presence of magnesium ions. From these results, Watanabe and Sleator (1957) concluded that calcium must act at a different site on the protein than magnesium in producing contraction. Ebashi et al. (1960) and Bendall (1964) have reported similar results with glycerinated muscle fibers.

Bendall (1953) showed that the addition of 20 mM of pyrophosphate resulted in slow lengthening of a glycerinated, previously shortened, muscle bundle. The addition of 4 mM $MgCl_2$ resulted in a five-fold increase in length. Ebashi et al. (1960) found that the lengthening of glycerinated muscle fibers by inorganic polyorthophosphate was markedly accelerated by EDTA and its analogues. In another study using glycerol-extracted rabbit psoas muscle, Bowen and Martin (1958) found that the tension which develops in isometric contraction and the shortening which occurs in isotonic contraction increased if the concentration of $MgCl_2$ was increased to 10^{-2} M. They showed that the ATPase activity of the fiber-bundles was not affected as the concentration of magnesium changed.

More recently, Watanabe et al. (1964) observed that magnesium had a triphasic effect on the ATP-induced tension of glycerinated fibers from rabbit psoas muscle. The first phase of the magnesium effect, the increase in tension with increasing concentrations of magnesium, was abolished by the metal chelators, DCTA and EDTA, but not by EGTA. They

pointed out that DCTA was much more effective than EDTA in abolishing the first phase. Watanabe et al. (1964) stated that the second phase of the magnesium effect consisted of a decrease in tension with increasing concentrations of magnesium between 25 uM and approximately 1 mM. They showed, however, that when the concentration of ATP was decreased from 5 mM to about 0.5 mM, the effect of magnesium on the second phase was reduced.

Watanabe et al. (1964) found that the third phase, the increase in tension with increasing concentrations of magnesium above 1 mM, was also reduced by metal chelators. They pointed out, however, that EGTA gave the greatest reduction of tension and DCTA the least. From these observations, these workers suggested that the first two phases are due to magnesium (probably in the form of a Mg-ATP complex) and that the third phase reflects the influence of a small amount of calcium.

Filo et al. (1965) found that the concentration of free calcium for contraction of glycerinated striated muscle fibers was 1.8×10^{-7} M. Maximum tension developed when the concentration was slightly greater than 10^{-6} M. The inhibition of contraction by magnesium concentrations greater than 0.1 M was completely reversed by the addition of 10^{-5} M free calcium. Martonosi and Feretos (1964) attributed the relaxing effect of chelating agents and of relaxing factor extract to their ability to lower the calcium concentration of the test system below 10^{-6} M in the presence of 5 mM ATP and 5 mM $MgCl_2$.

Using myofibrils, Perry and Grey (1956) found that EDTA strongly inhibited the magnesium-activated myofibrillar ATPase activity. They

showed, however, that the calcium activation of myofibrillar ATPase was much less sensitive to EDTA. More recent work by Weber and Herz (1963) showed that the addition of magnesium ions and EGTA to myofibrils reduced the amount of bound calcium. At an ionic strength of 0.1 M, these same workers found that a highly concentrated solution of myofibrils containing a considerable amount of bound calcium was superprecipitated, even if calcium had not been added. This shows that sufficient contaminating calcium was present to saturate the actomyosin of the myofibrils. When 75% of the bound calcium from the same preparation of myofibrils was removed by washing with EGTA and magnesium, no superprecipitation was observed.

Hasselbach (1957) and Kaldor and Gitlin (1964) have reported that magnesium ions inhibit myofibrillar ATPase activity, if the ionic strength of the solution exceeds 0.3 M KCl. In solutions of low ionic strength, however, magnesium ions activated myofibrillar ATPase.

Using DCTA and EGTA to control the concentrationsof free magnesium and calcium, Barron et al. (1966) studied the effect of adding actin to myosin on ATPase activity at 0.12 M KCl. At free magnesium concentrations of 2.8×10^{-9} M, they found that actomyosin ATPase activity was very low. As the free magnesium concentration was increased, the actomyosin ATPase activity reached a maximum at 2.8×10^{-6} M. Additional magnesium above this level decreased the enzyme activity. Perry and Cotterill (1965) suggested that modifications in the enzymatic properties of myosin by actin is of physiological significance.

Working with actomyosin at low ionic strengths (0.06 - 0.12 M), Weber and Herz (1962) and Yasui and Watanabe (1964) showed that low concentrations of magnesium (< 20 uM in the presence of 1 mM ATP) markedly increased the rate of superprecipitation. However, high concentrations of magnesium (in presence of 1 mM ATP) decreased both the speed and extent of superprecipitation. Earlier results of Tonomura and Yashimura (1960) indicated that a Mg-ATP complex inhibited the superprecipitation of actomyosin by binding to a specific site. As a consequence, ATPase activity diminished. Weber and Herz (1962) speculated that the inhibitory effect of magnesium on superprecipitation may be due to the bound Mg-ATP complex, since the inhibiting effect of high concentrations of magnesium is incomplete at low ATP concentrations.

Mommaerts and Hanson (1956) found that the addition of ATP to 0.6 M KCl actomyosin solution resulted in the dissociation of the complex into its component proteins. They estimated that 1 mole of ATP would dissociate one mole of actomyosin. These workers also showed that pyrophosphate, inorganic triphosphate in the presence of magnesium and a number of other triphosphorylated nucleotides have dissociating properties.

Noda and Maruyama (1958) stated that the best explanation of the change in flow birefringence properties on adding ATP (0.6 M KCl) to an actomyosin solution seemed to be the dissociation of the actomyosin into its component parts, actin and myosin. These workers also showed that at concentrations higher than 1 M, KCl alone had dissociating effects on actomyosin. Maximal dissociation occurred at 1.5 M KCl. When KOH or KI were added to solutions of actomyosin, the effect on flow birefringence

was much more pronounced than with ATP (Noda and Maruyama, 1958).

Marsh (1952) extracted rabbit muscle with 0.16 M KCl. Upon the addition of ATP, he noted an immediate increase in volume of the shrunken system, followed by superprecipitation. In the presence of 0.02% $CaCl_2$, the addition of ATP was followed by an immediate superprecipitation. On adding a water soluble protein factor from the supernatant to the extracted rabbit muscle, the addition of ATP resulted in an increase in volume of the shrunken system without any subsequent superprecipitation. However, the addition of $CaCl_2$ to this system resulted in superprecipitation. This factor has since been referred to as the relaxing factor in muscle contraction.

Hasselbach (1957) found that at a low ionic strength ATP caused dissociation of actomyosin in the presence of EDTA. In more recent work, Maruyama (1962) observed the clearing response of actomyosin solutions ($u = 0.06 - 0.08$, pH 7.0) if various chelating agents or muscle granules were present prior to the addition of ATP. In the absence of chelating agents, superprecipitation occurred immediately. He suggested that the mechanism of action of chelating agents appears to be the removal of trace amounts of calcium. To prove this, Maruyama (1962) showed that EDTA was not bound to the actomyosin. He also demonstrated that actomyosin in a calcium-free media showed a clearing response and low ATPase activity after ATP was added. The addition of a small amount of calcium resulted in superprecipitation with high ATPase activity. Similar results and conclusions have been reported by Bürany and Jaisle (1960) and Kaldor and Gitlin (1964) using EDTA or the natural relaxing factor.

Maruyama and Gergely (1962) presented data showing that the clearing of actomyosin (u = 0.1 - 0.15) on the addition of ATP in the presence of magnesium is due to its dissociation into the constituent proteins, F-actin and myosin. In interpreting the significance of the double refraction of flow, light scattering and viscosity data in terms of dissociation, these workers assumed that there is no interaction between dissociated myosin and actin particles. Using an electron microscope, Ikemoto et al. (1966) showed that on clearing a system of actomyosin (0.1 M) with ATP, the actin and myosin filaments are clearly distinguishable and show little or no association with each other. They pointed out that the separate existence of structures corresponding to actin and myosin, confirmed earlier conclusions that were deduced in a more indirect way regarding the dissociation of actomyosin during clearing.

Maruyama and Gergely (1962) explained the changes occurring in the ATPase activity of actomyosin during clearing and superprecipitation. They suggested that in the clear phase, actomyosin exists in a dissociated form. They pointed out that the ATPase activity of the dissociated form in the presence of magnesium is very low because it corresponds to that of myosin. As the ATP concentration decreases, the association of myosin and actin begins to take place again and the ATPase becomes activated by magnesium.

A number of investigators (Noda and Maruyama, 1958; Watanabe and Duke, 1960; Tonomura and Sekiza, 1961) have shown that pyrophosphate in the presence of magnesium is as effective in clearing actomyosin as ATP. Noda and Maruyama (1958) pointed out that the failure of some workers to

observe the effect of pyrophosphate may have been due to the absence of magnesium in the system. The results of Granicher-Frick (1965) indicate that the dissociating influence of the pyrophosphate system is conditioned by the Mg-pyrophosphate complex, while the Ca-pyrophosphate complex, free pyrophosphate ions, magnesium ions and calcium ions have no dissociating effect on the fibers.

Muscle Contraction

Hanson and Huxley (1955) proposed a sliding filament theory of muscle contraction. This theory states that muscle contracts without an over-all permanent change in the length of the constituent molecules. The theory was based on studies with the light microscope. Observations clearly indicated that shortening is brought about by a sliding movement of the actin filaments into the arrays of the myosin filaments in the A bands. The extension of the muscle reverses this movement. In a review on muscle contraction, Huxley (1960) stated that it is unlikely that muscle would contract by one process over one part of its range and by a different process over another. He thus concluded that the sliding process is the fundamental mechanism over the range of shortening, and that any folding up of actin or myosin filaments is probably the result of shortening rather than the cause.

From X-ray diffraction patterns of the sartorius muscle of the toad, Elliott et al. (1965) concluded that there is no overall change in the molecular arrangement within the actin or myosin filaments during contraction.

In a review of the muscle cell, Huxley (1960) pointed out that the results from early investigations with myosin, actomyosin, and glycerinated-muscle fibers suggested that ATP is intimately associated with contraction. This widely held theory has recently been confirmed by the direct demonstration of ATP breakdown as a result of work during single contractions of isolated intact muscle (Cain and Davies, 1962). The results of Cain and Davies (1962) also make it clear that in working muscle, creatine phosphoryltransferase can make the energy stored in CP available. Myokinase also operates in the system and can make twice as much energy available from ATP, while preventing large increases in ADP.

Huxley (1960) pointed out that in living muscle at rest, the presence of ATP keeps the actin and myosin complex dissociated. He further indicated that the cross links of the myosin filaments are not attached to the actin filaments. Therefore, the two sets of filaments can slide past each other easily, and the muscle is readily extensible.

In studying the role of calcium in muscle contraction, Niedergerke (1955) found that calcium injected into the sarcoplasm of a muscle fiber caused local contraction at the point of injection, but did not set up a wave of activity across the fibers. Protzell et al. (1964) used calcium buffers to stabilize the concentration of calcium in the solutions which they injected into the muscle fibers. Using EGTA as the buffer, these workers showed that threshold contraction effects were activated by a very low concentration of calcium (0.3 to 1.5 uM). The shortening resulting from injection of various solutions into single muscle fibers from the leg muscle of the crab was measured by Caldwell and Walster

(1963). Their results showed that contraction was produced on injecting either $CaCl_2$, $SrCl_2$, $BaCl_2$ or caffeine, and that contraction was only slight or absent after the injection of distilled water, $MgCl_2$, KCl, NaCl, ATP, AMP, EDTA or potassium phosphate.

Frank (1960) found that potassium-induced contraction of a muscle was reduced and eventually eliminated, if the muscle was kept in a calcium-free solution for various periods of time before testing. The speed of inhibition was increased by adding small quantities of EDTA to the calcium-free solution. Edman and Grieve (1964) showed similar results with individual muscle fibers immersed in calcium-free Ringer's solution and excited with a single current pulse.

Goodwall and Szent-Györgyi (1953) found that the contraction and relaxation of glycerinated muscle fibers in a solution containing CP and the relaxing factor could be brought about by increasing or decreasing pH.

Szent-Györgyi (1963) described muscle activity as the summation of the reactions taking place at the cross bridges, and indicated that each cross bridge was the site of a unit of activity. He stated that the cross bridge is the site of actomyosin formation and ATP hydrolysis during contraction.

A more detailed molecular theory of muscle contraction has been proposed by Davies (1966). This model is based on the present knowledge of muscle structure and the properties of the various muscle proteins and their interactions with ATP, calcium, and magnesium. The theory is based on the assumption that ATP is bound to the end of a mobile polypeptide, which is part of the H-meromyosin cross bridge. A fixed negative charge

inside the cross bridge repels the extra negative charge of the ionized ATP, resulting in extension of the polypeptide chain during the resting state. When the muscle is activated, depolarization of the sarcolemma and the sarcoplasmic reticulum liberates enough calcium to form chelate links between actin and myosin. These linkages cause the neutralization of the negative charge on the extended H-meromyosin, and thus the extended polypeptide in the cross bridge contracts, resulting in the development of tension and shortening. This process is repeated cyclically during muscle contraction. When the muscle is no longer depolarized, the calcium in the sarcoplasm is removed by the relaxing factor system and relaxation occurs.

Results of Infante et al. (1964) revealed that ATP was not the direct final energy source for muscle contraction. These workers suggested that the energy from ATP is used to extend the polypeptide in the cross bridges and that the actual energy to do work during muscle contraction comes from the formation of hydrogen and hydrophobic bonds as the extended polypeptide contracts.

Perry and Cotterill (1965) pointed out that the special features of the magnesium-activated ATPase of the natural actomyosin system enables the hydrolysis of ATP during the contraction-relaxation cycle to be regulated by the release and withdrawal of minute amounts of calcium by the sarcoplasmic reticular system.

Levy and Ryan (1966) proposed a model of muscular contraction indicating how the reaction of ATP at two sites on each cross bridge on myosin could cause actin and myosin to slide past each other.

Rigor Mortis

Physical changes

After death, muscle changes from the highly extensible plastic condition of freshly excised muscle to the inextensible and rigid condition observed in full rigor (Huxley, 1960). From a histological study of muscle in rigor, Bendall (1951) postulated that rigor mortis is essentially the same process as physiological contraction. He showed that the cross-striations of the fibers in rigor are packed at least twice as tightly as those in pre-rigor. Work by Marsh (1954) also tended to support the view that shortening in rigor may be regarded as a slow and irreversible physiological contraction. More recent results by a number of workers (Locker, 1959; Mauriello and Sandow, 1959; Partmann, 1963) also indicates that rigor mortis and muscular contraction develop through the same mechanism. Nauss and Davies (1966) pointed out that during normal contraction, the contractile components develop tension on stimulation. However, in rigor mortis contraction and tension maintenance are unusual, since the muscle contracts in the absence of external stimulation and can bear a load in the absence of ATP.

In his studies with rabbit muscle, Erdos (1943) found that full development of rigor was coincident with the complete disappearance of ATP. The results of a number of researchers (Bate-Smith and Bendall, 1947, 1949; Bendall, 1951; Lawrie, 1953) have confirmed the relationship between the onset and development of rigor mortis and the breakdown of ATP. Huxley (1960) pointed out that it is the loss of ATP that leads to the formation of fixed links between the actin and myosin filaments. The physical changes which occur during rigor have been explained by

Nauss and Davies (1966). They indicated that rigor is a transformation from a system of actin and myosin filaments, which slide freely along each other, to a rigid system consisting of actomyosin joined together by cross bridges between the actin and heavy meromyosin. The cross links, which are able to break and reform during shortening, can not be broken in the absence of ATP. This rigid system maintains continuous tension in the absence of ATP breakdown. Huxley (1960) pointed out that a muscle may contract slightly as it passes into rigor, but shortening can be prevented by a relatively small force.

A number of devices are available for measuring the time course of rigor mortis by following the changes in extensibility of muscle strips under controlled conditions (Bate-Smith and Bendall, 1949; Briskey et al., 1962; Partmann, 1963). Marsh (1954) and Bendall (1960) have shown that a short-time course of rigor is associated with greater contraction than a long-time course of rigor.

In a study of the striation patterns of ox muscle during rigor mortis, Locker (1959) established a relationship between the striation patterns and the sarcomere length during shortening. Sink et al. (1965) found that muscles having a long delay phase during rigor mortis have longer sarcomeres than muscles that have a short delay phase. Consequently, these workers stated that the amount of sarcomere shortening or contraction is highly dependent upon the course of rigor mortis.

Herring et al. (1964) showed that sarcomere length varies between muscles and can be affected by pre-rigor excision. Their results showed that sarcomere length can be markedly increased in stretched muscles and markedly decreased by thaw-rigor contracture.

Locker (1960) found that contraction of muscle progressively reduces tenderness. He concluded that the state of contraction is a significant factor in tenderness, where the effect of connective tissue is small. This has been confirmed by Locker and Hagyard (1963) while studying the cold shortening effect in beef muscle. Herring et al. (1965a, 1965b) have also shown that muscles, which shorten during rigor, have a corresponding decrease in sarcomere length, an increase in fiber diameter and a decrease in tenderness.

Marsh and Leet (1966a, 1966b) reported an interesting relationship between tenderness and cold shortening in beef. These workers showed that a decrease of up to 20% of the initial excised length did not exert a significant effect on tenderness, but toughness increased rapidly with further shortening beyond this point. It reached a maximum shear reading of several times the initial value at about 60% of its original length. With still further shortening, the meat became progressively more tender, until at about 40-45% of its resting length, it was cleaved as easily as the non-contracted muscle.

Ramsey and Street (1940) and Gordon et al. (1964) have shown that the maximum isometric tension can be controlled by the length of the muscle. They point out that when muscle is stretched so that no overlap occurs, tension can not be developed. When the muscle is contracted to the extent that the Z-line limits further shortening, then tension can not occur. These workers stressed that maximum tension development depends on the amount of overlap of the filaments in the sarcomere.

Chemical changes

The main chemical changes in muscle after death include the production of lactic acid by anaerobic glycolysis, the breakdown of CP and finally the breakdown of ATP (Bate-Smith and Bendall, 1956). Lawrie (1953) indicated that immediately after death, there is a rapid drop in CP, a slow decline and then a rapid decrease in ATP, which is accompanied by a parallel decline in pH. With few exceptions, data published since that time indicate that the change in extension of the muscle is not complete until the pH is at or near its ultimate value, and the ATP content is reduced to a low level.

Bendall and Davey (1957) reported that at 17°C, the loss of extensibility set in when about 3/4 of the ATP was broken down. At 37°C, however, development of inextensibility occurred when about 1/2 of the ATP was borken down. They showed that the change in extensibility occurred when the ATP level at 17°C and 37°C was 2 and 4 uM ATP per g of muscle, respectively. They pointed out that most of the remaining ATP is broken down as rigor is completed, but occasionally as much as 0.7 uM ATP/g may remain at room temperature for 10 to 12 additional hours. Using paper innophoresis, Fredholm (1963) reported 17% of the initial ATP in beef muscle was still present after 3-5 days of storage under refrigeration. The results of Newbold (1966), using an enzymatic method for ATP, indicated that about 0.2 uM of ATP/g of beef muscle was still present after reaching the ultimate pH.

Nauss and Davies (1966) induced rigor mortis in frog sartorius muscle by incubating the isolated muscle in 2-4-dinitrofluorobenzene (DFB) in Ringer's solution. They showed that as the muscle began to shorten, the rate of ^{45}Ca efflux increased, and that the ATP level fell from 3.8

to 0.0 uM/g. These results contradict Davies' (1963) theory of muscle contraction, which suggests that ATP is present in the actomyosin complex formed during contraction. Nauss and Davies (1966) suggested that the increased rate of calcium efflux probably resulted from the leakage of calcium from the sarcoplasmic reticulum as a consequence of prolonged exposure to DFB.

On the basis of their work, Bárány and Jaisle (1960) concluded that contraction is only produced when the mechanism of ATP splitting is based on the interaction between actin and myosin. Nauss and Davies (1966) postulated that the important chemical event determining the onset of the physical changes in a muscle passing into rigor seems to be the internal liberation of a sufficient amount of calcium to initiate the interaction between actin and myosin. Thus, the need for external stimulation would be bypassed.

The onset of shortening in rigor mortis following the release of calcium into the sarcoplasm can be accounted for by the cyclic formation and breakage of cross links between actin and myosin, which is accompanied by the enzymatic hydrolysis of ATP due to actomyosin ATPase (Nauss and Davies, 1966). In addition, further breakdown of ATP occurs once the calcium is released into the sarcoplasm as a result of the ATPase activity of the sarcoplasmic reticulum. This process is activated continuously during the development of rigor until the level of ATP is decreased. Newbold (1966) and Marsh (1966) also suggested that the release of calcium by the sarcoplasmic reticulum can result in ATPase activity and subsequent rigor development in muscle.

A somewhat different interpretation of the relationship of some of the changes in rigor has been suggested by Newbold (1966). Results from his work showed that post-mortem stiffening, as measured by changes in extension, reached completion in beef sternomandibularis muscle at 1°C, while there was still an appreciable amount of ATP. He further stressed that there is a close coincidence between the completion of the change in inextensibility of muscle and the disappearance of CP. He found that this coincidence is closer than that between the completion of the physical changes and the reduction of ATP to a low level. Similar results on the longissimus dorsi muscle of the pig were obtained by Lawrie (1960). This is especially interesting in light of the results of Brown et al. (1963) and Lee (1965), who showed that CP was necessary in order for the relaxing factor to take up calcium.

The formation of lactic acid from glycogen through glycolysis plays an important part in determining muscle pH and color. The rate of post-mortem glycolysis and its relationship to the ultimate properties of porcine muscle have recently been covered in a review by Briskey (1964). More recent work by Kastenschmidt et al. (1965) showed that a sudden ante-mortem temperature change from a warm to cold environment altered the post-mortem glycolytic rate and associated properties of porcine muscle. It also improved meat quality. Bodwell et al. (1966) found that holding pork carcasses at 37°C immediately post-mortem did not consistently produce soft, watery and pale muscle. From results with pigs pre-treated with curare, Bendall (1966) postulated that the extreme variability in the rate of pH decline is due to the variable intensity of the various stimuli reaching the muscles.

In studies with poultry, DeFremery and Pool (1963) and DeFremery (1966) found that post-mortem glycolysis was minimized by epinephrine injections, sodium iodoacetate injections or rapid cooking. The treated meat was tender without aging. DeFremery (1966) concluded that acceleration of post-mortem glycolysis induces toughness in poultry. The pH of the longissimus dorsi muscle from rabbits (Paul, 1964) showed no relation to the amount of struggle post-mortem.

Effect of Various Treatments on Muscle Characteristics

According to Howard and Lawrie (1956), preslaughter injection of beef with sufficient magnesium sulfate markedly prolonged the delay phase of rigor mortis. Magnesium sulfate injection resulted in a decreased rate of pH fall and breakdown of ATP, but the ultimate pH was not affected. The preslaughter injection of beef with calcium shortened the delay phase of rigor mortis considerably. This treatment resulted in a lower initial pH and reduced the levels of glycogen, ATP and CP.

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 sodium hexametaphosphate into pre-rigor hams. They showed that the hams injected with sodium hexametaphosphate were more tender ($P < .01$) than control hams injected with an equal volume of water. The treated hams also had higher pH values.

Carpenter *et al.* (1961) tried injecting rabbit legs with chelating compounds, but difficulty was encountered in obtaining an increase of 5% over the initial weight because of the massive tetany occurring upon

infusion. After failing in their study with rabbit muscle, the same workers conducted a study to determine the effect of an infusion of sodium hexametaphosphate solution into hot beef rounds. Tenderness, as recorded by taste panel and shear, was improved by pre-rigor infusions of 10, 15 and 20% solutions of sodium hexametaphosphate and a 15% solution plus lactic acid (pH 5.8). Extractable nitrogen was not a reliable indication of tenderness in this experiment. These workers also showed that the small differences between the pH values and the glycogen levels for the treated and control samples after 48 hours indicated that infusion did not interrupt glycolysis.

In a study of the ionization of some phosphates used in food products, Batra (1965) found that sodium orthophosphate completely dissociated in solution and that the degree of dissociation of the polyphosphates was inversely proportional to the number of phosphate atoms in the chain or ring. The addition of calcium enhanced the dissociation of the polyphosphate and lowered the pH in each case. Results showed that the calcium-complexing ability of sodium hexametaphosphate was the highest while that of orthophosphate was nil.

More recent work by Weiner et al. (1966) with hams removed from the carcass within 1 hr post-mortem and pumped with cold brine, showed lower total cooking losses and lower drip losses for treated than for control hams. Muscles from treated hams also had significantly lower shear values than the controls. In a similar study, Mandigo and Henrickson (1966) found that hams pumped, boned, smoked and chilled within 15 hours after slaughter were not significantly different than hams processed by conventional procedures.

Extractability and Fractionation of Muscle Proteins

One of the first studies reported concerning the extractability of muscle proteins was by Deuticke (1932). He found that muscles, which had been fatigued by stimulation, frozen and pulverized, had less extractable protein than those freshly extracted. Bate-Smith (1934) studied the effects of a series of extracting solutions. He found that no difference could be observed between the extractability of fresh muscle and muscle in rigor.

In a review of muscle chemistry, Dubuisson (1952) pointed out that the extractability of muscle proteins depends not only on the solubility characteristics of the proteins, but also upon the firmness with which the proteins are bound and upon the dissociation power of the salts used for extraction.

Dubuisson (1952) has also shown that the amount of extractable myosin is reduced, if the muscle is frozen in the contracted state or while contracted by rigor mortis. If the muscle was allowed to relax after being contracted, it yielded the same amount of extractable protein as normal muscle. He suggested, therefore, that the changes in extractability are rapidly reversible and that the change in the structural proteins reflects some change in the contractile elements themselves.

Wierbicki et al. (1954) were among the first to directly approach the study of quality of meat by protein fractionation. These workers attempted to determine the amount of actomyosin in meat and relate this to tenderness changes during aging. They said their extracting solution was designed to dissolve actin and myosin and other proteins, but not

actomyosin. They used a citric acid buffer of pH 5.6 with an ionic strength of 0.48. This included 0.22 M KCl. In a group of 48 beef animals, hydroxyproline values for connective tissue showed no relation to tenderness. However, a very good correlation between extractable nitrogen and tenderness was obtained. Using the same extraction procedure, Wierbicki et al. (1956) presented evidence indicating that tenderness may be related to the degree of hydration of meat proteins. They suggested that post-mortem tenderization may be due to certain ion-protein or protein-protein interactions rather than classical proteolysis or dissociation of actomyosin.

On the basis of present knowledge of the fibrillar proteins, Baliga (1962) pointed out that the citric acid buffer used by Wierbicki et al. (1954, 1956) would extract only the non-protein nitrogen and the sarcoplasmic proteins and would not extract myosin and actin as suggested by the experimenters. He explained that myosin can not be extracted by the potassium chloride-citrate buffer at pH 5.6, since this is near the isoelectric point of the proteins. Baliga (1962) also pointed out that in preparation of actin, Bailey (1954) first extracted the muscle mince with salt solution at pH 6.5 to remove the sarcoplasmic fraction, and the residue was then treated with a strongly alkaline (pH 10) salt solution to dissolve out the actin.

Weinberg and Rose (1960) extracted the pectoralis major muscles of young chickens with a phosphate-potassium chloride buffer (pH 7.5, ionic concentration 0.55). The supernatant was then diluted in steps to specific

ionic strengths so that the actomyosin, myosin and sarcoplasmic proteins could be separated. Using this procedure, these workers showed that the amount of nitrogen extracted increased on holding the carcass for 24 hours at 4°C. The increase was entirely accounted for by an increase in the actomyosin fraction.

Saffle and Galbreath (1964) showed that as pH increased from 5.5 to 6.5, the amount of protein extracted in a 3% saline solution (0.51 M NaCl) also increased. They also showed that the amount of salt-soluble protein was 50% greater in pre-rigor beef than at 48 hours post-mortem. They further demonstrated that freezing beef reduced the salt-soluble protein in comparison with beef held for 48 hours post-mortem. Scopes (1964) found that the myofibrillar proteins from ox muscle are most soluble in 1 M KCl at a pH of 6.0. The extractability was observed to drop sharply at a lower pH, owing to the insolubility of actomyosin near its isoelectric point.

In another study, the proteins of the longissimus dorsi muscle of rabbits were separated by their solubility properties into sarcoplasmic proteins, non-protein nitrogen (NPN), the myofibrillar proteins soluble in 0.6 M KI-borate buffer at pH 7.5, the remainder of the myofibrillar proteins soluble in 0.1 N NaOH and the stroma proteins (Paul et al., 1966). The average percentage of total nitrogen for the different fractions was reported to be: 27% sarcoplasmic, 12% NPN, 50% myofibrillar protein soluble in 0.6 M KI buffer, 10% of myofibrillar protein soluble in 0.1 N NaOH and less than 1% stroma protein.

Baliga et al. (1962) studied the protein solubility of fresh water fish in a 5% saline solution (0.85 M NaCl) buffered with 0.02 M sodium bicarbonate. They found that the initial protein solubility of 92% decreased to 82% after five days storage. This was followed by an increase in solubility, reaching a maximum of 97% after 12 days storage. The solubility of the proteins then decreased to 71% at 15 days. They found that the actomyosin fraction precipitated by dilution of the salt extract to an ionic strength of 0.225 rose to a high value after 1 to 3 days, and then declined to the initial level. In another study with fish using a similar extraction media, Partmann (1963) found that the extractability of structural proteins of rosefish and cod decreased at high storage temperatures and with advancing periods of time in freezer storage.

In studying the properties of the fibrillar proteins of normal and watery pork muscle, Bendall and Wismer-Pedersen (1962) determined the amount of protein extracted by a phosphate buffer (0.55 ionic strength, pH 6.5) from fibrils washed free of soluble sarcoplasmic protein with 0.04 M potassium phosphate (pH 6.5; ionic strength 0.05). Their results showed that normal fibrils were almost completely extracted, giving a highly viscous solution containing 88% of the fibrillar proteins. With "watery" fibrils, however, only 11% of the fibrillar proteins were extracted and the supernatant showed a low viscosity. From additional work, they concluded that in watery meat, the main fibrillar protein, actomyosin, is in the native form but has become covered with a layer of denatured sarcoplasmic protein. They suggested that the layer of denatured sarcoplasmic protein covering the fibrillar protein makes it resistant to extraction at high ionic strengths.

The extractability of pork muscle under various conditions using the procedure of Helander (1957) has been reported by several investigators (Sayre and Briskey, 1963; Briskey and Sayre, 1964; Borchert and Briskey, 1965). In this procedure, the sarcoplasmic proteins are extracted with 0.03 M potassium phosphate buffer at pH 7.4, and the total soluble proteins are extracted with 1.1 M KI in 0.1 M potassium phosphate at pH 7.4. The myofibrillar proteins were calculated by the difference between the amounts of the total soluble proteins and the sarcoplasmic proteins. These workers showed that muscle protein solubility was grossly altered by the temperature and pH existing during the onset of rigor mortis or during the first few hours after death.

Hill (1962) reported values for the amount of sarcoplasmic, myofibrillar and stroma protein as well as for NPN in porcine, bovine and ovine muscle, using a modification of Helander's (1957) extraction technique. His results showed that the amount of stroma protein was highest in bovine and lowest in porcine muscle. He also reported differences in extractability of the protein fractions between individual muscles.

The process of transformation of myosin and actin into actomyosin during the prolonged extraction of ground rabbit muscle with Weber-Edsall solution (0.6 M KCl, 0.04 M $KHCO_3$, 0.01 M K_2CO_3) has been recently investigated in detail using various physico-chemical techniques such as viscosity, turbidity, flow birefringence, ultracentrifugation and ATPase activity (Haga et al., 1965). Results of Haga et al. (1965) showed that

the formation of actomyosin began about 8-10 hours after the start of extraction and was completed in about 20 hours. In a similar study, Mikalyi and Rowe (1966) extracted actomyosin from rabbit muscle with the Weber-Edsall solution and followed the disappearance of ATP from the muscle mince. Their results also indicated that the splitting of ATP is not affected in the presence of 0.2 M phosphate, but the formation of actomyosin is strongly inhibited. They proposed that ATP, as well as inorganic phosphate, preserves the myofibrillar structure, and thereby prevents the extraction of actomyosin.

Hegarty et al. (1963) reported the relationship of some intracellular proteins to beef muscle tenderness. The sarcoplasmic proteins were extracted in a phosphate buffer (pH 7.6, ionic strength 0.05) and then the residue was extracted with 0.1 M NaOH to remove the total fibrillar proteins. Another sample of the same meat was extracted with Weber-Edsall solution. The soluble fibrillar protein nitrogen was obtained by subtracting the sarcoplasmic fraction from the Weber-Edsall extract. They reported that the fibrillar protein solubility was highly correlated with tenderness.

In the extraction of myosin from cod flesh, Connell (1960) observed that the only method available for the quantitative extraction of myosin was that of Hasselbach and Schneider (1951). In this method the finely minced muscle is extracted repeatedly with a slightly acid salt solution containing pyrophosphate ions. Connell (1960) further pointed out that pyrophosphate is believed to dissociate the actin bonds existing in the muscle and to inhibit the formation of such bonds during extraction. In

this way myosin alone is extracted, leaving the actin adhering to the muscle residue or stroma. During cold storage of cod, Connell (1960) reported that 70-80% of the myosin becomes non-extractable at a rate similar to that at which the total myofibrillar protein of the flesh becomes non-extractable. He then discussed the implications of these findings in regards to the mechanism of cold-storage deterioration. Baliga et al. (1962) added pyrophosphate to the salt solution during the extraction of fish proteins and found that it had a dissociating effect on the actomyosin.

Fukazawa et al. (1961) examined the effect of different inorganic phosphates on the extractability of the myofibrillar proteins. They found that the pyrophosphate showed the greatest improvement in the extractability of fibrillar proteins, while both triphosphate and hexametaphosphate increased the extractability of the fibrillar proteins to a lesser extent. These workers also showed that pyrophosphate decreased the viscosity of protein solutions extracted from intact fibrils with Weber-Edsall solution. Yasiu et al. (1964) have reported similar results on the extractability of muscle protein while using these phosphates.

The results of Bendall (1954) and Helandorn (1962) showed that low concentrations of pyrophosphate in combination with NaCl increase the water binding of cooked meat. In another experiment, Bendall (1962) showed that the maximum effect of pyrophosphate develops rather slowly. He also found that addition of calcium or magnesium ions at the concentrations found in tap-water did not significantly effect the action of pyrophosphate.

Fujimaki et al. (1965a, 1965b) have studied the changes in actomyosin during storage of rabbit muscle. Their results indicated that the approximate content of myosin and actin in actomyosin are variable. The maximum extractability of actin from actomyosin occurred 2 days after slaughter. The authors concluded that the interaction between actin and myosin in actomyosin becomes weaker as aging progresses. Recent studies by Scharph et al. (1966) followed the post-rigor changes occurring in the properties of the actomyosin fraction of turkey muscle. Their results gave some evidence that favors the concept of dissociation of actomyosin into actin and myosin with the resolution of rigor.

EXPERIMENTAL METHODS

General Methods

Preparation of Samples

All frozen muscle samples were powdered with pre-chilled equipment in a -20°C cold room. The frozen tissue was chipped into smaller pieces and placed in a Waring blender with an approximately equal weight of dry ice to prevent an increase in temperature. The blender was operated at full speed until the sample was finely powdered, usually 30 to 60 sec. The powdered sample was then passed through an 18 mesh screen and stored in glass jars at -30°C. At least 12 hours was allowed for the dry ice to sublime before using the sample for analysis.

Muscle pH

Approximately 1 g of powdered muscle was homogenized for 30 sec in a small Waring blender containing 25 ml of 0.005 M sodium iodoacetate. Where fresh muscle was used, approximately 2.5 g was homogenized for 1 min in a small Waring blender containing 25 ml of 0.005 M sodium iodoacetate. All pH estimates were performed in duplicate with a Corning, Model 12, expanded scale pH meter.

Adenosine Triphosphate

Muscle ATP was extracted from the frozen powdered muscle with hot (100°C) distilled water. A 0.3 to 0.6 g sample was accurately weighed and then carefully poured into a beaker of boiling water. The sample was

boiled about 30 sec and then placed in a cooler at 0°C to cool. The water extract was assayed for ATP by the bioluminescence method described by Strehler and Trotter (1952) and Strehler (1953).

The standard ATP enzyme system was prepared by extracting 100 mg of vacuum dried firefly lanterns (Sigma Chemical Co.) with 10 ml of ice-cold 0.1 M sodium arsenate for 10 min, while grinding in a mortar. The extract was then filtered through cheese cloth into an Erlenmeyer flask in an ice bath. The filtrate was then diluted with 15 ml of deionized water, and 48 mg $MgSO_4$ was dissolved in it. The enzyme preparation was stored at 0°C. Before assaying for ATP, 0.5 ml of the enzyme preparation was placed in a test tube with sufficient deionized distilled water so that after the dilution of the unknown or standard, the final volume would be 3 ml. All solutions were brought to room temperature. The reaction was started by addition of the desired volume of the unknown sample or standard to the diluted enzyme solution. Fluorescence was measured using an Aminco-Bowman Spectrophotofluorometer without a light source at 550 mu, 30 sec after starting the reaction. The standard curves were prepared each day with fresh solutions of the disodium salt of ATP (Sigma Chemical Co.).

Protein Fractionation

The protein fractionation procedure was adapted from that of Weinberg and Rose (1960). The principal change in adapting the procedure was the extraction of the sarcoplasmic proteins, first with 0.03 M potassium phosphate at pH 7.4. The residue from the sarcoplasmic extraction was

then extracted with a phosphate buffer (pH 7.5) in 0.4 M potassium chloride (total ionic strength = 0.55). All fractionation procedures were carried out at 0-3°C with cold extracting solutions (0-3°C). Details of the procedures are outlined in Figure 1.

In the scheme for the quantitative determination of the muscle proteins, approximately 5 g of the powdered sample was placed in a small Waring blender containing 50 ml of a 0.3 M phosphate buffer (pH 7.4). This was blenderized immediately for one minute at a speed of 8000 rpm (adjusted with a Powerstat transformer setting of 40). The sample weight was then determined by difference before the material was transferred into a 250 ml centrifuge tube. The Waring blender was then rinsed with 50 ml of the extracting solution, which was used for the second extraction. The material in the centrifuge tube was gently mixed with a magnetic stirrer for 30 min. It was then centrifuged at 2600 rpm for 20 min. The supernatant was retained. The residue was resuspended in 50 ml of the above rinse solution, stirred and centrifuged as described above. The volume of the combined supernatant was recorded and designated as solution A (protein solution extracted at low ionic strength). The filtrate resulting from the precipitation of a 15 ml aliquot of solution A with 15 ml of 10% TCA was used for the determination of NPN. The difference between solution A and the NPN (A-NPN) represented the sarcoplasmic protein nitrogen.

The residue A, remaining from the 0.03 M phosphate buffer extraction, was suspended in 50 ml of a mixture of phosphate buffer (pH 7.5) in 0.4 M KCl (total ionic strength = 0.55). The mixture was stirred gently for

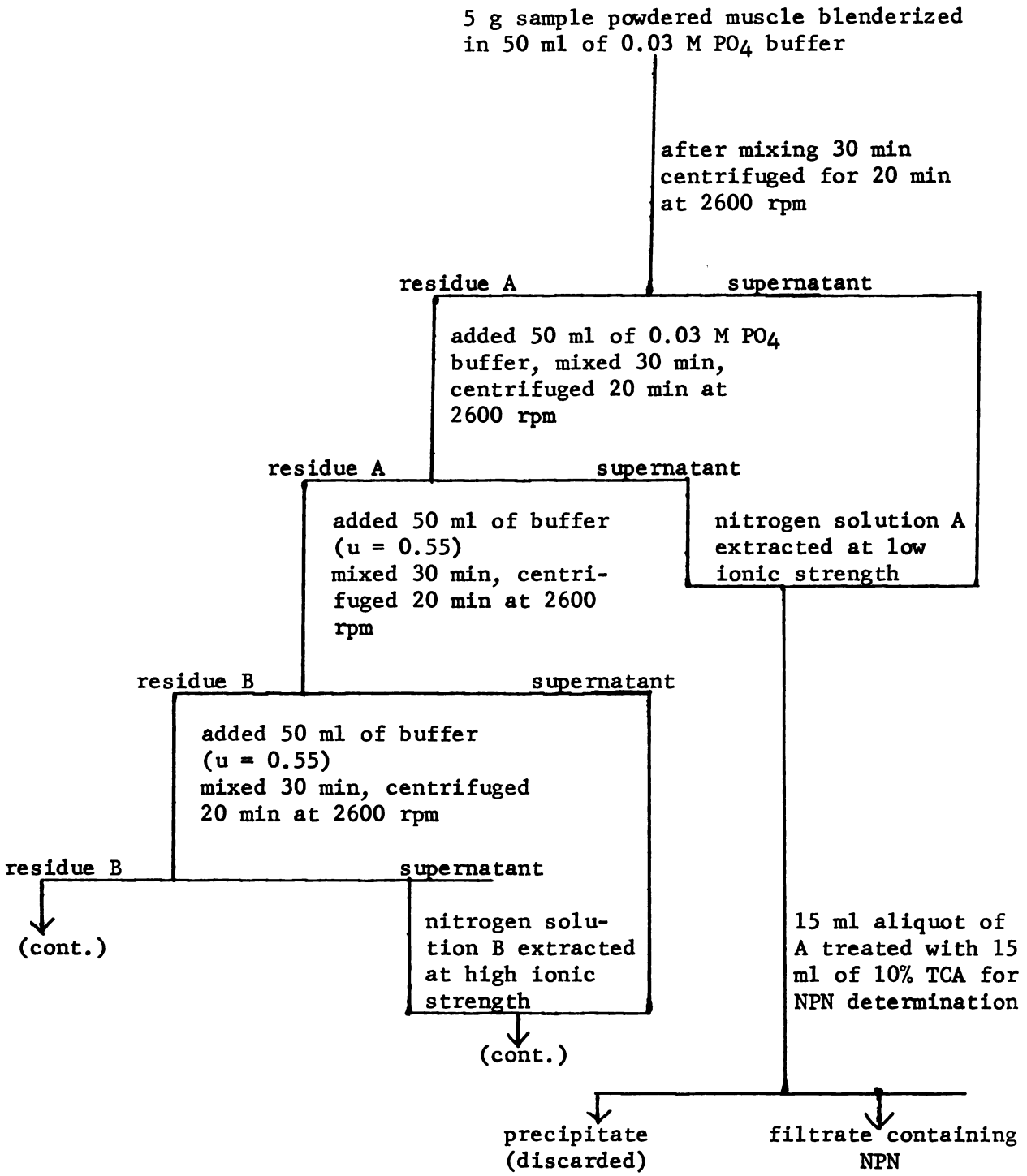

Figure 1. Scheme for the quantitative determination of the muscle proteins.

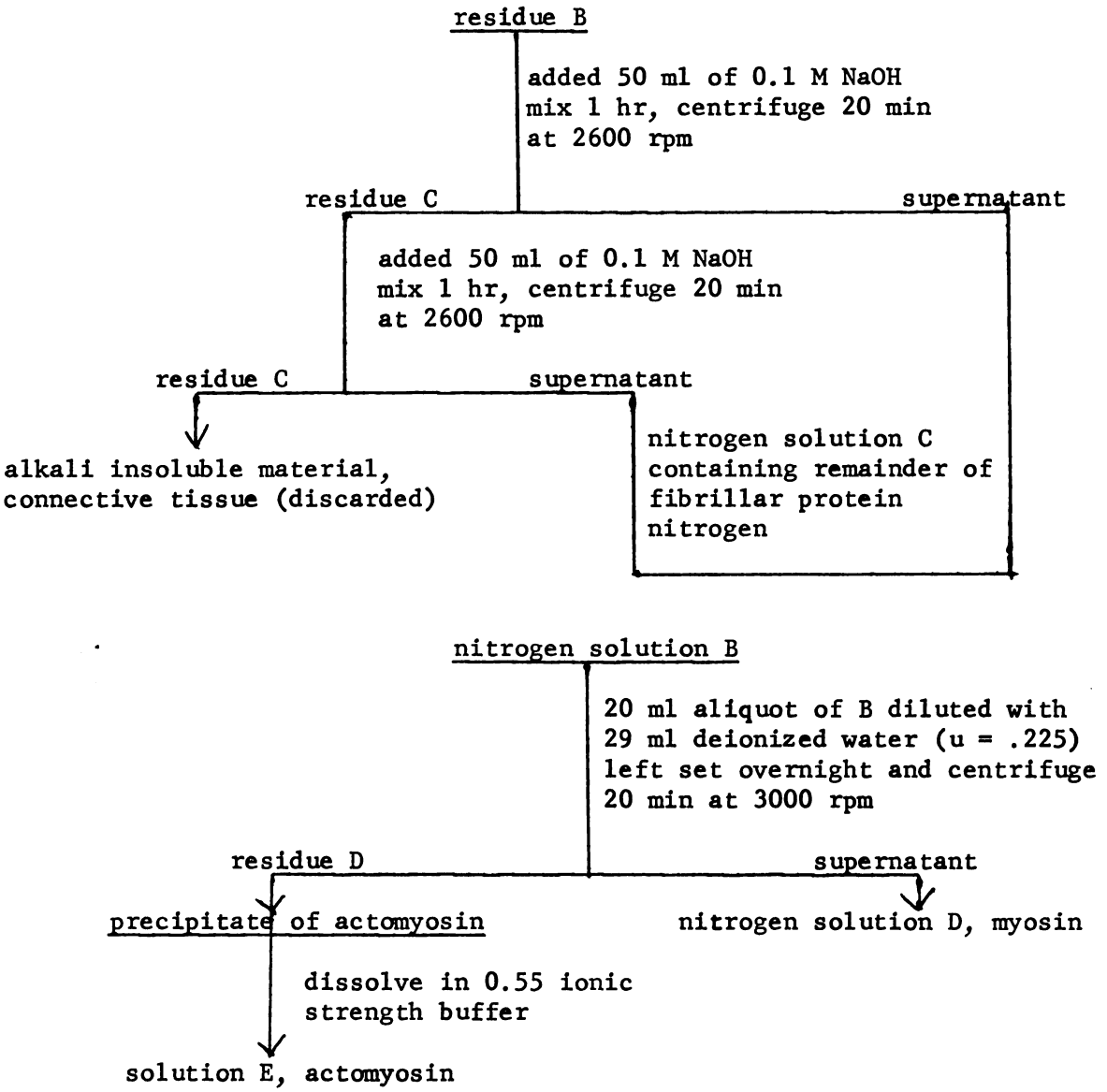

Figure 1 (cont.) Scheme for the quantitative determination of the muscle proteins.

30 min and then centrifuged at 2600 rpm for 20 min. The extraction and centrifugation was repeated. The volume of the combined supernatants were recorded and designated as solution B, the fibrillar protein extracted at high ionic strength. Twenty ml of solution B was then diluted with 29 ml of deionized water, left overnight and then centrifuged at 3000 rpm for 20 minutes. The volume of the supernatant was recorded and designated as solution D, the myosin fraction. The residue D was resuspended in 0.55 ionic strength buffer. The volume was recorded and designated as solution E, the actomyosin fraction.

Residue B from the 0.55 ionic strength buffer was resuspended in 50 ml of 0.1 M NaOH, stirred gently for 1 hr, and then centrifuged at 2600 rpm for 20 min. Extraction and centrifugation was again repeated. The volume of the combined supernatants was recorded and designated as solution C, the remaining soluble fibrillar protein. A very small amount of residue (alkali insoluble material) was discarded. All fractions were analysed for nitrogen.

Nitrogen Analysis

All nitrogen analyses were performed by the micro-Kjeldahl method as outlined by the American Instrument Co. (1961). For calculating protein concentrations, it was assumed that the fibrillar extract contained 16.15% nitrogen (Mikalyi and Rowe, 1966).

Preparation of Fibrillar Protein Extract

The fibrillar protein extract of the longissimus dorsi muscle of the rabbit was prepared according to Mihalyi and Rowe (1966). The muscle was cleared of fat and connective tissue and homogenized in a Waring blender with 4 volumes of Weber-Edsall solution (0.6 M KCl, 0.04 M $KHCO_3$, 0.1 M K_2CO_3). The suspension was poured into a 250 ml centrifuge tube and left for 24 hours. At the end of this time, 2 volumes of Weber-Edsall solution were added, and the sample was stirred thoroughly before centrifuging in an International Refrigerated Centrifuge at 2,600 rpm for 20 min.

The myosin components of the supernatant were precipitated by diluting with 10 volumes of deionized water to remove the water soluble muscle constituents. The precipitate was collected after centrifugation for 20 min at 2,600 rpm and dissolved in 0.6 M KCl. Following 3 precipitation steps, the myosin components dissolved in 0.6 M KCl were clarified by centrifugation for 10 min at 12,000 rpm with a Sorvall, Model SS1 centrifuge. The supernatant was then diluted to approximately 0.2% protein with 0.6 M KCl. Final protein concentrations were determined by nitrogen analysis as described above.

Viscosity

The viscosity of a 0.6 M KCl solution of the purified Weber-Edsall extract was estimated by an Ostwald viscometer at pH 6.4. The viscometer used in these experiments had an outflow time for deionized water of 62 sec at 23°C and 110 sec at 3°C. Preliminary experiments were run to compare the effect of adding 4 mM $MgCl_2$ and to compare the results at 3° and

23°C. The change in viscosity of the protein solution was measured following the addition of 1 ml of 0.1 M potassium pyrophosphate to a solution containing 1 ml of 0.01M $MgCl_2$ in 23 ml of the protein extract. The change in viscosity following the addition of ATP was measured in a similar manner.

ATPase Activity and Changes in Turbidity

For measuring changes in turbidity and ATPase activity, the purified myosin components were diluted with 0.6 M KCl to a final concentration of 0.675 mg of protein per ml. Reagent solutions needed for this reaction included 0.01 M ATP, buffered at pH 6.4 with tris buffer, and 0.01 M $MgCl_2$. For determining the enzymatic activity and the changes in turbidity, 1 ml of 0.01 M $MgCl_2$ was added to 23 ml of the diluted protein extract. The reaction was started by adding 1 ml of the 0.01 M ATP solution to 24 ml of the $MgCl_2$ protein solution. This was mixed immediately, poured into a cuvette and changes in turbidity were measured at 350 mu (Perry and Cotterill, 1965) with a Beckman DU spectrophotometer equipped with a Gilford, Model 220, absorbance indicator. The reference turbidity was taken from a solution containing 0.5 ml of 0.01 M $MgCl_2$, 0.5 ml of deionized water and 11.5 ml of the diluted muscle extract.

ATP was determined on 1 ml aliquots of the reaction mixture removed at appropriate times. The reaction mixture was immediately pipetted into boiling water to stop all enzymatic activity. The level of ATP was determined by the method previously described.

Water Holding Capacity

Measurement of water holding capacity was made by modifying the rapid method proposed by Grau and Hamm (1956). This modified procedure utilized Whatman #1 filter paper, 9.0 cm in diameter, dried in a 110°C drying oven for 12-14 hours and then placed in a desiccator until used. When the sample was prepared for measurement, the filter paper was removed from the desiccator and placed on a plexiglass plate. A fresh meat sample weighing 300 mg was immediately placed on the mid portion of the filter paper. It was covered with a second plexiglass plate and pressed in a Carver Press at 5,000 pounds pressure for three min. Subsequently, the moisture area and meat area were measured with a compensating polar planimeter. Results are expressed as a ratio of total area to meat area.

Cooking Procedure

All cuts were placed on broiling trays and cooked in an electric oven at 175°C to an internal temperature of 75°C.

Statistical Analysis

The data collected from Experiment II and Experiment V were punched onto IBM cards and analyzed in a CDC 3600. The data in Experiment II were analyzed for treatment differences by the least squares method. Duncan's multiple range test was used for comparing means. Correlation coefficients were also calculated between some of the variables in Experiment II. The data in Experiment V were subjected to analysis of variance. Correlation coefficients were also calculated between all the variables for the treated group and the control group. Levels of significance were used as indicated by Snedecor (1956).

Experimental Animals and Treatments

Experiment I

Eight rabbits weighing between 1.6 and 2.3 kg were used. They were randomly divided into two groups, one treated and one control with 4 animals in each group. The treatment consisted of an intravenous injection of a lethal dose of the tetrasodium salt of EDTA in isotonic saline solution. The dosage varied from 65 to 132 mg of EDTA per kg of body weight. The controls received an intravenous injection of 3 ml of an isotonic saline solution per kg body weight, so that both groups were injected with approximately the same volume.

All rabbits were bled within 5 min of injection. Following exsanguination, the intact semitendinosus muscle was removed from the right leg. Shortening was recorded on a kymograph with an isotonic lever loaded with approximately 8 gm/sq cm of muscle cross section. The muscle was held at 0°C in a vertical position with two clamps over calcium-free Ringer's solution with nitrogen bubbling through it. This provided a moist atmosphere and prevented the muscle from drying out.

The posterior portion of the longissimus dorsi muscle from the right side was removed immediately after bleeding and frozen in liquid nitrogen. The posterior portion of the longissimus dorsi muscle from the left side was removed after 20 hrs at 0°C and frozen in liquid nitrogen. The frozen muscle samples were then powdered and stored at -30°C for subsequent pH and ATP determinations. The anterior portion of the loin was removed after 20 hrs, wrapped in aluminum foil, immediately frozen at -30°C and stored for subsequent cooking and tenderness studies.

All loins were removed from the freezer 24 hrs before cooking and allowed to thaw at room temperature (approximately 23°C). The loins were weighed and cooked as described in general methods. After cooking, the samples were weighed and cooled overnight at room temperature. Warner-Bratzler shear values were recorded on 3 cores 12.5 mm in diameter removed parallel to the fibers of the longissimus dorsi muscle.

Experiment II

A total of 22 rabbits weighing between 1.5 and 3.0 kg were used. They were randomly divided into 5 groups -- 4 treated groups and one control group. There were 4 rabbits in group I, 6 in group II, 5 in group III, 2 in group IV, and 5 in group V. The groups consisted of the following treatments: group I - EGTA; group II - CDTA; group III - EDTA; group IV - sodium oxalate; and group V - control. All rabbits were treated and handled as in Experiment I.

Following exsanguination, the intact semitendinosus muscle was removed from both legs and shortening was recorded on separate kymographs with an isotonic lever loaded with a weight approximately equal to that of the muscle. The muscles were held under the same conditions as in Experiment I. After the semitendinosus muscle from the left leg was clamped into position, it received several micro-injections of a 0.1 M $CaCl_2$ solution. The total amount of $CaCl_2$ injected was equivalent to approximately 0.4 ml/kg of live weight. After 6-8 hrs, both semitendinosus muscles were removed from the kymograph and checked for extensibility. The muscle from the right leg was frozen in liquid nitrogen, powdered and stored at -30°C for ATP determination.

The posterior portion of the longissimus dorsi muscle was removed from the right side immediately after bleeding and frozen in liquid nitrogen. After the remainder of the carcass was chilled at 0°C for 24 hrs, the posterior portion of the longissimus dorsi muscle from the left side was removed and frozen in liquid nitrogen. The frozen muscle samples were powdered and stored at -30°C for subsequent pH and ATP determination and for protein fractionation studies. The anterior portion of the loin was removed after 24 hrs, wrapped in aluminum foil, immediately frozen and stored at -30°C until removed for cooking and tenderness studies.

All loins were removed from the freezer 24 hrs before cooking and allowed to thaw at room temperature. Before cooking as described in general methods, the longissimus dorsi muscle was removed from the left side of each loin, trimmed of external fat and weighed. Following cooking, the samples were cooled at room temperature overnight, weighed and shear values were recorded as in Experiment I.

Experiment III

Ten rabbits weighing between 1.5 and 3.0 kg were used. Four were randomly treated with EDTA as previously described, 1 with EGTA and 1 with CDTA, while the other 4 were treated as controls. All rabbits were treated and handled as in Experiment I.

The semitendinosus muscles were removed and shortening was recorded as in Experiment II. The semitendinosus muscle from the left leg of the #2 and #4 control rabbits received micro-injections of 0.01 M $CaCl_2$. The left semitendinosus muscle from the other 8 rabbits in this experiment

received micro-injections of 0.1 M $CaCl_2$. The semitendinosus muscle from the right leg of the #1 and #3 control rabbits received micro-injections of 0.1 M $MgCl_2$ in an amount equivalent to the 0.1 M $CaCl_2$ injected into the paired muscle of the same rabbit.

After 6-8 hrs both semitendinosus muscles were removed from the kymograph and checked for extensibility. The muscles from the #1 control rabbit were kept overnight for pictures. The 9 remaining semitendinosus muscles from the right legs and 3 semitendinosus muscles from the left leg of the last 3 rabbits treated with EDTA were then frozen in liquid nitrogen, powdered and stored at -30°C for pH and ATP determinations.

Experiment IV

A fibrillar protein extract was prepared from the longissimus dorsi muscle from the right side of the rabbit immediately after bleeding as described in the general methods. After the remainder of the carcass was chilled 24 hrs at 0°C, a fibrillar protein extract was prepared from the longissimus dorsi muscle from the left side by the same procedure.

The properties of the fibrillar protein extracts were investigated by the measurement of ATPase activity, and by changes in the turbidity and viscosity as described in the general methods. In addition, changes in turbidity and ATPase activity were also investigated in the absence of added $MgCl_2$. In this study, 1 ml of 0.01 M ATP was added to 24 ml of the diluted protein extract. All other procedures were carried out as described earlier herein.

Experiment V

Twelve crossbred (Hampshire X Yorkshire) barrows and gilts were randomly divided into two groups, one treated and one control with 6 animals in each group. The animals ranged in weight from 92 to 110 kg at slaughter. The treatment consisted of an intravenous injection of 250 to 310 ml of the tetrasodium salt of EDTA (0.1M) in isotonic saline solution. Following injection, the pigs were in a relaxed state. They were then shackled, hoisted and bled within 5 min. The controls were electrically stunned and bled. In all cases, a treated and control pig were slaughtered simultaneously.

The semitendinosus muscle from the right ham was removed as rapidly as possible about 10 min post-mortem. A sample from this muscle was excised and frozen in liquid nitrogen. The remainder of the muscle was hung in a vertical position at 0-3°C and shortening was recorded over a 24 hr period. After 24 hrs, another sample of the same muscle was removed and frozen in liquid nitrogen. The frozen muscle samples were then powdered and stored at -30°C until used for pH and ATP determinations.

After a 24 hr chill, the hams from all carcasses were removed and trimmed. A 4 cm thick center ham slice was removed by cutting perpendicular to the femur, 2.5 cm from the aitch bone toward the shank. Most of the external fat was removed. The weight of the center ham slice was recorded. Following this, the ham slice from the right ham of a treated and control pig were placed on a broiling rack and cooked as outlined under general methods. After cooking, the cuts were removed from the oven and cooked weights were recorded. The ham slices from the left hams were then cooked in the same manner.

All ham slices were cooled overnight at 0-3°C. Warner-Bratzler shear values were then determined on 15 mm diameter cores from the biceps femoris, semimembranosus, rectus femoris and the semitendinosus muscles.

At 36 hrs post-mortem, muscle samples from the biceps femoris were utilized to determine the pH and water holding capacity. Muscle color of the right ham was subjectively evaluated at 24 hrs post-mortem. Each ham was visually rated on a 5-point scale as follows: 1 - very pale, 2 - slightly pale, 3 - grayish pink, 4 - slightly dark, 5 - very dark.

RESULTS AND DISCUSSION

Effect of Chelating Agents, Calcium and Magnesium on Various Properties of Muscle

The effects of various chelating agents on the physical and chemical properties of muscles from rabbits and pigs were investigated in five different experiments. The effects of added calcium and magnesium on muscle properties were also studied. Results are discussed on the basis of shortening, ATP, muscle extensibility, pH, color, water holding capacity, cooking losses, shear values, protein fractionation, ATPase activity, turbidity and viscosity. Each will be discussed separately.

Shortening

Fig. 2 demonstrates that the intravenous injection of EDTA greatly inhibited shortening of the semitendinosus muscle in Experiment I. The shortening occurring in the muscle of the treated rabbits was completed at the end of 3 hrs, whereas, normal shortening, as shown by the controls, continued up to 7 hrs.

Davies (1963) suggested that the calcium-actomyosin complex develops tension during formation, and if the sub-total of the forces developed at any one time by the ultramicro-contractions is sufficient to overcome the extended load and the resistance of the series of elastic elements, the actin filaments will be pulled along the myosin filaments into the A band and the muscle will undergo quantal contraction. Hasselbach (1957) showed that the calcium content of the undissolved structural proteins

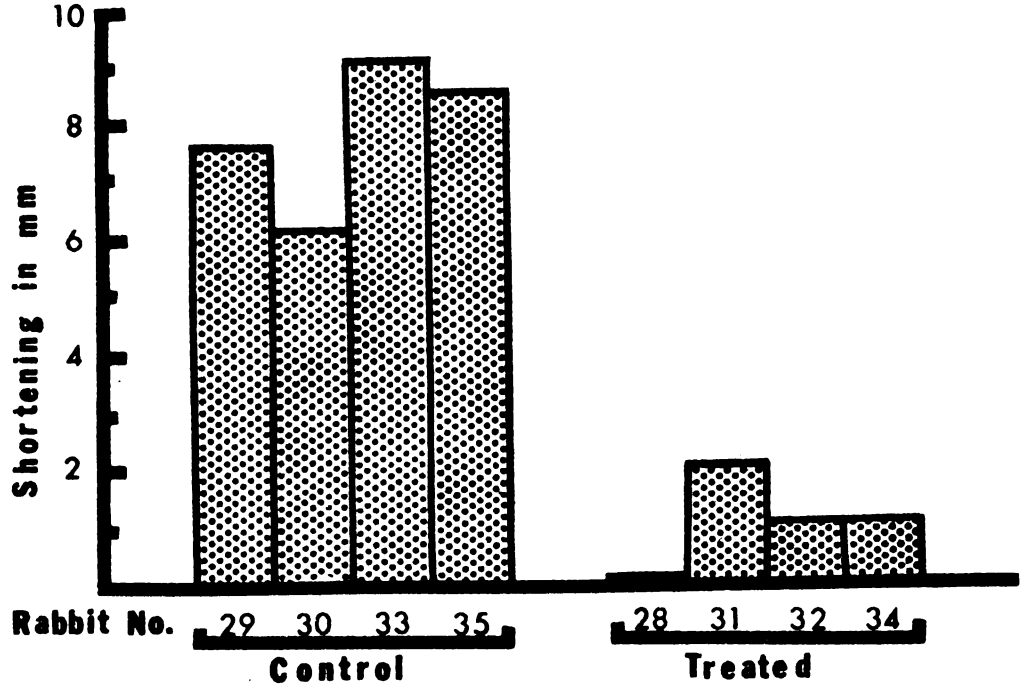

Fig. 2. Effect of EDTA on the shortening of the semitendinosus muscle. The control consisted of an intravenous antemortem injection of isotonic saline and the treatment consisted of an intravenous antemortem injection of EDTA in isotonic saline.

from fresh muscle was diminished 50% by washing with EDTA, but that the magnesium content was not altered. Several investigators (Watanabe and Sleator, 1957; Ebashi et al., 1960; Bendall, 1964) have shown that EDTA in the presence of $MgCl_2$ and ATP manifests a relaxing effect on glycerol treated fibers. Martonosi and Feretos (1964) explained the relaxing effect of chelating agents by their ability to lower the calcium concentration of the test system. In accord with the results cited above, the addition of EDTA in the present experiment appeared to form a complex with free calcium and thereby inhibited shortening.

Results from Experiment II show that the intravenous injection of either EDTA, Na Oxalate, EGTA, or CDTA significantly ($P < .05$) inhibited shortening of the semitendinosus muscle (Fig. 2). The decrease in the load on the isotonic lever of the kymograph in this experiment can account for the increased values for shortening in relation to those obtained in Experiment I. No significant difference was observed between the different treatments, but results show that CDTA (the poorest calcium chelator) had the least effect on decreasing shortening. In Experiment V, shortening (measured as the percentage of original length) was significantly less ($P < .05$) for pigs treated with EDTA (6.6%) than for control pigs (10.3%). These results support the premise that chelating agents complex enough free calcium to inhibit shortening.

Additional experiments were run to determine the effect of increased levels of calcium and magnesium upon shortening. The data in Table 1 show that micro-injections of $CaCl_2$ into the paired semitendinosus muscle

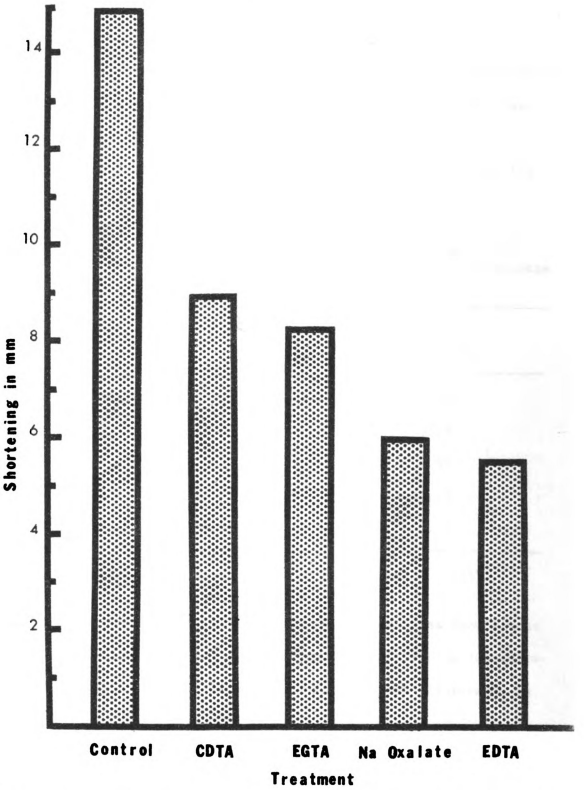

Fig. 3. Effect of chelating agents on the shortening of the semitendinosus muscle. The control consisted of an intravenous antemortem injection of isotonic saline and the treatments consisted of an intravenous antemortem injection of either CDTA, EGTA, Na oxalate or EDTA in isotonic saline.

(Experiment II) resulted in extensive shortening. Even after the injection of $CaCl_2$, however, the muscles from the treated animals shortened less than muscles from control animals. These results would suggest that chelating agents may form a complex with calcium thereby decreasing the shortening effect of the added calcium.

Table 1. Means and standard error of the means for shortening of the semitendinosus muscles of the rabbit for the various treatments in Experiment II.[1,2]

Treatment	Right semitendinosus uninjected	Left semitendinosus injected with $CaCl_2$
Control	14.96[a] ± 1.46	55.71 ± 7.13
CDTA	9.00[b] ± 1.33	32.17 ± 6.53
EGTA	8.25[b] ± 2.31	36.25 ± 7.99
Na Oxalate	6.00[b] ± 2.31	27.40 ± 11.33
EDTA	5.53[b] ± 1.46	31.88 ± 7.13

[1]Shortening in mm as measured on the kymograph.
[2]Means with different superscripts differ significantly ($P < .05$).

The values for shortening of the semitendinosus muscles from 4 control rabbits following micro-injections of $MgCl_2$ and different concentrations of $CaCl_2$ (Experiment III) are given in Table 2. The difference in shortening found on the addition of either 0.1M $MgCl_2$ or 0.1M $CaCl_2$ to the semitendinosus muscles from a rabbit are illustrated in Fig. 4. The data show that micro-injections of $MgCl_2$ had no significant effect on shortening during rigor mortis. These results are in agreement with those

of Caldwell and Walster (1963) who reported that an injection of $MgCl_2$ into the muscle fibers did not produce contraction.

Table 2. Effect of micro-injections of magnesium and calcium on shortening of the semitendinosus muscles of the control rabbits in Experiment III.[1]

Rabbit number	Right semitendinosus		Left semitendinosus	
	Uninjected	Injected with 0.1M $MgCl_2$	Injected with 0.01M $CaCl_2$	Injected with 0.1M $CaCl_2$
1		14		100
2	19		25	
3		18		54
4	13		18	
Average shortening	16	16	21.5	77

[1]Shortening in mm as measured on the kymograph.

The data in Table 2 demonstrate that an increased concentration of $CaCl_2$ within the muscle resulted in a definite increase in shortening during the development of rigor mortis. These results are in agreement with those of Niedergerke (1955) and Caldwell and Walster (1963), who showed that calcium injected into the sarcoplasm of a muscle fiber caused local contraction at the point of injection.

Results of the present experiments show that the reduction of free calcium in the muscle by chelating agents will reduce the shortening of the muscle during development of rigor mortis. On the other hand, it was also shown that increased levels of calcium resulted in increased shortening. However, increased levels of $MgCl_2$ had no effect on shortening.

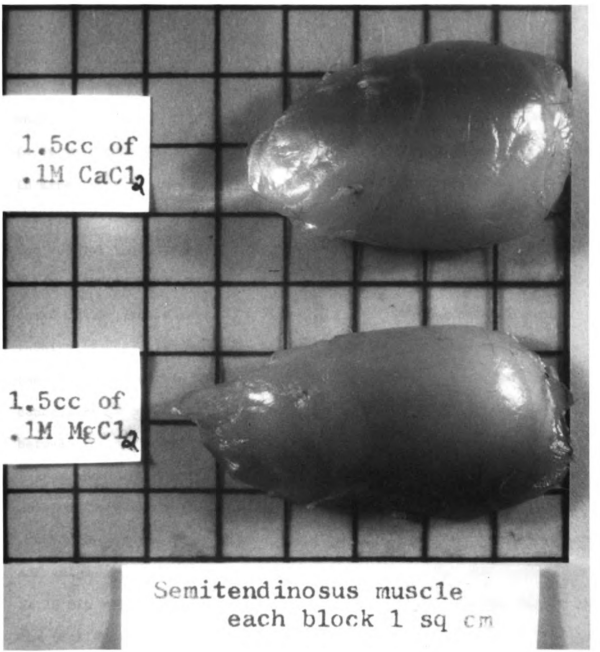

Fig. 4. The difference in shortening found following the addition of $MgCl_2$ and $CaCl_2$ to paired semitendinosus muscles. (Experiment III - rabbit number 1).

ATP

The data in Table 3 show that there was not any appreciable difference in ATP values of the longissimus dorsi muscle from EDTA treated (4.99 uM ATP/g) and control rabbits (5.13 uM ATP/g) at 0 hr post-mortem in Experiment I. Similar results were obtained in Experiment III where the muscles from the EDTA treated rabbits contained 4.34 uM ATP/g and the control rabbits contained 4.36 uM ATP/g. In Experiment II, the ATP values at 0 hr post-mortem ranged from 3.69 uM ATP/g in the longissimus dorsi muscle from the rabbits treated with EDTA to 8.12 uM ATP/g from rabbits treated with EGTA. However, because of the large variation in the ATP levels within treatments, these values only approached significance. At 20 or 24 hrs post-mortem, no consistent difference was observed between the different groups in either Experiment I, II or III. The values obtained in the present study are in agreement with the results obtained by Bendall (1951) and Bate-Smith and Bendall (1956).

In Experiment V, no significant difference was obtained between the ATP values of the semitendinosus muscle for the treated and control pigs at 10 min and 24 hrs post-mortem (Table 3). However, the ATP values for the pigs treated with EDTA were generally higher with values at 2.42 and 0.451 uM ATP/g at 10 min and 24 hr post-mortem, respectively, as compared to 1.95 and 0.308 uM ATP/g for the controls. The ATP values at 10 min post-mortem in this experiment (2.42 uM ATP/g for the EDTA treated and 1.95 uM ATP/g for the controls) are in accord with the value of 1.86 uM ATP/g reported by Aberle (1967) for the longissimus dorsi muscle of the pig at 15 min post-mortem. However, much higher values have been reported

Table 3. Means and standard error of the means for ATP for the various muscles and times in Experiment I, II, III and V.[1]

Experiment	Muscle	Time post-mortem	Treatment				
			Control	CDTA	EGTA	Na Oxalate	EDTA
I	Longissimus dorsi	0 hr	5.13				4.99
		20 hr	0.286				0.162
II	Longissimus dorsi	0 hr	5.972±1.04	6.872±0.95	8.120±1.16	5.515±1.64	3.69±1.04
		24 hr	0.142±0.015	0.121±0.015	0.105±0.018	0.131±0.026	0.131±0.015
	Right semitendinosus	7 hr	1.129±0.36	1.716±0.33	0.840±0.40	0.416±0.57	0.753±0.36
V	Semitendinosus	10 min	1.95 ± 0.33				2.42 ± 0.33
		24 hr	0.308± 0.13				0.451± 0.13
III	Longissimus dorsi	0 hr	4.36				4.34
		24 hr	0.126				0.108
	Right semitendinosus	7 hr	0.748				1.03
	Left semitendinosus[2]	7 hr					0.165

[1]u moles ATP/g muscle
[2]Injected with 0.1M $CaCl_2$ 15 min post-mortem

(5.6 - 8.0 uM ATP/g) for the longissimus dorsi muscle of the pig at the time of death (Lawrie, 1960; Bendall et al., 1963; Bodwell et al., 1966; Bendall, 1966). The time of post-mortem ATP extraction from the muscle could account for some of this difference. Furthermore, most of the high values for ATP were determined as labile phosphate, thus, part of the difference in these values may be explained by the lack of correspondence between ATP breakdown as determined by the enzymatic method and the appearance of inorganic phosphate (Nauss and Davies, 1966). Nauss and Davies (1966) explained the above discrepancy by showing an increase in the concentration of the other labile phosphate compounds, such as glucose-6-phosphate, fructose-6-phosphate and glucose-1-phosphate.

The ATP values reported in the present experiment with pork at 24 hrs post-mortem (0.451 and 0.308 uM ATP/g for the EDTA treated and control pigs, respectively) are consistently higher than those reported by Bodwell et al. (1966). These workers reported that one carcass contained 0.3 uM ATP/g at 24 hrs post-mortem, although all other carcasses showed a complete absence of ATP. The higher ATP levels observed in the present study may be the result of a different extraction procedure.

It should be mentioned that trouble was encountered during the injection of EDTA in the pigs #2 and #5. Consequently, these two pigs never obtained the relaxed state observed in the other treated pigs and were extremely excited at the time of slaughter. The values listed in Appendix III show that the ATP levels for these two pigs were the lowest of any of the treated animals at both 10 min and 24 hr post-mortem. A

comparison with other individual ATP values suggests that proper injection of EDTA resulted in higher ATP values at 10 min and 24 hrs post-mortem (Experiment V).

The difference in the effect of treatment on the ATP values observed for the rabbits and pigs may be explained by the fact that the injection of chelating agents into the rabbits resulted in extreme tetany with subsequent death. This was not observed for the treated pigs. They generally showed but slight tetany for a short period of time and then became very relaxed.

On using paired muscles from rabbits treated with EDTA (Experiment III), the data show a definite increase in the rate of ATP hydrolysis following the injection of $CaCl_2$ in the left semitendinosus muscle (Table 3). This effect may be explained by the presence of free calcium in the sarcoplasm resulting in the cyclic formation and breakage of the cross links between actin and myosin, which is accompanied by the enzymatic hydrolysis of ATP due to actomyosin ATPase (Nauss and Davies, 1966). In addition, Nauss and Davies (1966) pointed out that further breakdown of ATP occurs as a result of the ATPase activity of the sarcoplasmic reticulum, which is involved in the active uptake of free calcium from the sarcoplasm.

Extensibility of the semitendinosus muscle and its relationship to ATP level

Rigor has been described as a transformation from a system of actin and myosin, which is elastic, to a rigid system consisting of actomyosin, which is joined together by cross bridges between the actin and myosin (Nauss and Davies, 1966). Observations in Experiment I show that at 7 hrs post-mortem, the muscles from the control rabbits were rigid and inextensible (Table 4). However, the muscles from the treated rabbits were elastic and extensible and could be stretched easily to about 40% of their equilibrium length and would still snap back to their original length on being released.

Treatment had no consistent effect upon the elasticity or rigidity of the uninjected semitendinosus muscle as shown in Table 4 (Experiment II). Observations do show, however, that the paired left semitendinosus muscles from the rabbits in this experiment, which were injected with 0.1M $CaCl_2$, were always rigid and inextensible at 7 hrs post-mortem. Similar results were obtained in Experiment III (Table 4). These results indicate that an increase of free calcium in the sarcoplasm shortens the delay phase during the development of rigor mortis. Locker (1959) and Sink et al. (1965) reported that a short delay phase is associated with increased shortening of the muscle during the development of rigor. In accord with these results, it is concluded that an increase in the free calcium in the sarcoplasm is responsible for the shorter delay phase observed in the development of rigor and the increased shortening which occurred following the injection of $CaCl_2$.

Table 4. Effect of treatment on the elasticity or rigidity of muscle at 7 hrs post-mortem.

Experiment	Animal treatment	Leg	Treatment of semitendinosus muscle	Condition of muscle[1] Elastic	Rigid
I	Control	Right	uninjected		2
	EDTA	Right	uninjected	2	
II	Control	Right	uninjected	2	3
		Left	injected with 0.1M $CaCl_2$		5
	CDTA	Right	uninjected	3	3
		Left	injected with 0.1M $CaCl_2$		6
	EGTA	Right	uninjected	2	2
		Left	injected with 0.1M $CaCl_2$		4
	Na Oxalate	Right	uninjected	1	1
		Left	injected with 0.1M $CaCl_2$		2
	EDTA	Right	uninjected	3	2
		Left	injected with 0.1M $CaCl_2$		5
III	Control	Right	uninjected		2
		Right	injected with 0.1M $MgCl_2$	1	1
		Left	injected with 0.01M $CaCl_2$		2
		Left	injected with 0.1M $CaCl_2$		2
	EDTA	Right	uninjected	3	1
		Left	injected with 0.1M $CaCl_2$		4

[1]Number of semitendinosus muscles from each treatment possessing an elastic and extensible condition or a rigid and inextensible condition.

Huxley (1960) pointed out that the loss of ATP leads to the formation of fixed links between actin and myosin filaments as the muscle becomes rigid and inextensible. The rigid system maintains continuous tension in the absence of ATP breakdown (Nauss and Davies, 1966). In Experiment II, the level of ATP was compared between the elastic and the inextensible left semitendinosus muscles at 7 hrs post-mortem. It was found that the muscles which were elastic after 7 hrs post-mortem had significantly higher ($P < .01$) levels of ATP (1.544 uM/g) than those which were rigid and inextensible (0.388 uM/g). Bendall and Davey (1957) reported that with rabbit muscle held at 37°C, the onset of rigor occurred when 1/2 of the initial ATP was depleted. If held at room temperature, however, the onset of rigor occurred when 3/4 of the initial level was depleted.

Nauss and Davies (1966) found that as the muscle began to shorten, the rate of ^{45}Ca efflux increased and the ATP values fell. They suggested that the important chemical event determining the onset of the physical changes in a muscle passing into rigor seemed to be the internal liberation of a sufficient amount of calcium to initiate the interaction between actin and myosin. Results from the present experiments indicate that an intravenous injection of chelating agents will inhibit shortening, but does not always prevent the formation of some links between actin and myosin, which will eventually result in an inextensible condition of the muscle following the depletion of ATP.

The injection of $CaCl_2$ in these experiments increased shortening of the muscle and lowered the level of ATP at 7 hrs post-mortem. Thus, results support the postulation (Marsh, 1966; Newbold, 1966; Nauss and

Davies, 1966) that the release of calcium by the sarcoplasmic reticulum is responsible for the rapid ATP degradation and the subsequent stiffening of muscle during the development of rigor.

pH

Mean values for pH in Experiment I, II, III and V are listed in Table 5. The data from Experiment I and III show that there was no appreciable difference in pH values of the longissimus dorsi muscle from EDTA treated and control rabbits at either 0, 20 or 24 hr post-mortem. Injection of $CaCl_2$ into the muscle also had no significant effect upon pH values of the semitendinosus muscle at 7 hrs post-mortem.

In Experiment II, 0 hr post-mortem pH values for the longissimus dorsi muscle of rabbits treated with EDTA (6.31) were significantly lower ($P < .05$) than those recorded for the group treated with EGTA (6.67) and the control group (6.57). No significant differences in pH values were observed between the other groups at 0 hr post-mortem. The ultimate (24 hrs) pH values of the longissimus dorsi muscle did not differ significantly between treatments.

The data in Table 5 indicate that there was no significant difference in pH between the treated and untreated longissimus dorsi muscle of the pig at either 10 min or 24 hr post-mortem in Experiment V. The ultimate (36 hr) pH of the rectus femoris muscle did not differ significantly between treatments.

Table 5. Means and standard error of the means for pH of the various muscles and times in Experiment I, II, III and V.[1]

Experiment	Muscle	Time post-mortem	Treatments				
			Control	CDTA	EGTA	Na Oxalate	EDTA
I	Longissimus dorsi	0 hr	6.66				6.65
		20 hr	5.68				5.73
II	Longissimus dorsi	0 hr	$6.57^{a} \pm 0.07$	$6.49^{ab} \pm 0.06$	$6.67^{a} \pm 0.08$	$6.50^{ab} \pm 0.11$	$6.31^{b} \pm 0.07$
		24 hr	5.81 ± 0.04	5.77 ± 0.03	5.76 ± 0.05	5.72 ± 0.08	5.64 ± 0.04
V	Right semitendinosus	10 min	6.26 ± 0.09				6.34 ± 0.09
		24 hr	5.66 ± 0.08				5.69 ± 0.08
	Biceps femoris	36 hr	5.56 ± 0.07				5.55 ± 0.07
III	Longissimus dorsi	0 hr	6.48				6.37
		24 hr	5.82				5.65
	Right semitendinosus	7 hr	6.17				6.16
	Left semitendinosus[2]	7 hr					6.12

[1]Means with different superscripts are significantly different from each other ($P < .05$). Means without superscripts are not significantly different from each other.

[2]Injected with 0.1M $CaCl_2$ 15 min post-mortem.

Color score and water holding capacity

The data in Table 6 show that there was no significant difference between the subjective color score of the muscles from the pigs treated with EDTA (3.50) and from the control pigs (3.33). There was also no significant difference between the water holding capacity of the treated pigs (2.49) and the control pigs (2.60).

Table 6. Means and standard deviations for color score and water holding capacity ratios in pork muscle (Experiment V).

Treatment	Control	EDTA	Standard deviation
Color score[1]	3.33	3.50	0.67
WHC[2]	2.60	2.49	0.38

[1]Color was subjectively scored on the following 5-point scale: very pale (1); slightly pale (2); grayish pink (3); slightly dark (4); and very dark (5).
[2]Water holding capacity was determined by the press method. Results are expressed as a ratio of the total area to meat area.

Cooking losses and shear values

Mean values for cooking losses and shear force values in rabbit muscle from Experiment I and II are listed in Table 7. These data show that the difference in cooking losses between the rabbits treated with EDTA (22.3%) and the control rabbits (25.3%) in Experiment I approached significance at the 5% level. In Experiment II, however, the cooking losses for the rabbits treated with EDTA (30.0%) were the highest as compared to 27.6% for CDTA, 26.9% for Na Oxalate, 26.1% for the controls and 25.3% for EGTA.

Table 7. Means and standard error of means for cooking losses and shear values in rabbit muscle (Experiment I and II)

	Experiment I		Experiment II	
Treatment	Cooking loss[1]	Shear values[2]	Cooking loss	Shear values
Control	25.3 ± 0.92	6.38 ± 0.63	26.1 ± 1.16	5.64 ± 0.60
EDTA	22.3 ± 0.92	5.22 ± 0.63	30.0 ± 1.16	3.43 ± 0.60
CDTA			27.6 ± 1.06	4.76 ± 0.55
EGTA			25.3 ± 1.30	5.93 ± 0.68
Na Oxalate			26.9 ± 1.84	3.97 ± 0.96

[1]Expressed as % of uncooked weight.
[2]Shear force values expressed in pounds -- measured by the Warner-Bratzler shear using 12.5 mm cores.

No significant difference in shear values was observed between the different treatments in Experiment I and II. However, it should be noted that shear values of the longissimus dorsi muscle from the rabbits treated with EDTA (5.22 for Experiment I and 3.43 for Experiment II) were lower than the control groups (6.38 for Experiment I and 5.64 for Experiment II) in both experiments. The muscle from rabbits treated with CDTA (4.76) and Na Oxalate (3.97) also had slightly lower shear values than the control rabbits (5.64) in Experiment II.

Preliminary work using intravenous and intramuscular injections of EDTA solution into hams removed from pig carcasses at 30 min post-mortem (Experiment V) showed no effect on tenderness of the muscles as measured by the Warner-Bratzler shear. In an effort to overcome the apparent lack of permeability of the EDTA in this preliminary work, all additional experiments consisted of an intravenous injection of EDTA into the live animal.

The average cooking losses and shear force values for Experiment V are shown in Table 8. These data show no significant difference in cooking losses between treatments.

Table 8. Means and standard deviation for cooking losses and shear values in pork muscle in Experiment V.

Treatment	Control	EDTA	Standard deviation
Cooking Loss[1]			
Right ham slice	25.5	26.8	2.7
Left ham slice	27.6	27.7	3.7
Ave.	26.52	27.28	2.45
Shear Values[2]			
Ave. both hams	10.08	8.65**	1.22
Semimembranosus	9.54	7.93***	1.30
Rectus femoris	9.24	7.38*	1.78
Biceps femoris	11.31	10.50	1.33
Semitendinosus	10.32	8.93*	1.34

[1]Expressed as % of uncooked weight.
[2]Shear force values expressed in pounds -- measured by the Warner-Bratzler shear using 15 mm cores.
* $P < .10$
** $P < .05$
*** $P < .01$

The data in Table 8 show that the Warner-Bratzler shear values for the hams from the pigs treated with EDTA (8.65) were significantly lower ($P < .05$) than the shear values obtained for the control hams (10.08).

This is in agreement with the work of Locker (1960), Locker and Hagyard (1963) and Herring et al. (1965a) who have shown that beef muscles which shorten during rigor show a corresponding decrease in sarcomere length and tenderness. Marsh and Leet (1966a and 1966b) also reported that shortening of muscle during the development of rigor mortis results in a decrease in tenderness. In recalling the effect that EDTA had on the shortening of the semitendinosus muscle in these experiments, it may be stated that the improved tenderness of the treated hams is probably the result of less shortening of the muscle during rigor mortis.

Ramsey and Street (1940) and Gordon et al. (1964) have pointed out that the maximum tension that develops in muscles depends on the amount of overlap of the filaments. In accord with these results, the inhibition of shortening resulting from the removal of some of the free calcium by EDTA in these experiments most probably decreased the formation of complexes between actin and myosin and seems to be associated with the improvement in tenderness that was observed. This would support the postulation of Partmann (1963) suggesting that if the interaction between actin and myosin during rigor could be completely or partly impeded, tenderness may be improved.

The data in Table 8 also indicate that the effect of treatment on tenderness varied between muscles. The shear force values of the semimembranosus muscles from the pigs treated with EDTA (7.93) were significantly ($P < .01$) lower than the shear force values from the control pigs (9.54). A definite ($P < .10$) improvement in tenderness was also observed

for the rectus femoris (7.39) and the semitendinosus (8.93) muscles from the treated animals as compared to 9.24 and 10.32 for the controls, respectively. However, no significant difference was found between treatments for the biceps femoris muscle from the treated animals (10.50) and the controls (11.31).

From work with beef, Locker (1960), Herring et al. (1965a) and Marsh and Leet (1966a, 1966b) have concluded that the state of contraction is the major factor contributing to tenderness if the effect of connective tissue is small. The connective tissue may play a greater role in tenderness of the rectus femoris muscle (Experiment V) than for other muscles and therefore could explain the difference in the effect of EDTA treatment on tenderness of the different muscles. It is also possible that the difference in the effect of EDTA treatment on tenderness could be the result of differences in permeability of the EDTA in the different muscles.

Protein fractionation

Table 9 shows the averages for the nitrogen composition of the different fractions of the longissimus dorsi muscle of the rabbits for the five treatment groups in Experiment II at both 0 hr and 24 hr post-mortem. There was no appreciable change in the solubility of the sarcoplasmic fraction during the first 24 hrs post-mortem. There was, however, a definite increase in the amount of the fibrillar protein fraction extracted at high ionic strength (solution B) during the first 24 hrs post-mortem. According to Weinberg and Rose (1960), the precipitate

Table 9. Mean values and standard error of the mean for the nitrogen composition of the different fractions of the longissimus dorsi muscle from rabbits in Experiment II.[1,2]

Treatment	Sarcoplasmic protein nitrogen	Solution B[3]	Solution D[4]	Solution E[5]	NPN[6]	Solution C[7]
			0 hr post-mortem			
EDTA	10.65 ± 0.34	2.33[a] ± 0.11	1.92 ± 0.12	0.26 ± 0.03	4.61 ± 0.27	18.78 ± 0.61
Na Oxalate	10.45 ± 0.55	2.22[a] ± 0.17	1.85 ± 0.20	0.26 ± 0.06	4.59 ± 0.43	18.32 ± 0.96
EGTA	10.25 ± 0.38	2.01[ab] ± 0.12	1.76 ± 0.14	0.17 ± 0.04	4.96 ± 0.30	18.19 ± 0.68
Control	9.85 ± 0.34	1.93[ab] ± 0.11	1.63 ± 0.12	0.21 ± 0.03	4.85 ± 0.27	18.96 ± 0.61
CDTA	10.68 ± 0.34	1.77[b] ± 0.11	1.53 ± 0.12	0.25 ± 0.03	4.95 ± 0.27	18.75 ± 0.61
			24 hr post-mortem			
EDTA	10.64 ± 0.37	5.80 ± 1.75	2.00 ± 0.15	3.58 ± 1.53	4.56 ± 0.22	15.63 ± 1.82
Na Oxalate	9.75 ± 0.59	9.15 ± 2.78	1.99 ± 0.24	7.01 ± 2.43	4.40 ± 0.35	13.50 ± 2.9
EGTA	10.77 ± 0.41	2.48 ± 1.81	2.05 ± 0.17	0.56 ± 1.71	5.10 ± 0.25	19.53 ± 2.04
Control	9.65 ± 0.37	7.47 ± 1.75	2.10 ± 0.15	5.05 ± 1.53	4.78 ± 0.22	15.79 ± 1.82
CDTA	11.03 ± 0.37	5.66 ± 1.75	2.16 ± 0.15	3.49 ± 1.53	4.66 ± 0.22	17.36 ± 1.82

[1]Nitrogen composition mg N/g muscle.
[2]Means with different superscripts are significantly different from each other ($P < .05$). Means without superscripts are not significantly different from each other.
[3]The fibrillar protein fraction extracted at high ionic strength.
[4]The myosin fraction (soluble at u = 0.225)
[5]The actomyosin fraction (precipitated at u = 0.225)
[6]Non protein nitrogen
[7]The remaining soluble fibrillar protein fraction

formed upon the dilution of this fraction to an ionic strength of 0.225 is actomyosin. Thus, the increase in the amount of the fibrillar protein fraction extracted at high ionic strength can be accounted for by an increase in the formation of actomyosin (solution E).

Significantly less fibrillar protein extracted at a high ionic strength was obtained from the rabbits treated with CDTA (1.77 mg N/g) than for the rabbits treated with either EDTA (2.33 mg N/g) or Na Oxalate (2.22 mg N/g) at 10 min post-mortem. The other fractions at 10 min post-mortem did not differ significantly between treatments. The data in Table 9 indicate that there was no significant difference between treatments for any of the muscle fractions at 24 hrs post-mortem.

It should be pointed out, however, that there was a big difference in the actomyosin fraction of the various treatments at 24 hrs post-mortem. The rabbits treated with EGTA had the lowest concentration of actomyosin nitrogen with a value of 0.56 mg N/g as compared to 3.49 for the CDTA treated rabbits, 3.58 for the EDTA treated rabbits and 5.05 mg N/g for the control rabbits. These results suggest that the formation of actomyosin was inhibited by injecting chelating agents into the live animal.

Correlations

Simple correlation coefficients between pH and ATP in rabbit muscle (Experiment II) are presented in Table 10. These data indicate that significant relationships exist between 0 hr ATP values and pH values at slaughter ($P < .01$) and at 24 hr post-mortem ($P < .05$). A significant

relationship was also observed between pH at 0 hr and 24 hrs ($P < .05$). However, the data indicate that the relationships were not statistically significant between the ATP levels at 24 hrs post-mortem and the ATP levels at slaughter or pH at either 0 hr or 24 hrs post-mortem.

Table 10. Simple correlation coefficients between pH and ATP for rabbit muscle (Experiment II).

	pH 0 hr	pH 24 hr	ATP 0 hr
pH - 24 hr	.50*	--	--
ATP - 0 hr	.78**	.45*	--
ATP - 24 hr	.07	.22	-.01

* $P < .05$
**$P < .01$

Table 11 contains the simple correlation coefficients between pH and ATP for muscle from the pigs treated with EDTA and the controls in Experiment V. The pH values 10 min post-mortem for pigs treated with EDTA showed no significant relationship with pH and ATP values at 24 hrs post-mortem. The pH values at 24 hrs post-mortem also showed no significant relationship with the ATP values at 10 min and 24 hrs post-mortem for the treated pigs. For control pigs, however, 24 hr post-mortem pH values were significantly related ($P < .05$) to 10 min pH values and to ATP values at 10 min post-mortem. Also, for control pigs, a low correlation was observed between ATP at 24 hrs post-mortem and pH values at 10 min and 24 hrs post-mortem. The data in Table 11 also indicated that a significant relationship existed between ATP values at 10 min and 24 hrs for both treatments.

Table 11. Simple correlation coefficients between pH and ATP for muscle from pigs treated with EDTA and controls (Experiment V).

	pH - 10 min		pH - 24 hr		ATP - 10 min	
Treatment	EDTA	Control	EDTA	Control	EDTA	Control
pH - 24 hr	.33	.76*	--	--		
ATP - 10 min	.91**	.92**	-.04	.83*	--	--
ATP - 24 hr	.61	.47	-.02	.70	.78*	.76*

* $P < .05$
**$P < .01$

Correlation coefficients (Table 11) show significant relationships between ATP values at 10 min post-mortem and pH values at 10 min post-mortem ($P < .01$) for both the treated and control pigs. The high relationship found between ATP and pH values within 10 min post-mortem in Experiment II and V verify the results reported by Kastenschmidt et al. (1964) and Aberle (1967).

In attempting to assess the significance of the results of this experiment in relation to the development of rigor mortis, the role of ATP and its hydrolytic products on glycolysis should be established. Phosphofructokinase, the glycolytic enzyme catalyzing the conversion of fructose-1-phosphate to fructose-1,6-diphosphate, has been shown to be a rate limiting step in glycolysis (Regen et al., 1964; Özand and Narahara, 1964). Passonneau and Lowry (1962) and Mansour (1963) reported that the activity of this enzyme is strongly inhibited by ATP at concentrations normally present in the cell. However, the results of Passonneau and

Lowry (1962) and Regen et al. (1964) indicated that phosphofructokinase activity and subsequent glycolysis will increase if either the ATP concentration falls or the concentration of inorganic phosphate, adenosine diphosphate (ADP) or adenosine monophosphate (AMP) increases.

Regen et al. (1964) showed that a reduction of the contractile activity of the muscle as a result of a reduced concentration of calcium was always associated with a decrease in glycolysis. However, these workers found that when contractile activity is increased by electrical stimulation, phosphofructokinase inhibition was relieved and glycolysis increased. This was accompanied by a reduction in the ATP concentration as the amount of AMP and inorganic phosphate increased. A strong tetanic contraction on stimulation was observed by Hallund and Bendall (1965) and Bendall (1966) to result in a more rapid rate of glycolysis as indicated by an increase in the rate of pH fall. The same workers suggested that the high rates of glycolysis with the associated pH drop and occurrence of poor quality in pork is determined to a large extent by the long term after effect of the nervous stimuli which reached the muscle during the sticking process. The present experiment has shown that calcium chelating agents inhibited shortening while micro-injections of $CaCl_2$ resulted in increased shortening and an increased rate of ATP hydrolysis. Results of the present experiment suggest that the amount of calcium released following the nervous stimuli on slaughtering and the subsequent hydrolysis of ATP by the actomyosin and sarcoplasmic ATPase systems could be associated with the rate of ATP degradation and glycolysis.

Simple correlation coefficients between pH and ATP to muscle shortening, cooking losses and tenderness for rabbit muscle are presented in Table 12. The data indicate that no significant relationship existed between shortening of the right, uninjected semitendinosus muscle and pH and ATP values at either slaughter or at 24 hrs post-mortem. Similarly, the relationships between shortening of the left semitendinosus muscle injected with 0.1M $CaCl_2$ and of shear values to pH and ATP values were not significant at either 10 min or 24 hrs post-mortem. However, cooking losses were significantly related to pH and ATP values at slaughter ($P < .01$) and pH values at 24 hr post-mortem ($P < .05$). On the other hand, cooking losses were not related to 24 hr ATP values.

Table 12. Simple correlation coefficients between pH, ATP, muscle shortening, cooking losses and tenderness for rabbit muscle (Experiment II).

	pH - 0 hr	pH - 24 hr	ATP - 0 hr	ATP - 24 hr
Shortening right S.T.[1]	.24	.34	.16	.06
Shortening left S.T.[2]	.34	.21	.05	-.05
Cooking loss	-.72**	-.45*	.58**	.11
Shear values	.39	.16	.36	-.19

* $P < .05$
** $P < .01$
[1]Right semitendinosus muscle - uninjected.
[2]Left semitendinosus muscle - injected with 0.1M $CaCl_2$.

Table 13 contains simple correlation coefficients between pH, ATP, muscle shortening, color, cooking losses and tenderness for muscle from pigs treated with EDTA and the untreated controls (Experiment V). These

data indicate that there was no significant relationship between color score and pH and ATP values at either 10 min or 24 hrs post-mortem. The cooking losses for the control pigs were significantly related to pH values at both 10 min and 24 hrs post-mortem. This indicates that higher cooking losses were associated with lower pH values.

The data in Table 13 also show that pH values at 10 min and 24 hrs post-mortem were not significantly related to muscle shortening or shear values of the right and left hams. For the control pigs, no significant relationship was observed between ATP values at 10 min and 24 hrs post-mortem and muscle shortening or shear values of the right and left hams. However, the pigs treated with EDTA did show a significant relationship between ATP values at 24 hrs post-mortem and muscle shortening as well as with shear values of the right and left hams. These results indicate that the higher ATP values at 24 hrs post-mortem in the treated pigs was associated with less shortening of the semitendinosus muscle and a more tender product.

Table 13. Simple correlation coefficients between pH, ATP, muscle shortening, color, cooking losses and tenderness for muscle for control pigs or pigs treated with EDTA (Experiment V).

	pH - 10 min		pH - 24 hr		ATP - 10 min		ATP - 24 hr	
Treatment	EDTA	Control	EDTA	Control	EDTA	Control	EDTA	Control
Muscle shortening[1]	-.23	.12	.19	-.36	-.36	.34	-.83*	.64
Color score	.36	.20	.72	.16	.00	-.12	-.35	-.29
Cooking loss	-.37	-.97**	-.10	-.81*	-.62	.48	-.49	-.60
Shear values - right ham	-.50	.04	-.06	.31	-.68	.06	-.81*	.14
Shear values - left ham	-.56	-.06	-.12	.07	-.78*	-.15	-.78*	-.32

[1]Muscle shortening as a % of original length of the semitendinosus muscle.
* $P < .05$
** $P < .01$

Properties of Weber-Edsall Extracts in Experiment IV

ATPase activity and turbidity

The ATPase activity and changes in turbidity following the addition of ATP to Weber-Edsall extract were measured in the presence of 0.6M KCl with and without added $MgCl_2$. In the present experiment, the addition of ATP to the Weber-Edsall extract was followed by a high initial rate of ATPase activity, during which 50-75% of the ATP was hydrolyzed within 1 min. As shown in Fig. 5, the rapid hydrolysis of ATP was accompanied by an almost instantaneous decrease in the turbidity of the solution. Following this, the ATPase activity declined to a low rate. The turbidity remained relatively constant until the level of ATP decreased below 15%, at which time the turbidity increased. These results are in agreement with Weber and Hasselbach (1954) who reported a high initial ATPase activity in isolated myofibrils of rabbit muscle. They indicated that the high initial ATPase activity was not affected by the accumulation of end products, decreasing concentrations of ATP, impurity in the ATP solutions or by the concentration of myofibrils.

In Fig. 5 it can be seen that addition of $MgCl_2$ suppressed the ATPase activity of Weber-Edsall extract and extended the period of time during which the actomyosin was dissociated (clear phase). Kaldor and Gitlin (1963) also found that magnesium inhibited the myofibrillar ATPase activity, if 0.3M KCl was incorporated into the buffer. Present results agree with those of Noda and Maruyama (1958) who showed that addition of magnesium to a cleared actomyosin solution extended the time period for the clear phase.

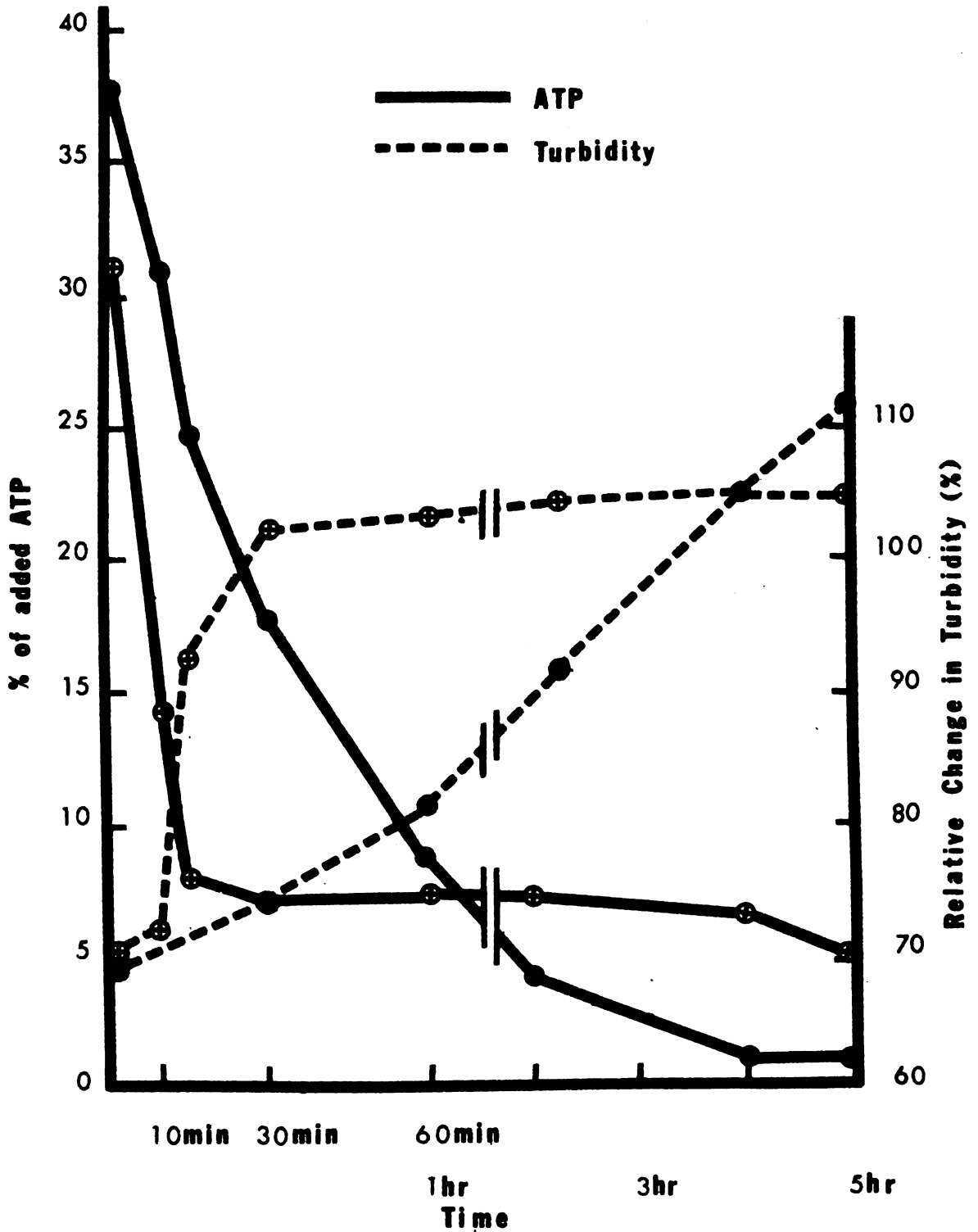

Fig. 5. Effect of $MgCl_2$ on the ATPase activity and relative changes in turbidity of pre-rigor Weber-Edsall extract at 25°C. (⊕) The reaction was started by adding 1 ml of 0.01M ATP (pH 6.4) to 24 ml of the diluted protein extract. (●) One ml of 0.01M $MgCl_2$ was added to 24 ml of the diluted protein extract and the reaction was started by adding 0.01M ATP.

Maruyama and Gergely (1962) and Ikemoto (1966) suggested that the low ATPase activity of the actomyosin solution in the presence of magnesium, as found in the present experiment, is attributable to the magnesium inhibited ATPase of the myosin moiety.

From the data in Table 14, it can be seen after five hours that the concentration of ATP in the absence of $MgCl_2$ was about five times as high as the level in the presence of $MgCl_2$. This may be explained by the high affinity magnesium has for myosin if present in high concentrations (Szent-Györgyi, 1951). Szent-Györgyi (1951) further indicated that bound magnesium ions result in the adsorption of ATP to the myosin molecule. Results of Levy and Ryan (1966) indicated that magnesium and ATP are bound to the hydrolytic site on myosin. Therefore, more complete hydrolysis of ATP in the presence of $MgCl_2$ may be the result of increased binding of ATP to the hydrolytic site.

Table 14. Effect of $MgCl_2$ on the ATPase activity of the Weber-Edsall extracts at 25°C. The reaction was started by adding 1 ml of 0.01M ATP (pH 6.4) to 24 ml of the diluted protein extract. When $MgCl_2$ was added, 1 ml of 0.01M $MgCl_2$ was added to 23 ml of the diluted protein extract and the reaction was started by adding ATP[1].

Post-mortem time of extraction	Added $MgCl_2$	Time after adding ATP[1]							
		1 min	10 min	15 min	20 min	1 hr	2 hr	4 hr	5 hr
0 hr	Yes	37.8	31.0	24.8	17.6	8.87	4.2	1.2	1.2
0 hr	No	31.2	14.6	8.1	7.0	7.8	7.5	6.6	5.0
24 hr	Yes	39.8	33.0	26.8	18.4	8.5	4.8	1.8	1.0
24 hr	No	32.6	22.5	11.8	9.1	10.1	5.5		6.2

[1]Values expressed as % of added ATP.

The ATPase and turbidity values for the different extraction times are shown in Tables 14 and 15. Except for the lower turbidity values at 1, 10 and 15 min for samples extracted 24 hrs post-mortem, no differences were observed in ATPase activity and turbidity between the Weber-Edsall extract isolated from pre-rigor muscle and muscle in rigor. Although there was some variation among the preparations from the six rabbits, there was not any consistent difference between samples from different treatments. These results suggest that differences observed between the properties of pre-rigor and rigor muscle are not a result of a change in the actomyosin ATPase activity or the ability of actomyosin to dissociate and the actin and myosin to form complexes with each other. Robson et al. (1966) also found little difference in enzymatic activity between myosin B isolated from pre-rigor, rigor or post-rigor muscle.

Table 15. Effect of $MgCl_2$ on the relative change in turbidity of the Weber-Edsall extracts at 25°C following the addition of ATP. The reaction was started by adding 1 ml of 0.01M ATP (pH 6.4) to 24 ml of the diluted protein extract. When $MgCl_2$ was added, 1 ml of 0.01M $MgCl_2$ was added to 23 ml of the diluted protein extract and the reaction was started by adding ATP.

Post-mortem time of extraction	Added $MgCl_2$	Time after adding ATP[1] 1 min	10 min	15 min	30 min	1 hr	2 hr	4 hr	5 hr
0 hr	Yes	68.9		72.6	74.3	81.8	91.5	105.8	112
0 hr	No	71.0	73.6	92.7	102.6	103.6	104.8	105.0	
24 hr	Yes	62.8	66.5	69.6	74.3	81.2	91.9	107.7	
24 hr	No	63.9	67.6	73.0	102.9	105.0	106.9		

[1]Values expressed as relative change in turbidity.

However, somewhat different results have been reported by Fujimaki et al. (1965). At low ionic strength, they showed that extracts of post-rigor muscle stored at 4°C for 2 days had higher ATPase activity than extracts of pre-rigor muscle.

Viscosity

The changes in viscosity following the addition of potassium pyrophosphate to the Weber-Edsall extract was measured in the presence of 0.6M KCl, with and without added $MgCl_2$. Preliminary results, (Fig. 6) showed that the decrease in viscosity was faster and more complete on adding $MgCl_2$. The change in viscosity was also faster and more complete at 3°C than at 23°C (Fig. 6). The data in Table 16 indicates very little difference in viscosity between Weber-Edsall extracts from pre-rigor and rigor muscle following the addition of pyrophosphate.

Table 16. The relative changes in viscosity of the Weber-Edsall extracts from pre-rigor and rigor muscle following the addition of pyrophosphate. The changes in viscosity were recorded at 3°C following the addition of 1 ml of 0.1M potassium pyrophosphate to 24 ml of the Weber-Edsall extract containing approximately 0.2% protein and 0.4 mM added $MgCl_2$.

Post-mortem time of extraction	Time		
	5 min	1 hr	5 hr
0 hr	47.0	47.0	47.0
24 hr	47.9	46.4	47.0

A number of investigators (Noda and Maruyama, 1958; Watanabe and Duke, 1963; Tonomura and Sekiza, 1961; Yasui et al., 1964; Mihalyi and

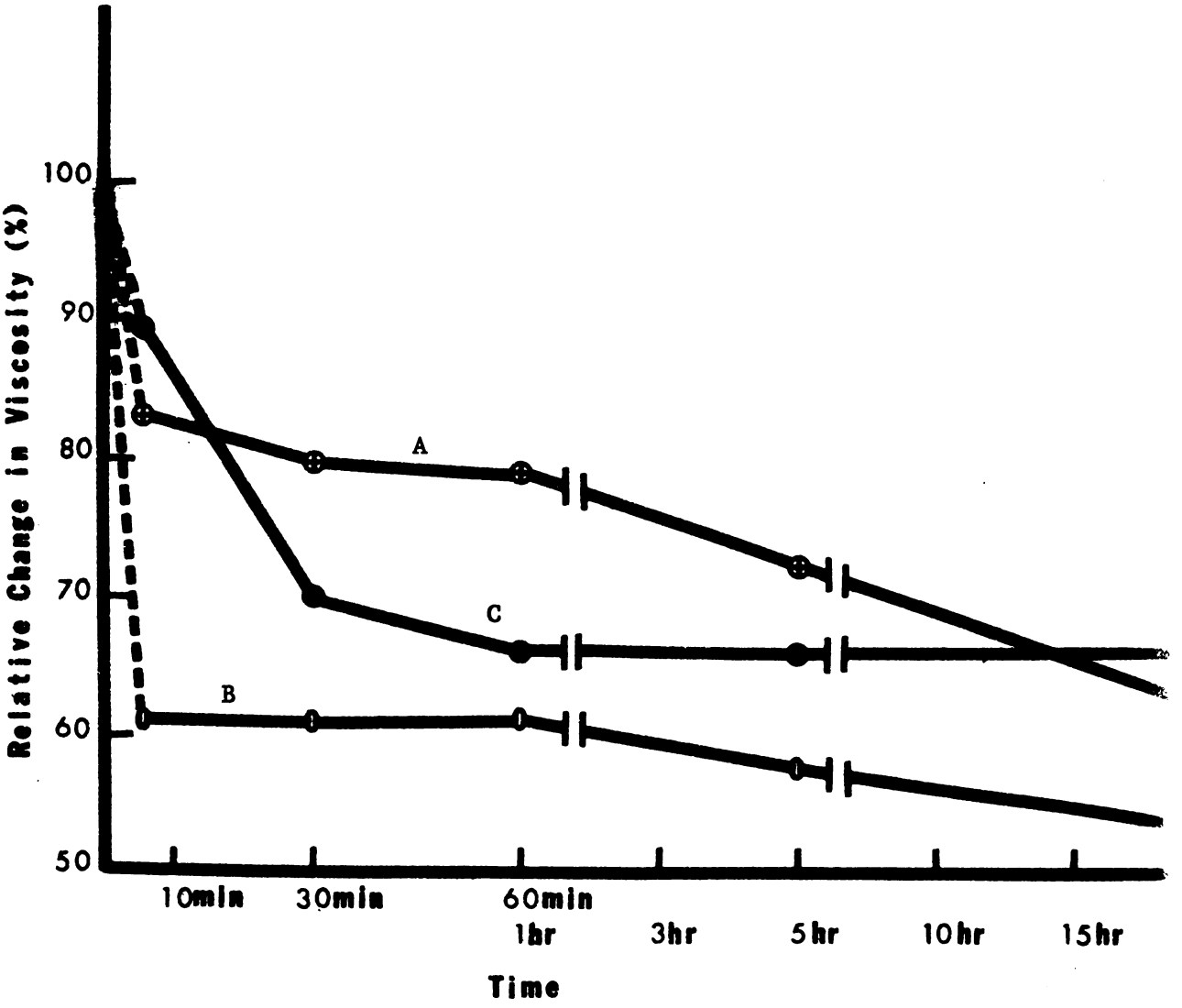

Fig. 6. Effect of $MgCl_2$ and temperature on the relative change in viscosity of 24 hr post-mortem Weber-Edsall extract (approximately 0.2% protein). (A) Viscosity was measured at 3°C following the addition of 1 ml of 0.1M potassium pyrophosphate to 24 ml protein extract. (B) One ml of 0.1M potassium pyrophosphate was added to 24 ml of the protein extract containing 1 ml of 0.01M $MgCl_2$. Viscosity was recorded at 3°C. (C) Same solution as for (B) but viscosity was recorded at 23°C.

Rowe, 1966) have shown that pyrophosphate in the presence of magnesium is as effective in clearing actomyosin as ATP. Results from this experiment (Fig. 7) also indicate that pyrophosphate in the presence of magnesium is as effective in clearing actomyosin as ATP. As the ATP was hydrolyzed, however, the viscosity of the solution returned to values greater than those obtained before the addition of ATP. On the other hand, samples cleared with pyrophosphate in the presence of magnesium indefinitely retained low viscosity characteristics.

The dissociating influence of the pyrophosphate system, as observed in this experiment, could be conditioned by the Mg-pyrophosphate complex (Granicher and Frick, 1965). Martonosi and Meyer (1964) concluded that the binding site for the pyrophosphate on myosin is identical with a portion of the ATPase center of myosin. Results of the present experiment suggest that pyrophosphate may be bound to the site on myosin that is responsible for the splitting of the actomyosin complex, but it is not involved in the formation of the complex formed between actin and myosin.

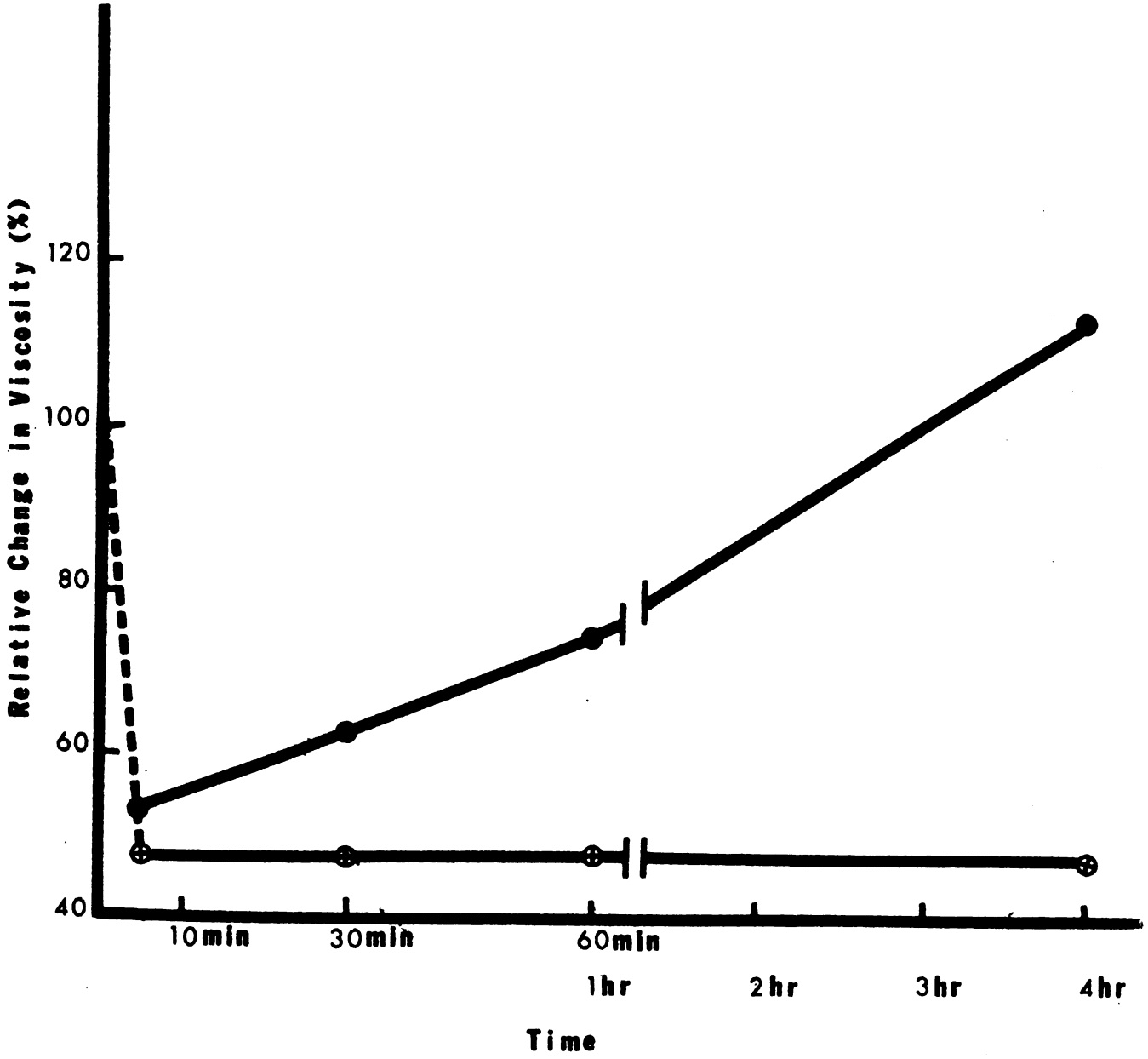

Fig. 7. The relative changes in viscosity of Weber-Edsall extracts from rigor muscle. One ml of 0.1M potassium pyrophosphate or 0.01M ATP (pH 6.4) was added to 24 ml of Weber-Edsall extract containing approximately 0.2% protein and 0.4 mM added $MgCl_2$. Changes in viscosity were recorded at 23°C for ATP (●) and at 3°C for potassium pyrophosphate (⊕).

SUMMARY

The effects of various chelating agents on the physical and chemical properties of muscles from rabbits and pigs were investigated in five different experiments. The effect of added calcium and magnesium on muscle properties was also studied.

The intravenous antemortem injection of various chelating agents (EDTA, CDTA, EGTA or Na Oxalate) significantly inhibited shortening of the semitendinosus muscle during the development of rigor in both rabbits and pigs. On the other hand, micro-injections of $CaCl_2$ into muscle resulted in a definite increase in shortening during the development of rigor. However, increased levels of $MgCl_2$ had no effect on shortening.

Chelating agents had no consistent effect on ATP levels of rabbit muscle. However, the ATP values for the pigs treated with EDTA were generally higher than for the controls, but the differences were not significant. There was a definite increase in the rate of ATP hydrolysis following the micro-injection of $CaCl_2$ into the semitendinosus muscle.

Antemortem injection of chelating agents had no consistent effect upon either the elasticity or rigidity of uninjected muscles at 7 hrs post-mortem. Muscles, which were elastic after 7 hrs post-mortem, had significantly higher ($P < .01$) levels of ATP than those that were rigid and inextensible. Paired semitendinosus muscles injected with 0.1M $CaCl_2$ were always rigid and inextensible at 7 hrs post-mortem.

No consistent differences in pH values were observed between treatments. In Experiment II, however, pH values for muscles from rabbits

treated with EDTA were significantly lower ($P < .05$) than for the controls, or those treated with EGTA. Treatment had no significant effect on subjective color score or water holding capacity.

Antemortem treatment had no significant effect upon cooking losses. Shear values of the longissimus dorsi muscle for EDTA-treated rabbits were lower than those of the control groups, but the differences were not significant. Antemortem injection of pigs with EDTA resulted in a marked improvement in tenderness as shown by significantly lower ($P < .05$) shear values of the ham muscles. The effect of treatment on tenderness varied between muscles.

Although there was no appreciable change in the solubility of the sarcoplasmic protein fraction during the first 24 hrs post-mortem, there was a definite increase in the amount of fibrillar protein extracted at high ionic strength. This could be accounted for by an increase in the formation of actomyosin. Muscles treated with either EGTA, CDTA or EDTA had less actomyosin at 24 hrs post-mortem than the controls, but the differences were not significant.

A highly significant relationship ($P < .01$) was found between ATP and pH values within 10 min post-mortem for muscles from rabbits and from both treated and control pigs. The treated pigs had higher ATP values at 24 hrs post-mortem, which were significantly ($P < .05$) and negatively related to shortening and shear values.

No consistent differences were observed in ATPase activity, turbidity and viscosity between the Weber-Edsall extract isolated from pre-rigor

muscle or muscle in rigor. The addition of $MgCl_2$ decreased the rate of ATP hydrolysis and extended the clear phase of the Weber-Edsall extract. Pyrophosphate in the presence of magnesium was as effective in clearing actomyosin as ATP. As the ATP was hydrolyzed, the viscosity of the solution returned to values greater than those obtained before the addition of ATP. However, samples cleared with pyrophosphate retained their low viscosity characteristics indefinitely.

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APPENDIX

Appendix I. Experiment II - Physical and chemical properties of rabbit muscle.

Treatment	Animal No.	X(13)[1]	X(14)[2]	X(15)[3]	X(16)[4]	X(17)[5]	X(18)[6]	X(19)[7]	X(20)[8]	X(23)[9]
EGTA	46	6.75	4.0	15.0	11.41	.106	0.375	6.80	5.80	24.2
	47	6.90	10.0	20.0	8.12	.109	0.347	6.70	5.65	27.6
	48	5.85	9.0	45.0	6.74	.093	1.213	6.50	5.80	27.4
	60	4.23	10.0	65.0	6.21	.112	1.424	6.70	5.80	21.8
CDTA	50	4.32	10.0	12.0	6.06	.120	3.870	6.50	5.90	30.1
	52	4.93	4.5	20.0	4.91	.073	1.136	6.35	5.75	26.0
	53	2.95	18.0	47.0	7.94	.118	3.390	6.50	5.80	29.2
	58	5.27	7.0	34.0	12.65	.182	0.747	6.70	5.90	24.3
	59	4.97	6.5	41.0	2.75	.117	0.383	6.30	5.60	29.6
	61	6.27	8.0	39.0	6.92	.116	0.775	6.60	5.70	26.5
EDTA	66	3.02	6.0	30.0	3.59	.139	0.375	6.30	5.80	28.8
	68	2.92	7.0	40.0	4.15	.146	2.370	6.45	5.60	31.6
	69	3.90	6.0	16.0	1.43	.118	0.165	6.05	5.70	31.7
	72	3.45	3.0	61.0	5.72	.102	0.516	6.60	5.70	26.1
	73	3.52	6.5	18.0	3.31	.141	0.920	6.15	5.40	31.8
Na Oxalate	70	3.50	6.0	52.0	4.95	.107	0.383	6.50	5.75	29.6
	71	4.45	6.0	29.0	6.08	.124	0.449	6.50	5.70	24.2
Control	49	4.33	19.0	72.0	7.03	.101	2.140	6.55	5.80	23.8
	56	7.93	14.0	46.0	5.60	.124	0.818	6.40	5.80	28.0
	57	4.00	11.0	40.0	4.39	.256	0.411	6.60	5.90	27.3
	62	8.40	17.0	55.0	5.80	.116	0.402	6.70	5.80	23.6
	65	3.88	17.0	60.0	7.29	.129	1.296	6.60	5.80	27.5

[1]Shear force values
[2]Shortening of right semitendinosus muscle.
[3]Shortening of left semitendinosus muscle injected with 0.1M $CaCl_2$.
[4]ATP values (uM/g) longissimus dorsi 0 hr
[5]ATP values (uM/g) longissimus dorsi 24 hr
[6]ATP values (uM/g) semitendinosus 7 hr
[7]pH 0 hr
[8]pH 24 hr
[9]Cooking loss expressed as % of original weight

Appendix II. Experiment II - Protein fractionation of the longissimus dorsi muscle of the rabbit at 0 and 24 hr post-mortem expressed as mg N/g tissue.

Treatment	Animal No.	0 hr post-mortem						24 hrs post-mortem					
		X(1)[1]	X(2)[2]	X(3)[3]	X(4)[4]	X(5)[5]	X(6)[6]	X(7)[1]	X(8)[2]	X(9)[3]	X(10)[4]	X(11)[5]	X(12)[6]
EGTA	46	10.94	1.83	1.80	0.11	4.85	17.39	10.02	2.48	2.47	0.54	5.08	19.67
	47	10.25	2.01	1.76	0.17	4.96	18.19	11.43	2.32	2.18	0.34	5.21	18.34
	48	9.83	2.23	1.96	0.22	4.92	17.59	10.43	2.18	1.61	0.39	4.91	20.32
	60	9.98	1.96	1.51	0.18	5.12	19.58	11.24	2.92	1.94	0.98	5.18	19.84
CDTA	52	10.00	1.85	1.68	0.20	5.40	17.20	10.48	4.65	2.07	2.57	4.68	18.96
	53	11.11	1.60	1.37	0.32	3.28	18.60	10.52	8.62	1.65	6.26	3.94	14.68
	58	10.17	2.09	1.80	0.18	5.40	19.60	10.90	7.27	2.72	4.99	5.32	14.32
	59	11.13	1.80	1.39	0.44	5.94	17.07	11.39	5.68	2.66	2.94	5.29	17.32
	61	11.05	1.46	1.27	0.11	4.79	21.18	11.83	2.47	1.82	1.08	4.18	20.55
EDTA	66	10.24	2.21	1.80	0.21	3.73	16.45	10.60	4.20	1.84	2.18	4.19	14.43
	68	10.04	2.23	1.92	0.34	4.73	19.87	11.11	12.21	2.44	8.71	4.73	10.11
	69	11.45	2.06	1.45	0.23	4.75	19.13	10.75	2.40	1.72	0.94	4.05	19.23
	72	10.79	2.84	2.49	0.31	5.18	20.50	10.54	4.57	2.07	2.44	5.01	18.44
	73	10.66	2.33	2.05	0.23	4.62	18.10	10.26	5.21	1.81	3.27	4.70	16.96
Na Oxalate	70	9.78	2.07	2.01	0.27	4.61	18.22	9.45	5.69	2.03	3.37	4.42	17.48
	71	10.60	2.41	1.71	0.24	4.56	18.43	10.05	12.60	2.42	10.66	4.36	9.53
Control	49	10.34	2.01	1.86	0.15	4.60	19.16	9.93	2.36	2.22	0.63	3.70	19.26
	56	10.51	1.63	1.75	0.22	5.07	19.03	11.11	5.52	1.77	3.78	4.93	19.52
	57	10.71	1.94	1.01	0.20	4.85	18.53	10.45	13.46	2.40	10.25	4.98	8.53
	62	7.79	1.97	1.52	0.23	5.05	20.19	7.68	2.56	2.05	.52	5.21	21.41
	65	9.95	2.14	1.84	0.17	4.69	17.76	9.04	13.78	2.23	10.52	5.20	9.20

[1]Sarcoplasmic protein fraction
[2]Fibrillar protein fraction extracted at high ionic strength
[3]Myosin fraction (soluble at u = 0.225)
[4]Actomyosin fraction (precipitate at u = 0.225)
[5]Non protein nitrogen
[6]Remaining fibrillar protein fraction soluble in 0.1N NaOH

Appendix III. Experiment V - Physical and chemical properties of pig muscle.

Animal No.	Color score	pH			ATP (uM/gm)		WHC[2]	Shortening[3]
		0 hr	24 hr	B.F.[1]	0 hr	24 hr		
				Pigs treated with EDTA				
1	3	6.45	5.85	5.6	3.079	.939	2.60	1.9
2	5	6.42	6.11	5.7	2.003	.146	2.72	10.4
3	4	6.55	5.57	5.3	3.268	.597	2.40	7.2
4	3	6.19	5.55	5.5	2.250	.223	2.52	10.4
5	3	5.94	5.50	5.6	.859	.118	2.24	6.0
6	3	6.53	5.54	5.6	3.081	.613	2.46	3.7
				Control pigs				
7	3	6.22	5.72	5.5	2.195	.747	3.01	12.9
8	4	6.37	5.80	5.5	2.106	.319	2.39	8.8
9	3	6.08	5.60	5.4	1.557	.107	2.56	10.7
10	3	6.57	5.75	5.5	2.757	.481	2.72	10.6
11	3	6.05	5.50	5.7	1.443	.099	2.27	8.1
12	4	6.24	5.56	5.8	1.641	.093	2.70	10.8

[1]pH of ficeps femoris 36 hrs post-mortem
[2]Water holding capacity expressed as a ratio of the total area to meat area.
[3]Shortening measured as a % of original length.

Appendix IV. Experiment V - Cooking data and shear values of the center ham slice.

Animal No.	Right ham				Left ham				
	% cooking[1] loss	Shear values			% cooking[1] loss	Shear values			
		B.F.[2]	R.F.[3]	S.M.[4]		B.F.[2]	R.F.[3]	S.M.[4]	S.T.[5]
				Pigs treated with EDTA					
1	21.50	9.40	6.40	7.20	26.0	7.20	6.70	7.60	7.40
2	28.50	13.12	5.70	8.12	29.4	12.90	7.60	8.08	8.97
3	24.40	10.52	7.17	8.95	28.5	9.00	7.97	8.42	9.72
4	27.10	12.22	5.60	7.70	20.5	10.65	6.83	6.80	8.76
5	28.60	10.30	9.80	7.60	35.0	10.50	10.40	10.05	10.00
6	30.90	10.20	6.87	7.00	26.9	10.00	7.60	7.60	8.73
				Control pigs					
7	22.40	12.70	9.90	9.50	30.4	10.50	8.00	8.20	10.2
8	26.30	11.25	8.63	11.05	24.6	10.82	8.67	10.28	10.97
9	26.70	13.22	11.57	9.65	29.8	12.67	12.90	10.60	12.12
10	24.40	11.62	7.23	10.45	23.4	11.22	8.63	9.27	9.73
11	25.60	10.15	6.73	7.60	30.0	9.90	8.50	6.95	8.00
12	27.40	11.00	10.30	10.70	27.2	10.70	9.77	10.25	10.90

[1]Cooking loss expressed as % of original weight
[2]Biceps femoris
[3]Rectus femoris
[4]Semimembranosus
[5]Semitendinosus